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6-Aminopenicillanic acid (6-APA) derivatives equipped with anchoring arms

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ABSTRACT

6-APA derivatives were considered as selective labels for the construction of bifunctional linkers dedicated to the oriented immobilization of proteins on materials. Sulbactam-like compounds (i.e., 6-β-sulfonamido-penam sulfones) and penicillin G—like compounds (i.e., *para*-substituted 6-β-phenylacetamido-penams) were prepared and tested as irreversible inhibitors of representative β-lactamases and D,D-peptidases, respectively. The activity of the modified antibiotics was preserved despite their substitution with various anchoring arms. The (2-nitro-4,5-dimethoxy)-benzyl esters revealed of particular interest due to their capacity to acylate BlaR-CTD without deprotection.

1. Introduction

The ability to pattern surfaces with functional organic (mono) layers has became the focus of numerous studies because of the potential applications in various fields, such as microelectronics and (bio)sensors.[1] Several diagnosis methods and drug discovery techniques are currently based on the immobilization of proteins on solid supports.[2–5] The design of protein-chips requires the development of efficient attachment methods, and for the array format, the concomitant development of patterning methods.[6,7] Generally, proteins are fixed in random orientations. However, increasing attention is put on the controlled orientation issue. For instance, the use of recombinant affinity tags and expressed protein ligation have been proposed.[8]

We are interested in the oriented immobilization of class A and class C β-lactamases (BLs) and Penicillin-Binding Proteins (PBPs) (or selected mutants thereof) onto metal or silicon supports for the construction of novel protein-arrays. These proteins are bacterial serine-enzymes interacting with β-lactam antibiotics.[9,10] This interaction leads to acyl-enzyme intermediates, which are very unstable in the case of BLs and are naturally quite stable for PBPs. For some β-lactam compounds the acyl-enzyme intermediate rearranges into permanently stable entities, in the case of so-called 'suicide-inhibition' (irreversible inhibition) for both BLs and PBPs. Hence, the active site could be considered as a valuable anchoring point for the regular arrangement of such proteins on surfaces. This strategy relies upon the availability of dedicated molecular tools, i.e., heterotelechelic spacers[11] featuring at one terminus a reactive function for grafting on the support, and at the other terminus a β-lactam motif susceptible to fix on the serine residue of the active site.

In this article, we fully describe the construction of original 6-APA (6-aminopenicillanic acid) derivatives designed to interact with BLs and PBPs in this context of tools for materials science. Accordingly, the C_6 lateral chain typical of antibiotics has been replaced with different spacer-arms for surface-grafting. The effect of the C_6 modifications on the activity of the resulting penicillin-like compounds versus representative target enzymes has been examined.

2. Synthesis

2.1. Sulbactam-like compounds

6-β-Sulfonamido-penam sulfones are structurally related to sulbactam and behave similarly as suicide inhibitors of β-lactamases, the defence enzymes produced by resistant bacteria.[12] We have thus decided to equip the bicylic framework with spacer-arms positioned on the C_6 sulfonamide lateral chain. Our starting material was the pivaloyloxymethyl ester (PIV) or the ethoxymethyl ester (EOM) of 6-APA, which could be deprotected in situ by esterases or hydrolysis in water–methanol medium,[13,14] respectively. Indeed, the chemistry of penam derivatives is easier to perform with C_3 ester compounds than with C_3 free acids.[15,16] The selected

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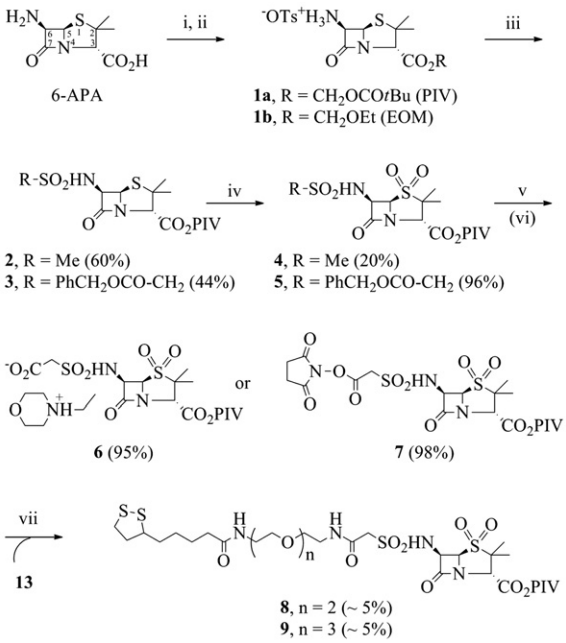
function for further immobilization on Au and Ag supports was the disulfide function of thioctic acid (TA).[17,18] The two end-motifs, namely penam-sulfone and TA, were linked via a few ethylene glycol units (named PEG, for polyethylene glycol) to ensure water-solubility of the final construct.[11]

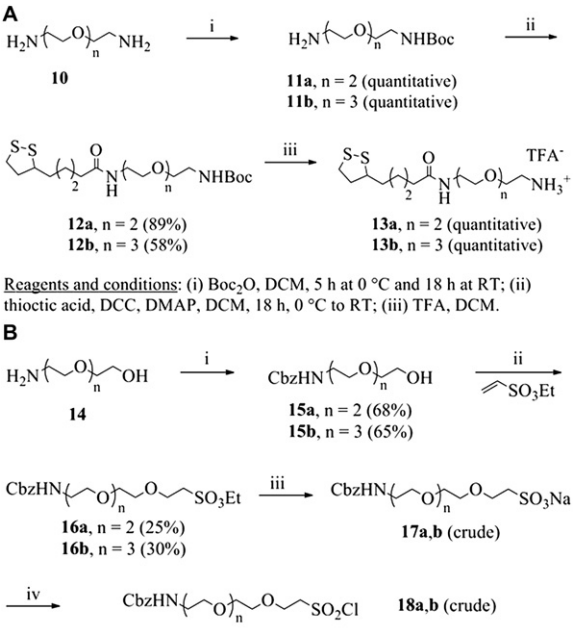
The first strategy we envisaged (method A; Scheme 1) was based on 6-[(hydroxycarbonyl)methylsulfony]-aminopenicillinate-*S*,*S*-dioxide (**6**)[19] as key intermediate for the coupling with the TA spacers (**13**) independently prepared (Scheme 2A). The pivaloyloxymethyl (PIV) ester of 6-APA (**1a**), crystallized as the tosylate salt, reacted readily with methanesulfonyl chloride and triethylamine to afford the methanesulfonamide **2** in 60% yield. The oxidation with $KMnO_4$ in aqueous acetic acid, at low temperature, led to the expected sulfone **4**, but in low yield due to loss of product during the work-up. A similar sequence of reactions was applied using benzyl chlorosulfonylacetate freshly prepared from sulfoacetyl dichloride and benzyl alcohol.[12] We isolated 6-[(benzyloxycarbonyl)methylsulfonyl]-aminopenicillinate **3** and the corresponding sulfone **4** in 44% and 96% yields, respectively. Hydrogenolysis was performed in the presence of *N*-ethylmorpholine (NEM) to deprotect the benzyl ester; the salt **6** could be stored in the freezer without degradation. Hydrogenation of **5** in the absence of NEM, immediately followed by reaction with *N*-hydroxysuccinimide (NHS) and dicyclohexylcarbodiimide (DCC) furnished quantitatively the activated ester **7**, which was stable under careful storage conditions.



Reagents and conditions: (i) $ClCH_2OCOtBu$, Et_3N, DMF, 4 h, RT or $ClCH_2OEt$, Et_3N, CH_3CN, 4 h, RT; (ii) PTSA, acetone; (iii) $MeSO_2Cl$ or BnO_2C-CH_2SO_2Cl, Et_3N, DCM, 20 min at 0 °C and 5 min at RT; (iv) $KMnO_4$, AcOH / H_2O (4:1), 1 h, -10 °C; (v) H_2, Pd / C, EtOH / EtOAc (4:1), 1 h, RT; (vi) N-ethylmorpholine EtOH / EtOAc (4:1) or NHS, DCC, DCM, 16 h, RT; (vii) HBTU, N-ethylmorpholine, DMF, 3 h, TA.

Scheme 1. Synthesis of 6-β-sulfonamido-penam sulfones equipped with a side-chain for Au (Ag) surface-grafting (method A).

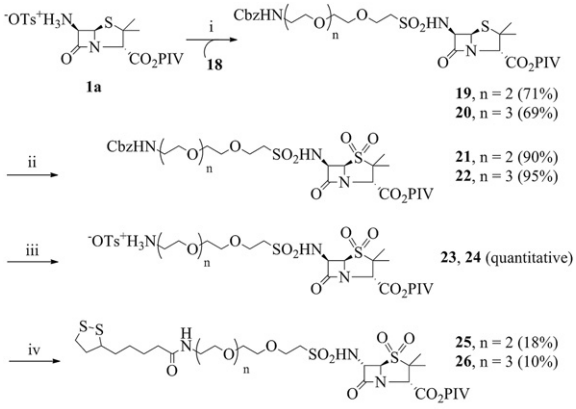
The thioctic spacers were prepared in three steps, from the commercially available diamines **10**: Boc-monoprotection, coupling to TA with DCC and dimethylaminopyridine (DMAP) as the catalyst, and Boc-deprotection with trifluoroacetic acid (TFA) gave **13a** and **13b** in about 90% and 60% yields, respectively. Several attempts of coupling the fragments **13** and **7** were performed, under various experimental conditions (different bases, different solvents, variation of time and temperature), but without success. Similar



Scheme 2. Synthesis of spacer-arms (part I).

disappointing results were collected when applying peptide synthesis methods to the coupling of **13** and **6**: reactions in the presence of PyBOP ((benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate), EDCI (1-(3-dimethylamino-propyl)-3-ethylcarbodiimide hydrochloride) or HBTU (*O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate), with triethylamine (TEA) or NEM, in dichloromethane (DCM), dimethylformamide (DMF) or mixtures of DCE and DMF, at low temperature (−20 °C to 0 °C) or room temperature, led to complex crude mixtures that were very difficult to purify by chromatography. Tenuous yields of target-molecules **8** and **9** were recovered by using HBTU as coupling agent. From the ^1H NMR data, a 80:20 mixture of epimers was visible (5,6-*cis* and 5,6-*trans*).

Therefore, we considered a second strategy (method B, Scheme 3) based on the coupling of short sulfonated hetero-telechelic PEG spacers (**18**, Scheme 2B) to 6-APA, followed by oxidation into sulfones and fixation of thioctic acid in the last step. Thus the required sulfonyl-terminated PEGs were independently prepared in four steps from the amino-alcohols **14**. The *N*-benzyloxycarbonyl-protected molecules **15**, reacted with ethyl vinylsulfonate (Michael addition) to furnish **16** in moderate yields (Scheme 2B). Then, the sulfonate esters were transformed into sodium sulfonates (**17**) and sulfonyl chlorides (**18**) according to a protocol from the literature.[20] These reactive species were directly engaged with the 6-APA derivative **1a** to afford the sulfonamides **19**,**20** in good yields (~70% from **6a**,**b**). $KMnO_4$ oxidation (**21**,**22**) followed by hydrogenolysis led quantitatively to the key-intermediates **23**,**24**, which are stable only in the form of tosylate salts. The final coupling was performed with the *N*-hydroxysuccinimide ester of TA,[11] in the presence of NEM as base. Due to material loss during the chromatographic purifications, low yields of target-molecules **25**,**26** were obtained. From the ^1H NMR data, the 5,6-*trans* epimers were isolated. Indeed the coupling constant of the β-lactamic protons H_5 and H_6 (see Table S1 in SD) was in the range of 1.3–1.5 Hz (trans relationship) instead of 4.3–4.6 Hz (cis relationship). The intrinsic tendency of penam-sulfones to epimerize[15,16] is enhanced in the basic medium used for the TA coupling.



Reagents and conditions: (i) Et_3N, DCM, 1 h, 0 °C to RT; (ii) $KMnO_4$, AcOH / H_2O (4:1), 1 h, -10 °C; (iii) H_2, Pd / C, APTS, EtOH / AtOAC (4:1), 1 h, RT; (iv) NHS ester of TA, N-ethylmorpholine, DMF, 24 h, RT.

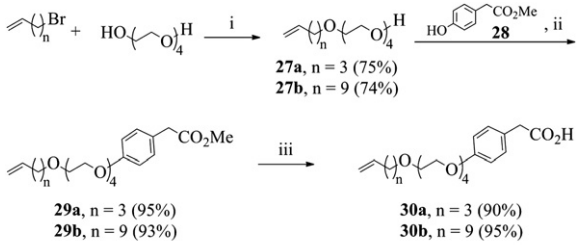
Scheme 3. Synthesis of 6-β-sulfonamido-penam sulfones equipped with a side-chain for Au (Ag) surface-grafting (method B).

We tested the use of ethoxymethyl (EOM) ester of 6-APA (**1b**, see Supplementary data) as starting material for the application of Schemes 1 and 3. The problems encountered in the PIV series (low yields, hard purifications) were even more serious in the EOM series! The synthesis of the target-molecules (i.e., **8**,**9** and **25**,**26** in series b) could not be achieved efficiently. However, some intermediates were obtained (see Supplementary data) and considered for the biological evaluations.

2.2. Benzylpenicillin-like compounds

Benzylpenicillin, also named Pen G: the molecule discovered by A. Fleming in 1928, is an inhibitor of D,D-carboxypeptidases involved in the bacterial cell-wall biosynthesis. All the semi-synthetic penicillins differ by the nature of their C_6 aminoacyl side-chains.[15] Here, we propose to substitute the *para*-position of the Pen G benzyl group with the anchoring arms. We considered as starting material, a photo-cleavable ester derivative of 6-APA, namely the 2-nitro-4,5-(dimethoxy)-benzyl ester.[21] The application of UV light is a mild technique of deprotection compatible with the penam stability. This also offers the possibility of surface patterning by irradiation through a mask or by lithography.[22,23] The selected function for Si or Ge supports derivatization was an alkene group susceptible to make a hydrosilylation (hydrogermylation) reaction with reduced Si (Ge) surfaces.[24,25] This function could also be involved in thiol-ene click chemistry.[26,27] As previously, the link between the penam motif and the alkene ending was made of a short PEG chain.

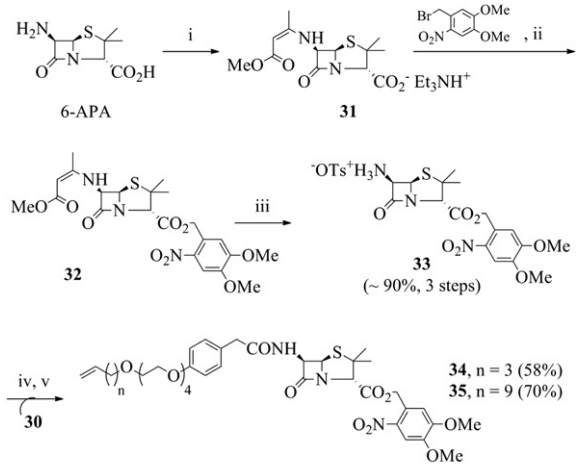
Methyl (4-hydroxyphenyl)acetate (**28**) was used as precursor for the synthesis of modified Pen G side-chains (Scheme 4). Indeed, alcohol derivatives (**27**) can be easily coupled to the phenol moiety via a Mitsunobu reaction. Tetraethylene glycol was monoalkylated with 1-bromo-ϖ-alkenes in concentrated sodium hydroxide solution as previously described in the literature.[28] We considered two different lengths of alkenyl chain, giving the spacers **27a** (n=3) and **27b** (n=9). Their reaction with **28** in the presence of diisopropyl azodicarboxylate (DIAD) and triphenylphosphine gave the intermediates **29a**,**b**, which saponification led to the corresponding acids **30a**,**b** with 64% and 65% overall yields (three steps). In the Mistsunobu coupling, the order of addition of reagents could be of prime importance. Here, we have modified a protocol developed by Townsend and Salituro[29] in the case of nocardicin side-chain synthesis, for obtaining >90% yield of **29a**,**b**. It is worthy of note that the Williamson coupling between **28** and the mesylates derived from alcohols **27a**,**b** (K_2CO_3, CH_3CN, reflux, several days) gave low yields of **29a**,**b** (<30%).



Reagents and conditions: (i) NaOHaq, THF, reflux; (ii) Ph_3P, DIAD, THF, 20 °C; (iii) NaOH, MeOH, 20 °C.

Scheme 4. Synthesis of spacer-arms (part II) coupled to the side-chain of Penicillin.

6-APA was *N*-protected using ethyl acetoacetate (**31**)[30] and then esterified with (2-nitro-4,5-dimethoxy)benzyl bromide and triethylamine in DMF (**32**). Treatment with *p*-toluenesulfonic acid (PTSA) liberated the amine function of the 6-APA ester **33**. The photochemical cleavage[31,32] of the (2-nitro-4,5-dimethoxy)benzyl moiety under UV-irradiation at 420 nm, without destruction of the penam core has been verified at this stage of the synthesis and also later on (see Supplementary data). For the next coupling with acids **30a**,**b**, the tosylate salt **33** was neutralized and the corresponding free amine was added to a pre-formed mixture of acid and DCC in dichloromethane. Purification by preparative thin layer chromatography afforded the target compounds **34** and **35** in, respectively, 58% and 70% yield (Scheme 5). In order to facilitate the purification step, we considered the use of water soluble carbodiimides as coupling agents, namely 1,3-bis(2,2-dimethyl-1,3-dioxolan-4-ylmethyl)-carbodiimide (BDDC)[33] and 1-(3-dimethylamino-propyl)-3-ethyl carbodiimide hydrochloride (EDCI),[34] but without significant improvement of isolated yields.



Reagents and conditions: (i) $MeCOCH_2CO_2Me$, Et_3N, DCM, RT; (ii) DMF, RT; (iii) $TsOH.H_2O$, acetone, RT; (iv) Et_3N and NaOH; (v) DCC, DCM, RT.

Scheme 5. Synthesis of Penicillin G photo-cleavable ester equipped with a side-chain for Si (Ge) surface-grafting.

All the synthesized compounds have been characterized by NMR spectroscopy and HRMS. The ^1H and ^{13}C data of the common penam framework are collected in Tables S1 and S2 (see Supplementary data). The most typical features are summarized here: (i) the *gem*-dimethyl substituents at C_2 give signals at ~32 and ~27 ppm in the penam series, and at ~20 and ~17 ppm in the penam-sulfone series (^{13}C NMR data); (ii) the H_3 proton is a singlet at ~4.4 ppm in the penam series and at ~4.5 ppm in the penam-sulfone series; (iii) the H_5 proton appears as a doublet ($J_{5,6}$=4.3–4.7 Hz typical of the cis configuration) at 4.5–4.6 ppm for

the 6-APA derivatives, at 5.5–5.6 ppm for the 6-β-aminosulfonyl derivatives, and 5.4–5.5 ppm for the 6-β-aminocarbonyl derivatives; (iv) the H_6 proton features a doublet of doublet ($J_{5,6}$=4.3–4.7 Hz and $J_{6,NH}$=9–11 Hz) at ~5.5 ppm for the 6-APA derivatives, at ~5.2 ppm for the 6-β-aminosulfonyl derivatives, and 5.6 ppm for the 6-β-aminocarbonyl derivatives; (v) after oxidation to sulfone (6-β-aminosulfonyl series), H_5 and H_6 give signals at 4.8–4.9 ppm and 5.4–5.6 ppm, respectively. The epimerization leading to 5,6-*trans* compounds (most probably via the H_6 enolisation in basic conditions) is easily detected from the H_5–H_6 chemical shifts and coupling constant values: $J_{5,6}$ *trans*=0–1.5 Hz and $J_{6,NH}$=8–10 Hz; H_5 at 4.9–5.2 ppm and H_6 at 5.1 ppm (**8**-epimer, **25**, **26**).

3. Biological evaluation

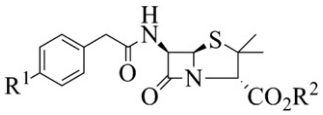
The capacity of the synthesized molecules to form stable acyl-enzyme complexes with bacterial enzymes of interest has been verified in vitro. Two β-lactamases, namely BlaP (class A)[35] and P99 (class C),[36] were pre-incubated with the different tested compounds (at different concentrations) and the residual enzymic activity was determined with nitrocefine, a chromogenic substrate of β-lactamases, which hydrolysis rate can be followed in solution by spectrophotometry at 482 nm. Two penicillin-binding proteins were also considered: the model D,D-carboxypeptidase R39 (from *Actinomadura*)[37] and BlaR-CTD.[38,39] As above, the tested compounds (at different concentrations) were pre-incubated with the PBPs, and then fluorescent ampicillin was added in order to label the residual active enzymes. Reading was made, after denaturation and SDS-PAGE analysis, with a Phospho-Imager.

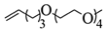
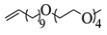
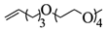
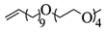
The results obtained with the penam-sulfone derivatives are collected in Table 1. The activities versus BlaP and P99 β-lactamases are given as percentages of residual enzyme activity for a given final concentration of tested molecule. The activities versus BlaR and R39 are given as scores: –, inactive; +, acylation at 100 μM; ++, acylation at 10 μM; +++, acylation at 1 μM.

The 6-sulfonamido-penam sulfones (**4**, **5**, **6′**, **7**) are good inhibitors of the class C β-lactamase P99 and this activity is not disturbed by the presence of a spacer-arm (**21**). In the case of the class A β-lactamase BlaP, only the 6-methylsulfonamido derivatives (**4**) are modestly active. The penam precursors (**2**, **3**, **19**, **20**) showed also anti-β-lactamase activity (vs P99). All the compounds in this series were able to form acyl-enzyme complexes with BlaR-CTD.

The Table 2 summarizes the inhibition activity of the Penicillin G derivatives versus BlaR-CTD and R39. Surprisingly, the 2-nitro-4,5-(dimethoxy)-benzyl esters **34**,**35** are able to acylate BlaR-CTD, although this ester function cannot be hydrolyzed under the assay conditions, and the corresponding free acids **36**,**37**, resulting from the photochemical deprotection, do not acylate BlaR-CTD. The inverse situation is observed in the case of R39. The activity of Pen G is not impaired by the presence of a spacer-arm fixed on the aromatic ring.

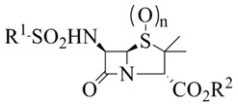
Table 2
Evaluation of Pen G derivatives



Entry	R^1	R^2	Compd	BlaR-CTD[b]	R39[b]
1	H	PIV		++	+
2	H	NV-ester[a]	**38** (see SD)	+++	++
3	H	H		+++	+++
4		NV-ester[a]	**34**	+++	–
5		NV-ester[a]	**35**	++	–
6		H	**36** (see SD)	–	++
7		H	**37** (see SD)	–	+

[a] 2-Nitro-4,5-(dimethoxy)-benzyl (i.e., nitroveratryl (NV)) ester.
[b] See Table 1.

Table 1
Evaluation of penam-sulfone derivatives and their penam precursors



Entry	R^1	R^2	*n*	Compd	BlaP[a] (μM)	P99[a,c] (μM)	BlaR-CTD[b,c]	R39[b]
1	Me	PIV	0	**2a**	10 (10^3)	15 (10)	++	+++
2	Me	EOM	0	**2b**	80 (10^2)	50 (10^2)	–	
3	Me	PIV	2	**4a**	35 (10^3)	10 (10)	+++	++
4	Me	EOM	2	**4b**	30 (10^2)	20 (10)	+++	
5	$BnO_2C–CH_2–$	PIV	0	**3a**	90 (10^3)	25 (10^2)	++	–
6	$BnO_2C–CH_2–$	EOM	0	**3b**	80 (10^2)	25 (10)	+++	
7	$BnO_2C–CH_2–$	PIV	2	**5a**	90 (10^3)	25 (10^2)	+++	+
8	$BnO_2C–CH_2–$	EOM	2	**5b**	80 (10^2)	0 (10^2)	+++	
9	$NEM^+–O_2C–CH_2–$	PIV	2	**6**	90 (10^3)	25 (10^3)	+	–
10	$HO_2C–CH_2–$	PIV	2	**6′**		40 (10^2)	+++	
11	$NHS–O_2C–CH_2–$	PIV	2	**7**		25 (10^2)	+++	
12	$CbzNH(CH_2CH_2O)_3CH_2CH_2–$	PIV	0	**19**		30 (10^2)	++	
13	$CbzNH(CH_2CH_2O)_4CH_2CH_2–$	PIV	0	**20**		25 (10^2)	++	
14	$CbzNH(CH_2CH_2O)_3CH_2CH_2–$	PIV	2	**21**		20 (10^2)	+++	

[a] The enzyme was incubated 60 min with the tested compound (10–10^3 μM final concentration, indicated into parenthesis); then nitrocefine was added for measuring the residual hydrolytic activity, expressed as percentage (%) of the initial activity. Low value indicates a very active compound.
[b] The enzyme was incubated 15 min with the tested compound (1–10^3 μM); then fluorescent ampicillin was added to form a fluorescent acyl-enzyme complex with the residual active enzyme. The absence of fluorescence indicates a very active compound: –inactive compd;+enzyme acylation at 100 μM; ++enzyme acylation at 10 μM; +++enzyme acylation at 1 μM.
[c] In situ ester hydrolysis gave similar results (data not shown).

4. Conclusion

We have investigated the synthesis of two series of bifunctional ligands for the construction of oriented protein-chips using the penam framework to covalently bind the active site of serine-enzymes, namely β-lactamases and Penicillin-Binding Proteins that we have selected on the basis of an easy access to various active mutants.[40,41] A similar strategy has been recently disclosed by Funeriu et al.[42] for the oriented immobilization of cysteine-proteases via epoxy-succinyl-based peptides as affinity labels incorporated into their bifunctional ligands.

Our attempts to incorporate 6-β-sulfonamido-penam sulfones into bifunctional linkers featuring a thioctic acid terminus were disappointing. Very poor yields of the final targets were recovered by using either an amide bond (method A, molecules **8**, **9**) or an ether bond (method B, molecules **25**, **26**) to connect the oligoethyleneglycol spacer. Moreover, due to the enhanced acidity of the H_6 proton in the 6-sulfonamido-penam sulfone moiety (regarding the 6-APA and penicillin G frameworks), epimerization occurred during the last step of the total synthesis, leading to 80:20 mixtures of 5,6-*cis* and 5,6-*trans* azetidinones (**8**, **9**) or to exclusively 5,6-*trans* azetidinones (**25**, **26**). Thus the idea to use penam-sulfones, like **8**,**9** and **25**,**26**, as irreversible inhibitors (i.e., suicide-substrates) to label β-lactamases and fix these enzymes on supports in a regular manner, has to be abandoned.

Fortunately, the alternative approach we examined, based on the benzylpenicillin (i.e., Pen G) framework to label PBPs via stable acyl-enzyme complexes, was successful. We could prepare the final targets **34**,**35** in good yields and without epimerization. The linker, anchored on the *para*-position of the aromatic ring of the penicillin G side-chain, should allow materials derivatization via 'click' chemistry or hydrosilylation reaction. The full construct is compatible with the UV-deprotection of a masked ester.

From the biological evaluations of several intermediates and final targets on representative BLs and PBPs, it clearly appeared that BlaR-CTD (or mutants thereof) is the candidate of choice for the oriented immobilization on supports. This protein could be efficiently acylated by all tested compounds. Moreover, the deprotection of the C_3 ester functions was not required to observe BlaR-CTD inhibition. Indeed, after chemical hydrolysis of the EOM esters and pre-incubation of the PIV esters with serum (data no shown), similar results were collected. Yet, the behaviour of the 2-nitro-4,5-(dimethoxy)-benzyl ester derivatives appeared quite unexpected because this ester was not susceptible to be in situ deprotected during the assays. The specific recognition of *ortho*-nitrobenzyl esters of various antibiotics by BlaR-CTD is currently under investigation. The possibility to 'orthogonally' label the active site of PBPs with penam esters (acylation of BlaR-CTD) and penam-acids (acylation of R39) opens new perspectives for the construction of oriented protein-arrays.

5. Experimental section

5.1. General

The reactions were performed in anhydrous solvents and under argon atmosphere. The reagents were purchased from commercial suppliers and used as received. TLC was performed on Merck silica gel 60 F_{254} plates, using an ethanolic solution of phosphomolybdic acid for the visualization of spots. Column-chromatography was made on 'flash' silica gel from Merck (40–60 μM). The NMR spectra were recorded at room temperature on Bruker DPX-500, Bruker Avance-300, Bruker Avance-250, or Varian Gemini-200 spectrometers. Chemical shifts are reported in parts per million values using tetramethylsilane as reference. The IR spectra were recorded on FTIR-8400S Shimadzu apparatus; the most significant bands are reported. Rotations were measured at 20°C on Perkin–Elmer 241HC polarimeter; concentration is given in % (g/100 mL solvent). HRMS data were obtained from the analytical service of the University College (London, UK). Most of the compounds were isolated as oils (or gums) and amorphous solids, which decompose under heating.

Protocols and spectroscopic characterizations of compounds **11** to **13a**,**b** and **15** to **18a**,**b** described in Scheme 2 (respectively, part A and part B) are given as Supplementary data (SD). Compounds **1b** to **5b** (EOM ester series of Scheme 1) are described in SD. Protocols for **27** (Scheme 4) and Pen G esters (Table 2), adapted from the literature, are given in SD, as well as the free acids **36** and **37** (Table 2) prepared directly from 6-APA.

5.2. Synthesis of 6-β-sulfonamido-penam sulfones of Scheme 1 (first strategy)

*5.2.1. Pivaloyloxymethyl 6-β-aminopenicillinate, tosylate salt (**1a**).* To a suspension of 6-APA (1 g, 4.64 mmol) in anhydrous DMF (9 mL) was added triethylamine (906 μL, 6.50 mmol) and, after stirring for 30 min, chloromethylpivalate (1.34 mL, 9.28 mmol). After stirring at 25 °C for 4 h, the mixture was diluted with EtOAc (70 mL). The precipitate was filtered off and the filtrate was washed with H_2O and brine to remove DMF and unreacted 6-APA. The organic layer was dried over $MgSO_4$ and concentrated under vacuum. The yellow oil was solubilised in EtOAc (5 mL) and freshly recrystallised *p*-toluenesulfonic acid (PTSA) was added (800 mg, 4.64 mmol). The precipitate was filtered and washed with cold EtOAc. The ammonium salt **1a** was obtained as a white solid (1.793 g, 77%) and stored at −20 °C; IR (film): ν 1778, 1760, 1740 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 1.22 (s, 9H, *Me*(^{t}Bu)), 1.53 (s, 3H, Me), 1.65 (s, 3H, Me), 4.42 (s, 1H, NC*H*CO_2), 4.60 (d, 1H, *J*=4.3 Hz, NH_2*CH*CH), 5.51 (d, 1H, *J*=4.3 Hz, NH_2CH*CH*), 5.77 (d, 1H, *J*=5.5 Hz, O*CH*$_2$O), 5.88 (d, 1H, *J*=5.5 Hz, O*CH*$_2$O) (*p*TosO$^-$ signals omitted); ^{13}C NMR (75 MHz, $CDCl_3$): δ 26.7, 26.8, 31.2, 38.6, 62.8, 63.5, 69.5, 70.0, 79.6, 166.7, 176.5, 177.8 (*p*TosO$^-$ signals omitted).

*5.2.2. Pivaloyloxymethyl 6-β-(methylsulfonamido)-penicillinate (**2a**).* To a solution of **1a** (191 mg, 0.69 mmol) in anhydrous DCM (3 mL) at 0 °C, were added triethylamine (97 μL, 0.69 mmol) and methane sulfonylchloride (54 μL, 0.69 mmol). After 20 min at 0 °C and 5 min at room temperature, water (5 mL) was added. The aqueous phase was extracted with DCM and the organic phases were dried over $MgSO_4$ and concentrated. The crude product was purified by column-chromatography on silica gel (cyclohexane/EtOAc (1:1), R_f=0.54) to give **2a** as a yellow amorphous solid (148 mg, 60%); $[\alpha]_D$ +138.3 (*c* 3.0, $CHCl_3$); IR (film): ν 3280, 1785, 1765, 1740 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 1.25 (s, 9H, *Me*(^{t}Bu)), 1.54 (s, 3H, Me), 1.65 (s, 3H, Me), 3.15 (s, 3H, SO_2*Me*), 4.49 (s, 1H, NC*H*CO_2), 5.17 (dd, 1H, *J*=4.5, 10.5 Hz, NHC*H*CH), 5.61 (d, 1H, *J*=4.5 Hz, NHCHC*H*), 5.35 (d, 1H, *J*=10.5 Hz, N*H*), 5.79 (d, 1H, *J*=5.5 Hz, O*CH*$_2$O), 5.88 (d, 1H, *J*=5.5 Hz, O*CH*$_2$O); ^{13}C NMR (75 MHz, $CDCl_3$): δ 26.9, 28.1, 29.4, 38.8, 44.8, 52.5, 64.2, 66.2, 71.2, 80.0, 166.6, 167.0, 176.8.

*5.2.3. Pivaloyloxymethyl 1,1-dioxide-6-β-(methylsulfonamido)-penicillinate (**4a**).* The penam **2a** (342 mg, 0.83 mmol) dissolved in a 4:1 mixture of AcOH and H_2O (28 mL) was cooled at −15 °C under vigorous stirring. $KMnO_4$ (277 mg, 1.74 mmol) dissolved in water (10 mL) was added very slowly (over about 30 min). The mixture was further stirred for 1 h at −10 °C, then 10% H_2O_2 was added to discolour the solution. DCM (40 mL) was added. The organic phase was washed twice with 5% $NaHCO_3$, dried over $MgSO_4$ and concentrated. The penam-sulfone **4a** was obtained as a colourless solid (70 mg, 20%); $[\alpha]_D$ +68.0 (*c* 1.6, $CHCl_3$); IR (film): ν 3315, 1807, 1768, 1743 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 1.25 (s, 9H, *Me*(^{t}Bu)), 1.45

(s, 3H, Me), 1.61 (s, 3H, Me), 3.15 (s, 3H, SO_2Me), 4.54 (s, 1H, NC*H*CO_2), 4.88 (d, 1H, *J*=4.7 Hz, NHCHC*H*), 5.46 (dd, 1H, *J*=4.7, 11.5 Hz, NHC*H*CH), 5.75 (d, 1H, *J*=5.5 Hz, OCH_2O), 5.98 (d, 1H, *J*=5.5 Hz, OCH_2O), 6.18 (d, 1H, *J*=11.5 Hz, N*H*); ^{13}C NMR (75 MHz, $CDCl_3$): δ 17.7, 19.9, 26.7, 29.6, 42.2, 60.3, 63.6, 64.6, 65.5, 80.5, 165.2, 173.2, 176.7.

*5.2.4. Pivaloyloxymethyl 6-β-[(benzyloxycarbonyl)-methylsulfonamido]-penicillinate (**3a**).* Sulfoacetyl dichloride[43] (105 μL, 0.99 mmol) in anhydrous DCM (1 mL) was added at 0 °C the benzyl alcohol (103 μL, 0.99 mmol) in DCM (1 mL). After 1 h, this mixture was slowly added to a solution of **1a** (328 mg, 0.99 mmol) in anhydrous DCM (2 mL) previously treated with triethylamine (276 μL, 1.98 mmol). After 15 min at 0 °C and 1 h at room temperature, water (4 mL) was added and the aqueous phase was extracted with DCM. The organic phase was then washed with brine. After concentration under vaccum, the crude product was purified by column-chromatography on silica gel (hexane/EtOAc (3:1), R_f=0.80 in (1:1)) to give **3a** as a colourless oil (240 mg, 44%); $[\alpha]_D$ +76.4 (*c* 4.0, $CHCl_3$); IR (film): ν 1788, 1759, 1713, 1524, 1454, 1339, 1281, 1252, 1148, 1107, 1026, 978, 721 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.24 (s, 9H, *Me*(^{t}Bu)), 1.53 (s, 3H, Me), 1.61 (s, 3H, Me), 4.22 (d, 1H, *J*=15.8 Hz, $SO_2CH_aH_bCO$), 4.47 (s, 1H, NC*H*CO_2), 4.48 (d, 1H, *J*=15.8 Hz, $SO_2CH_aH_bCO$), 5.17 (dd, 1H, *J*=4.6, 11.0 Hz, NHC*H*CH), 5.23 (s, 2H, Ph–CH_2), 5.61 (d, 1H, *J*=4.6 Hz, NHCHC*H*), 5.81 (d, 1H, *J*=5.5 Hz, OCH_2O), 5.87 (d, 1H, *J*=5.5 Hz, OCH_2O), 5.93 (d, 1H, *J*=11.0 Hz, N*H*), 7.36–7.39 (m, 5H, Ph); ^{13}C NMR (75 MHz, $CDCl_3$): δ 26.7, 26.9, 32.5, 38.8, 57.5, 62.3, 64.8, 67.5, 65.1, 69.9, 79.8, 128.5, 128.7, 134.5, 163.9, 166.2, 172.7, 176.8; HRMS (ESI) *m*/*z*: calcd for $C_{23}H_{30}N_2O_9S_2$: 541.1314 $[M-H]^-$. Found: 541.1318.

*5.2.5. Pivaloyloxymethyl 1,1-dioxide-6-β-[(benzyloxycarbonyl)-methylsulfonamido]-penicillinate (**5a**).* The penam **3a** (221 mg, 0.40 mmol) dissolved in a 4:1 mixture of AcOH and H_2O (20 mL) was cooled at −15 °C, under vigorous stirring. $KMnO_4$ (61 mg, 0.85 mmol) dissolved in water (10 mL) was added very slowly (30 min). The mixture was further stirred for 1 h at −10 °C, then 10% H_2O_2 was added to discolour the solution. DCM (40 mL) was added. The organic phase was washed twice with 5% $NaHCO_3$, dried over $MgSO_4$ and concentrated. The penam-sulfone **5a** was obtained as a colourless oil (224 mg, 96%); R_f=0.46 (hexane/EtOAc (3:1)); $[\alpha]_D$ +105.0 (*c* 0.2, $CHCl_3$); IR (film): ν 1809, 1774, 1747, 1456, 1358, 1325, 1281, 1159, 1115, 978, 732 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.25 (s, 9H, *Me*(^{t}Bu)), 1.43 (s, 3H, Me), 1.60 (s, 3H, Me), 4.25 (sharp q_{AB}, 2H, SO_2CH_2CO), 4.50 (s, 1H, NC*H*CO_2), 4.82 (d, 1H, *J*=4.6 Hz, NHCHC*H*), 5.26 (sharp q_{AB}, 2H, Ph–CH_2), 5.48 (dd, 1H, *J*=4.6, 11.1 Hz, NHC*H*CH), 5.74 (d, 1H, *J*=5.5 Hz, OCH_2O), 5.77 (d, 1H, *J*=5.5 Hz, OCH_2O), 6.55 (d, 1H, *J*=11.1 Hz, N*H*), 7.39–7.40 (m, 5H, Ph); ^{13}C NMR (75 MHz, $CDCl_3$): δ 17.6, 20.0, 26.8, 38.8, 57.7, 61.5, 63.6, 64.6, 66.0, 68.4, 80.6, 128.7, 128.9, 134.4, 163.6, 165.4, 173.1, 176.8; HRMS (ESI) *m*/*z*: calcd for $C_{23}H_{30}N_2O_{11}S_2Na$: 597.1189 $[M+Na]^+$. Found: 597.1197.

*5.2.6. Pivaloyloxymethyl 1,1-dioxide-6-β-[hydroxycarbonyl-methylsulfonamido]-penicillinate, N-ethylmorpholine salt (**6**) and free acid (**6′**).* To a solution of sulfone **5a** (50 mg, 0.087 mmol) in EtOH/EtOAc (4:1) (5 mL) were added successively *N*-ethylmorpholine (11 μL, 0.087 mmol) and Pd on activated carbon (0.0087 mmol). The mixture was submitted to hydrogenation for 1 h at room temperature (pH_2=1 atm). Filtration on Celite and concentration under vacuum gave the salt **6** as a colourless oil (49 mg, 95%); ^{1}H NMR (300 MHz, acetone-d_6): δ 1.21 (s, 9H, *Me*(^{t}Bu)), 1.33 (t, 3H, *J*=6.7 Hz, *Me*-morph), 1.45 (s, 3H, Me), 1.56 (s, 3H, Me), 3.04–3.11 (m, 6H, NH^+(morph)-CH_2, CH_2–CH_3), 3.92–3.93 (m, 4H, O(morph)-CH_2), 4.06 (sharp q_{AB}, 2H, SO_2CH_2CO), 4.53 (s, 1H, NC*H*CO_2), 4.35 (d, 1H, *J*=4.7 Hz, NHCHC*H*), 5.66 (d, 1H, *J*=4.7 Hz, NHC*H*CH), 5.82 (d, 1H, *J*=5.7 Hz, OCH_2O), 6.01 (d, 1H, *J*=5.7 Hz, OCH_2O); ^{13}C NMR (75 MHz, acetone-d_6): δ 8.5, 16.8, 19.3, 26.2, 38.4, 50.9, 51.6, 59.6, 61.0, 63.3, 64.0, 64.1, 66.2, 80.5, 165.6, 167.6, 174.5, 176.3.

Hydrogenation as above in the absence of NEM gave the free acid **6′** (unstable compound), as a colourless oil (44 mg, quantitative yield); $[\alpha]_D$ +53.40 (*c* 0.4, $CHCl_3$); ^{1}H NMR (300 MHz, acetone-d_6): δ 1.21 (s, 9H, *Me*(^{t}Bu)), 1.45 (s, 3H, Me), 1.57 (s, 3H, Me), 4.40 (sharp q_{AB}, 2H, CH_2SO_2), 4.57 (s, 1H, NC*H*CO_2), 5.32 (d, 1H, *J*=4.7 Hz, NHCHC*H*), 5.73–5.74 (m, 1H, NHC*H*CH), 5.82 (d, 1H, *J*=5.7 Hz, OCH_2O), 6.01 (d, 1H, *J*=5.7 Hz, OCH_2O), 7.02 (d, 1H, *J*=10.2 Hz, N*H*); ^{13}C NMR (75 MHz, acetone-d_6): δ 17.9, 20.3, 27.3, 39.2, 57.3, 61.3, 63.9, 65.2, 66.7, 81.2, 166.1, 168.2, 174.3, 177.4.

*5.2.7. Pivaloyloxymethyl 1,1-dioxide-6-β-[succinimidyloxycarbonyl-methylsulfonamido]-penicillinate (**7**).* To a solution of **6′** (44 mg, 0.09 mmol) in anhydrous DCM (3 mL) were added successively at 0 °C *N*-hydroxysuccinimide (10 mg, 0.09 mmol) and DCC (19 mg, 0.092 mmol). After 16 h at room temperature, the solvent was evaporated and the crude product was triturated with EtOAc. After a filtration on Celite and evaporation, the product **7** was used without purification (60 mg, quantitative yield); $[\alpha]_D$ +42.50 (*c* 0.1, $CHCl_3$); IR (film): ν 1811, 1782, 1742, 1628, 1574, 1450, 1323, 1157, 1117, 1068, 980, 760 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.23 (s, 9H, *Me*(^{t}Bu)), 1.29 (s, 3H, Me), 1.41 (s, 3H, Me), 2.88 (br s, 4H, $NCOC_2H_4$), 4.52 (br s, 3H, NC*H*CO_2, CH_2SO_2), 4.90 (d, 1H, *J*=4.6 Hz, NHCHC*H*), 5.64 (dd, 1H, *J*=4.6, 10.9 Hz, NHC*H*CH), 5.71 (d, 1H, *J*=5.5 Hz, OCH_2O), 5.95 (d, 1H, *J*=5.5 Hz, OCH_2O), 6.76 (br d, 1H, *J*=10.9 Hz, N*H*); ^{13}C NMR (75 MHz, acetone-d_6): δ 17.7, 20.2, 26.4, 27.2, 39.4, 55.5, 61.6, 64.4, 65.3, 67.0, 81.5, 160.7, 166.4, 170.1, 174.1, 177.2.

*5.2.8. Coupling of the spacer-arm **13** to **6′**.* To a solution of freshly prepared **6′** (1 equiv) and **13** (1 equiv) in DMF (3 mL, 0.1 mmol) were added NEM (2.2 equiv) and HBTU (1.2 equiv). The mixture was stirred for 3 h at room temperature. EtOAc (15 mL) was added and the organic phase was washed with brine (3×), dried over $MgSO_4$ and concentrated. The crude product was purified by column-chromatography on silica gel (3x), using EtOAc as the eluant. About 5% yield of pure **8** (or **9**) could be recovered. Compound **8** (gum): IR (film): ν 2958, 2956, 2854, 1801, 1778, 1759, 1647 (br), 1537, 1461, 1327, 1159, 1115 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.24 (s, 9H, *Me*(^{t}Bu)), 1.44, (s, 3H, Me), 1.48 (m, 2H, $NHCOC_2H_4CH_2$), 1.59 (s, 3H, Me), 1.70 (m, 4H, $NHCOCH_2CH_2$+$NHCOC_3H_6CH_2$), 1.93 (m, 1H, S_2CHCH_2), 2.23 (m, 2H, $NHCOCH_2CH_2$), 2.45 (m, 1H, S_2CHCH_2), 3.16 (m, 2H, S_2CH_2), 3.49 (m, 4H, $CONHCH_2$), 3.62 (m, 9H, S_2CH, OCH_2-CH_2O), 4.15 (d, 1H, *J*=14.2 Hz, SO_2CH_2), 4.20 (d, 1H, *J*=14.2 Hz, SO_2CH_2), 4.52 (s, 1H, NHC*H*CO_2), 4.99 (d, 1H, *J*=4.5 Hz, NHCHC*H*), 5.55 (dd, 1H, *J*=4.5 and 10.8 Hz, NHC*H*CH), 5.73 (d, 1H, *J*=5.5 Hz, OCH_2O), 5.96 (d, 1H, *J*=5.5 Hz, OCH_2O), 6.13 (br t, 1H, CON*H*), 6.75 (d, 1H, *J*=10.8 Hz, SO_2NH), 7.21 (br t, 1H, N*H*CO); *Epimer*: δ 1.43 (s, 3H, Me), 1.59 (s, 3H, Me), 4.09 (d, 1H, *J*=14 Hz, SO_2CH_2), 4.14 (d, 1H, *J*=14 Hz, SO_2CH_2), 4.42 (s, 1H, NHC*H*CO_2), 4.93 (br s, 1H, NHCHC*H*), 5.15 (br d, 1H, *J*=8.1 Hz, NHC*H*CH), 5.74 (d, 1H, *J*=5.5 Hz, OCH_2O), 5.96 (d, 1H, *J*=5.5 Hz, OCH_2O), 6.37 (br t, 1H, CON*H*), 7.12 (br t, 1H, N*H*CO), 7.39 (br d, 1H, *J*=8.1 Hz, SO_2NH); ^{13}C NMR (125 MHz, $CDCl_3$): δ 176.8, 173.4, 168.0, 165.4, 161.9, 80.5, 70.5–69.0, 66.3, 64.6, 63.6, 61.5, 59.6, 56.5, 40.3, 39.9, 39.5, 39.2, 38.6, 36.4, 34.6, 28.9, 26.9, 25.4, 20.1, 17.6; *Epimer*: 69.7, 62.4, 61.3, 19.9, 18.3; MS (ESI): *m*/*z*=824.95 $[M+Na]^+$ 100%, 802.83 $[M+H]^+$, 18%. **9**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.24 (s, 9H, *Me*(^{t}Bu)), 1.44 (s, 3H, Me), 1.48 (m, 2H, $NHCOC_2H_4CH_2$), 1.60 (s, 3H, Me), 1.70 (m, 4H, $NHCOCH_2CH_2$+$NHCOC_3H_6CH_2$), 1.92 (m, 1H, S_2CHCH_2), 2.22 (m, 2H, $NHCOCH_2CH_2$), 2.48 (m, 2H, S_2CHCH_2), 3.17 (m, 2H, S_2CH_2), 3.45–3.57 (m, 4H, $CONHCH_2$), 3.60–3.70 (m, 13H, S_2CH, OCH_2-CH_2O), 4.12 (d, 1H, *J*=14.5 Hz, SO_2CH_2), 4.23 (d, 1H, *J*=14.5 Hz, SO_2CH_2), 4.52 (s, 1H, NHC*H*CO_2), 5.03 (d, 1H, *J*=4.5 Hz, NHCHC*H*), 5.54 (dd, 1H, *J*=4.5 Hz and 10.7 Hz, NHC*H*CH), 5.74 (d, 1H, *J*=5.5 Hz,

OCH_2O), 5.97 (d, 1H, J=5.5 Hz, OCH_2O), 6.24 (br t, 1H, CONH), 6.75 (d, 1H, J=10.7 Hz, SO_2NH), 7.36 (br t, 1H, NHCO); ^{13}C NMR (75 MHz, $CDCl_3$): δ 176.8, 173.3, 168.0, 165.4, 162.1, 80.6, 70.1–69.2, 66.3, 64.5, 63.5, 61.6, 59.4, 56.5, 40.2, 39.9, 39.1, 38.8, 38.4, 36.2, 34.6, 29.7, 26.8, 25.4, 20.0, 17.6; MS (ESI): m/z=869.04 $[M+Na]^+$ 100%, 846.9 $[M+H]^+$ 25%.

5.3. Synthesis of 6-β-sulfonamido-penam sulfones of Scheme 3 (second strategy)

*5.3.1. Coupling of the spacer-arm **18** to **1a**.* To a solution of **1a** (127 mg, 0. 25 mmol) in DCM (3 mL) at 0 °C, was added triethylamine (71 μL, 0.5 mmol). After 10 min, a solution of sulfonylchloride **18a** (104 mg, 0.25 mmol) in DCM (2 mL) was added. After 10 min at 0 °C and 40 min at room temperature, the mixture was washed by water and brine, dried over $MgSO_4$ and concentrated. The crude product was purified by column-chromatography on silica gel (EtOAc) to give **19** (127 mg, 71%) as a yellow oil; R_f=0.25 (EtOAc); $[\alpha]_D$ +62.4 (c 0.17, $CHCl_3$); IR (film): ν 1786, 1757, 1705, 1529, 1456, 1331, 1279, 1254, 1145, 1111, 1026, 982, 733 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.21 (s, 9H, Me(^{t}Bu)), 1.50 (s, 3H, Me), 1.63 (s, 3H, Me), 3.27 (m, 1H, $SO_2CH_2CH_2$), 3.38–3.41 (m, 2H, CONHCH_2), 3.57–3.70 (m, 11H, $SO_2CH_2CH_2$, OCH_2CH_2O), 3.86–3.88 (m, 2H, $SO_2CH_2CH_2$), 4.43 (s, 1H, NCHCO_2), 5.10 (s, 2H, CH_2Ph), 5.20 (dd, 1H, J=3.6 Hz, 10.0 Hz, NHCHCH), 5.44 (br s, 1H, O_2CNH), 5.52 (d, 1H, J=3.6 Hz, NHCHCH), 5.75 (d, 1H, J=5.3 Hz, OCH_2O), 5.86 (d, 1H, J=5.3 Hz, OCH_2O), 5.95 (d, 1H, J=10.0 Hz, SO_2NH), 7.30–7.33 (m, 5H, Ph); ^{13}C NMR (75 MHz, $CDCl_3$): δ 26.8, 26.9, 31.5, 38.8, 40.9, 53.8, 62.0, 64.6, 65.1, 66.6, 67.9, 69.7, 69.9, 70.2, 70.3, 79.8, 128.1, 128.2, 128.5, 136.6, 156.5, 166.4, 173.4, 176.8; HRMS (ESI) m/z: calcd for $C_{30}H_{45}N_3O_{12}S_2Na$: 726.2342 $[M+Na]^+$. Found: 726.2369.

Compounds **20** was similarly prepared from **1a** (145 mg, 0.028 mmol) and **18b** (146 mg, 0.31 mmol). Column-chromatography afforded **20** (171 mg, 79%) as a yellow oil; $[\alpha]_D$ +110.0 (c 0.17, $CHCl_3$); IR (film): ν 1788, 1759, 1713, 1524, 1454, 1338, 1281, 1252, 1148, 1107, 1026, 978, 721 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.23 (s, 9H, Me(^{t}Bu)), 1.52 (s, 3H, Me), 1.64 (s, 3H, Me), 3.30 (m, 1H, $SO_2CH_2CH_2$), 3.40–3.43 (m, 2H, CONHCH_2), 3.56–3.66 (m, 15H, $SO_2CH_2CH_2$, OCH_2CH_2O), 3.91–3.92 (m, 2H, $SO_2CH_2CH_2$), 4.44 (s, 1H, NCHCO_2), 5.11 (s, 2H, CH_2Ph), 5.22 (dd, 1H, J=4.4, 10.0 Hz, NHCHCH), 5.40 (br s, 1H, O_2CNH), 5.55 (d, 1H, J=4.4 Hz, NHCHCH), 5.77 (d, 1H, J=5.5 Hz, OCH_2O), 5.87 (d, 1H, J=5.5 Hz, OCH_2O), 5.92 (d, 1H, J=10.0 Hz, SO_2NH), 7.30–7.36 (m, 5H, Ph); ^{13}C NMR (75 MHz, $CDCl_3$): δ 26.8, 26.9, 31.6, 38.8, 40.9, 53.8, 62.1, 64.6, 65.2, 66.6, 68.0, 69.7, 70.0, 70.2, 70.4, 70.5, 79.8, 128.1, 128.2, 128.5, 136.6, 156.5, 166.4, 173.4, 176.9; HRMS (ESI) m/z: calcd for $C_{32}H_{49}N_3O_{13}S_2Na$: 770.2605 $[M+Na]^+$. Found: 770.2595.

*5.3.2. Oxidation of the penams **19**,**20**.* The penam **19** (43 mg, 0.06 mmol) dissolved in a 4:1 mixture of AcOH and H_2O (4 mL) was cooled at −15 °C, under vigorous stirring. $KMnO_4$ (20 mg, 0.13 mmol) dissolved in water (2 mL) was added very slowly (30 min). The mixture was further stirred for 1 h at −10 °C, then 10% H_2O_2 was added to discolour the solution. DCM (15 mL) was added. The organic phase was washed twice with 5% $NaHCO_3$, dried over $MgSO_4$ and concentrated. The penam-sulfone **21** was obtained as a colourless oil (42 mg, 90%); R_f=0.46 (hexane/EtOAc (3:1)); $[\alpha]_D$ +72.1 (c 0.19, $CHCl_3$); IR (film): ν 1809, 1776, 1757, 1705, 1521, 1456, 1325, 1281, 1149, 1113, 1028, 980, 737 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.23 (s, 9H, Me(^{t}Bu)), 1.39 (s, 3H, Me), 1.55 (s, 3H, Me), 3.37 (m, 1H, $SO_2CH_2CH_2$), 3.39–3.40 (m, 2H, CONHCH_2), 3.43 (m, 1H, $SO_2CH_2CH_2$), 3.55–3.62 (m, 10H, OCH_2CH_2O), 3.82 (m, 1H, $SO_2CH_2CH_2$), 3.92 (m, 1H, $SO_2CH_2CH_2$), 4.47 (s, 1H, NCHCO_2), 4.88 (d, 1H, J=4.6 Hz, NHCHCH), 5.11 (s, 2H, CH_2Ph), 5.50 (br s, 1H, O_2CNH), 5.56 (dd, 1H, J=4.6, 11.2 Hz, NHCHCH), 5.69 (d, 1H, J=5.4 Hz, OCH_2O), 5.94 (d, 1H, J=5.4 Hz, OCH_2O), 6.36 (d, 1H, J=11.2 Hz, SO_2NH), 7.30–7.37 (m, 5H, Ph); ^{13}C NMR (75 MHz, $CDCl_3$): δ 17.5, 20.0, 26.8, 38.8, 40.9, 54.5, 60.9, 63.5, 64.4, 65.1, 66.1, 66.6, 69.6, 69.9, 70.1, 70.3, 70.7, 80.5, 128.1, 128.2, 128.5, 136.7, 156.5, 165.5, 173.9, 176.8; HRMS (ESI) m/z: calcd for $C_{30}H_{45}N_3O_{14}S_2Na$: 758.2241 $[M+Na]^+$. Found: 758.2250.

Compound **22** was similarly prepared from **20** (116 mg, 0.15 mmol) and $KMnO_4$ (51 mg, 0.325 mmol). After work-up, the penam-sulfone **22** was recovered as a colourless oil (120 mg, 95%); $[\alpha]_D$ 65.4 (c 0.81, $CHCl_3$); IR (film): ν 1807, 1778, 1755, 1711, 1520, 1456, 1325, 1259, 1149, 1107, 1026, 976, 737 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.22 (s, 9H, Me(^{t}Bu)), 1.39 (s, 3H, Me), 1.55 (s, 3H, Me), 3.38 (m, 1H, $SO_2CH_2CH_2$), 3.39–3.41 (m, 2H, CONHCH_2), 3.47 (m, 1H, $SO_2CH_2CH_2$), 3.55–3.63 (m, 14H, OCH_2CH_2O), 3.82 (m, 1H, $SO_2CH_2CH_2$), 3.93 (m, 1H, $SO_2CH_2CH_2$), 4.47 (s, 1H, NCHCO_2), 4.96 (d, 1H, J=4.6 Hz, NHCHCH), 5.10 (s, 2H, CH_2Ph), 5.46 (br s, 1H, O_2CNH), 5.58 (dd, 1H, J=4.6, 11.1 Hz, NHCHCH), 5.70 (d, 1H, J=5.4 Hz, OCH_2O), 5.94 (d, 1H, J=5.4 Hz, OCH_2O), 6.33 (d, 1H, J=11.1 Hz, SO_2NH), 7.32–7.36 (m, 5H, Ph); ^{13}C NMR (75 MHz, $CDCl_3$): δ 17.5, 20.0, 26.8, 38.8, 40.9, 54.6, 60.9, 63.5, 64.4, 65.1, 66.1, 66.6, 69.7, 70.0, 70.2, 70.3, 70.4, 70.5, 70.7, 80.5, 128.0, 128.1, 128.5, 136.7, 156.5, 165.5, 174.0, 176.8; HRMS (ESI) m/z: calcd for $C_{32}H_{50}N_3O_{15}S_2$: 780.2683 $[M+H]^+$. Found: 780.2676.

*5.3.3. Hydrogenolysis of penam-sulfones **21**,**22**.* To a solution of **21** (65 mg, 0.088 mmol) in EtOH/EtOAc (4:1) (6.5 mL) were added Pd on activated carbon (0.0088 mmol) and *p*-toluenesulfonic acid (15 mg, 0.088 mmol). The mixture was submitted to hydrogenation for 1 h at room temperature (pH_2=1 atm). Filtration on Celite, washing with acetone and concentration under vacuum gave the deprotected product **23** (n=2) as a colourless oil, which was used without further purification.

The deprotected compound **24** (n=3) was similarly prepared. In both cases, the complete disappearance of the Cbz group was verified by ^{1}H NMR.

*5.3.4. Coupling of **23**,**24** to thioctic acid.* To a solution of **23** (PTSA salt) (68 mg, 0.088 mmol) and *N*-hydroxysuccinimide ester of TA (26 mg, 0.088 mmol) in anhydrous DMF (3 mL) was added *N*-ethylmorpholine (0.176 mmol). The mixture was stirred for 24 h at room temperature. EtOAc was added (5 mL). The organic phase was washed with brine (6×), dried over $MgSO_4$ and concentrated. The crude product was purified by column-chromatography on silica gel to give **25** as a yellow oil (12 mg, 18%); R_f=0.27 (EtOAc); $[\alpha]_D$ +51.2 (c 0.2, $CHCl_3$); IR (film): ν 1801, 1772, 1757, 1659, 1634, 1541, 1468, 1423, 1331, 1277, 1149, 1115, 978, 735 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.24 (s, 9H, Me(^{t}Bu)), 1.43 (s, 3H, Me), 1.45–1.47 (m, 2H, NHCO$C_2H_4CH_2$), 1.59 (s, 3H, Me), 1.67–1.69 (m, 4H, NHCOCH_2CH_2, NHCOC_3H_6CH_2), 1.94 (m, 1H, S_2CHCH_2), 2.25 (t, 2H, J=7.4 Hz, NHCOCH_2CH$_2$), 2.47 (m, 1H, S_2CHCH_2), 3.05–3.22 (m, 2H, S_2CH_2), 3.37 (m, 1H, $SO_2CH_2CH_2$), 3.42–3.49 (m, 3H, $SO_2CH_2CH_2$, CONHCH_2), 3.59–3.97 (m, 11H, OCH_2CH_2O, S_2CH), 3.96–3.98 (m, 2H, $SO_2CH_2CH_2$), 4.43 (s, 1H, NCHCO_2), 5.12 (dd, 1H, J=1.3, 10.1 Hz, NHCHCH), 5.14 (d, 1H, J=1.3 Hz, NHCHCH), 5.73 (d, 1H, J=5.4 Hz, OCH_2O), 5.98 (d, 1H, J=5.4 Hz, OCH_2O), 6.48 (br s, 1H, CONH), 7.02 (d, 1H, J=10.1 Hz, SO_2NH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 18.2, 20.0, 25.4, 26.8, 28.9, 34.6, 36.1, 38.4, 38.8, 38.9, 40.2, 52.1, 56.5, 61.6, 62.5, 63.2, 65.2, 68.1, 69.5, 69.7, 69.8, 70.1, 70.5, 80.4, 165.3, 168.6, 173.5, 176.8; HRMS (ESI) m/z: calcd for $C_{30}H_{50}N_3O_{13}S_4$: 788.2227 $[M-H]^-$. Found: 788.2234.

Compound **24** was similarly treated to furnish **26** as a yellow oil (10% yield after column-chromatography); R_f=0.27 (EtOAc); $[\alpha]_D$ +64.8 (c 0.15, $CHCl_3$); IR (film): ν 1803, 1774, 1755, 1660, 1635, 1540, 1469 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.22 (s, 9H, Me(^{t}Bu)), 1.40 (s, 3H, Me), 1.45–1.50 (m, 2H, NHCO$C_2H_4CH_2$), 1.58 (s, 3H, Me), 1.67–1.73 (m, 2H, NHCOCH_2CH$_2$), 1.74 (br s, 2H, NHCOC_3H_6CH_2), 1.90–1.96 (m, 1H, S_2CHCH_2), 2.22 (t, 2H, J=7.5 Hz, NHCOCH_2CH$_2$),

2.44–2.51 (m, 1H, S_2CHCH_2), 3.13 (m, 1H, S_2CH_2), 3.21 (m, 1H, S_2CH_2), 3.30–3.36 (m, 1H, $SO_2CH_2CH_2$), 3.40–3.52 (m, 3H, $SO_2CH_2CH_2$, $CONHCH_2$), 3.58–3.83 (m, 15H, OCH_2CH_2O, S_2CH), 3.92–4.01 (m, 2H, $SO_2CH_2CH_2$), 4.39 (s, 1H, $NCHCO_2$), 5.07 (dd, 1H, J=1.5, 10.1 Hz, NHCHCH), 5.29 (d, 1H, J=1.5 Hz, NHCHCH), 5.70 (d, 1H, J=5.4 Hz, OCH_2O), 5.96 (d, 1H, J=5.4 Hz, OCH_2O), 6.39 (br s, 1H, CONH), 7.04 (d, 1H, J=10.1 Hz, SO_2NH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 18.3, 20.0, 25.5, 27.0, 29.0, 34.8, 36.4, 38.6, 39.3, 40.3, 40.2, 52.6, 56.6, 61.7, 62.5, 63.2, 65.4, 68.3, 69.7, 69.9, 70.0, 70.2, 70.4, 70.5, 70.7, 80.5, 165.5, 169.3, 173.2, 176.9.

5.4. Synthesis of benzylpenicillin derivatives of Scheme 5

*5.4.1. Synthesis of the O-substituted 4-hydroxyphenylacetic side-chains **29**,**30**. Mitsunobu coupling:* To the mixture of DIAD (429 μL, 2.16 mmol) and triphenylphosphine (568 mg, 2.16 mmol) in anhydrous THF (5 mL) was added at room temperature methyl 4-hydroxyphenylacetate **28** (264 mg, 1.58 mmol). The mixture was stirred for 30 min, then **27a** or **27b** (1.44 mmol) was added. After stirring for 20 h, the mixture was concentrated and purified by flash chromatography on silica gel (cyclohexane/EtOAc (1:1)) to give **29a** or **29b** (>90% yield) as a translucent oil. Compound **29a**: IR (film): ν 1745 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.65 (quint, 2H, J=8.5 Hz, $=CHCH_2CH_2$), 2.08 (q, 2H, J=8.5 Hz, $=CHCH_2CH_2$), 3.44 (t, 2H, J=8.3 Hz, $=CHC_2H_4CH_2$), 3.53–3.68 (m, 17H, OCH_2CH_2O+ O*Me*), 3.81 (s, 2H, $PhCH_2$), 4.08 (t, 2H, J=5.6 Hz, $PhOCH_2$), 4.95 (dd, 1H, J=1.8, 10.3 Hz, CH_2=), 5.01 (dd, 1H, J=1.8, 16.9 Hz, CH_2=), 5.79 (ddt, 1H, J=16.9, 10.3, 6.7 Hz, CH=), 6.85 (d, 2H, J=8.8 Hz, Ph), 7.16 (d, 2H, J=8.8 Hz, Ph); ^{13}C NMR (50 MHz, $CDCl_3$): δ 28.8, 30.1, 40.2, 51.8, 67.5, 69.7, 70.1, 70.6, 70.8, 114.5, 114.8, 126.2, 130.1, 138.2, 157.9, 172.1; HRMS (ESI) m/z: calcd for $C_{22}H_{34}O_7Na$: 433.2202 $[M+Na]^+$. Found: 433.2213. Compound **29b**: IR (film): ν 1745 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.24–1.42 (m, 12H, $=CHC_2H_4C_6H_{12}$), 1.51 (m, 2H, $=CHCH_2CH_2$), 1.95 (q, 2H, J=7.1 Hz, $=CHCH_2CH_2$), 3.39 (t, 2H, J=6.7 Hz, alkCH_2O), 3.36–3.67 (m, 17H, OCH_2CH_2O+ O*Me*), 3.78 (s, 2H, $PhCH_2$), 4.05 (t, 2H, J=5.4 Hz, $PhOCH_2$), 4.90 (dd, 1H, J=1.8, 10.3 Hz, CH_2=), 4.94 (dd, 1H, J=1.8, 16.9 Hz, CH_2=), 5.75 (ddt, 1H, J=16.9, 10.3, 6.7 Hz, CH=), 6.81 (d, 2H, J=8.7 Hz, Ph), 7.12 (d, 2H, J=8.7 Hz, Ph); ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 21.7, 25.8, 28.7, 28.9, 29.2, 33.6, 40.0, 51.7, 67.2, 69.5, 69.8, 70.3, 71.3, 113.9, 114.5, 126.0, 130.0, 139.0, 157.7, 172.1; HRMS (ESI) m/z: calcd for $C_{28}H_{46}O_7Na$: 517.3141 $[M+Na]^+$. Found: 517.3146.

Saponification: The ester **29a** or **29b** (2.29 mmol) was treated with NaOH 1 M (2 mL) in MeOH (5 mL) at room temperature for 2 h, then the mixture was acidified by addition of HCl 1 M. The aqueous phase was extracted with EtOAc (3×). The organic layer was washed with brine, dried over $MgSO_4$ and concentrated to furnish **30a** or **30b** as a translucent oil (>90% yield). Compound **30a**: IR (film): ν 3200, 1710 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.65 (quint, 2H, J=8.5 Hz, CH_2), 2.08 (q, 2H, J=8.5 Hz, CH_2), 3.44 (t, 2H, J=8.3 Hz, CH_2), 3.53–3.68 (m, 14H, CH_2), 3.81 (s, 2H, CH_2Ph), 4.08 (t, 2H, J=5.6 Hz, CH_2CH_2O), 4.90–5.03 (m, 2H, CH_2=), 5.79 (ddt, 1H, J=16.9, 10.3, 6.7 Hz, CH=), 6.85 (d, 2H, J=8.8 Hz, Ph), 7.16 (d, 2H, J=8.8 Hz, Ph); ^{13}C NMR (50 MHz, $CDCl_3$): δ 28.9, 30.3, 40.2, 67.9, 69.8, 70.2, 70.7, 70.9, 114.8, 114.9, 125.8, 130.4, 138.3, 158.1, 176.7; HRMS (ESI) m/z: calcd for $C_{21}H_{32}O_7Na$: 419.2046 $[M+Na]^+$. Found: 419.2013. **30b**: IR (film): ν 3200, 1710 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.24–1.42 (m, 12H, CH_2), 1.51 (m, 2H, CH_2), 1.95 (q, 2H, J=7.1 Hz, CH_2C=C), 3.39 (t, 2H, J=6.7 Hz, CH_2), 3.36–3.67 (m, 14H, CH_2), 3.78 (s, 2H, CH_2Ph), 4.05 (t, 2H, J=5.4 Hz, CH_2CH_2O), 4.85–4.96 (m, 2H, CH_2=), 5.75 (ddt, 1H, J=16.9, 10.3, 6.7 Hz, CH=), 6.81 (d, 2H, J=8.7 Hz, Ph), 7.12 (d, 2H, J=8.7 Hz, Ph); ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 21.9, 26.0, 28.9, 29.1, 29.3, 29.4, 33.7, 40.1, 67.5, 69.7, 70.0, 70.5, 70.8, 71.5, 114.0, 114.8, 125.9, 130.3, 139.1, 158.0, 176.4; HRMS (ESI) m/z: calcd for $C_{27}H_{44}O_7Na$: 503.2985 $[M+Na]^+$. Found: 503.2953.

*5.4.2. Coupling of **30** to the photo-cleavable ester of 6-APA. 2-Nitro-4,5-(dimethoxy)-benzyl 6-β-aminopenicillinate* (**33′**): 6-APA (1 g, 4.6 mmol) and triethylamine (1.30 mL, 9.2 mmol) in DCM (10 mL) were stirred at room temperature until total dissolution. Methyl acetoacetate (502 μL) was added and the mixture was stirred for 3 h. The solvent was evaporated and the crude **31** was dissolved in dry DMF (10 mL) with 4,5-dimethoxy-2-nitrobenzyl bromide (1.28 g, 4.6 mmol). The mixture was stirred overnight, then diluted with EtOAc (100 mL). The solution was washed with brine (3×), dried over $MgSO_4$ and concentrated under vacuum. The solid residue (**32**) was dissolved in acetone (10 mL) with *p*-toluenesulfonic acid monohydrate (967 mg). Within 15 min a solid appeared (**33**). It was filtered and washed with EtO_2. Finally, crude **33** was solubilized with triethylamine (1.30 mL) in DCM (50 mL) and stirred at room temperature for 2 h. A solution of NaOH 1 M was added and the organic layer was separated. The aqueous layer was extracted with DCM. The combined organic layer was washed with brine, dried over $MgSO_4$ and concentrated under vacuum to give the free amine (**33′**) as a yellow solid (86% yield); Mp=81 °C; IR (film): ν 3338, 1778, 1743, 1522 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.47 (s, 3H, Me), 1.61 (s, 3H, Me), 3.92 (s, 3H, OMe), 3.95 (s, 3H, OMe), 4.41 (s, 1H, $NCHCO_2$), 4.54 (d, 1H, J=5.7 Hz, NHCHCH), 5.47 (d, 1H, J=5.7 Hz, NHCHCH), 5.53 (s, 2H, CH_2Ph) 6.98 (s, 1H, MeOCCH(Ph)), 7.68 (s, 1H, NO_2CCH(Ph)); ^{13}C NMR (50 MHz, $CDCl_3$): δ 27.3, 31.6, 56.8, 63.1, 64.2, 64.4, 70.2, 70.5, 77.9, 108.8, 110.6, 125.7, 140.6, 149.0, 153.9, 167.8, 178.1; Anal. Calcd for $C_{17}H_{21}N_3O_7S$: C 49.63, H 5.14, N 10.21; found: C 49.61, H 5.13, N 9.98%.

*Coupling of the side-chains **30** to **33′***: The side-chain **30a** or **30b** (0.396 mmol) in dry DCM (5 mL) was treated with dicyclohexylcarbodiimide (90 mg, 0.436 mmol) at room temperature for 30 min. After filtration on Celite, **33′** (163 mg, 0.396 mmol) was added to the solution. The mixture was stirred for 6 h, then concentrated and purified by preparative TLC (DCM/EtOAc (1:1)) to furnish **34** or **35** as a yellow oil (58% and 70% yield, respectively); **34**: IR (film): ν 3328, 1775, 1740, 1512 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.40 (s, 3H, Me), 1.55 (s, 3H, Me), 1.65 (m, 2H, $=CHCH_2CH_2$), 2.08 (q, 2H, J=6.6 Hz, $=CHCH_2CH_2$), 3.44 (t, 2H, J=6.8 Hz, $=CHC_2H_4CH_2$), 3.54–3.71 (m, 14H, OCH_2CH_2O), 3.83 (s, 2H, $PhCH_2$), 3.94 (s, 3H, OMe), 3.95 (s, 3H, OMe), 4.09 (t, 2H, J=4.7 Hz, $PhOCH_2$), 4.38 (s, 1H, $NCHCO_2$), 4.92 (dd, 1H, J=1.8, 9.0 Hz, CH_2=CH), 4.98 (dd, 1H, J=1.8, 16.9 Hz, CH_2=CH), 5.47 (d, 1H, J=4.2 Hz, NHCHCH), 5.53 (s, 2H, OCH_2Ph), 5.62 (dd, 1H, J=9.0, 4.2 Hz, NHCHCH), 5.78 (ddt, 1H, J=16.9, 9.0, 6.6 Hz, CH=), 6.11 (d, 1H, J=9.0 Hz, NH) 6.88 (d, 2H, J=8.6 Hz, Ph), 6.96 (s, 1H, MeOCCH(Ph)), 7.14 (d, 2H, J=8.6 Hz, Ph), 7.69 (s, 1H, NO_2CCH(Ph)); ^{13}C NMR (125 MHz, $CDCl_3$): δ 26.5, 28.6, 30.0, 31.2, 42.3, 56.3, 56.4, 58.5, 64.2, 67.3, 67.8, 69.5, 69.9, 70.3, 70.4, 70.5, 70.7, 108.2, 111.1, 114.5, 115.1, 125.1, 125.7, 130.4, 138.1, 140.2, 148.6, 153.4, 158.2, 166.9, 170.6, 173.6; HRMS (ESI) m/z: calcd for $C_{38}H_{51}N_3O_{13}SNa$: 812.3040 $[M+Na]^+$. Found: 812.3010; **35**: IR (film): 3328, 1774, 1740, 1512 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.24–1.38 (m, 12H, $=CHC_2H_4C_6H_{12}$), 1.32 (s, 3H, Me), 1.53 (s, 3H, Me), 1.54 (m, 2H, $=CHCH_2CH_2$), 2.00 (q, 2H, J=6.6 Hz, $=CHCH_2CH_2$), 3.41 (t, 2H, J=6.8 Hz, alkCH_2O), 3.54–3.71 (m, 14H, OCH_2CH_2O), 3.83 (s, 2H, $PhCH_2$), 3.94 (s, 3H, OMe), 3.95 (s, 3H, OMe), 4.09 (t, 2H, J=4.9 Hz, $PhOCH_2$), 4.38 (s, 1H, $NCHCO_2$), 4.89 (dd, 1H, J=1.8, 9.0 Hz, CH_2=CH), 4.96 (d, 1H, J=1.8, 16.9 Hz, CH_2=CH), 5.47 (d, 1H, J=4.2 Hz, NHCHCH), 5.53 (s, 2H, OCH_2Ph), 5.62 (dd, 1H, J=9.0, 4.2 Hz, NHCHCH), 5.78 (ddt, 1H, J=16.9, 9.0, 6.6 Hz, CH=), 6.14 (d, 1H, J=9.0 Hz, NH) 6.88 (d, 2H, J=8.6 Hz, Ph), 6.96 (s, 1H, MeOCCH(Ph)), 7.14 (d, 2H, J=8.6 Hz, Ph), 7.69 (s, 1H, NO_2CCH(Ph)); ^{13}C NMR (125 MHz, $CDCl_3$): δ 26.5, 28.6, 30.0, 31.2, 42.2, 56.2, 56.4, 58.5, 64.2, 67.3, 67.8, 69.5, 69.8, 70.3, 70.4, 70.6, 71.3, 108.2, 111.1, 113.9, 115.1, 125.0, 125.7, 130.4, 139.0, 140.1, 148.5, 153.3, 158.1, 167.0, 170.6, 173.5; HRMS (ESI) m/z: calcd for $C_{44}H_{63}N_3O_{13}SNa$: 896.3979 $[M+Na]^+$. Found: 896.3946.

5.5. Biological evaluations

5.5.1. Assay with BlaP (class A β-lactamase). BlaP (5 μL, solution of 42 ng/mL) in buffer (50 μL, NaPi buffer 50 mM, with ovalbumin 1 mg/mL, pH 7.2) and tested molecule (5 μL solution at different concentrations) were incubated at 30 °C (multi-well plate) for 60 min. Nitrocefine (50 μL, at 100 μM) was added just before reading at 482 nm (Power-Wave microplate-reader, BioTek). The nitrocefine hydrolysis by the residual active enzyme (i.e., BlaP non acylated by the tested compound) gives immediately a coloured product. Pre-incubation of EOM esters with buffer (4 h, 20 °C for ester hydrolysis) afforded similar results (incubation with the enzyme for 15 min).

5.5.2. Assay with P99 (class C β-lactamase). P99 (5 μL, solution of 400 ng/mL) in buffer (50 μL, HEPES buffer 50 mM with NaCl 200 mM and ovalbumin 1 mg/mL, pH 8) and tested molecule (5 μL solution at different concentrations) were incubated at 30 °C (multi-well plate) for 60 min. Reading at 482 nm is performed as above. Previous hydrolysis of EOM esters (see above) gave similar results.

5.5.3. Assay with R39 D,D-carboxypeptidase. R39 (5 μL, solution of 100 μg/mL) in buffer (5 μL, NaPi buffer 50 mM with NaCl 200 mM and ovalbumine 1 mg/mL, pH 8), water (4 μL) and tested molecule (5 μL solution at different concentrations) were incubated at 25 °C during 15 min (Table 1) or 90 min (Table 2). Fluorescent ampicillin (1 μL, 100 μM) was added and after 5 min or 15 min, the protein was denaturated and deposited on the SDS-PAGE 15% gel. After migration, the fluorescence was measured with a Phospho-Imager (Molecular Image FX equipment and Quantity One Software, Biorad, Hercules, CA, USA). The fluorescence intensity is proportional to the residual active protein (i.e., protein non acylated by the tested compound).

5.5.4. Assay with BlaR-CTD receptor. BlaR-CTD (5 μL, solution of 150 μg/mL) in buffer (5 μL, Hepes buffer 50 mM with EDTA 10 mM and DMSO 10%, pH 7.2), water (4 μL) and tested molecule (5 μL solution at different concentrations) were incubated at 25 °C during 15 min (Table 1) or 90 min (Table 2). Labelling and fluorescence measurement were performed as above.

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Supplementary data

The syntheses of the spacer-arms (Schemes 2A, B and 4) and of the EOM ester series (Scheme 1), the deprotection of the penam esters, the reference compounds of Table 2, and Tables of ^{1}H and ^{13}C NMR data, are provided as Supplementary data. Supplementary data related to this article can be found online at doi:10.1016/j.tet.2011.10.100.

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