Pergamon

Tetrahedron, Vol. 52, No. 15, pp. 5591-5606, 1996
Copyright © 1996 Elsevier Science Ltd
Printed in Great Britain. All rights reserved
0040-4020/96 $15.00 + 0.00

S0040-4020(96)00197-4

Design and Synthesis of a Bifunctional Label for Selection of ß-Lactamase displayed on Filamentous Bacteriophage by Catalytic Activity.

Jacqueline Marchand-Brynaert [a*], Michèle Bouchet [b], Roland Touillaux [c], Cécile Beauve [b] and Jacques Fastrez [b].

[a] Laboratoire de Chimie Organique de Synthèse. [b] Laboratoire de Biochimie Physique et des Biopolymères.
[c] Laboratoire de Chimie Physique et Cristallographie.
Département de Chimie, Université Catholique de Louvain, Place Louis Pasteur, 1,
B-1348 Louvain-la-Neuve, Belgium.

Abstract : A bifunctional activity label **1c** has been constructed for the selection of active ß-lactamases displayed on filamentous bacteriophage. It features an original 6-sulfonylamido-penam sulfone moiety, as ß-lactamase suicide-inhibitor, and a biotinyl residue, for separation by affinity chromatography, connected through a linker including a cleavable disulfide bond. The inhibitor **28** resulted from coupling of methoxymethyl 6-aminopenicillinate **8** with N-protected (aminoethoxy)ethoxyethanesulfonyl chloride **23**, followed by oxidation into the corresponding sulfone **25**, and usual deprotections. The biotinyl ester **32** reacted with 3-(2-aminoethyldithio)propanoic acid **31** as linker, to give **33** which was further activated as pentafluorophenol ester **34b**. Final coupling of the building blocks **28** and **34b** gave the target label **1c**.
Copyright © 1996 Elsevier Science Ltd

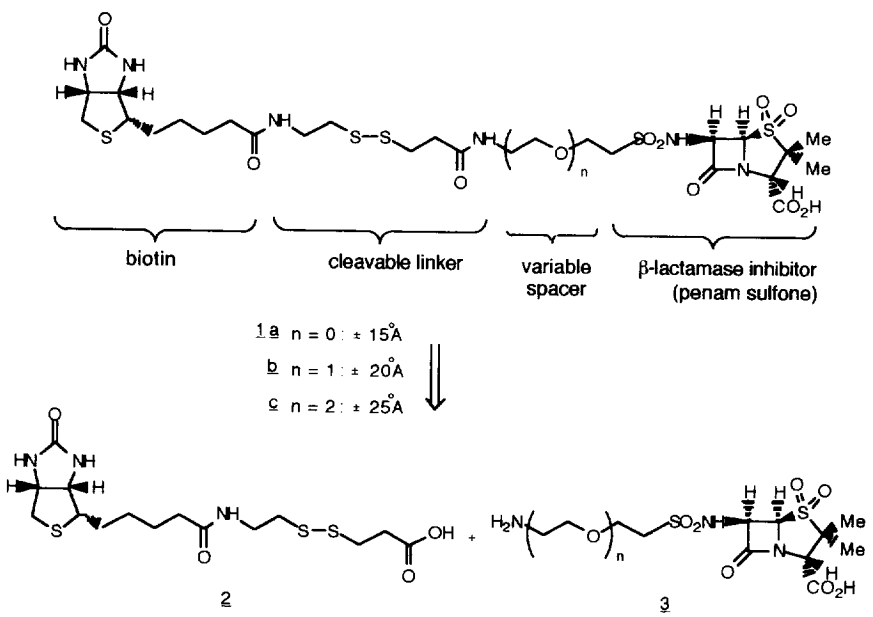
INTRODUCTION

Recently, the display of repertoires of peptides and proteins on the surface of filamentous bacteriophages, and the selection of phages by binding to a ligand, has allowed the isolation of peptides and proteins with rare binding affinities (for reviews, see references 1 and 2). Enzymes have also been displayed on phages and shown to retain their catalytic activities.[3-7] Accordingly, filamentous bacteriophages provide attractive vehicles for the selection of new enzymes from libraries of mutants by proper choice of the ligands.[8-11] By using mechanism-based (or suicide) inhibitors, we planned to base the selection process not simply on binding affinity, but on catalytic activity. [8,12] To develop the selection technology, we have chosen the RTEM ß-lactamase whose the three-dimensional structure[13] and catalytic mechanism are known.[14] A catalytically inactive mutant in which the essential serine of the active site was mutated to alanine was also prepared. [15,16] The corresponding phage-enzymes were constructed by fusion to the minor coat protein (g3p) of the fd phage.[8-10]

For the selective labelling of active (but not the inactive) phages, we designed a bifunctional activity label **1** including a penam sulfone moiety as ß-lactamase suicide-inhibitor,[17,18] and a biotinyl residue allowing subsequent *in vitro* separation by affinity chromatography on a streptavidin support[19] (Scheme 1). The two entities were connected through a linker including a disulfide bond, which can be reductively cleaved[20,21] after immobilisation of the active phages, and a polyether spacer, allowing the variation of the distance between the two recognition entities (arm of 10 to 16 atoms, *i.e.* about 15 to 25 Å length).

On incubation with the label **1c**, the active phages could be selected from mixtures with inactive phages, by irreversible binding and elution from streptavidin-coated beads, thus proving the validity of our selection principle.[8]

In this synthetic report, we describe in detail the preparation of novel ß-lactamase inhibitors **3**, and the construction of the related bifunctional activity label successfully used for the phage-enzymes selection. Our strategy was based on the coupling, *via* a peptide bond, of two building blocks : the biotinyl derivative **2** equipped with the cleavable linker, and the 6-sulfonylamidopenicillanic acid sulfone[22] **3** bearing the variable spacer-arm (Scheme 1).



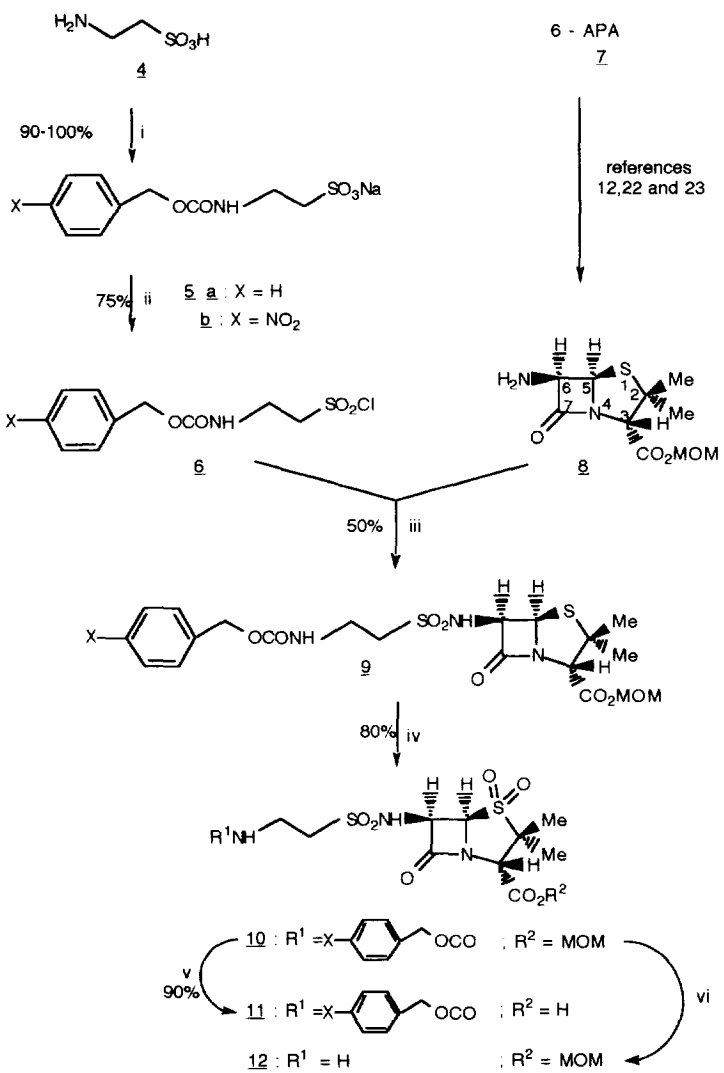
Scheme 1 : Bifunctional activity label for selection of β - lactamase displayed on filamentous bacteriophage

RESULTS AND DISCUSSION

We first considered the synthesis of the penam sulfone derivative **3a** (n=0) devoid of a polyether spacer-arm. This compound formally results from the coupling of taurine **4** and 6-amino-penicillanic acid **7** (6-APA).

Protection of the C-3 carboxyl function of **7** as methoxymethyl (MOM) ester **8** was realised according to previously described procedures.[12,22-23] Using p-toluenesulfonic acid as a model compound, we were unable to perform the coupling reaction to the amine **8** with dicyclohexylcarbodiimide, a classical dehydrating agent;[24]

no reaction was observed after several days, but progressive degradation of the ß-lactam **8** occurred. However, p-toluenesulfonyl chloride reacted easily with **8**, in the presence of triethylamine, to give the expected sulfonylamide. Therefore, we selected the N-protected aminoethanesulfonyl chloride **6** as a useful reagent for the synthesis of 6-sulfonylamido-penicillinate **9** (Scheme 2).



Reagents and conditions: (i) $ArCH_2OCOCl$, NaOH, H_2O, 0°C to 20°C; (ii) $OPCl_3$, sulfolane, CH_3CN, 70°C, 1h; (iii) Et_3N, CH_2Cl_2, 0°C to 20°C; (iv) $KMnO_4$, AcOH - H_2O (4:1), 1h, 0°C; (v) MeOH - H_2O (3 : 2), 20°C, 17h; (vi) H_2, Pd - C, EtOAc, 20°C, 3h.

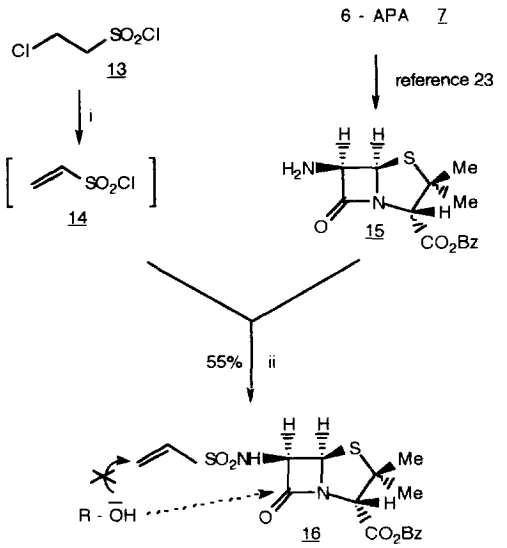
Scheme 2

Reaction of taurine **4** with benzyl chloroformate under Schotten-Baumann conditions yielded sodium 2-N-(benzyloxycarbonyl)aminoethanesulfonate **5a**. Under acidification (pH 0-1) to recover the corresponding sulfonic acid, about 20% of the protecting group was hydrolyzed (^{1}H NMR analysis). Moreover, treatment of this crude sulfonic acid with thionyl chloride[25,26] or phosphorus pentachloride[27,28] led to cleavage of the benzyl group and formation of unidentified degradation products. Treatment of the sulfone salt **5a** with $SOCl_2$[29] or PCl_5[30] gave the same results. Fortunately, the method of Fujita[31] could be readily applied; thus, the salt **5a** dissolved in sulfolane and acetonitrile was treated at 70°C with phosphorus oxychloride to give the sulfonyl chloride **6a** in 75% yield from **4**. Reaction of MOM 6-APA **8** with the chloride **6a** and triethylamine in dichloromethane furnished the sulfonamide **9a** in 50% yield after column-chromatography. Subsequent oxidation[32,33] with potassium permanganate in aqueous acetic acid yielded the corresponding sulfone **10a**. Finally, the MOM protecting group was removed, by smooth solvolysis in aqueous methanol[12], in order to evaluate the activity of the new 6-sulfonylamido-penicillanic acid sulfone **11a** against a ß-lactamase.

Incubation of the *E. coli* RTEM ß-lactamase (4×10^{-8} M in phosphate buffer at pH 7) with compound **11a** (2×10^{-5} to 5×10^{-4} M) leads to a fast (< 30 min.) irreversible inactivation. But, as the inhibitor is simultaneously destroyed by the enzyme, the residual activity after inhibition depends on the ratio between the inhibitor and enzyme concentrations as observed with other suicide inhibitors.[17,22] A plot of the fraction of activity after inhibition versus this ratio allows to determine the number of hydrolytic events before complete inactivation[34]; it measures the quality of the inhibitor. With compound **11a**, this ratio equals 1250.

The construction of the bifunctional activity label **1a** (Scheme 1) required the deprotection of the terminal amine function of the penam sulfone **10a**. When submitted to hydrogenation in the presence of palladium catalyst, the benzylcarbamate **10a** was found to react very sluggishly. After several consecutive runs of hydrogenation, we observed an extensive degradation of the ß-lactam ring (IR and ^{1}H NMR analysis). Therefore, we prepared the p-nitrobenzylcarbamate **10b** which should be more sensitive to hydrogenolysis.[35] This compound was obtained in three steps from 2-N-(p-nitrobenzyloxycarbonyl)aminoethanesulfonate **5b**, according to the procedures previously described for the preparation of **10a**. The cleavage of the N-protecting group occurred easily under hydrogenation in the presence of 10% Pd on carbon, for 3 h in ethyl acetate or ethanol (MS(FAB) m/e 400 ($C_{12}H_{21}N_3O_8S_2$, M + 1)). However, the free amine **12** appeared very unstable; upon concentration of the crude hydrogenation solution, the ß-lactam ring was destroyed (IR and ^{1}H NMR analysis), most probably by intramolecular nucleophilic attack of the amine residue on the azetidinone carbonyl. This degradation was also observed when acetic acid (1 or 2 equivalents) was introduced to the hydrogenation mixture. The preparation of the activity label **1a**, including the ß-lactamase inhibitor **3a** (n=0, Scheme 1) was therefore abandoned, and we turned to the synthesis of the inhibitors **3b** (n=1) and **3c** (n=2).

The first strategy examined towards **3b**,**c** was based on the possibility to perform Michael additions on vinylsulfonylamides.[36-38] Thus, the penam derivative **16** (Scheme 3) was designed as the key-intermediate for the anchorage of various spacer-arms (for instance : N-protected 2-ethanolamine and N-protected 2-aminoethoxy-2-ethanol). 6-N-(Vinylsulfonyl)amino-penicillinate **16** was prepared, in a one-pot process,[39] from 2-chloroethanesulfonyl chloride **13** and benzyl 6-amino-pencillinate **15**.[23] The reactive intermediate, the vinylsulfonyl chloride **14**,[40-41] was formed in situ, at low temperature, by dehydrohalogenation of **13** with triethylamine; coupling of **14** to the amine **15** gave **16** in 55% yield after washing and precipitation from CH_2Cl_2-hexane. Several attempts to add simple alcohols (ethanol, butanol, benzyl alcohol) on the vinylic moiety of **16** were unsuccessful : without catalyst, no reaction occurred, even when we used an excess of



Reagents and conditions : (i) Et_3N, CH_2Cl_2, -60°C to 0°C, 1h; (ii) Et_3N, CH_2Cl_2, -60°C to 0°C, 1h.

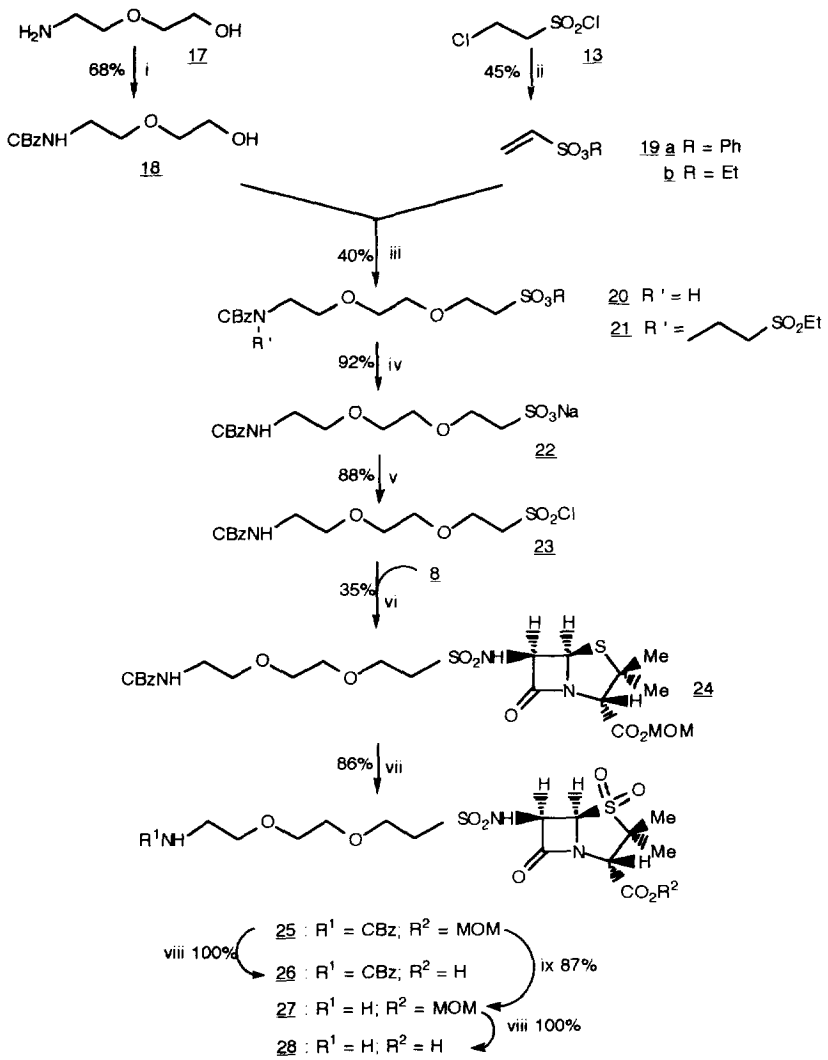
Scheme 3

alcohol in polar solvents. In the presence of p-toluene sulfonic acid as catalyst (2% -5%), the ß-lactam ring gradually disappeared (IR analysis); in the presence of sodium alkoxide (as catalyst or reagent), the ß-lactam ring was rapidly destroyed (IR analysis).

These failures prompted us to consider an alternative strategy using the same building blocks (6-APA, **13** and **17**), but combining them in a different order (Scheme 4). The spacer-arm **20** was independently constructed by Michael addition[42-45] of the alcohol **18** on the vinyl sulfonate **19**. After appropriate activation, the sulfonylated spacer **23** was coupled to the 6-APA derivative **8**. The advantage of this route is to introduce the sensitive ß-lactam in the last step.

2-(2-Aminoethoxy)ethanol **17** was protected by reaction with benzylchloroformate, under standard Schotten-Baumann conditions. The resulting compound **18** was reacted with the commercially available phenyl vinylsulfonate **19a**, in refluxing acetonitrile, for 24 h and in the presence of potassium hydrogenocarbonate as catalyst. The expected Michael adduct **20a** (R=Ph) could be isolated in 60% yield. Unfortunately, all attempts to hydrolyse the sulfonate ester **20a** into the salt **22** failed : retroaddition rapidly occurred, giving back the starting materials **18** and **19a** (NaOH, aqueous dioxane, 1 h, 20°C). Therefore, we used, as Michaël acceptor, ethyl vinylsulfonate **19b**[46-48] whose ester group could be cleaved under neutral conditions. An equimolar mixture of alcohol **18** and sulfonate **19b** in CH_3CN was heated at 80°C with $KHCO_3$ (5-10%) for 3 days. Pure coupling product **20b** (R=Et) was isolated in 40% yield after column-chromatography. Using KOH as catalyst (pH>8), we obtained a 1:2 mixture of mono-adduct **20b** (R_F=0.55; SiO_2, CH_2Cl_2-EtOAc, 50:50) and bis-adduct **21** (R_F=0.70) resulting from the Michaël addition of the carbamate NH-function of **20b** on the vinylsulfonate (MS(EI) m/e=511 for $C_{20}H_{33}NO_{10}S_2$). The action of sodium iodide in acetone on ethyl sulfonate **20b** readily converted the ester function into the corresponding sodium sulfonate **22** and ethyliodide

by nucleophilic substitution.[49] Finally, the acid chloride **23** was formed, as previously described for compound **6**, by reaction of **22** with $OPCl_3$ in sulfolane-acetonitrile[31] (overall yield from **20b**: 81%).



Reagents and conditions: (i) $PhCH_2COCl$, H_2O, NaOH, THF, 0°C to 20°C; (ii) ROH, CH_2Cl_2, Et_3N, 0°C to 20°C; (iii) $KHCO_3$, CH_3CN, 80°C, 3 days; (iv) NaI, acetone, 20°C, 1 day; (v) $OPCl_3$, CH_3CN, sulfolane, 1h, 70°C; (vi) Et_3N, CH_2Cl_2, 1h, 20°C; (vii) $KMnO_4$ (2.1 equiv.), HOAc - H_2O (4 : 1), -10°C, 1h; (viii) MeOH - H_2O (3 : 2), 17h,20°C; (ix) H_2, Pd - C, pTosOH, EtOH, 1h30, 20°C.

Scheme 4

Coupling of the sulfonyl chloride **23** with MOM 6-APA **8**[12,22] gave the penam derivative **24**. Subsequent oxidation by $KMnO_4$[32,33] furnished the sulfone **25** (overall yield from **8**: 30%). MOM deprotection, either by treatment with magnesium bromide etherate[50], or by mild hydrolysis,[12] quantitatively yielded the free acid **26**.

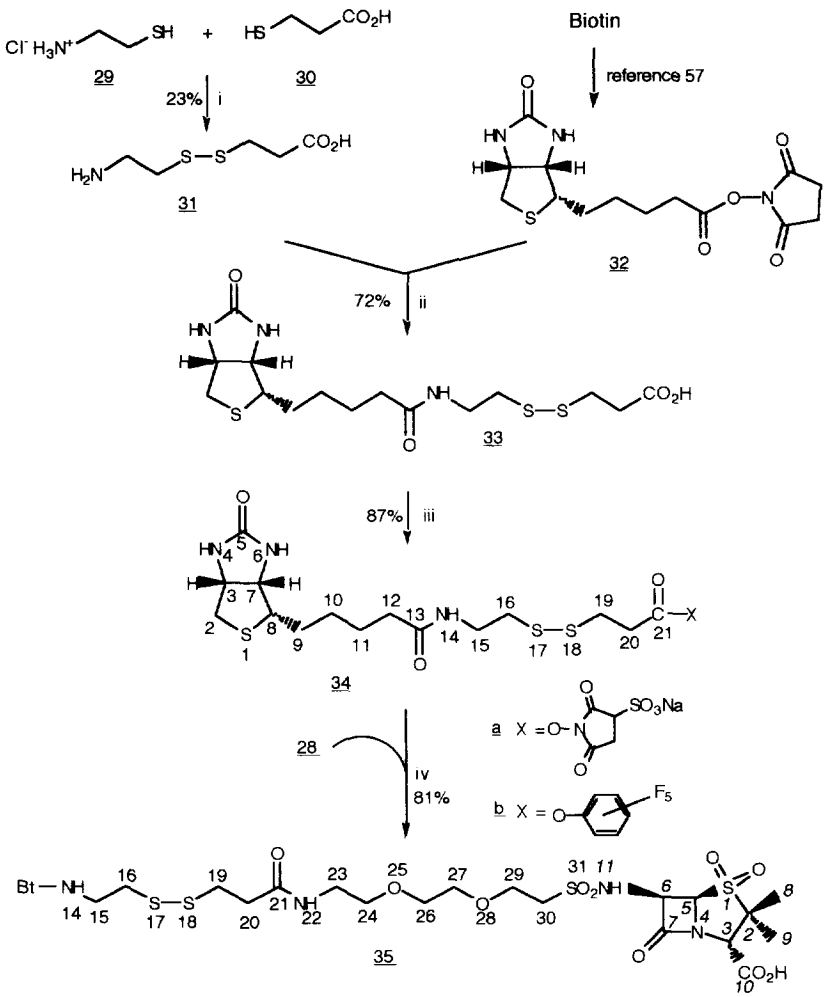
This novel compound was tested as inhibitor (5×10^{-4} M to 10^{-3} M) against RTEM ß-lactamase (4×10^{-8} M) as described above for the sulfone **11a**. **26** was less efficient than **11a**, with a number of hydrolytic events before inhibition of 25000.

Selective deprotection of the benzyloxycarbonyl group of **25** was first attempted under acidic conditions: no reaction occurred in HCO_2H or CF_3CO_2H at 0°C to 20°C; in the presence of HBr dissolved in HOAc, the ß-lactam ring was rapidly destroyed (IR analysis). Catalytic hydrogenation (Pd on carbon) in ethyl acetate, ethanol or acetic acid gave the free amine **27**, together with some degradation products arising from the ß-lactam ring opening. When the hydrogenation was performed in ethanol, in the presence of one equivalent of p-toluenesulfonic acid, we could isolate the amine **27** as its p-toluenesulfonate salt in 87% yield. Treatment of **27** (p.TosOH salt) in aqueous methanol smoothly cleaved the MOM ester[12] to give the totally deprotected penam sulfone **28**.

At this stage, we could envisage rapidly obtaining the designed bifunctional activity label **1c** (Scheme 1), since the biotinyl derivative **34b**[51,52] equipped with the 3-(2-aminoethyldithio)propanoic chain, activated as 3-sodium sulfonate-N-hydroxysuccinimidyl ester, is commercially available (Scheme 5). Unfortunately, several attempts for coupling the synthons **34a** and **28** failed, or furnished very low yields of the final target **35**. Careful spectroscopic analysis (IR, ^{1}H NMR and ^{13}C NMR) of the reagent **34a** (purchased from Pierce Chemical Co.) showed that the real content of the expected product was significantly different from one batch to another, and generally low, ranging between 10% to 30% ! Therefore, we decided to prepare the related biotinyl reagent **34b** (Scheme 5), activated as pentafluorophenyl ester; this compound can be stored at -20°C without degradation (purity ≥ 90%).

Few methods are known for the practical synthesis of unsymmetrical dialkyl disulfides.[53-55] We choose to couple cysteamine **29** and 3-mercaptopropionic acid **30** by oxidation with hydrogen peroxide.[56,57] The symmetrical coupling products (diacid and diamine) were separated from the desired unsymmetrical product **31** by crystallisation (diacid) followed by chromatography on ion-exchange resin. 3-(2-Aminoethyldithio)-propanoic acid **31** reacted with biotin N-hydroxysuccinimidyl ester **32**[58,59] in aqueous $NaHCO_3$ and DMF. The acid **33**, isolated by precipitation with ether, was esterified with pentafluorophenol in DMF, using dicyclohexylcarbodiimide as dehydrating agent. The crude ester **34b** was pure enough to be directly coupled with the penam sulfone **28** in the final step. The reaction was conducted in DMF at room temperature, in the presence of N-ethylmorpholine. The bifunctional activity label **35** could be purified by preparative thin-layer chromatography on reversed-phase silicagel plate. This compound has been successfully used for the phage-enzyme selection based on catalytic activity.[8] Mixtures of active (fd-bla$^+$) and inactive phages (fd-bla$^-$) were incubated with the label **35**. The active phages were labelled and selected from the mixtures by binding on streptavidin coated beads. They were further recovered from the beads on elution by reductive cleavage of the S-S bond of the spacer.[8]

In this technique, the enzyme is lost as it is irreversibly blocked by reaction with the suicide inhibitor, however, the corresponding gene is recovered; it can be recloned to produce the active enzyme in quantity for characterisation and use.



Reagents and conditions: (i) Et_3N, H_2O_2 - H_2O, $FeSO_4$ (catal.), 1h, 0°C; (ii) 0.1M $NaHCO_3$ - DMF (2:5), 1h30, 20°C; ((iii) F_5C_6OH, DCC, DMF, 19h, 20°C; (iv) N-ethylmorpholine, DMF, 30 min., 20°C.

Scheme 5

CONCLUSION

New representatives **3** of the 6-sulfonylamido-penam sulfone[22] family have been prepared and shown to retain the anti-ß-lactamase activity, independently of the nature and the length of the 6-side chain. One compound (**28**) has been involved, as the "reactive head", in the construction of a bifunctional label useful for the selection of phages displaying active ß-lactamase. For that purpose, we have developed a practical synthesis of pentafluorophenyl 3-[2-N-(biotinyl)aminoethyldithio]propanoate. This activated biotinyl spacer **34b** could be used for the anchorage of various enzyme inhibitors.

EXPERIMENTAL SECTION

The reagents were purchased from Janssen Chimica, Aldrich or Fluka. The solvents were dried as follows, then distilled : acetonitrile, N,N-dimethylformamide (DMF) and dichloromethane over phosphorous pentoxide; ether and tetrahydrofuran (THF) over sodium/benzophenone; ethanol over sodium/diethylsuccinate. N-ethylmorpholine and triethylamine were freshly distilled over calcium hydride. Sulfolane was distilled under vacuum.

The column-chromatographies were carried out with silicagel 60, 70-230 mesh ASTM, supplied by Merck. The analytical thin-layer chromatographies were run on silicagel 60 plates F254 (Merck, 0.2 mm thick), on which the compounds were detected with ultraviolet light, iodine vapour and a spray of p-dimethylaminocinnamaldehyde (DMACA), a reagent specific for biotin derivatives.[60, 61] The stock solution was obtained by dissolving DMACA (2g) into ethanol (50 mL) and 6N HCl (50 mL).The spray-solution was prepared by dilution of the stock-solution (1 mL) in ethanol (5 mL). Amino derivatives was detected, as usual, with ninhydrin. The preparative thin-layer chromatographies were realised on RP-18 $F_{254}S$ plates (5 cm x 10 cm; 0.25 mm thick) supplied by Merck.

The optical rotations (± 0.1°) were determined on a Perkin-Elmer 241 MC polarimeter. The IR spectra were recorded with a Perkin-Elmer 1710 instrument (Fourier Transformed Infra Red Spectrometer), only the most significant and diagnostic absorption bands being reported. The ^1H and ^{13}C NMR spectra were recorded on Varian Gemini-200, Varian Gemini-300 or Bruker AM-500 spectrometers. Chemical shifts are reported in ppm (δ) downfield from internal TMS. The atom numbering used for the description of the spectra is shown in scheme 5. The Mass spectra were obtained on a Finnigan MAT TSQ-70 instrument.

The microanalyses were performed at the University College of London, Chemistry Department, Christopher Ingold Laboratories (Dr. Alan Stones).

Sodium 2-N-(benzyloxycarbonyl)aminoethanesulfonate **5a** : taurine **4** (1.46 g, 11.7 mmol) was dissolved in 1N NaOH (11.7 mL, 11.7 mmol) at 0°C. Benzyl chloroformate (2 mL, 14 mmol, 1.2 equiv.) was added dropwise to the cold solution which was neutralised by the simultaneous addition of 1N NaOH (11.7 mL). The mixture was stirred for 30 min at 0°C and 2 h at 20°C, then submitted to lyophilization to give the crude salt **5** (100% yield, white powder) which was further used as such : ^1H NMR (200 MHz, D_2O) δ 2.95 (t, J = 8 Hz, 2H, COND-CH_2), 3.40 (t, J = 8 Hz, 2H, CH_2-SO_3Na), 5.04 (s, 2H, $PhCH_2O$), 7.3 (s, 5H, Ph).

2-N-(benzyloxycarbonyl)aminoethanesulfonyl chloride **6a** : the crude salt **5a** (800 mg, 2.28 mmol, taking into account the presence of 1.2 equiv. of NaCl), suspended in sulfolane (1.2 mL) and CH_3CN (2.5 mL), was treated with phosphorous oxychloride (665 mg, 4.33 mmol, 1.9 equiv.) and heated at 70°C during 40 min. After cooling, the mixture was put into ice-water (10 mL). The oily phase was recovered and washed rapidly with cold water (3 x), then dissolved in CH_2Cl_2 (10 mL). Drying over $MgSO_4$ and concentration gave **6a** (475 mg, 75% yield) as a pale yellow oil : ^1H NMR (200 MHz, $CDCl_3$) δ 3.85 (m, 4H, N-$CH_2CH_2SO_2$), 5.12 (s, 2H, $PhCH_2O$), 5.40 (br s, 1H, NHCOO), 7.36 (s, 5H, Ph).

Methoxymethyl 6-N-[2'-N-(benzyloxycarbonyl) aminoethanesulfonyl]amino-penicillinate **9a** : methoxymethyl 6-amino-pencillinate **8**[12,22] (390 mg, 1.5 mmol) dissolved in CH_2Cl_2 (5 mL) was treated with **6a** (416 mg, 1.5 mmol) and triethylamine (210 µL, 1.5 mmol) at 0°C. The mixture was stirred for 20 min at 20°C, then diluted with CH_2Cl_2 (20 mL) and washed with water. Drying over $MgSO_4$, concentration and column-chromatography on silica gel gave **9a** (316 mg, 42% yield) as a pale yellow amorphous foam : R_F = 0.73 (CH_2Cl_2-EtOAc, 50:50); IR (CH_2Cl_2) ν 3382 (NH), 3290 (NH), 1785 (CO ß-lactam), 1750 (CO ester), 1719 (CO carbamate), 1526, 1456, 1373, 1341, 1310, 1262, 1207, 1150, 1096, 927 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.55 (s, 3H, CH_3), 1.64 (s, 3H, CH_3), 3.37 (t, J = 5.7 Hz, 2H, N-CH_2), 3.51 (s, 3H, OCH_3), 3.72 (m, 2H, CH_2SO_2), 4.47 (s, 1H, H-3), 5.11 (s, 2H, $PhCH_2O$), 5.14 (dd, 1H, J = 10 and 4 Hz, H-6), 5.31 (sharp ABq, 2H, OCH_2O), 5.43 (m, 1H, OCONH), 5.61 (d, 1H, J = 4 Hz, H-5), 5.66 (d, 1H, J = 10 Hz, SO_2NH), 7.34 (s,

5H, Ph); MS (FAB) m/e 502 (M+1), 458, 307, 259, 204, 91; Anal. calcd for $C_{20}H_{27}N_3O_8S_2$ (501) : C, 47.89%; H, 5.43%; N, 8.38% - Found : C, 47.77%; H, 5.31%; N, 8.20%.

Methoxymethyl 6-N-[2'-N(benzyloxycarbonyl)aminoethanesulfonyl]amino-penicillinate sulfone **10a** : the penam **9a** (150 mg, 0.3 mmol) dissolved in a 4:1 mixture of acetic acid and water (10 mL) was treated at -12°C, under vigorous stirring, with $KMnO_4$ (100 mg, 2.1 equiv.) dissolved in water (1 mL) and added very slowly (1 h). The mixture was further stirred for 1 h at -10°C, then extracted with CH_2Cl_2 (2 x 25 mL). Washing with water (2 x 20 mL) and 5% aqueous $NaHCO_3$ (1 x 20 mL), drying over $MgSO_4$ and concentration gave the sulfone **10a** (115 mg, 72% yield) as a white amorphous foam : $[\alpha]_D^{20}$ + 108.6 (CH_3OH, c=1); R_F = 0.60 (SiO_2; CH_2Cl_2-EtOAc, 50:50); IR (CH_2Cl_2) ν 3387 (NH), 3313 (NH), 1807 (CO ß-lactam), 1758 (CO ester), 1718 (CO carbamate), 1526, 1455, 1325, 1264, 1201, 1151, 1118 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.43 (s, 3H, CH_3), 1.62 (s, 3H, CH_3), 3.35 (t, J = 6.2 Hz, 2H, N-CH_2), 3.52 (s, 3H, OCH_3), 3.75 (m, 2H, CH_2SO_2), 4.49 (s, 1H, H-3), 4.83 (d, J = 4.5 Hz, 1H, H-5), 5.11 (s, 2H, $PhCH_2O$), 5.25 (ABd, J = 5.9 Hz, 1H, OCHO), 5.38 (dd, J = 10 Hz and 4.5 Hz, 1H, H-6), 5.40 (m, 1H, OCONH), 5.43 (ABd, J = 5.9 Hz, 1H, OCHO), 6.36 (d, J = 10 Hz, 1H, SO_2NH), 7.34 (s, 5H, Ph); Anal. calcd. for $C_{20}H_{27}N_3O_{10}S_2$ (533) : C, 45.02%; H, 5.10%; N, 7.88% - Found : C, 44.88%; H, 5.12%; N, 7.60%.

Methoxymethyl 6-N-[2'-N-(p-nitrobenzyloxycarbonyl)aminoethanesulfonyl]amino-penicillinate sulfone **10b** : the procedures described for the preparation of **10a** were applied starting from the protected taurine **5b**.
5b : ^1H NMR (200 MHz, D_2O) δ 2.99 (t, 2H), 3.43 (t, 2H), 5.12 (s, 2H), 7.46 (d, J = 7 Hz, 2H), 8.11 (d, J = 7 Hz, 2H). **6b** : ^1H NMR (200 MHz, $CDCl_3$) δ 3.90 (m, 4H), 5.25 (s, 2H), 5.48 (br s, NH), 7.50 (d, 2H), 8.23 (d, 2H). **9b** : R_F = 0.45 (SiO_2; CH_2Cl_2-EtOAc, 50:50); ^1H NMR (200 MHz, $CDCl_3$) δ 1.55 (s, 3H), 1.63 (s, 3H), 3.40 (t, 2H), 3.51 (s, 3H), 3.75 (m, 2H), 4.48 (s, 1H), 5.15 (dd, J = 8 Hz and 4 Hz, 1H), 5.21 (s, 2H), 5.31 (sharp ABq, 2H), 5.55 (br s, NHCOO), 5.62 (d, J = 4 Hz, 1H), 5.70 (d, J = 8 Hz, SO_2NH), 7.50 (d, J = 7 Hz, 2H), 8.20 (d, J = 7 Hz, 2H). **10b** : R_F = 0.36 (SiO_2; CH_2Cl_2-EtOAc, 50:50); ^1H NMR (500 MHz, $CDCl_3$) δ 1.37 (s, 3H, CH_3), 1.54 (s, 3H, CH_3), 3.31 (m, 2H, N-CH_2), 3.45 (s, 3H, OCH_3), 3.66 (m, 2H, CH_2SO_2), 4.43 (s, 1H, H-3), 4.82 (d, J = 4.5 Hz, 1H, H-5), 5.14 (sharp ABq, 2H, $PhCH_2O$), 5.19 (ABd, J = 6 Hz, 1H, O-CH-O), 5.36 (dd, J = 10 Hz and 4.5 Hz, 1H, H-6), 5.36 (ABd, J = 6 Hz, 1H, O-CH-O), 5.56 (br s, 1H, NHCOO), 6.40 (d, J = 10 Hz, 1H, $NHSO_2$), 7.43 (d, J = 8 Hz, 2H, Aryl), 8.12 (d, J = 8 Hz, 2H, aryl); ^{13}C NMR (125 MHz, $CDCl_3$) δ 17.59 (CH_3), 19.90 (CH_3), 35.82 (N-CH_2), 53.84 (SO_2CH_2), 58.40 (OCH_3), 60.48 (C-6), 63.71 (C-3) , 64.67 (C-2), 65.36 (Ph-CH_2), 65.70 (C-5), 92.36 (O-CH_2-O), 123.60, 128.01, 143.63 and 147.53 (Aryl), 155.88 (CO carbamate), 165.98 (CO ß-lactam), 173.28 (CO ester); MS (FAB) m/e 579 ($C_{20}H_{26}N_4O_{12}S_2$ + 1), 563, 547, 535, 519, 489, 419, 291, 287.

6-N-[2'-N-(benzyloxycarbonyl)aminoethanesulfonyl]amino-pencillanic acid sulfone **11a** : MOM ester **10a** (15 mg, 0.028 mmol) was dissolved in aqueous methanol (2.5 mL; 40:60, v/v) and left overnight at room temperature. Concentration under vacuum and lyophilization gave the acid
11a : 12 mg (90%); IR (film) ν 3400 (br), 1808, 1705 (br), 1528, 1445, 1323, 1270, 1149, 1116 cm^{-1}. ^1H NMR (500 MHz, CD_3OD) δ 1.43 (s, 3H, CH_3), 1.57 (s, 3H, CH_3), 3.36 (m, J_{AB} = 13.8 Hz, 2H, N-CH_2), 3.60 (m, J_{AB} = 14.5 Hz, 2H, CH_2SO_2), 4.45 (s, 1H, H-3), 5.08 (s, 2H, $PhCH_2O$), 5.09 (d, J = 4.55 Hz, 1H, H-5), 5.54 (d, J = 4.55 Hz, 1H, H-6), 7.34 (br s, 5H, Ph); ^{13}C NMR (125 MHz, CD_3OD) δ 17.82 (CH_3), 20.24 (CH_3), 36.79 (N-CH_2), 54.0 (CH_2SO_2), 61.23 (C-6), 65.24 (C-3), 65.7 (C-2), 67.38 (CH_2O), 67.69 (C-5), 128.83, 128.98, 129.45 and 138.19 (Ph), 158.72 (CO carbamate), 169.79 (CO β-lactam), 175.35 (CO acid); MS (FAB) m/e 488 ($C_{18}H_{23}N_3O_9S_2$ - 1), 444, 242.

Benzyl 6-N-(vinylsulfonyl)amino-pencillinate **16** : The reaction was conducted under argon atmosphere. 2-Chloroethanesulfonyl chloride **13** (0.37 mL, 3.5 mmol) dissolved in CH_2Cl_2 (5 mL) was treated at -60°C (dry-ice/acetone cooling bath) with Et_3N (490 µL, 3.5 mmol) in CH_2Cl_2 (5 mL). The mixture was stirred for 30 min

at -60°C, and then for 45 min at 0°C. To the crude vinylsulfonyl chloride **14**, cooled again at -60°C, was added a mixture of 6-APA benzyl ester **15**[23] (1.064 g, 3.5 mmol) and Et_3N (490 µL, 3.5 mmol) in CH_2Cl_2 (5 mL). The mixture was stirred for 30 min at -60°C, 45 min at 0°C, and 20 min at 20°C. Washing with 0.01 M aqueous HCl (2 x 20 mL), drying over $MgSO_4$ and concentration gave crude **16** as an oil. The product was dissolved in CH_2Cl_2 and precipitated by addition of hexane to yield **16** as an amorphous yellow solid : 762 mg (55%); IR (KBr) ν 1785 (CO ß-lactam), 1742 (CO ester), 1686 (C=C) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.40 (s, 3H, CH_3), 1.59 (s, 3H, CH_3), 4.47 (s, 1H, H-3), 5.12 (dd, J = 10 Hz and 4.2 Hz, 1H, H-6), 5.27 (s, 2H, $PhCH_2O$), 5.53 (d, J = 4.2 Hz, 1H, H-5), 5.81 (d, J = 10 Hz, 1H, $NHSO_2$), 5.96 (d, J = 9.7 Hz, 1H, HC=C), 6.29 (d, J = 16.5 Hz, 1H, HC=C), 6.63 (dd, J = 16.5 Hz and 9.7 Hz, 1H, HC=C), 7.36 (s, 5H, Ph); MS (FAB^+) m/e 397 ($C_{17}H_{20}N_2O_5S_2$ + 1).

2-[2-N-(benzyloxycarbonyl)aminoethoxy]ethanol **18** : 2-(2-aminoethoxy)ethanol **17** (3.92 mL, 0.04 mol) was dissolved in water (100 mL) containing NaOH (3.2 g, 0.08 mol). Benzyl chloroformate (6.85 mL, 0.048 mol) in THF (10 mL) was added dropwise, at 0°C under stirring. The mixture was left for 1 h at 0°C and 30 min at 20°C. 1N HCl was added to reach pH 4. Extraction with ethyl acetate (3 x 50 mL), drying over $MgSO_4$ and concentration gave crude **18** that was purified by column-chromatography on silicagel; yield : 6.5 g (68%); R_F = 0.2 (CH_2Cl_2-EtOAc, 50:50). IR (CH_2Cl_2) ν 3300-3450 (NH, OH), 1703 (CO carbamate), 1536, 1261, 1126 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 2.40 (br s, 1H, OH), 3.40 (m, 2H), 3.55 (t, 4H), 3.72 (m, 2H), 5.11 (s, 2H), 5.38 (br s, 1H, NH), 7.33 (s, 5H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 41.30 (NCH_2), 61.89 (OCH_2), 67.15 (OCH_2), 70.49 (OCH_2), 72.73 (CH_2 Cbz), 128.58, 128.98 and 137.06 (Ph), 157.36 (CO); MS (FAB) m/e 240 ($C_{12}H_{17}NO_4$ + 1), 196 , 91.

Vinyl sulfonates **19** : phenyl vinylsulfonate was purchased from Aldrich. Ethyl vinyl sulfonate was prepared from 2-chloroethanesulfonyl chloride **13**[46]. **13** (11.5 mL, 0.11 mol) dissolved in CH_2Cl_2 (75 mL) and ethanol (5.74 mL, 0.11 mol) was treated, at 0°C, with triethylamine (31 mL, 0.22 mol) added dropwise during 1 h. The mixture was stirred for 30 min at 20°C, then washed with 0.01 N HCl and water. Drying over $MgSO_4$, concentration and distillation gave **19b** as a colourless oil (6.73 g, 45%) : Eb. : 88°C (0.6 mm Hg); R_F = 0.87 (SiO_2; CH_2Cl_2-EtOAc, 50:50); ^1H NMR (200 MHz, $CDCl_3$) δ 1.40 (t, J = 7.1 Hz, 2H), 4.20 (q, J = 7.1 Hz, 2H), 6.13 (d, J = 9.2 Hz, 1H), 6.40 (d, J = 16.6 Hz, 1H), 6.56 (dd, J = 9.2 Hz and 16.6 Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.3, 67.0, 130.1, 132.3.

Ethyl 2-[2-[2-N-(benzyloxycarbonyl)aminoethoxy]]-ethoxyethanesulfonate **20b** : a mixture of **19b** (680 mg, 5 mmol), **18** (1.2 g, 5 mmol) and finely powdered $KHCO_3$ (100 mg) in acetonitrile (10 mL) was heated at 80°C, under stirring, for 3 days. Filtration, concentration and column-chromatography on silica gel gave **20b** (750 mg, 40% yield) as a colourless oil; R_F = 0.55 (CH_2Cl_2-EtOAc, 50:50); IR (film) ν 3388 (NH), 1719 (CO carbamate), 1530, 1353, 1251, 1167, 1114, 1004, 922 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.38 (t, J = 7 Hz, 3H), 3.37 (m, 4H), 3.50-3.70 (m, 6H), 3.90 (t, J = 6.4 Hz, 2H), 4.29 (q, J = 7 Hz, 2H), 5.11 (S, 2H), 5.32 (br s, 1H, NH), 7.35 (s, 5H); Anal. Calcd. for $C_{16}H_{25}NO_7S$ (375) : C, 51.19%; H, 6.71%; N, 3.73% - Found : C, 51.11%; H, 6.74%; N, 3.77%.

2-[2-[2-N-(benzyloxycarbonyl)aminoethoxy]]-ethoxyethanesulfonyl chloride **23** : a mixture of ethyl sulfonate **20b** (0.98 g, 2.61 mmol) and sodium iodide (0.394 g, 2.62 mmol) in acetone (10 mL) was stirred at 20°C during 24 h. Concentration under vacuum, and washing of the oily residue with hexane gave the sodium sulfonate **22** (0.886 g, 92% yield) as a white powder : IR (KBr) ν 3430 (br), 1692, 1546, 1456 cm^{-1}; ^1H NMR (200 MHz, D_2O) δ 3.00 (t, 2H, ND-$\underline{CH_2}$-CH_2-O), 3.14 (t, 2H, ND-CH_2-$\underline{CH_2}$-O), 3.40 (t, 2H, O-$\underline{CH_2}$-CH_2-SO_3), 3.45 (s, 4H, O-CH_2-CH_2-O), 3.68 (t, 2H, O-CH_2-$\underline{CH_2}$-SO_3), 4.95 (s, 2H, Ph$\underline{CH_2}$O), 7.25 (s, 2H, Ph). The sulfonate **22** (0.369 g, 1 mmol) dissolved in sulfolane (0.5 mL) and acetonitrile (0.5 mL) was treated with $OPCl_3$ (0.17 mL, 1.9 mmol) for 50 min at 70°C. The mixture was cooled and ice-water was added (10 mL).

The oil formed was rapidly washed with cold water (3 x 5 mL), then dissolved in CH_2Cl_2 (10 mL). The organic phase was washed with water, dried over $MgSO_4$ and concentrated to give the sulfonyl chloride **23** (0.322 g, 88% yield) as an oil : IR (film) ν 3417-3348 (br), 1718, 1525, 1455, 1371, 1256, 1167, 1113, 1028 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 3.35 (m, 2H), 3.47-3.70 (m, 6H), 3.92 (m, 2H), 4.03 (m, 2H), 5.06 (s, 2H), 5.18 (br, s, 1H, NH), 7.30 (s, 5H).

Methoxylmethyl 6-[2-[2-[2-N(benzyloxycarbonyl)aminoethoxy]]ethoxyethanesulfonyl]amino-penicillinate **24** : methoxymethyl 6-aminopenicillinate **8**[12,22] (163 mg, 0.6 mmol) dissolved in CH_2Cl_2 (2 mL) was treated with triethylamine (84 μL, 0.6 mmol) and then, with the sulfonyl chloride **23** (219 mg, 0.6 mmol) in CH_2Cl_2 (1 mL). The mixture was stirred for 20 min at 20°C, then washed with water, dried over $MgSO_4$ and concentrated. The crude product was purified by column-chromatography on silica gel to give the sulfonamide **24** (124 mg, 35% yield) : R_F = 0.35 (CH_2Cl_2-EtOAc, 50:50); IR (film) ν 3379 (br, NH), 1785 (CO ß-lactam), 1730 (CO ester), 1719 (CO carbamate), 1526, 1456, 1340, 1299, 1254, 1149, 1097, 1026, 923 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.45 (s, 3H, CH_3), 1.57 (s, 3H, CH_3), 3.35 (m, 2H), 3.45-3.63 (m, 8H), 3.44 (s, 3H, OCH_3), 3.81-3.89 (m, 2H), 4.35 (s, 1H, H-3), 5.04 (sharp ABq, 2H, O-CH_2Ph), 5.10 (dd, J = 4 Hz and 10 Hz, 1H, H-6), 5.20 (ABd, J = 6 Hz, 1H, O-CH-O), 5.24 (ABd, J = 6 Hz, 1H, O-CH-O), 5.41 (d, J = 4 Hz, 1H, H-5), 5.49 (br t, 1H, NH-COO), 5.90 (d, J = 10 Hz, 1H, NH-SO_2), 7.20-7.30 (m, 5H, Ph); ^{13}C NMR (125 MHz, $CDCl_3$) δ 26.68 (*C-8*), 31.67 (*C-9*), 40.69 (C-23), 53.62 (C-30), 58.10 (*OCH_3*), 61.95 (*C-6*), 64.49 (*C-2*), 65.06 (Cbz), 66.38 (C-29), 67.74 (*C-5*), 69.16 (C-24), 69.96 (C-27), 70.04 (C-26), 70.37 (*C-3*), 91.57 (*O-CH_2-O*), 127.9, 128.3, 136.61 (Ph), 156.41 (CO carbamate), 167.08 (CO ß-lactam), 173.25 (CO ester); MS (DCI/CH_4-N_2O) m/e 590 ($C_{24}H_{35}N_3O_{10}S_2$ + 1).

Methoxymethyl 6-[2-[2-[2-N(benzyloxycarbonyl)aminoethoxy]]ethoxyethanesulfonyl]amino-pencillinate sulfone **25** : the penam **24** (294 mg, 0.5 mmol) dissolved in a 4:1 mixture of HOAc and H_2O (15 mL) was treated at -12°C, under vigorous stirring, with $KMnO_4$ (166 mg, 2.1 equiv.) dissolved in water (2 mL), and added very slowly (2 h). The mixture was further stirred for 1 h at -10°C; 10% H_2O_2 was added to discolour the solution. CH_2Cl_2 (75 mL) was added; the organic phase was washed twice with 5% $NaHCO_3$, dried over $MgSO_4$ and concentrated. The residue was purified by column-chromatography on silica gel to give the sulfone **25** (267 mg, 86% yield) as a colourless oil : $[\alpha]_D^{20}$ + 78.3 (CH_3OH, c = 0.9); R_F = 0.50 (CH_2Cl_2-EtOAc, 50:50); IR (film) ν 3400-3950 (br, NH), 1808 (CO ß-lactam), 1764 (CO ester), 1718 (CO carbamate), 1526, 1455, 1325, 1258, 1151, 1117, 937, 896 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.40 (s, 3H, *H-9*), 1.61 (s, 3H, *H-8*), 3.29-3.46 (m, 4H, H-23 + H-24), 3.53 (s, 3H, *OCH_3*), 3.56 (m, 2H, H-26), 3.61-3.70 (m, 4H, H-27 + H-29), 3.86-3.96 (m, 2H, H-30), 4.48 (s, 1H, *H-3*), 4.75 (d, J = 4.5 Hz, 1H, *H-5*), 5.08 and 5.15 (two ABd, J = 12 Hz, 2H, CH_2Ph), 5.24 and 5.42 (two ABd , J = 6 Hz, 2H, *O-CH_2-O*), 5.48 (dd, J = 4.5 Hz and 10.5 Hz, 1H, *H-6*), 5.61 (br s, 1H, NHCOO), 6.50 (d, J = 10.5 Hz, 1H, *$NHSO_2$*), 7.30-7.40 (m, 5H, Ph); ^{13}C NMR (125 MHz, $CDCl_3$) δ 17.84 (*C-9*), 20.03 (*C-8*), 41.12 (C-23), 54.57 (C-30), 58.21 (*OCH_3*), 60.94 (*C-6*), 64.07 (*C-3*), 64.61 (*C-2*), 65.28 (Cbz), 66.19 (*C-5*), 66.55 (C-29), 69.76 (C-27), 70.03 (C-26), 70.69 (C-24), 92.40 (*O-CH_2-O*), 127.86, 127.93, 128.37 and 136.98 (Ph), 156.46 (CO carbamate), 166.24 (CO β-lactam), 173.32 (CO ester); MS (DCI/CH_4-N_2O) m/e 622 (M+1); Anal. Calcd. for $C_{24}H_{35}N_3O_{12}S_2$(621) : C, 46.37%; H, 5.67%; N, 6.76% - Found : C, 46.35%; H, 5.85%; N, 6.54%.

6-[2-[2-[2-N(benzyloxycarbonyl)aminoethoxy]]ethoxyethanesulfonyl]amino-penicillanic acid sulfone **26** : method A : the MOM ester **25** (31 mg, 0.049 mmol) dissolved in CH_2Cl_2 (1.5 mL) was treated with $MgBr_2$.ether (13 mg, 2 equiv.) for 1 h at 20°C under stirring. Concentration and washing of the solid residue with ether gave **26** (bromo magnesium salt, 30 mg, 100% yield) : IR (KBr) ν 3425 (br), 1804, 1710, 1634 (COO^-), 1550, 1480, 1320, 1152, 938, 603 cm^{-1}.
Method B : the MOM ester **25** (17 mg, 0.024 mmol) was dissolved in aqueous methanol (2.5 mL; 40:60, v/v) and left overnight at 20°C. Concentration and lyophilization gave the free acid **26** : 15 mg (100%); IR (KBr) ν

3400 (br), 1806 (CO β-lactam), 1711 (br), 1640, 1534, 1454, 1324, 1151, 1115 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 1.40 (s, 3H, *H-9*), 1.53 (s, 3H, *H-8*), 3.30 (m, 2H, H-23), 3.43-3.48 (m, 2H, H-30), 3.52 (m, 2H, H-24), 3.62 (m, 4H, H-26 + H-27), 3.86 (m, 2H, H-29), 4.43 (s, 1H, *H-3*), 5.07 (d, 1H, J = 4.4 Hz, *H-5*), 5.06 (ABq, 2H, CH_2Ph), 5.55 (d, 1H, J = 4.4 Hz, *H-6*), 7.25-7.35 (m, 5H, Ph); ^{13}C NMR (125 MHz, CD_3OD) δ 17.85 (*C-9*), 20.27 (*C-8*), 41.82 (C-23), 55.01 (C-30), 61.43 (*C-6*), 65.24 (*C-3*), 65.64 (*C-2*), 66.28 (C-29), 67.50 (*C-5*), 67.50 (Cbz), 70.85 (C-24), 70.92 (C-27), 71.61 (C-26), 128.89, 128.99, 129.48 and 138.35 (Ph), 158.86 (CO carbamate), 169.90 (*C-7*), 175.65 (*C-10*); MS (FAB) m/e 578 ($C_{22}H_{32}N_3O_{11}S_2$ + 1).

Methoxymethyl 6-[2-[2-(2-aminoethoxy)]ethoxyethanesulfonyl]amino-penicillinate sulfone **27** : to the sulfone **25** (113 mg, 0.182 mmol) dissolved in ethanol (18 mL) were added successively pTosOH (31 mg, 1 equiv.) and 10% Pd on activated carbon (100 mg). The mixture was submitted to hydrogenation (Parr apparatus, P_{H2} = 40 psi), under shaking, for 1 h 30 min at 20°C. Filtration, concentration under vacuum and drying under high vacuum gave the amine **26** as p.toluenesulfonate salt (107 mg, 87% yield) : IR (KBr) ν 3500-3200 (br), 1806 (CO β-lactam), 1760 (CO ester), 1630, 1456, 1324, 1153, 1118, 1035, 1010, 926 cm^{-1}; ^1H NMR (200 MHz, acetone-d6) δ 1.45 (s, 3H, *H-8*), 1.56 (s, 3H, *H-9*), 2.32 (s, 3H, Ph-CH_3), 3.30-3.95 (m, 12H), 3.67 (s, 3H, *OCH_3*), 4.46 (s, 1H, *H-3*), 4.63 (d, J = 4 Hz, 1H, *H-5*), 5.30 (sharp ABq, 2H, *O-CH_2-O*), 5.70 (dd, J = 4 Hz and 10 Hz, 1H, *H-6*), 6.65 (d, J = 10 Hz, 1H, $NHSO_2$), 7.20 (d, J = 8 Hz, 2H, Tosyl), 7.70 (d, J = 8 Hz, 2H, Tosyl). MS (FAB) m/e 490 ($C_{16}H_{30}N_3O_{10}S_2$ + 1), 460, 444, 307, 289. The MOM ester **27** was unstable in the presence of pTosOH; after storage at -20°C for 3 months, the MOM was cleaved.

6-[2-[2-(2-aminoethoxy)]ethoxyethanesulfonyl]amino-penicillanic acid sulfone **28** : the MOM ester **27** (p-TosOH salt; 68 mg, 0.109 mmol) was dissolved in MeOH/H_2O (60:40, v/v; 2.5 mL) and left for 18 h at 20°C. Concentration and lyophilization gave **28** : 60 mg (p-TosOH salt, 100%); IR (film) ν 3430 (br), 1804 (CO β-lactam), 1737 (CO acid), 1639, 1460, 1323, 1151, 1124, 1036, 1011 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 1.45 (s, 3H, *H-8*), 1.57 (s, 3H, *H-9*), 2.36 (s, 3H, CH_3-Ar), 3.12 (m, 2H, H-23), 3.67 (m, 8H, H-24 + H-26 + H-27 + H-30), 3.91 (m, 2H, H-29), 4.46 (s, 1H, *H-3*), 5.12 (d, J = 4.64 Hz, 1H, *H-5*), 5.59 (d, J = 4.64 Hz, 1H, *H-6*), 7.23 (d, J = 8.1 Hz, 2H, $ArSO_3^-$), 7.70 (d, J = 8.1 Hz, 2H, $ArSO_3^-$); ^{13}C NMR (125 MHz, CD_3OD) δ 17.82 (*C-8*), 20.22 (CH_3-Ar), 21.27 (*C-9*), 40.70 (C-23), 54.66 (C-30), 61.30 (*C-6*), 65.21 (*C-3*), 65.69 (*C-2*), 66.27 (*C-5*), 67.41 (C-24), 67.68 (C-29), 70.99 (C-27), 71.43 (C-26), 126.96, 129.79 and 141.64 (Ar), 169.79 (*C-7*), 175.61 (*C-10*); MS (FAB) m/e 444 ($C_{14}H_{26}N_3O_9S_2$), 391, 307, 289.

3-(2-aminoethyldithio)propanoic acid **31** : to a mixture of cysteamine hydrochloride **29** (4.54 g, 40 mmol), 3-mercaptopropionic acid **30** (4.24 g, 40 mmol) and triethylamine (8.36 mL, 60 mmol) in water (40 mL) were added successively, at 0°C, one crystal of $FeSO_4$ (indicator) and 16% H_2O_2 (± 6.8 mL); H_2O_2 was added dropwise under stirring. At the end of the addition, the pink solution became pale yellow. The cold solution was acidified to pH 2 by addition of ccHCl, and stirred for 30 min. The crystallised diacid (HO_2C-CH_2-CH_2-S)$_2$ was filtered off, and washed with cold 0.01 N HCl [R_F = 0.95 (SiO_2; EtOAc-HOAc-H_2O, 8:2:1)]. The filtrate was extracted with EtOAc (40 mL). The aqueous phase was made neutral by addition of Et_3N, concentrated to a volume of 20 mL, and chromatographed on a column of Amberlite ^{R} IRC-50S ion-exchange resin (25 g). Elution with water (pH 5.5), furnished first the diamine (H_2N-CH_2-CH_2-S)$_2$ [R_F = 0.03 (SiO_2; EtOAc-HOAc-H_2O, 8:2:1)], and then the amino acid **31** [R_F = 0.25 (SiO_2; EtOAc-HOAc-H_2O, 8:2:1)]. Concentration gave 1.7 g of crude **31** (23% yield). Dissolution in aqueous HOAc followed by evaporation to dryness and crystallisation from 90% aqueous n-propanol gave pure **31** : mp 155-156° C; IR (KBr) ν 2500-3200 (br), 1630 (CO_2H), 1544, 1511, 1478, 1434, 1407, 1305, 1249 cm^{-1}; ^1H NMR (200 MHz, D_2O) δ 2.52 (t, J = 6.4 Hz, 2H, CH_2COOD), 2.78 and 2.80 (two t, J = 6.4 Hz, 4H, CH_2-S), 3.19 (t, J = 6.4 Hz, 2H, CH_2-ND_2).

3-[2-N-(biotinyl)aminoethyldithio]propanoic acid **33** : a solution of **31** (382 mg, 2.11 mmol, 1.2 equiv.) in 0.1 M aqueous $NaHCO_3$ (3.6 mL) was added to a solution of biotin N-hydroxysuccinimidyl ester **32** [57] (600 mg, 1.76 mmol) in DMF (10 mL). The mixture was stirred for 1 h 30 min at 20°C. The oil obtained after addition of ether (30 mL) was washed with ether, then extracted with 0.01 N HCl (trituration of the solid), and dried under high vacuum to give **33** (516 mg, 72% yield) :IR (KBr) ν 3290 (br), 1698, 1655, 1543, 1464, 1420, 1327, 1266, 1204, 1188 cm^{-1}; ^1H NMR (500 MHz, DMSO-d6) δ 1.26 (m, 2H, H-10), 1.42 (m, 1H, H-9), 1.49 (m, 2H, H-11), 1.60 (m, 1H, H-9'), 2.03 (t, 2H, H-12), 2.56 (d, 1H, H-2), 2.60 (t, 2H, H-20), 2.75 (t, 2H, H-16), 2.81 (m, 1H, H-2'), 2.86 (t, 2H, H-19), 3.07 (m, 1H, H-8), 3.28 (m, 2H, H-15), 4.11 (m, 1H, H-7), 4.28 (m, 1H, H-3), 6.36 (br s, 1H, H-4), 6.43 (br s, 1H, H-6), 7.95 (br s, 1H, H-14), 12.32 (br s, 1H, CO_2H); ^{13}C NMR (125 MHz, DMSO-d6) δ 25.17 (C-11), 27.97 (C-9), 28.12 (C-10), 33.07 (C-19), 33.62 (C-20), 35.10 (C-12), 37.33 (C-16), 37.80 (C-15), 39.59 (C-2), 55.35 (C-8), 59.17 (C-7), 61.00 (C-3), 162.68 (C-5), 172.15 (C-13), 172.62 (C-21); MS (FAB) m/e 408 ($C_{15}H_{25}N_3O_4S_3$ + 1), 307, 289, 227, 165.

Pentafluorophenyl 3-[2-N-(biotinyl)aminoethyldithio]propanoate **34b** : to a mixture of pentafluorophenol (177 mg, 0.96 mmol) and acid **33** (334 mg, 0.8 mmol) in DMF (10 mL) was added dicyclohexylcarbodiimide (214 mg, 1.04 mmol). The mixture was stirred for 19 h at 20°C. Filtration of urea, and concentration under high vacuum gave a residue which was precipitated from ether (one night, at 0°C). Filtration and drying gave **34b** (400 mg, 87% yield) : R_F = 0.73 (SiO_2; CH_2Cl_2-CH_3OH, 4:1); IR (KBr) ν 3310 (br), 1786, 1742, 1703, 1642, 1521, 1460, 1425, 1359, 1266, 1244, 1204, 1103, 1004 cm^{-1}; ^1H NMR (500 MHz, DMSO-d6) δ 1.28 (m, 2H, H-10), 1.44 (m, 1H, H-9), 1.47 (m, 2H, H-11), 1.59 (m, 1H, H-9'), 2.05 (t, 2H, H-12), 2.56 (d, 1H, H-2), 2.79-2.81 (m, 3H, H-16 + H-2'), 3.04 (t, 2H, H-19), 3.08 (m, 1H, H-8), 3.21 (t, 2H, H-20), 3.31 (m, 2H, H-15), 4.11 (m, 1H, H-7), 4.25 (m, 1H, H-3), 6.36 (br s, 1H, H-4), 6.43 (br s, 1H, H-6), 7.96 (br s, 1H, H-14); ^{13}C NMR (125 MHz, DMSO-d6) δ 25.17 (C-11), 27.98 (C-9), 28.12 (C-10), 32.04 (C-19), 32.63 (C-20), 35.09 (C-12), 37.33 (C-16), 37.67 (C-15), 39.59 (C-2), 55.34 (C-8), 59.15 (C-7), 60.98 (C-3), 162.62 (C-5), 167.84 (C-21), 172.13 (C-13), C-F carbons not visible; Anal. Calcd. for $C_{21}H_{24}F_5N_3O_4S_3$ (573): C, 43.97%; H, 4.22%; N, 7.33%. Found : C, 45.45%; H, 4.68%; N, 8.22%.

6-[2-[2-[2-[3-(2-N-biotinyl)aminoethyldithio)propanoyl]aminoethoxy]ethoxy]ethanesulfonyl-amino-pencillanic acid sulfone **35** : to a mixture of ester **34b** (46.5 mg, 0.0812 mmol) and penam sulfone **28** (50 mg, 0.0812 mmol, p-toluene sulfonyl salt) in DMF (2 mL) was added N-ethyl morpholine (21 µL, 0.162 mmol, 2 equiv.). The mixture was stirred for ~ 30 min at 20°C, then the solvent was evaporated under high vacuum. The residue was washed with ether (trituration of the oil), dissolved in water (5 mL + one drop of DMF) and extracted with CH_2Cl_2 (elimination of unreacted **34b**). The aqueous phase was lyophilised to give crude **35** (65 mg, 90% yield) as N-ethyl morpholinium salt. An aliquot (10 mg) was purified by thin-layer chromatography on reversed-phase silica gel using a mixture of H_2O-CH_3CN (60:40; 50 mM NaCl) as eluent (R_F = 0.5). The support containing adsorbed **35** was extracted with the elution mixture (5 mL), and the extract was evaporated to dryness; **35** was recovered as sodium salt, contaminated with NaCl : IR (KBr) ν 3300-3500 (br), 1798 (CO β-lactam), 1695, 1647 (br), 1549, 1461, 1316, 1266, 1210, 1150, 1113 cm^{-1}; 1H NMR (500 MHz, D_2O) δ 1.18 (s, 3H, *H-8*), 1.28 (m, 2H, H-10), 1.33 (s, 3H, *H-9*), 1.44 (m, 1H, H-9), 1.47 (m, 2H, H-11), 1.59 (m, 1H, H-9'), 2.03 (t, 2H, H-12), 2.44 (t, 2H, H-20), 2.56 (d, 1H, H-2), 2.63 (t, 2H, H-16), 2.72 (t, 2H, H-19), 2.81 (dd, 1H, H-2'), 3.08 (m, 1H, H-8), 3.15 (t, 2H, H-23), 3.27 (t, 2H, H-15), 3.39 (m, 4H, H-24 + H-30), 3.46 (m, 4H, H-26 + H-27), 3.72 (t, 2H, H-29), 4.09 (s, 1H, *H-3*), 4.11 (m, 1H, H-7), 4.28 (m, 1H, H-3), 4.96 (d, 1H, J = 4.3 Hz, *H-5*), 5.40 (d, 1H, J = 4.3 Hz, *H-6*); MS (FAB) m/e 840 ($C_{29}H_{42}N_6O_{12}S_5D_6$+1).

Enzyme inhibition[22]

RTEM ß-lactamase was obtained from Sigma and used without further purification. The active ß-lactamase concentration in solution was determined by measuring the activity on dilution in 50 mM phosphate buffer at pH 6.86, using the known specific activity on benzylpenicillin (k_{cat}) of 2000 sec^{-1} as reference[62]. 20 µL of the

inhibitor solution (10^{-2} M solution in DMSO) were added to 1.8 mL of the enzyme solution (4.10^{-8} M in 50 mM phosphate buffer at pH 6.86) at room temperature. 200 μL portions were taken off at different times and diluted into 2.5 mL of benzylpenicillin (sodium salt, S.K. Beecham) solution (10^{-3} M in phosphate buffer). The enzyme activity was recorded until complete disappearance of the substrate (spectrophotometric measurements at 232 nm with a Varian Cary 210 apparatus). The remaining enzyme activity was calculated from the linear portion of the hydrolysis curve.

ACKNOWLEDGEMENTS

The authors thank Daniela Werding and Manuel Powis for technical assistance. This work was supported by the Fonds National de la Recherche Scientifique (FNRS, Belgium) and the Fonds du Développement des Sciences (FDS, UCL).

REFERENCES

1. Gallop, M.A.; Barrett, R.W.; Dower, W.J.; Fodor, S.P.A.; Gordon, E.M. *J. Med. Chem.* **1994**, *37*, 1233-1251.
2. Gordon, E.M.; Barrett, R.W.; Dower, W.J. ; Fodor, S.P.A.; Gallop, M.A. *J. Med. Chem.* **1994**, *37*, 1385-1401.
3. McCafferty, J.; Jackson, R.H.; Chiswell, D.J. *Protein Eng.* **1991**, *4*, 955-961.
4. Corey, D.R.; Shiau, A.K.; Yang, Q.; Janowski, B.A.; Craik, C.S. *Gene* **1993**, *128*, 129-134.
5. Maruyama, I.N.; Maruyama, H.J.; Brenner, S. *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 8273-8277.
6. Janda, K.D.; Lo, C.-H.L.; Li ,T.; Barbas III, C.F., Wirshing, P.; Lerner, R.A. *Proc. Natl. Acad. Sci. USA* **1994** , *91*, 2532-2536.
7. Ku, J.; Schultz, P.G. *Bioorg. Med. Chem.* **1994**, *2*, 1413-1415.
8. Soumillion, P.; Jespers, L.; Bouchet, M.; Marchand-Brynaert, J.; Winter, G.; Fastrez, J. *J. Mol. Biol.* **1994**, *237*, 415-422.
9. Soumillion, P.; Jespers, L.; Bouchet, M.; Marchand-Brynaert, J.; Sartiaux, P.; Fastrez, J.*Appl. Biochem. Biotechnol.* **1994**, *47*, 175-190.
10. Soumillion, P.; Sartiaux, P.; Bouchet, M.; Marchand-Brynaert, J.; Fastrez, J. *The Royal Society of Chemistry ,Special Publication* **1995**, *148*, 149-160.
11. Light, J.; Lerner, R.A. *Bioorg. Med. Chem.* **1995**, *3*, 955-967.
12. Vanwetswinkel, S.; Touillaux, R.; Fastrez, J.; Marchand-Brynaert, J. *Bioorg. Med. Chem.* **1995**, *3*, 907-915.
13. Jelsch, C.; Lenfant, F.; Masson, J.M.; Samama, J.P. *FEBS Lett.* **1992**, *299*, 135-142.
14. Ghuysen, J.-M. *Annu. Rev. Microbiol.* **1991**, *45*, 37-67.
15. Dalbadie-McFarland, G.; Neitzel, J.J.; Richards, J.H. *Biochemistry* **1986**, *25*, 332-338.
16. Mazella, L.J.; Pazhanisamy, S.; Pratt, R.F. *Biochem. J.* **1991**, *274*, 855-859.
17. Pratt, R.F., *β-Lactamase Inhibition*, in *The Chemistry of β-Lactams*, Page, M.I.; Editor, Blackie Academic and Professional, London, **1992**, 239-271.
18. Mascaretti, O.A.; Boschetti, C.E.; Danelon, G.O.; Mata, E.G.; Roveri, O.A. *Curr. Med. Chem.* **1995**, *1*, 441-470.
19. Bayer, E.A.; Wilchek, M. *Methods Biochem. Anal.* **1980**, *26*, 1-45.
20. Shimkus, M.; Levy, T.; Herman, T. *Proc. Natl. Acad. Sci. USA* **1985**, *82*, 2593-2597.
21. Gretch, D.R.; Suter, M.; Stinski, M.F. *Anal. Biochem.* **1987**, *163*, 270-277.
22. Vanwetswinkel, S.; Fastrez, J.; Marchand-Brynaert, J. *J. Antibiot.* **1994**, *47*, 1041-1051.
23. Manhas, M.S.; Gala, K.; Bari, S.S.; Bose, A.K. *Synthesis* **1983**, 549-552.
24. Khorana, H.G. *Can. J. Chem.* **1953**, *31*, 585-588.
25. Noller, C.R.; Hearst, P.J. *J. Am. Chem. Soc.* **1948**, *70*, 3955.

26. Sutherland, H.; Shriner, R.L. *J. Am. Chem. Soc.* **1936**, *58*, 62-63.
27. Carius, Z. *Liebigs Ann.* **1860**, *114*, 142.
28. Barco, A.; Benetti, S.; Pollini, G.P.; Taddia, R. *Synthesis* **1974**, 877-878.
29. Orazi, O.O.; Corral, R.A.; Bravo, R. *J. Heterocyclic Chem.* **1986**, *23*, 1701-1708.
30. Adams, R.; Marvel, C.S. *Org. Synth. Coll. Vol.I*, **1947**, 84-85.
31. Fujita, S. *Synthesis* **1982**, 423-424.
32. Johnson, D.A.; Panetta, C.A.; Cooper, D.E. *J. Org. Chem.* **1963**, *28*, 1927-1928.
33. Mezes, P.S.F.; Clarke, A.J.; Dmitrienko, G.I.; Viswanatha, T. *FEBS Lett.* **1982**, *143*, 265- 267.
34. Fisher, J.; Charnas, R.L.; Knowles, J.R. *Biochemistry* **1978**, 2180-2184.
35. Greene, T.W.; Wuts, P.G.M. *Protectiv Groups in Organic Synthesis* (second Edition), John Wiley, New York, **1991**.
36. Etienne, A.; Lonchambon, G. *C.R. Acad. Sci. Paris, Serie C*, **1972**, *275*, 375-378.
37. Le Berre, A.; Porte, C. *Bull. Soc. Chim. Fr.* **1976**, 476-482.
38. Le Berre, A.; Porte, C. *Bull. Soc. Chim. Fr.* **1978**, II 602-608.
39. Changov, L.S.; Vassileva-Lukanova, B.K.; Angelova-Galabova, A.; Pavlova, A.V.; Spassov, S.L.*Arzneim.-Forsch.* **1994**, *44*, 856-858.
40. Etienne, A.; Lonchambon, G.; Benard, C. *Bull. Soc. Chim. Fr.* **1976**, 483-486.
41. Distler, H. *Angew. Chem. Internat. Edit. Engl.* **1965**, *4*, 300-311.
42. Shenhav, H.; Rappoport, Z.; Patai, S. *J. Chem. Soc. (B)* **1970**, 469-476.
43. Kropf, H.; Ball, M. *Tetrahedron* **1972**, *28*, 1391-1401.
44. Le Berre, A.; Delacroix, A. *Bull. Soc. Chim. Fr.* **1973**, 640-647.
45. Le Berre, A.; Etienne, A.; Delacroix, A.; Proust, A. *Bull. Soc. Chim. Fr.* **1975**, 2531-2537.
46. Whitmore, W.F.; Landau, E.F. *J. Am. Chem. Soc.* **1946**, *68*, 1797-1798.
47. Gilbert, E.E. *Synthesis* **1969**, 3-10.
48. King, J.F.; Aslam, M. *Synthesis* **1980**, 285-287.
49. Tipson, S.R.; Clapp, M.A.; Cretcher, L.H. *J. Org. Chem.* **1947**, *12* 133-137
50. Kim, S.; Park, Y.H.; Kee, I.S. *Tetrahedron Lett.* **1991**, *32*, 3099-3100.
51. Shimkus, M.; Levy, T.; Herman, T. *Proc. Natl. Acad. Sci. USA* **1985**, *82*, 2593-2597.
52. Gretch, D.R.; Suter, M.; Stinski, M.F. *Anal. Biochem.* **1987**, *163*, 270-277.
53. Van Rensburg, N.J.J.; Swanepoel, O.A. *Arch. Biochem. Biophys.* **1967**, *118*, 531-535.
54. Khim, Y.H.; Field, L. *J. Org. Chem.* **1972**, *37*, 2714-2720.
55. Alonso, M.E.; Aragona, H. *Org. Synth. Coll. Vol. VI* **1988**, 235-239.
56. Eager, J.E.; Savige, W.E. *Photochem. Photobiol.* **1963**, *2*, 25-37.
57. Schnaar, R.L.; Langer, B.G.; Brandley, B.K. *Anal. Biochem.* **1985**, *151*, 268-281.
58. Parameswaran, K.N. *Org. Prep. Proced. Internat.* **1990**, *22*, 119-121.
59. Anderson, G.W.; Zimmerman, J.E.; Callahan, F.M. *J. Am. Chem. Soc.* **1964**, *86*, 1839-1842.
60. McCormick, D.B.; Roth, J.A. *Anal. Biochem.* **1970**, *34*, 226-231.
61. McCormick, D.B.; Roth, J.A. *Methods Enzymol.* **1970**, *18*, 383-385.
62. Zafaralla, G.; Manavathu, E.K.; Lerner, S.A.; Mobashery, S. *Biochemistry* **1992**, 3847-3852.

(*Received in UK* 31 *December* 1995; *revised* 12 *February* 1996; *accepted* 16 *February* 1996)