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Acyloxymethyl as a Drug Protecting Group. Part 7: Tertiary Sulfonamidomethyl Ester Prodrugs of Benzylpenicillin: Chemical Hydrolysis and Anti-Bacterial Activity

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Abstract—Tertiary sulfonamidomethyl esters of benzylpenicillin (**4**) were synthesised and evaluated as a new class of potential prodrugs for β-lactam antibiotics. Their hydrolysis in aqueous buffers was studied by HPLC and reveal a U-shaped pH–rate profile with a pH-independent process extending from ca. pH 2 to ca. pH 10. This pathway is characterised by kinetic data that are consistent with a unimolecular mechanism involving rate-limiting iminium ion formation and penicillinoate expulsion. Benzylpenicillin and the corresponding sulfonamide are the ultimate products detected and isolated, indicating that β-lactam ring opening is much slower than ester hydrolysis. As expected from the high reactivity, benzylpenicillin esters (**4**) displayed similar in vitro antibacterial activity to benzylpenicillin itself. Compared to the benzylpenicillin derivatives, sulfonamidomethyl esters of benzoic, clofibric and valproic acids display a much higher stability, giving rise to a Brønsted β_{lg} value of −0.96 and suggesting that tertiary sulfonamidomethyl esters may be useful prodrugs for carboxylic acid drugs with $pK_a > 4$.

Introduction

Many drugs bearing a carboxylic acid group, including penicillins and cephalosporins, display limited oral bioavailability.[1] Transformation of the drug molecule into a prodrug, the main requisite of which is the in vivo quantitative release either chemically or enzymatically of the parent drug, has proved a useful approach for the improvement of physicochemical properties and oral drug delivery.[2] Simple esters of β-lactam antibiotics are too stable in vivo to be used as prodrugs.[3,4] A more suitable strategy is to prepare (acyloxy)alkyl derivatives **1**, for which hydrolysis to the parent drug is enzymatically triggered.[5,6] However, some (acyloxy)alkyl esters of neutral β-lactam antibiotics are also poorly active orally because of their low aqueous solubility.[7] The introduction of a basic α-amino group into the acyl pro-moiety increases the water-solubility but generally reduces in vitro stability.[8,9] Unfortunately, esterification at the C-3 carboxylic acid group of penicillins generally increases the rate of alkaline β-lactam hydrolysis,[10] therefore contributing to the reduction of in vitro stability.

Recently, we reported that amidomethylation to yield tertiary *N*-acyloxymethylamides **2**, is a potential approach for prodrug development of drugs that contain free carboxylic acid groups.[11–13] Specifically, benzylpenicilloates **2** (R^3 = benzylpenicillin, **3**) undergo spontaneous hydrolysis without suffering β-lactam ring opening to any detectable extent.[12] This is a clear advantage over the majority of α-acyloxyalkyl double prodrugs **1** of β-lactam antibiotics, which can suffer β-lactam ring opening depending on the substrate and the pH of the reaction.[14] However, due to the fact that compounds **2** contain a good carboxylate leaving group of pK_a ca. 3, they hydrolyse very rapidly[12] making pharmaceutical formulation a more difficult task. As the reactivity of amidomethyl esters **2** is largely depressed by electron withdrawing substitutents in the amide pro-moiety, we anticipated that using a sulfonamide pro-moiety would lead to substantially more stable prodrugs. We now report upon the effect of the sulfonamide pro-moiety on the reactivity and mechanism of chemical hydrolysis of tertiary sulfonamidomethyl benzylpenicilloates **4**, as well as on the extent of

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competitive β-lactam ring opening. This study is directed toward evaluating the suitability of sulfonamidomethyl esters as prodrugs for β-lactam antibiotics and the data are compared with the sulfonamidomethyl esters of benzoic, clofibric, and valproic acids.

Chemistry

The strategy for the synthesis of tertiary *N*-acyloxymethylsulfonamides **4a**–**k** involves the preparation of *N*-chloromethylsulfonamide intermediates **5** by reaction of the appropriate secondary sulfonamide with chlorotrimethylsilane and paraformaldehyde (Scheme 1). This reaction allows the synthesis of compounds **5** in excellent yield, even when the starting material is an *N*-arylsulfonamide. Reaction of intermediates **5** with the sodium salt of the corresponding carboxylic acids afforded **4** in ca. 30% yield. The same approach was used with cefotaxime (**6**). However, in this case, the reaction of *N*-chloromethyl-*N*-phenylmethanesulfonamide with **6** yielded a mixture of the Δ^3 and Δ^2 isomers, **7a** and **7b**, respectively (Scheme 2). These two isomers could be easily distinguished in the ^{1}H NMR spectra.[15,16] While the Δ^3 isomer presented two doublets at δ 3.47 and 3.59 ppm ($^2J = 18$ Hz) corresponding to the C_2-Hs, the Δ^2 isomer presented two singlets at 5.32 and 5.33 ppm and a broad singlet at 6.55 ppm, corresponding to the C_4-H and C_2-H, respectively. All attempts to separate the two isomers have so far resulted in the decomposition of products.

The structure of compounds **4** follows from their spectroscopic and analytical data. The spectra of esters **4h**–**k** exhibit a characteristic singlet at ca. δ 5.5 ppm due to the NCH_2O group. For the penicilloates **4a**–**g**, the NCH_2O signal appears as two doublets, reflecting the diastereotopic nature of the methylene protons ($^2J = 11$–12 Hz).

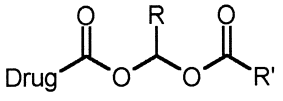
Results and Discussion

Kinetics of hydrolysis and pH–rate profiles

The pH–rate profiles for the hydrolysis of sulfonamidomethyl esters of benzylpenicillin, e.g., **4g**, exhibit a broad U-shape (Fig. 1), indicative of the presence of acid-catalysed, base-catalysed and pH-independent processes (eq (1)). These profiles are analogous to those of simple acyloxymethylsulfonamides,[17] e.g., **4k**, and similar pH–rate profiles have been previously described for their amidomethyl counterparts.[11–13]

$$k_{obs} = k_o + k_{H^+}[H^+] + k_{OH^-}[OH^-] \quad (1)$$

A striking feature of these pH–rate profiles is the unusually broad plateau extending from ca. pH 2 to ca. pH 10, indicating that the pH-independent hydrolysis is the dominant process at all physiologically relevant pH values. This contrasts markedly with simple ester hydrolysis, for which specific-base catalysis usually starts at ca. pH 6–7.[18] Moreover, the pH–rate profiles of the penicilloates observed here differ significantly from those of the hydrolysis of penicillins, which either have no significant spontaneous reaction (e.g., benzylpenicillin[19]) or have only small plateaus around neutral pH (e.g., phenethicillin[20]). The apparent first-order rate



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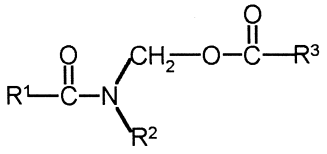

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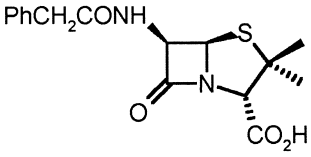

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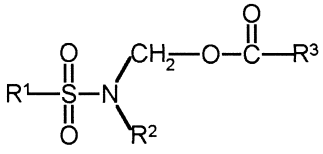

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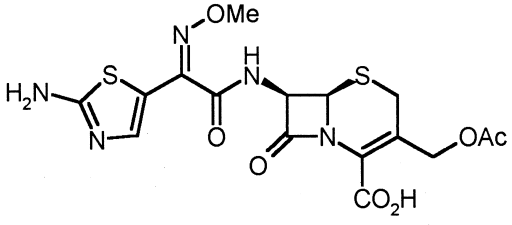

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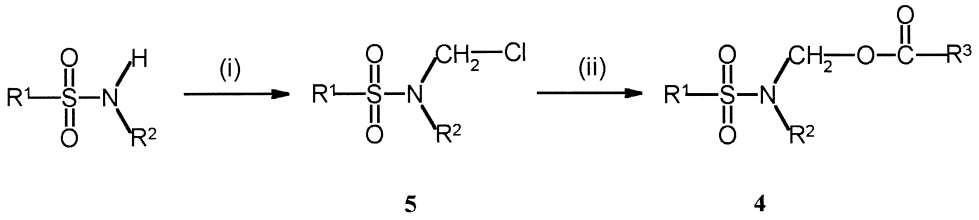
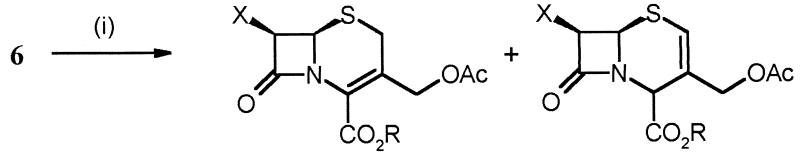
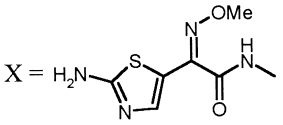


(i) Me_3SiCl, paraformaldehyde; (ii) R^3CO_2Na, THF

Scheme 1.





Scheme 2.

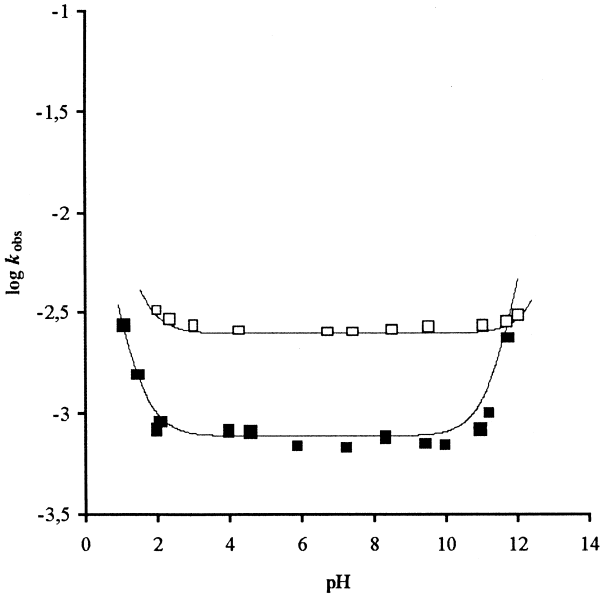


Figure 1. pH–rate profiles for penicilloate **4g** (■) at 37 °C, and for benzoate **4k** (□) at 25 °C.

constants for the pH-independent reactions for these latter penicillins are ca. 10^{-7}–10^{-6} s^{-1} at 30–35 °C, which are ca. 10^3 smaller than the values obtained for the sulfonamidomethyl esters **4a**–**g** at 37 °C. The apparent first-order rate constants, k_o, for the pH-independent hydrolyses of compounds **4** are listed in Table 2. Unfortunately, sulfonamidomethyl esters of benzylpenicillin are too reactive to study in the acid- and base-catalysed regions of the pH–rate profiles. For the more stable compound, **4g**, the best computer fit (solid lines) to the experimental data (individual points), using eq (1), gave values for the catalytic second-order rate constants, k_{H^+} and k_{OH^-}, of 0.0214 and 0.187 $dm^3\,mol^{-1}\,s^{-1}$, respectively.

pH-Independent region

HPLC analysis of the hydrolyses of the benzylpenicillin esters **4a**–**g** in the pH-independent region reveals that the parent drug and the corresponding secondary sulfonamide are released in quantitative yield. No lag time was observed for the formation of the sulfonamide and benzylpenicillin; neither was the corresponding *N*-hydroxymethylsulfonamide detected at any pH value, consistent with its extremely rapid decomposition to the parent sulfonamide. The rate of decomposition of *N*-hydroxymethyl derivatives of NH-acidic compounds depends on the pK_a of the parent NH-compound, with a Brønsted β_{lg} value of −0.8.[21] For an *N*-hydroxymethylsulfonamide derived from a parent sulfonamide that has a pK_a of 11 a half-life of 80 s can be calculated. Over the timescale of the hydrolysis reactions, no decomposition of benzylpenicillin to penicilloic acid, through β-lactam ring opening, was detected.

The pH–rate profiles with broad plateaus, the high values of the apparent first-order rate constants for the pH-independent process and the quantitative formation of benzylpenicillin clearly indicate that the source of reactivity of the sulfonamidomethyl derivatives **4a**–**g** is

Table 1. Structures of compounds **4**

Compound	R^1	R^2	R^3CO_2H
4a	4-MeC_6H_4	Me	Benzylpenicillin
4b	4-BrC_6H_4	Me	Benzylpenicillin
4c	4-ClC_6H_4	Me	Benzylpenicillin
4d	4-$NO_2C_6H_4$	Me	Benzylpenicillin
4e	Me	C_6H_5	Benzylpenicillin
4f	Me	4-MeC_6H_4	Benzylpenicillin
4g	4-MeC_6H_4	C_6H_5	Benzylpenicillin
4h	4-$NO_2C_6H_4$	Me	$OCOC(Me_2)C_6H_4$-4-Cl[a]
4i	4-$NO_2C_6H_4$	Me	$OCOC_6H_5$
4j	4-$NO_2C_6H_4$	Me	$OCOCHPr_2$[b]
4k	4-MeC_6H_4	Pr^n	$OCOC_6H_5$

[a]Clofibrate derivative.
[b]Valproate derivative.

neither β-lactam ring opening nor conventional ester hydrolysis. From inspection of the k_o values in Table 2 the following observations may be made. First, the pH-independent pathway is subject to the electronic effect of the substituents in the benzenesulfonamide moiety. Thus, for compounds **4a**–**d** a plot of log k_o against the Hammett σ values yields a ρ value of −0.93 (eq (2)). This value indicates positive charge development in the sulfonamide moiety in the transition state.

$$\log k_o = -0.93(\pm 0.11)\sigma - 2.06(\pm 0.05)$$
$$n = 4,\ r^2 = 0.97,\ s = 0.073 \qquad (2)$$

Second, the temperature dependence of the reaction for compound **4b** (Table 2) gives rise to a value of ΔS of -43 ± 5 J K^{-1} mol^{-1}. This value is well within the range observed for unimolecular ionisation reactions,[22] including those reported for other sulfonamidomethyl benzoates.[17] Third, the solvolysis of **4b** proceeds slightly faster in H_2O than in D_2O, giving a solvent kinetic isotope effect, k_{H_2O}/k_{D_2O}, of 1.21. Again, this value is consistent with a unimolecular ionisation process being the rate-limiting step,[23,24] and contrasts sharply with the value of 4.5 associated with the hydrolysis of the β-lactam ring in penicillins.[10] Fourth, there is no nucleophilic or general base-catalysed hydrolysis, as indicated by the absence of a buffer effect (monochloroacetate, acetate and phosphate) on the decomposition of **4g** (Table 3). The absence of buffer catalysis also contrasts with the hydrolysis of benzylpenicillin itself, which is buffer-catalysed and characterised by a Brønsted β value of 0.39 that is consistent with general base catalysis.[10] Buffer catalysis has also been reported for the hydrolysis of other penicillins (e.g., pheneticillin[20]), as well as for the decomposition of both talampicillin and bacampicillin, which are acyloxymethyl prodrugs of ampicillin.[14]

These data provide strong support for a dissociative mechanism for the pH-independent hydrolysis of esters **4a**–**g** to generate both the penicilloate anion and an *N*-sulfonyliminium ion (Scheme 3), in which the positive charge is stabilised by electron-donating substituents in the sulfonamide pro-moiety. The greater reactivity of esters of benzylpenicillin over those of simple carboxylic acids can also be accommodated by the mechanism depicted in Scheme 3. The observed rate constants for compounds **4d,h**–**j** follow a Brønsted relationship with the pK_a of the carboxylate leaving group, yielding a β_{lg} value of −0.92 (eq (3)).

$$\log k_o = -0.92(\pm 0.11)pK_a - 0.06(\pm 0.44)$$
$$n = 4,\ r^2 - 0.97,\ s = 0.19 \qquad (3)$$

The Brønsted β_{lg} value of ca. −1.0 indicates that the reactivity of these esters is highly dependent on the nucleofugacity of the carboxylate, a trend expected for the S_N1 mechanism of solvolysis of tertiary *N*-acyloxymethylsulfonamides via iminium ion formation. Furthermore, the fact that the highly sterically hindered esters of benzylpenicilloic, clofibric and valproic acids are included in this correlation clearly demonstrates that neither conventional ester hydrolysis via nucleophilic attack at the ester carbonyl carbon atom nor β-lactam ring opening is a major pathway for the hydrolysis of compounds **4a**–**g**.

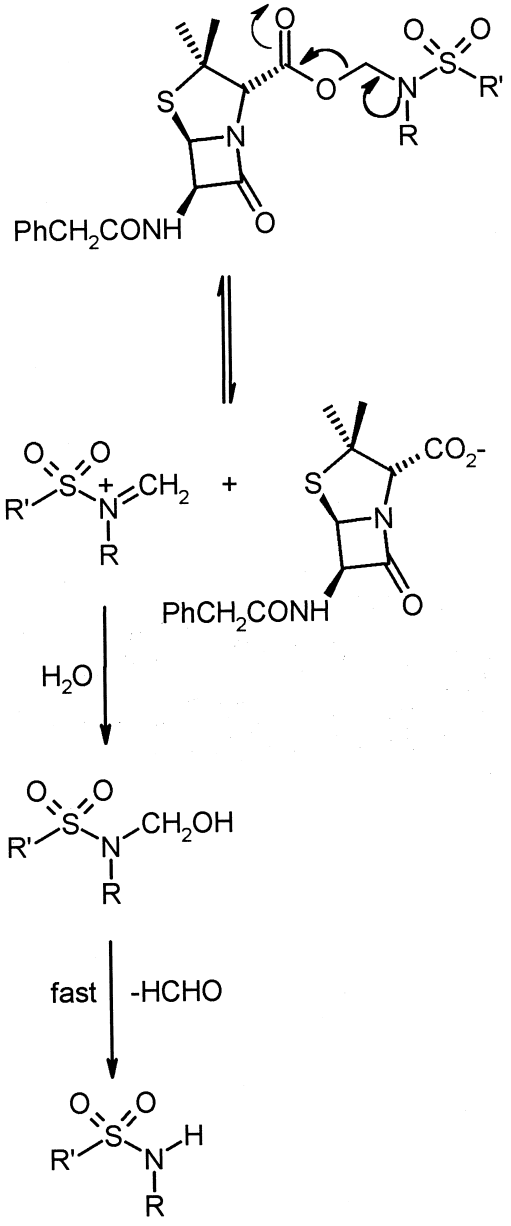
Table 2. Lipophilicity, molar refractivity and pseudo-first-order rate constants at 37 °C for the pH-independent pathway for compounds **4a**–**k**, together with the pK_as of the parent sulfonamides

Compound	pK_a^{NH} [a]	Log *P*	MR/cm^3	$k_0/10^{-4}$ s^{-1}
4a	11.63	3.47	137.85	126
4b	11.06	4.04	140.94	62.5; 12.8[c]; 9.97[d]; 3.40[e]; 2.80[f]; 2.07[g]; 1.51[h]
4c	10.94	3.87	138.05	42.9
4d	10.26	3.21	139.26	16.4
4e	8.98[b]	2.05	133.22	38.3
4f	9.23	2.51	137.85	38.8
4g	8.55[b]	4.12	157.93	7.76
4h	10.26	4.84	106.41	7.79
4i	10.26	4.00	85.94	1.62
4j	10.26	4.98	93.62	0.130
4k	12.35	5.32	93.79	185

[a]pK_a of the parent secondary sulfonamide. Unless otherwise stated, these pK_a values were calculated.[31]
[b]From ref 32. [c]30 °C; [d]26 °C; [e]20 °C; [f]20 °C in D_2O; [g]13.6 °C; [h]10 °C.

Table 3. Effect of buffer concentration on the pseudo-first order rate constants, k_o, for the pH-independent hydrolysis of **4g** at 37 °C and $\mu = 0.50$ mol dm^{-3}

Buffer	[Buffer]/10^{-2} mol dm^{-3}	pH	$k_{obs}/10^{-4}$ s^{-1}
Monochloroacetate	2.00	2.13	9.94
	4.00	2.02	8.71
	8.00	1.97	8.45
	10.0	1.95	9.34
Acetate	3.75	3.96	8.23
	7.50	3.96	8.69
	15.0	3.97	8.53
	22.5	3.99	8.08
Phosphate	1.67	6.97	6.90
	3.33	7.17	7.15
	5.00	7.26	6.61
	10.0	7.36	6.60
	15.0	7.35	6.70



Scheme 3.

It has recently been suggested that a key factor determining the chemical stability, and thus the design, of *N*-acyloxymethyl prodrugs is the pK_a of NH-acidic parent compounds.[25] For example, the high stability of *N*-acyloxymethyl derivatives of 5-fluorouracil, **8** (R = Me),[26] and phenytoin, **9** (R = CH_2NMe_2 or $CH_2CH_2NEt_2$),[27] at pH 4–8, when compared to the reactivity of *N*-acyloxymethylamides **2**,[11–13] has been ascribed to the much lower pK_a values of the imide functionality (ca. 8–10) when compared to the amide group (ca. 15).[25] However, in the present study the calculated pK_a values of the sulfonamide pro-moiety range from 8.5 to 12.3, and yet compounds **4** are ca. 10^3 times more reactive than either **8** or **9**. We have also recently reported a k_o value of 10^{-5} s^{-1} for the *N*-acyloxymethyl derivative **10**[28] (the pK_a value for the parent sulfonamide is 7.38), which is ca. 10^2 times more reactive than **8** or **9**. Clearly, our results indicate that the pK_a of the parent NH compound is not the only factor contributing to the stability of *N*-acyloxymethyl prodrugs.

Also worth noting, the rate constants for the sulfonamides **4** are very similar to those of the corresponding acyloxymethylamides **2**: at 37 °C the value of 4.3×10^{-3} s^{-1} for **4c** compares with 5.8×10^{-3} s^{-1} obtained for its amide analogue.[29] Similarly, the rates of the pH-independent hydrolysis of esters **4g–j** correlate with the corresponding values for the equivalent esters based on the ethyl hippurate carrier[13] **2**, R^1 = Ph, $R^2 = CH_2CO_2Et$, according to eq (4). This correlation clearly indicates that the same order of reactivity is obtained with both types of carrier and that similar factors must affect the hydrolysis of both series of prodrugs. This surprising finding has been supported by semiempirical SCF-MO calculations performed using the PM3 method.[17]

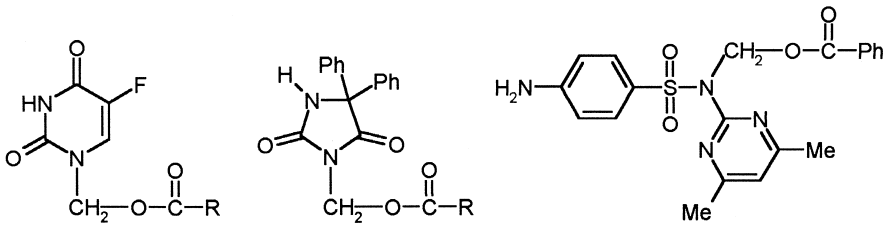
$$\log k_o^{sulf} = 0.94(\pm0.13)\log k_o^{amide} + 0.48(\pm0.57)$$
$$n = 4,\ r^2 = 0.96,\ s = 0.21 \qquad (4)$$

In vitro antibiotic activity

The in vitro antibiotic activities of compounds **4c–e**,**g** and benzylpenicillin against a range of Gram positive and Gram negative bacteria (Table 4) show that, in general, the sulfonamidomethyl esters of benzylpenicillin have a similar activity profile to the parent drug. This is particularly true of compounds **4c–e**. Compound **4g**, however, appears to be at least 16 times less active than benzylpenicillin and the other derivatives. Although this prodrug is chemically more stable than the others at pH 7, it is unlikely that this effect alone is responsible for the diminished antibiotic activity since such activity was measured over 24 h, whereas the half-life of **4g** is 15 min. Currently, it is unclear why **4g** should display reduced biological activity. Moreover, there is no obvious correlation between either antibiotic activity or chemical reactivity with the log *P* values of these compounds (Table 2).

Conclusions

Sulfonamidomethylation of the carboxyl group of benzylpenicillin with the appropriate *N*-chloromethylsulfonamide affords the corresponding sulfonamidomethyl esters in reasonable yields. Sulfonamidomethyl esters of benzylpenicillin hydrolyse at pH 2–10 via cleavage of the ester group to release the parent drug quantitatively. This is a clear advantage over the majority of α-acyloxyalkyl double prodrugs of β-lactam antibiotics, which can suffer β-lactam ring opening depending on the substrate and the pH.[14] For example, at 25 °C and pH 7.5 hydrolysis of bacampicillin involves 22% β-lactam ring opening and 78% ester hydrolysis, whereas at pH 3.5, β-lactam hydrolysis accounts for 74% of the total hydrolysis reaction.[14] Even so, simple sulfonamidomethyl esters **4a–f** are clearly too unstable to be of general use as prodrugs. However, compound **4g**, which contains an *N*-phenylbenzenesulfonamide promoiety, is a promising candidate, presenting a half-life



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of 15 min at pH 2–10. This value compares to the half-life of 15–20 min at pH 4–8 and 35 °C reported for the hydrolysis of hetacillin, a 4-imidazolidinone prodrug of ampicillin.[30] Improved stability might be expected by introducing electron withdrawing substituents either in the benzenesulfonyl *N*-aryl moieties of the carrier. The sulfonamidomethyl derivatives of other acid moieties, **4h–j**, are much more stable at pH 7.4, with half-lives ranging from 15 min to 15 h. Thus tertiary sulfonamidomethyl esters may be useful prodrugs for carboxylic acid drugs with $pK_a > 4$, as well as for secondary sulfonamide drugs.

Experimental

Elemental analyses were obtained from ITQB (Oeiras, Portugal) and from Medac (Englefield Green, UK) laboratories. ^1H and ^{13}C NMR spectra were recorded as $CDCl_3$ solutions on General Electric QE-300 or Jeol JNM-EX400 spectrometers using Me_4Si as internal standard; coupling constants, *J*, are quoted in Hz. FTIR spectra were recorded on a Nicolet Impact 400 spectrophotometer. Electron-impact ionisation (EI) and fast-atom bombardment (FAB) mass spectra were recorded on a VG Mass Lab 20-250 spectrometer. Melting points were determined using a Buchi 510 instrument and are uncorrected. HPLC was performed using a system comprising a Shimadzu LC-9A pump coupled to a variable wavelength Shimadzu SPD-6AV UV–Vis detector, a 20 μL loop injection valve and a Merck LiChrospher® 100 RP-8 5 μm 125×4 mm column.

Table 4. In vitro antibacterial activity of sulfonamidomethyl esters **4c–e,g** and of benzylpenicillin (Pen G)

	MIC 10^{-3}/μmol dm^{-3}				
Bacteria	**4c**	**4d**	**4e**	**4g**	Pen G
P. aeruginosa ATCC 27853	> 500	> 500	> 500	> 500	> 500
K. pneumonia[a]	> 500	> 500	> 500	> 500	> 500
K. pneumonia ATCC 10031	62.5	125	62.5	250	62.5
E. coli[a]	> 500	> 500	> 500	> 500	> 500
E. coli ATCC 25922	62.5	125	62.5	> 500	62.5
S. typhimurium ATCC 43971	3.9	7.8	3.9	250	3.9
S. enteritidis	7.8	62.5	7.8	62.5	7.8
S. flexneri	31.2	31.2	31.2	62.5	31.2
S. dysenteriae ATCC 13313	15.6	31.2	15.6	125	15.6
S. marcescens NCTC 1377	> 500	> 500	> 500	> 500	> 500
P. mirabilis	61.2	125	61.2	250	7.8
S. aureus ATCC 25923	< 0.24	0.48	< 0.24	31.2	< 0.24
S. faecalis ATCC 10541	15.6	125	15.6	> 500	15.6

[a]β-Lactamase producing bacteria.

Benzylpenicillin was purchased commercially. All chemicals were reagent grade except those for kinetic studies and HPLC, which were analytical or LiChrosolv (Merck) grade. Column chromatography was performed using silica gel 60 mesh 70-230 (Merck). Compounds **4h–j** were obtained from a previous study.[17]

(*N*-Methyl-4-toluenesulfonamido)methyl (2*S*,5*R*,6*R*)-1-aza-3,3-dimethyl-7-oxo-6-phenylacetamido-4-thiabicyclo-[3.2.0]heptane-2-carboxylate 4a. A solution of *N*-methyl-4-toluenesulfonamide (6 mmol) and paraformaldehyde (0.3 g) in chlorotrimethylsilane (20 mL) was refluxed during 2 h. Removal of the solvent yielded *N*-chloromethyl-*N*-methyl-4-methylbenzenesulfonamide **5a**; 99%; mp 83–87 °C; δ_H/ppm: 2.38 (3H, s), 2.85 (3H, s), 5.34 (2H, s), 7.12–7.74 (4H, AA′BB′). A solution of **5a** (1.1 equiv) in dry THF (1 mL) was added to a suspension of sodium benzylpenicilloate (5 mmol) in THF (5 mL) at room temperature. Upon completion of the reaction, the solvent was evaporated, and the residue treated with ice-water and extracted with ethyl acetate. The organic extracts were washed with ice-water, sodium hydrogen carbonate, brine and dried over magnesium sulfate. Evaporation of the solvent gave pure **4a** as a cream gum (68%); ν_{max} (cm^{-1}): 3300, 1770, 1720, 1650; δ_H/ppm: 1.32 (3H, s, C_3Me), 1.34 (3H, s, C_3Me), 2.64 (3H, s, Ar*Me*), 2.92 (3H, s, N*Me*), 3.63 (2H, s, PhCH_2), 4.17 (1H, s, C_2H), 5.15 (1H, d, $J = 4.0$ Hz, C_5H), 5.42 (1H, d, $J = 11.4$ Hz, NCH_2O), 5.52 (1H, dd, $J = 4.0$, 9.0 Hz, C_6H), 5.54 (1H, d, $J = 11.4$ Hz, NCH_2O), 6.07 (1H, d, $J = 9.0$ Hz, N*H*), 7.25–7.72 (9H, m, 2×*Ar*). Found C, 56.3; H, 5.8; N, 7.6; $C_{25}H_{29}N_3O_6S_2$ requires C, 56.5; H, 5.5; N, 7.9.

(*N*-Methyl-4-bromobenzenesulfonamido)methyl (2*S*,5*R*,6*R*)-1-aza-3,3-dimethyl-7-oxo-6-phenylacetamido-4-thiabicyclo[3.2.0]heptane-2-carboxylate 4b. Synthesised as **4a** starting from *N*-methyl-4-bromobenzenesulfonamide: cream gum (66%); ν_{max} (cm^{-1}): 3300, 1775, 1725, 1650; δ_H/ppm: 1.34 (3H, s, C_3Me), 1.35 (3H, s, C_3Me), 2.95 (3H, s, N*Me*), 3.65 (2H, s, PhCH_2), 4.20 (1H, s, C_2H), 5.28 (1H, d, $J = 4.0$ Hz, C_5H), 5.45 (1H, d, $J = 12.0$ Hz, NCH_2O), 5.52 (1H, d, $J = 12.0$ Hz, NCH_2O), 5.60 (1H, dd, $J = 4.0$, 9.0 Hz, C_6H), 6.06 (1H, d, $J = 9.0$ Hz, N*H*), 7.27–7.71 (9H, m, 2×*Ar*). Found C, 47.9; H, 4.7; N, 6.9; $C_{24}H_{26}BrN_3O_6S_2$ requires C, 48.3; H, 4.4; N, 7.0.

(*N*-Methyl-4-chlorobenzenesulfonamido)methyl (2*S*,5*R*,6*R*)-1-aza-3,3-dimethyl-7-oxo-6-phenylacetamido-4-thiabicyclo[3.2.0]heptane-2-carboxylate 4c. Synthesised as **4a** starting from *N*-methyl-4-chlorobenzenesulfonamide:

cream gum (66%); ν_{max} (cm^{-1}): 3300, 1775, 1725, 1640; δ_H/ppm: 1.34 (6H, s, C_3Me_2), 2.93 (3H, s, N*Me*), 3.64 (2H, s, PhCH_2), 4.19 (1H, s, C_2H), 5.28 (1H, d, $J=4.2$ Hz, C_5H), 5.44 (1H, d, $J=11.4$ Hz, NCH_2O), 5.51 (1H, d, $J=11.4$ Hz, NCH_2O), 5.60 (1H, dd, $J=4.2$, 9.0 Hz, C_6H), 6.13 (1H, d, $J=9.0$ Hz, NH), 7.27–7.42 (9H, m, 2×*Ar*). Found C, 52.6; H, 5.0; N, 7.3; $C_{24}H_{26}ClN_3O_6S_2$ requires C, 52.2; H, 4.7; N, 7.6.

(*N*-Methyl-4-nitrobenzenesulfonamido)methyl (2*S*,5*R*, 6*R*)-1-aza-3,3-dimethyl-7-oxo-6-phenylacetamido-4-thiabicyclo[3.2.0]heptane-2-carboxylate 4d. Synthesised as **4a** starting from *N*-methyl-4-nitrobenzenesulfonamide: slightly yellow gum (83%); ν_{max} (cm^{-1}): 3300, 1775, 1725, 1630; δ_H/ppm: 1.34 (6H, s, C_3Me_2), 2.99 (3H, s, N*Me*), 3.64 (2H, s, PhCH_2), 4.16 (1H, s, C_2H), 5.34 (1H, d, $J=4.0$ Hz, C_5H), 5.48 (1H, d, $J=11.0$ Hz, NCH_2O), 5.52 (1H, d, $J=11.0$ Hz, NCH_2O), 5.59 (1H, dd, $J=4.0$, 9.0 Hz, C_6H), 6.09 (1H, d, $J=9.0$ Hz, N*H*), 7.25–7.37 (5H, m, Ar), 7.95-8.42 (4H, AA′BB′, *Ar*SO_2). Found C, 51.1; H, 4.8; N, 9.7; $C_{24}H_{26}N_4O_8S_2$ requires C, 51.2; H, 4.6; N, 10.0.

(*N*-Phenylmethanesulfonamido)methyl (2*S*,5*R*,6*R*)-1-aza-3,3-dimethyl-7-oxo-6-phenylacetamido-4-thiabicyclo[3.2.0]-heptane-2-carboxylate 4e. Synthesised as **4a** starting from *N*-phenylmethanesulfonamide: cream gum (56%); ν_{max} (cm^{-1}): 3320, 1778, 1740, 1630; δ_H/ppm: 1.48 (6H, s, C_3Me_2), 3.02 (3H, s, *Me*SO_2), 3.65 (2H, s, PhCH_2), 4.41 (1H, s, C_2H), 5.48 (1H, d, $J=4.0$ Hz, C_5H), 5.67–5.71 (3H, m, NCH_2O and C_6H), 6.15 (1H, d, $J=9.0$ Hz, N*H*), 7.27–7.42 (10H, m, 2×*Ar*). Found C, 51.1; H, 5.4; N, 7.9; $C_{24}H_{27}N_3O_6S_2$ requires C, 51.7; H, 5.2; N, 8.1.

(*N*-4-Tolylmethanesulfonamido)methyl (2*S*,5*R*,6*R*)-1-aza-3,3-dimethyl-7-oxo-6-phenylacetamido-4-thiabicyclo[3.2.0]-heptane-2-carboxylate 4f. Synthesised as **4a** starting from *N*-4-tolylmethanesulfonamide: cream gum (27%); ν_{max} (cm^{-1}): 3300, 1770, 1730, 1625; δ_H/ppm: 1.48 (3H, s, C_3Me), 1.49 (3H, s, C_3Me), 2.39 (3H, s, Ar*Me*), 3.00 (3H, s, *Me*SO_2), 3.65 (2H, s, PhCH_2), 4.40 (1H, s, C_2H), 5.49 (1H, d, $J=4.2$ Hz, C_5H), 5.64–5.70 (3H, m, NCH_2O and C_6H), 6.15 (1H, d, $J=9.0$ Hz, N*H*), 7.21–7.40 (9H, m, 2×*Ar*). Found C, 56.7; H, 5.6; N, 7.8; $C_{25}H_{29}N_3O_6S_2$ requires C, 56.5; H, 5.5; N, 7.9.

(*N*-Phenyl-4-toluenesulfonamido)methyl (2*S*,5*R*,6*R*)-1-aza-3,3-dimethyl-7-oxo-6-phenylacetamido-4-thiabicyclo-[3.2.0]heptane-2-carboxylate 4g. Synthesised as **4a** starting from *N*-phenyl-4-toluenesulfonamide: cream gum (66%); ν_{max} (cm^{-1}): 3360, 1780, 1720, 1680; δ_H/ppm: 1.41 (6H, s, C_3Me_2), 2.44 (3H, s, Ar*Me*), 3.64 (2H, s, PhCH_2), 4.27 (1H, s, C_2H), 5.20 (1H, d, $J=4.0$ Hz, C_5H), 5.56 (1H, dd, $J=4.0$, 9.0 Hz, C_6H), 5.65 (1H, d, $J=11.0$ Hz, NCH_2O), 5.81 (1H, d, $J=11.0$, NCH_2O), 6.08 (1H, d, $J=9.0$, N*H*), 7.26–7.54 (14H, m, 3×*Ar*). Found C, 60.3; H, 5.3; N 6.9; $C_{30}H_{31}N_3O_6S_2$ requires C, 60.7; H, 5.2; N 7.1.

Kinetic studies

All kinetic experiments were carried out in aqueous buffers (borate, phosphate, acetate and chloroacetate) with an ionic strength maintained at 0.5 mol dm^{-3} using $NaClO_4$. A 20 μL aliquot of a 10^{-2} mol dm^{-3} stock solution of substrate in acetonitrile was added to 10 mL of the appropriate thermostatted buffer solution. At regular intervals, 50 μL samples of the reaction mixture were quenched with ice-cold acetonitrile (400 μL) and the resulting solution analysed by HPLC using the following conditions: detector wavelength, 230 nm (290 nm for **4d** and **4h–j**); mobile phase, methanol–water containing 0.1 mol dm^{-3} tetrabutylammonium phosphate (55:45 to 60:40%). Compound **4k** was studied by UV spectroscopy using a previously described protocol.[11]

Computations

Molecular refractivities (MR) were calculated with the software ChemSketch v. 4.01 from Advanced Chemistry Development (ACD) Inc. Octanol–water partition coefficients (log *P*) were obtained using the ACD/I-lab service (log *P* v. 3.6). No reliable experimental log *P* values could be obtained as result of extensive hydrolysis of penicillin derivatives when the shake-flask method was used. Calculation of the kinetic parameters in eq (1) was performed using the Solver option of the Microsoft Excel® software package. The regression analysis of kinetic data in Table 1 was performed using the statistical package of SigmaPlot® 5.0 from SSPS Inc.

Antimicrobial susceptibility tests

The minimum inhibitory concentrations (MICs) of benzylpenicillin and its sulfonamidomethyl esters were determined by the Agar Dilution Method (Mueller-Hinton) using a multipoint inoculator. The plates were incubated in aerobic atmospheres at 37 °C for 24 h. MICs were recorded as the lowest concentration completely inhibiting visible bacterial growth. The range of concentrations used for all the compounds was 2.24×10^{-10}–0.50×10^{-6} mol ml^{-1}. The following reference bacterial organisms were used: *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 10031, *Escherichia coli* ATCC 25992, *Salmonella typhimurium* ATCC 43971, *Shigella dysenteriae* ATCC 13313, *Serratia marcescens* NCTC 1377, *Staphylococcus aureus* ATCC 25923 and *Streptococcus faecalis* ATCC 10541. The following bacterial organisms belong to the collection of the Faculty of Pharmacy: β-lactamase producing *Klebsiella pneumoniae*, β-lactamase producing *Escherichia coli*, *Salmonella enteritidis*, *Shigella flexneri*, and *Proteus mirabilis*.

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