Studies on the reactivity of azetidin-2-ones in phosphate buffer

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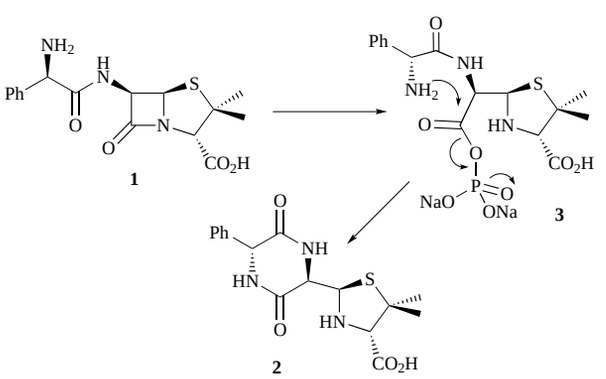
^{1}H NMR (500 MHz) analyses reveal that in phosphate buffer, *N*-arylsulfonyl β-lactams such as ethyl *N*-(*p*-nitrophenylsulfonyl)-2-oxoazetidine-4-carboxylate and (4*S*)-ethyl 3-ethyl-*N*-(*p*-nitrophenylsulfonyl)-2-oxoazetidine-4-carboxylate undergo hydrolysis through (minimally) two mechanisms: *via* direct hydrolysis and *via* an unstable acyl phosphate intermediate. The acyl phosphate intermediates can be trapped using hydrazine.

Since the antibacterial mode of action of β-lactams involves the acylation of specific enzymes involved in cell wall biosynthesis, mechanistic studies on the cleavage of β-lactams are of considerable interest.[1] Nucleophilic catalysis of penicillin degradation in neutral or alkaline aqueous solutions, containing carbohydrates or monohydrogen phosphate ion (HPO_4^{2-}), has been the subject of several recent reports.[2,3] In particular, product analysis studies on the degradation of ampicillin **1** (Scheme 1) in neutral phosphate buffer led to the isolation of the



Scheme 1

piperazine-2,5-dione **2**.[2] This observation supports indirectly the intermediacy of an acyl phosphate intermediate **3** since direct intramolecular aminolysis of the β-lactam moiety in **1** by the side chain amino group does not occur to a significant extent in neutral phosphate free aqueous solution.[4]

During the course of studies concerning the mechanism of inhibition of porcine pancreatic elastase by azetidin-2-ones of the general type **4**,[5,6] the stabilities of β-lactams **5** and **6** in deuterated phosphate buffer at pD 7.4 were investigated using ^{1}H NMR spectroscopy and other analytical techniques. The results of this study are presented below and provide direct evidence in support of a phosphate-mediated mechanism for the hydrolysis of monocyclic β-lactams.

^{1}H NMR (500 MHz) spectral analysis of a freshly prepared solution of **5** in D_2O–CD_3CN (10:1) reveals a set of AA′BB′ resonances in the 8.7–7.9 ppm region of the spectrum [Fig. 1(*a*)]. Re-analysis of the sample after standing at room temperature for 4 [Fig. 1(*b*)] and 6 days [Fig. 1(*c*)] demonstrated that **5** was unstable in solution, apparently decomposing to give a *ca.* 2:3 mixture of **5** and **7**, the product of β-lactam hydrolysis. Comparison of the resonances in Fig. 1(*b*) and 1(*c*) with the ^{1}H NMR spectrum of synthetic **7** in D_2O–CD_3CN (10:1) [Fig. 1(*d*)] combined with doping experiments confirmed the identity of **7**.

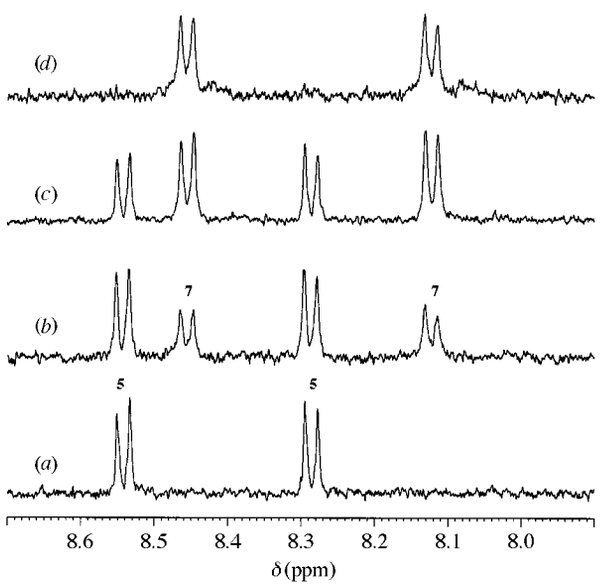
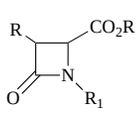
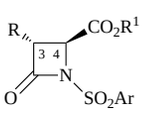


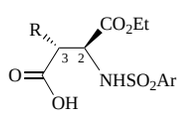
Fig. 1 ^{1}H NMR (500 MHz) spectra obtained on analysis of a solution of **5** in D_2O–CD_3CN (10:1) after standing at room temperature (295 K) for (*a*) 10 min, (*b*) 4 days and (*c*) 6 days, and (*d*) ^{1}H NMR (500 MHz) spectrum of synthetic **7** in D_2O–CD_3CN (10:1)



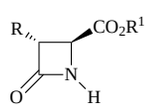
4 R^1 = COPh or SO_2Ar



5 R = R^1 = Et
6 R = H, R^1 = Et
12 R = R^1 = H
11 R = Et, R^1 = H



7 R = Et
10 R = H



9 R = R^1 = Et
8 R = Et, R^1 = H

Ar = *p*-$NO_2C_6H_4$

For an assignment of other resonances present in the ^{1}H NMR spectrum of **5** and **7** in D_2O–CD_3CN (10:1) see Table 1.

β-Lactam **5** was also found to be unstable with respect to β-lactam ring hydrolysis in deuterated phosphate buffer (0.1 mM) at pD 7.4. Fig. 2 shows a portion of the ^{1}H NMR spectrum

Table 1 ^{1}H NMR data for **5** and **7**; δ_H(integration, multiplicity, coupling constant/Hz)

	5	Assignment	**7**	Assignment
1	8.54, 8.28 (4H, AA′BB′)	Ar-H	8.45, 8.11 (4H, AA′BB′)	Ar-H
2	4.28–4.19 (2H, m)	OCH_2CH_3	3.94–3.83 (2H, m)	OCH_2CH_3
3	under HOD peak	C(4)H	4.14 (1H, d, *J* 7.0)	C(2)H
4	3.62–3.56 (1H, m)	C(3)H	2.60–2.54 (1H, m)	C(3)H
5	1.86–1.75 (2H, m)	CH_3CH_2CH	1.61–1.52 (1H, m)	1 × CH_3CH_2CH
6	1.22 (3H, t, *J* 7.0)	OCH_2CH_3	1.43–1.34 (1H, m)	1 × CH_3CH_2CH
7	0.92 (3H, t, *J* 7.5)	CH_3CH_2CH	1.06 (3H, t, *J* 7.0)	OCH_2CH_3
			0.87 (3H, t, *J* 7.5)	CH_3CH_2

Table 2 ^{1}H NMR for **13** and **14**[a]

	13	Assignment	**14**	Assignment
1	8.46, 8.13 (4H, AA′BB′)	Ar-H	8.44, 8.12 (4H, AA′BB′)	Ar-H
2	4.34 (1H, d, *J* 5.0)	C(2)H	4.45 (1H, t, *J* 6.0)	C(2)H
3	3.98–3.82 (m)[b]	OCH_2CH_3	3.98–3.90 (m)[c]	OCH_2CH_3
4	3.03–2.99 (1H, m)	C(3)H	3.01 (1H, dd, *J* 17.5, 6.0)	1 × $C(3)H_2$
5	—[b]	CH_3CH_2CH	2.88 (1H, dd, *J* 17.5, 5.0)	1 × $C(3)H_2$
6	1.02 (3H, t, *J* 7.5)	OCH_2CH_3	1.05 (3H, t, *J* 7.5)	OCH_2CH_3
7	0.98 (3H, t, *J* 7.5)	CH_3CH_2CH		

[a] Spectra recorded in deuterated phosphate buffer–CD_3CN (10:1). [b] Resonances present under multiplets assigned to **7**. [c] Resonances present under multiplets assigned to **10**. Note that the chemical shift data (compare Table 2, entry 1 and Table 1, entry 1) supports the proposal that **13** and **14** have a ring opened structure, *i.e.* they are formed by cleavage of the β-lactam rings of **5** and **6** respectively.

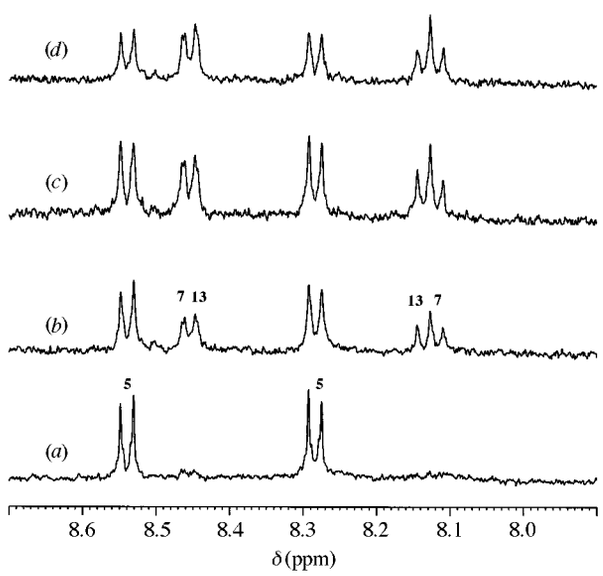
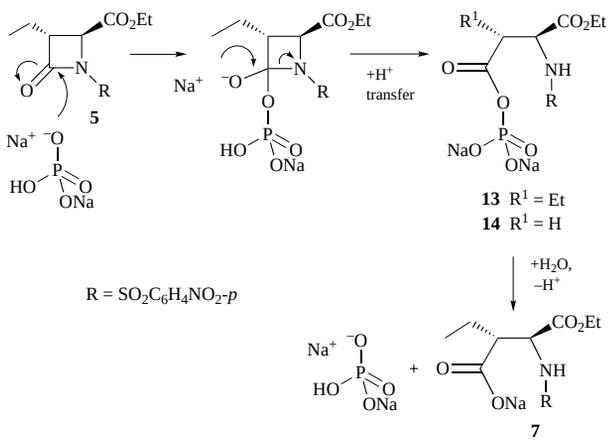


Fig. 2 ^{1}H NMR (500 MHz) spectrum obtained on analysis of a solution of **5** in deuterated phosphate buffer–CD_3CN (10:1) at (*a*) 0, (*b*) 5, (*c*) 10 and (*d*) 14 h



Scheme 2

obtained on analysis of a solution of **5** in buffer with increasing incubation times. During the course of the experiment, two new sets of AA′BB′ resonances were observed in addition to those corresponding to **5**. One set of AA′BB′ resonances was assigned to **7** resulting from hydrolysis of **5** (assignment confirmed by doping experiments). Analysis by ^{1}H NMR spectroscopy after overnight incubation of **7** in the same buffer demonstrated that **7** was stable under these conditions (<5% decomposition), hence ruling out the possibility that the second new compound observed was derived from **7**.

The rate of hydrolysis of **5** in buffer was, however, significantly faster than that observed in D_2O (as demonstrated by the relative intensities of **5** and **7** observed in the ^{1}H NMR spectra at comparable incubation times for the two solvent systems). This observation may be (at least in part) a result of the effect of the pD difference of the two solutions on the rate of hydrolysis of **5** (phosphate buffer at pD 7.4 compared with D_2O where the pD of the incubation falls as **7** is produced). Alternatively, the observed difference in rates of hydrolysis of **5** may reflect an alternative or additional mechanism for β-lactam ring hydrolysis occurring in phosphate buffer that is not operative in D_2O. A plausible mechanism (Scheme 2) involves nucleophilic attack at the β-lactam carbonyl carbon by a monohydrogen phosphate dianion[2] and collapse of the resulting tetrahedral intermediate to give an acyl phosphate **13**. Hydrolysis of this intermediate would result in the formation of **7** as its carboxylate salt.†,[7]

It is therefore proposed that the second new compound observed by ^{1}H NMR analysis of a solution of **5** in phosphate buffer corresponds to the acyl phosphate intermediate **13**. The observation that resonances corresponding to **13** and **7** appeared at a similar rate probably reflects the fact that competing direct hydrolysis of **5** also occurs to a significant extent in buffer at pD 7.4. For other resonances assigned to **13** see Table 2.

† Koshland[7] has demonstrated that at neutral pH the overall rate of hydrolysis of acetyl phosphate is determined by the rate of hydrolysis of the dianion ($CH_3COPO_3^{2-}$). It should be noted that two mechanisms for acyl phosphate hydrolysis may occur depending on whether attack of water occurs at the carbonyl carbon or at the phosphorous. However, due to the transient nature of **13** it was difficult to envisage readily carrying out further mechanistic studies (such as experiments in $H_2{}^{18}O$) that may address this question.

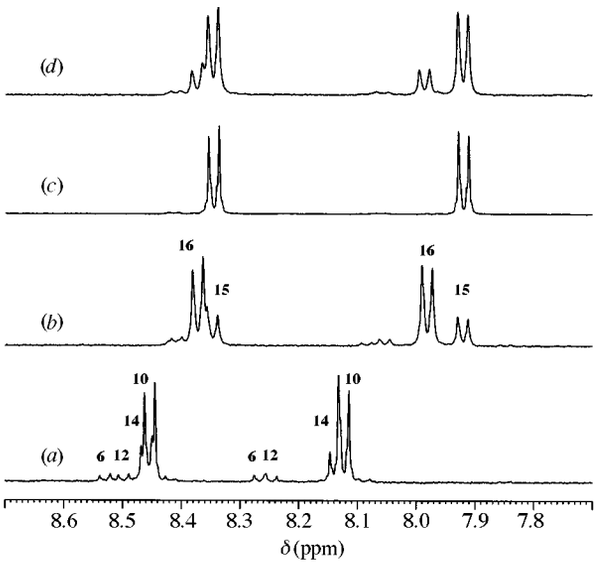
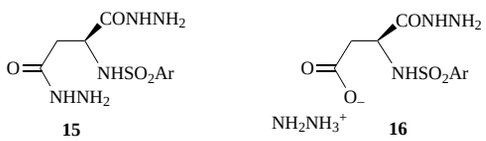


Fig. 3 ^{1}H NMR (500 MHz) spectra obtained on analysis of (*a*) the 20 h incubation of **6** at room temperature (295 K) in deuterated phosphate buffer–CD_3CN (10:1), (*b*) the solution from (*a*) after addition of NH_2NH_2 and 30 min incubation, (*c*) the overnight incubation of **6** with NH_2NH_2 in deuterated phosphate buffer–CD_3CN (10:1) and (*d*) a mixture of the solutions from (*b*) and (*c*)

Experiments aimed at providing further evidence in support of this assignment were carried out. Attempts to isolate **13** by reversed-phase HPLC were unsuccessful, consistent with the expected stability of an acyl phosphate intermediate. Analysis by ^{13}C or ^{31}P NMR spectroscopy of this system was precluded by the low solubility of **5** in phosphate buffer–CD_3CN (10:1) and hence the low levels of the transient species **13** present in solution. Further experiments also appeared to indicate that an activated β-lactam ring was required for the efficient formation of an acyl phosphate intermediate since no evidence for the hydrolysis of the β-lactam ring of **9** in phosphate buffer could be obtained by ^{1}H NMR spectral analysis after a one week incubation.‡

Attempts at trapping an acyl phosphate intermediate, as observed during the hydrolysis of **5**, with hydrazine[7,8] were carried out using the more readily available **6**. Analysis by ^{1}H NMR spectroscopy of a solution of **6** in deuterated phosphate buffer at pD 7.4 after a 20 h incubation at 295 K gave the spectrum shown in Fig. 3(*a*). The major set of AA′BB′ resonances observed corresponded to the formation of **10** from **6** by β-lactam ring hydrolysis [structural assignment based on comparison with the product from the incubation of **6** in D_2O–CD_3CN (10:1) for 4 days]. A second set of AA′BB′ resonances was assigned to the formation of the acyl phosphate **14** analogous to **13** (Scheme 2, Table 2 for assignment of other resonances corresponding to **14**). Weak resonances corresponding to **6** were also observed. A further set of weak AA′BB′ resonances observed were assigned to the formation of **12** formed by hydrolysis of the C_4-ester functionality of **6**. Subsequent addition of excess hydrazine to this solution, followed by ^{1}H NMR spectral analysis, resulted in the spectrum shown in Fig. 3(*b*). The resonances assigned to **10** and **14** were no longer present and two further sets of AA′BB′ resonances were observed corresponding to compounds apparently formed from **10** and **14**.§

Treatment of a solution of **6** in phosphate buffer–CD_3CN (10:1) with excess hydrazine followed by ^{1}H NMR spectral analysis resulted in the spectrum shown in Fig. 3(*c*), indicating that a single compound was produced in addition to ethanol [not shown in Fig. 3(*c*)]. Repetition of this experiment on a larger scale in water–acetonitrile and spectroscopic analysis of the product indicated that it had structure **15**. Comparison of



Ar = *p*-$NO_2C_6H_4$

the spectrum shown in Fig. 3(*b*) with that in Fig. 3(*c*) [see Fig. 3(*d*) for ^{1}H NMR analysis of a mixture of the two solutions] demonstrated that **15** was produced both on reaction of **6** and **14** with hydrazine. The other major set of resonances in Fig. 3(*b*) was assigned to the formation of **16** from **10**. The ratio of **16**:**15** produced on the addition of hydrazine to the incubation of **6** in buffer approximately equalled the ratio of **10**:**14** prior to the hydrazine addition.

These results imply that **14** reacts with hydrazine in an analogous manner to **6** and differently from **10**, consistent with a structure for **14** which contains an acylating functionality. In addition, the degree of β-lactam ring hydrolysis in **6** was shown to increase with increasing phosphate concentration at the same pD, consistent with a phosphate-mediated hydrolysis process.[2]

In summary, the azetidin-2-ones **5** and **6** were shown to be unstable with respect to β-lactam hydrolysis in phosphate buffer. Spectroscopic evidence for the formation of an acyl phosphate intermediate from both **5** and **6** supports the proposal[2] that phosphate-mediated nucleophilic catalysis of β-lactam hydrolysis occurs under these conditions.

Experimental

All solvents were distilled before use. All other reagents were purchased from commercial sources and purified in accordance with the recommendations of Perrin and Armarego,[9] or used as obtained. Flash chromatography was accomplished on silica gel using Sorbsil™ C60 (mpd 60 Å) silica gel (30–60 μm) by the method of Still.[10] Thin layer chromatography was performed using aluminium sheets coated with Merck Silica gel 60F254 which were visualised by UV fluorescence (λ_{max} 254 nm) and by 10% w/v ammonium molybdate in 2 M sulfuric acid or ninhydrin followed by heating.

Melting points were measured using a Büchi 510 capillary melting point apparatus. Values are quoted to the nearest 0.5 °C and are uncorrected. Optical rotations were measured using a Perkin-Elmer 241 polarimeter at 589 nm (Na D-line) at 20–25 °C, with a path length of 10 cm. Concentrations (*c*) are quoted in 10^{-2} g ml^{-1}. Retention factors are quoted to the nearest 0.1. Fourier Transform Infrared (FT IR) spectra were recorded as KBr discs on a Perkin-Elmer 1750 Fourier Transform spectrometer. Only selected absorptions are reported. Chemical shifts (δ_C) are quoted in ppm and referenced to $CDCl_3$ unless otherwise stated. Low resolution mass spectra

‡ During the course of these studies several examples of C_4-ester hydrolyses in phosphate buffer were obtained: (i) ^{1}H NMR analysis of the incubation of **9** in phosphate buffer after one week at room temperature demonstrated that formation of **8** and ethanol from **9** had occurred (**9**:**8**:ethanol = 2:1:1); (ii) analysis of a more concentrated sample of **5** after overnight incubation in phosphate buffer enabled the observation of a low intensity set of AA′BB′ resonances to be observed corresponding to the formation of **11** (structural assignment confirmed by comparison with synthetic **11**). Resonances at δ_H 3.64 (q, *J* 7.0, OCH_2CH_3) and 1.18 (t, *J* 7.0, OCH_2CH_3) corresponding to the formation of ethanol were also observed.

§ Compounds **6** and **12** also reacted with hydrazine (as expected) as judged by the disappearance of the minor sets of AA′BB′ resonances present in Fig. 3(*a*). It was shown that **6** reacts with hydrazine to form **15**. However, the amount of **15** formed by the reaction of hydrazine with the mixture of **6** and **14** resulting from overnight incubation of **6** in phosphate buffer (as judged by the intensities of the resonances corresponding to **15**) cannot have resulted solely from the reaction of residual **6** with hydrazine, *i.e.* **15** is formed from **14** as well.

(*m*/*z*) were recorded on a V.G. Micromass ZAB 1F (FAB/CI/DCI), V.G. Masslab 20-250 (CI/DCI/EI), a V.G. TRIO 1 (GCMS) or a V.G. BIO-Q (electrospray) spectrometer. Major peaks only are reported and intensities are quoted as percentages of the base peak. High resolution mass spectra were recorded by the EPSRC Mass Spectrometry Service Centre at the University of Wales, Swansea.

^{1}H NMR experiments were performed at 499.98 MHz on a Bruker AMX500 instrument equipped with a Nalorac MID500-3B 3 mm microprobe. Sample volumes were 130–150 μl and the sample temperature was regulated at 303 K. All spectra were referenced to internal MeCN at 2.05 ppm.

Light petroleum refers to the fraction boiling in the range 40–60 °C.

The phosphate buffer (0.1 M, pD 7.4) was prepared by dissolution of Na_2DPO_4 (1.107 g) and NaD_2PO_4 (276 mg) in D_2O (100 ml).

Synthesis of the azetidin-2-one **5** was carried out in accordance with literature precedent.[5] A modification[11] of the *N*-sulfonation procedure was made as detailed below.

(3*R*,4*S*)-Ethyl 3-ethyl-*N*-(*p*-nitrophenylsulfonyl)-2-oxoazetidine-4-carboxylate 5

Sodium bis(trimethylsilyl)amide (1.0 M in THF; 452 μl, 0.45 mmol, 0.90 equiv.) was added to a stirred solution of (3*R*,4*S*)-ethyl 3-ethyl-2-oxoazetidine-4-carboxylate **9** (86 mg, 0.50 mmol) in dry THF (10 ml) at −78 °C under argon. The reaction was stirred at −78 °C for 5 min and then a solution of *p*-nitrobenzenesulfonyl chloride (145 mg, 0.65 mmol; 1.3 equiv.) in dry THF (3 × 1 ml) was added dropwise. The reaction was stirred overnight at −78 °C. Saturated aqueous $NaHCO_3$ (20 ml) was added and the reaction extracted with DCM (2 × 50 ml). The combined organic extracts were dried ($MgSO_4$), filtered and concentrated *in vacuo* to give a yellow suspension (163 mg). The suspension was purified by flash column chromatography eluting with Et_2O–light petroleum (1:1) to give **5** as a colourless oil which solidified on standing (76 mg, 0.213 mmol, 47%); mp 52–52.5 °C; $[\alpha]_D^{24.5}$ −101.8 (*c* 1.0 in $CHCl_3$); [R_f 0.4 (Et_2O–light petroleum, 1:1)]; ν_{max}(KBr disc)/cm^{-1} 3110w, 3120w, 2979w, 1933w, 1805s (CONS), 1745s (CO_2R), 1609w, 1536s (NO_2), 1369s (NO_2), 1357m (SO_2), 1213m, 1171m (SO_2) and 857m (*p*-subst. ArCH); δ_H(200 MHz; $CDCl_3$) 1.07 (3H, t, *J* 7.5, C*H*$_3$CH$_2$CH), 1.29 (3H, t, *J* 7.0, OCH$_2$C*H*$_3$), 1.79–1.92 (2H, m, CH$_3$C*H*$_2$CH), 3.13–3.22 [1H, m, C(3)H], 4.20–4.27 (2H, m, OC*H*$_2$CH$_3$), 4.42 [1H, d, *J* 3.0, C(4)H] and 8.27 and 8.42 (4H, AA′BB′, Ar-H); δ_C(125 MHz; $CDCl_3$) 10.7 (*C*H$_3$CH$_2$), 14.0 (OCH$_2$*C*H$_3$), 21.3 (CH$_3$*C*H$_2$), 57.3 [C(3)], 58.3 [C(4)], 62.5 (OCH$_2$CH$_3$), 124.2, 129.3 (Ar-C), 144.1, 150.9 (2 × *ipso*-C) and 164.3 and 168.2 (2 × *C*O); *m*/*z* (CI, NH_3) 344 [MNH$_4^+$ − 30, ArNO$_2$ ⟶ ArOH, 100%], 327 [MH$^+$ − 30, ArNO$_2$ ⟶ ArOH, 15], 299 [35], 198 [10], 190 [12], 189 [18], 156 [23], 144 [12], 126 [21] and 94 [22] [Found: MNH$_4^+$, 374.1022. $C_{14}H_{16}N_2O_7S$ requires MNH$_4^+$, 374.1021(9)].

(4*S*)-Ethyl *N*-(*p*-nitrophenylsulfonyl)-2-oxoazetidine-4-carboxylate 6

Sodium bis(trimethylsilyl)amide (1.0 M solution in THF; 356 μl, 0.36 mmol, 1.0 equiv.) was added to a stirred solution of (4*S*)-ethyl 2-oxoazetidin-4-carboxylate[12] (51 mg, 0.36 mmol) in dry THF (7 ml) under argon at −78 °C. The reaction mixture was stirred for 10 min. A solution of *p*-nitrobenzenesulfonyl chloride (237 mg, 1.07 mmol; 3.0 equiv.) in dry THF (3 × 1 ml) was then added dropwise over 2 min and the reaction stirred at −78 °C for 1.5 h. Saturated aqueous NH_4Cl (30 ml) was added and the reaction mixture was extracted with Et_2O (3 × 50 ml). The combined organic extracts were dried ($MgSO_4$), filtered and concentrated *in vacuo* to give a yellow oil which was purified by flash column chromatography eluting with Et_2O–light petroleum (1:1) to give **6** as a colourless oil which solidified on standing to give a white solid (79 mg, 0.24 mmol, 68%); mp 106–107 °C; $[\alpha]_D^{22}$ −113.7 (*c* 0.9 in $CHCl_3$); [R_f 0.2 (Et_2O–light petroleum, 2:1)]; ν_{max}(KBr disc)/cm^{-1} 3114w, 2993w, 1817s (CONSO$_2$R), 1743s (CO_2R), 1613w, 1553s (NO_2), 1405m, 1371s (SO_2), 1353s (NO_2), 1219s, 1177s, 1138s (SO_2), 1075m and 853m (*p*-subst. ArCH); δ_H(200 MHz; $CDCl_3$) 1.29 (3H, t, *J* 7.0, OCH$_2$CH$_3$), 3.00–3.10 [1H, m, A part of ABX, J_{AB} 16.0, 1 × C(3)H$_2$], 3.41–3.52 [1H, m, B part of ABX, J_{AB} 16.0, 1 × C(3)H$_2$], 4.18–4.31 (2H, m, OC*H*$_2$CH$_3$), 4.71–4.79 [1H, m, X part of ABX, J_{AX}, J_{BX} 10.5, C(4)H], 8.19 and 8.50 (4H, AA′BB′, Ar-H); δ_C(50 MHz; $CDCl_3$) 13.9 (OCH$_2$*C*H$_3$), 41.8 [C(3)], 52.6 [C(4)], 62.7 (O*C*H$_2$CH$_3$), 124.5, 129.6 (Ar-C), 144.1, 151.2 (2 × *ipso*-C) and 161.5 and 168.6 (2 × CO); *m*/*z* (CI, NH_3) 346 [MNH$_4^+$, 100%], 316 [MNH$_4^+$ − 30, ArNO$_2$ ⟶ ArOH, 81], 298 [MH$^+$ − 30, ArNO$_2$ ⟶ ArOH, 14], 271 [38], 190 [31], 161 [36], 156 [55], 126 [29], 116 [22], 93 [39], 82 [19], 65 [17] and 55 [30].

(2*S*,3*R*)-Ethyl 3-ethyl-*N*-(*p*-nitrophenylsulfonyl)aspartate 7

A solution of (3*R*,4*S*)-ethyl 3-ethyl-*N*-(*p*-nitrophenylsulfonyl)-2-oxoazetidin-4-carboxylate **5** (20 mg, 56 μmol) in acetonitrile–water (1:9, 10 ml) was heated at 63 °C for 60 h. The reaction mixture was allowed to cool and was concentrated *in vacuo* to give **7** as a white solid (13 mg, 35 μmol, 62%); mp 89–90 °C; $[\alpha]_D^{22}$ +36.3 (*c* 0.6 in $CHCl_3$); ν_{max}(KBr disc)/cm^{-1} 3272br s (CO_2H), 2974w, 1710br s (CO_2H), 1530s (NO_2), 1353s (NO_2, SO_2), 1173s (SO_2) and 853m (*p*-subst. ArH); δ_H(500 MHz; CD_3OD) 0.97 (3H, t, *J* 7.5, C*H*$_3$CH$_2$), 1.06 (3H, t, *J* 7.0, OCH$_2$C*H*$_3$), 1.51–1.58 (1H, m, 1 × CH$_3$C*H*$_2$), 1.68–1.76 (1H, m, 1 × CH$_3$C*H*$_2$), 2.68 [1H, q, *J* 7.0, C(3)H], 3.89 (2H, q, *J* 7.0, OC*H*$_2$CH$_3$), 4.16 [1H, d, *J* 5.5, C(2)H] and 8.09 and 8.37 (4H, AA′BB′, Ar-H); δ_C(125 MHz, $CDCl_3$) 11.8 (*C*H$_3$CH$_2$), 13.8 (OCH$_2$*C*H$_3$), 21.7 (CH$_3$*C*H$_2$), 48.7 [C(3)], 55.7 [C(2)], 62.3 (O*C*H$_2$CH$_3$), 124.1, 128.5 (Ar-C), 146.0, 150.1 (2 × *ipso*-C), 170.0 and 177.3 (2 × CO); *m*/*z* (negative electrospray ionisation, cone voltage 50 V) 373 [M − H$^+$, 100%] and 345 [21].

(*S*)-*N*-(*p*-Nitrophenylsulfonyl)aspartodihydrazide 15

Hydrazine monohydrate (18 mg, 18 ml, 0.37 mmol) was added to a suspension of (4*S*)-ethyl 2-oxoazetidin-4-carboxylate **6** (12 mg, 37 μmol) in acetonitrile–water (1:10, 11 ml) at room temperature. The reaction was stirred until a clear solution was formed (30 min) which was then lyophilised to give **15** as a white solid (10 mg, 0.029 mmol, 80%); mp 170–171 °C (decomp.); $[\alpha]_D^{24}$ −5.3 (*c* 0.08 in MeOH); ν_{max}(KBr disc)/cm^{-1} 3321br s, 1658br s, 1525m (NO_2), 1350m (SO_2), 1332m (NO_2), 1308m and 1159 (SO_2); δ_H(500 MHz, D_2O) 2.41–2.46 [1H, m, A part of ABX, J_{AB} 14.5, 1 × C(3)H$_2$], 2.62–2.66 [1H, m, B part of ABX, J_{AB} 14.5, 1 × C(3)H$_2$], 4.02–4.05 [1H, m, X part of ABX, J_{AX} + J_{BX} 14.0, C(2)H] and 8.08 and 8.43 (4H, AA′BB′, Ar-H); *m*/*z* (positive electrospray ionisation, cone voltage 50 V) 347 [MH$^+$, 100%], 333 [10] and 315 [22].

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