Synthesis of a Monocyclic β-Lactam Stereospecifically Labelled at C-4

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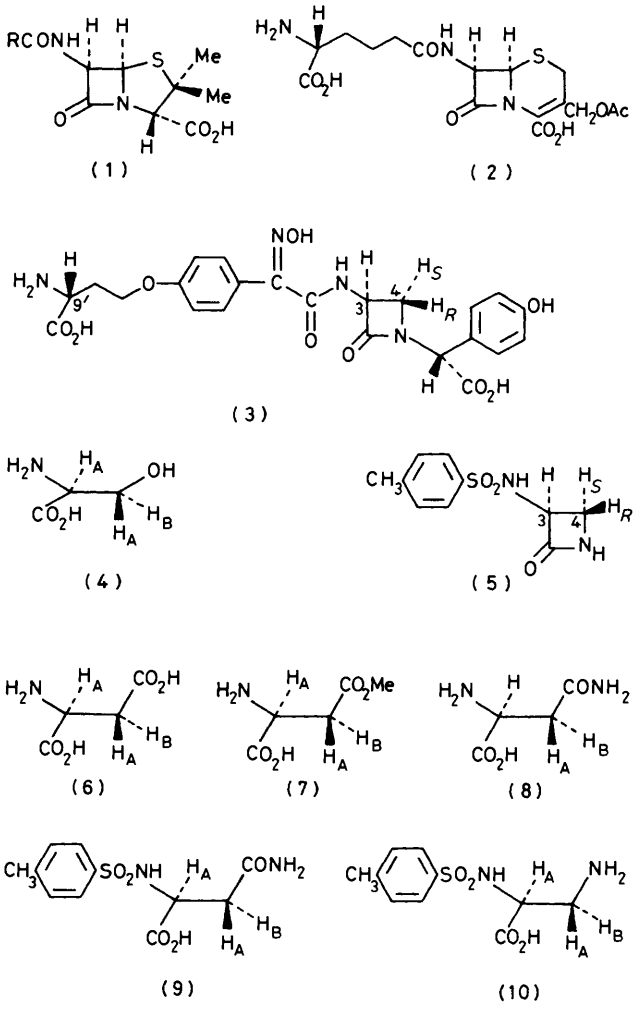
In connection with biosynthetic studies, the β-lactams (5; 4-H_R = ^2H) and (5; 3-H = 4-H_S = ^2H) have been synthesized. The ^1H and ^2H n.m.r. spectra of these compounds confirm the assignment of the stereochemistry to the two hydrogens at C-4 of monocyclic β-lactams such as nocardicin A. Samples of the amino-acid L-asparagine stereospecifically labelled at C-3 have been made in the course of this work.

The stereochemistry of the formation of the bond which gives rise to β-lactam rings in nature is of considerable interest. Some years ago we elucidated the stereochemistry of this process in the biosynthesis of the classical β-lactam antibiotics, namely the penicillins (1) [1] and cephalosporin C (2).[2] More recently new families of medically important β-lactams have been discovered and these involve very different biosyntheses from the penicillins and cephalosporin C. The β-lactam ring of nocardicin A (3) is known [3] to be derived from L-serine (4) and, since we had synthesized samples of serine stereospecifically deuteriated at C-3,[4] we were in a position to examine the overall stereochemistry of the β-lactam ring-forming process in nocardicin A biosynthesis. During the course of our work, Townsend [5a] published his findings on this problem and we now report the work done in our laboratories, some of which complements Townsend's results.

Before investigating feeding of the stereospecifically labelled samples of serine (4; H_B = ^2H) and (4; H_A = ^2H) to *Nocardia* species we felt that it was necessary to have a totally unambiguous assay for the chirality at C-4 of the resultant samples of [4-$^2H_1$]nocardicin A. This assay should also prove useful for biosynthetic studies on some of the monobactam [6] group of antibiotics. Hashimoto [7] noted that the protons on C-3 and C-4 of nocardicin A (3) appeared in the ^1H n.m.r. spectrum as an AMX system, δ 3.14 and 3.97 (4-H) and 5.01 (3-H) with the proton on C-9′ giving rise to a signal at δ 3.81. Townsend [5] reversed the assignments of one of the protons at C-4 and of 9′-H but the couplings J_{cis} 5 Hz and J_{trans} 2 Hz were in keeping with expectation [8] based empirically on values found for a large number of other β-lactams. The stereochemistry of the C-4 hydrogens can therefore be deduced from coupling constant data. A more unambiguous assignment of the stereochemistry of these hydrogens might be made, however, if we were to synthesize a monocyclic β-lactam stereospecifically labelled at C-4 with deuterium.

The β-lactams (5; H_R = ^2H) and (5; H_S = ^2H) were chosen as the targets for this synthesis and, since the stereospecifically labelled aspartic acids (6; H_B = ^2H) and (6; H_A = ^2H) could be prepared in synthetically useful quantities,[4] these were used as starting materials. The β-carboxylic acid group of aspartic acid (6) has been specifically esterified using methanol and thionyl chloride.[9] The labelled esters (7; H_B = ^2H) and (7; H_A = ^2H) were therefore prepared in this way from the corresponding samples of deuteriated aspartic acid. Treatment of these esters with ammonia gave samples of the labelled essential amino acid L-asparagine (8). The ^1H n.m.r. spectrum of unlabelled L-asparagine (8) in 10% NaO^2H–^2H_2O appeared as an ABX system, δ 1.6, 1.81, and 2.74, J_{AB} 14.7, J_{AX} 8.9, J_{BX} 4.8 Hz. The sample derived from (2*S*,3*R*)-[3-$^2H_1$]aspartic acid



(6; H_B = ^2H) had two doublets, δ 1.57 and 2.72, in the ^1H n.m.r. spectrum and that derived from (2*S*,3*S*)-[2,3-$^2H_2$]-aspartic acid (6; H_A = ^2H) had but a singlet, δ 1.78, in the ^1H n.m.r. spectrum. The synthetic samples were therefore stereospecifically labelled and the 3-*pro*-*R* hydrogen resonated at δ 1.8 and the 3-*pro*-*S* at δ 1.6 in the ^1H n.m.r. spectrum in 10% NaO^2H–^2H_2O.

The samples of deuteriated asparagine (8; H_B = ^2H) and (8; H_A = ^2H) were separately converted into the corresponding toluene-*p*-sulphonamides (9). These underwent Hofmann re-

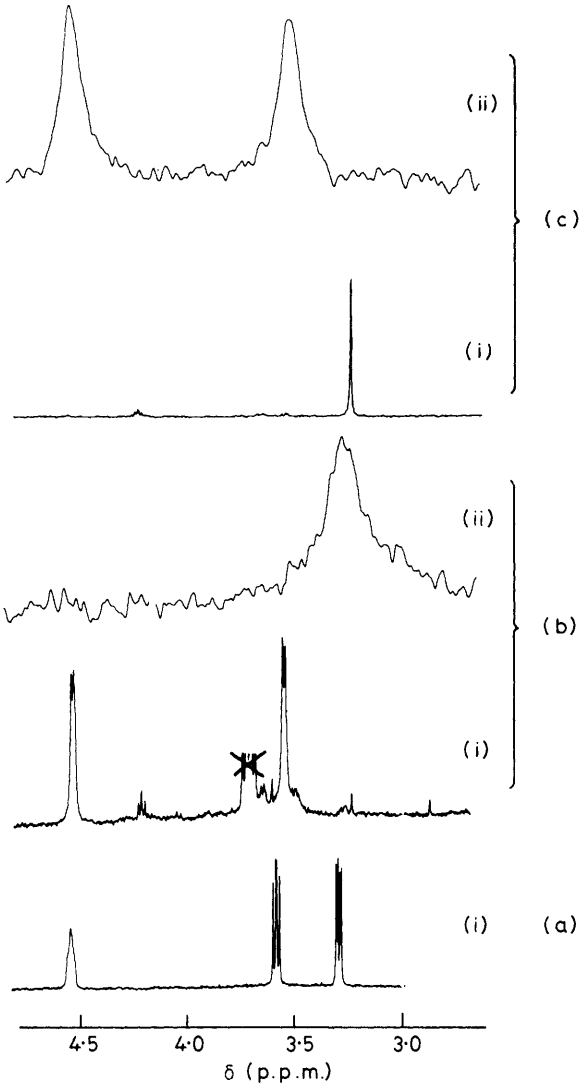


Figure. (i) 360 MHz ^1H n.m.r. spectra in C^2HCl_3, (ii) 55.28 MHz ^2H n.m.r. spectra in $CHCl_3$ of (a) the β-lactam (5); (b) (3*S*,4*R*)-[4-$^2H_1$]-(5); and (c) (3*S*,4*S*)[3,4-$^2H_2$]-(5)

arrangement to yield the amines (10). The ^1H n.m.r. spectra of the amines (10) showed selective omissions in the ABX system corresponding to the protons on C-3 and C-4 so that labelling was stereospecific. Since Hofmann rearrangement occurs with retention of stereochemistry at the migrating centre,[10,11] the sample derived from (2*S*,3*R*)-[3-$^2H_1$]asparagine (8; $H_B = {}^2H$) must be the (2*S*,3*R*)-[3-$^2H_1$]amine (10; $H_B = {}^2H$) and that derived from (2*S*,3*S*)-[2,3-$^2H_2$]asparagine (8; $H_A = {}^2H$) must be the (2*S*,3*S*)-[2,3-$^2H_2$]amine (10; $H_A = {}^2H$). The β-amino acids (10) were now cyclised using triphenylphosphine and 2,2′-dipyridyl disulphide. The β-lactams (5), obtained in low yield, had carbonyl absorptions at 1 750 cm^{-1} in the i.r. spectrum and ^1H and ^2H n.m.r. spectra as shown in the Figure. These spectra indicate that the 4-*pro-R* hydrogen absorbs at δ 3.29, J_{AB} 6, J_{AX} 2.5 Hz whilst the 4-*pro-S* hydrogen absorbs at δ 3.58, J_{AB} 6, J_{BX} 5 Hz. The synthesis has therefore confirmed the assignments made on the basis of coupling constant data. It is of interest to note that the 4-*pro-R* hydrogen resonates to lower frequency than the 4-*pro-S* hydrogen in both our model compound (5) and in nocardicin A (3).[7]

Having assured ourselves of an unambiguous method of determining the stereochemistry of labelling at C-4 of nocardicin A (3), we now fed (2*S*,3*R*)-[3-$^2H_1$]- and (2*S*,3*S*)-[2,3-$^2H_2$]-serine [(4; $H_B = {}^2H$) and (4; $H_A = {}^2H$) respectively] separately to *Nocardia uniformis* var. *tsuyamanensis*. Incorporation was, however, not sufficient to allow us to observe a deuterium signal in the ^2H n.m.r. spectrum even with prolonged acquisition times. At this point Townsend [5] reported that a 3*S*-deuteriated serine gave rise to nocardicin A with a deuterium signal to lower frequency than the signal from a sample derived from 3*R*-deuteriated serine. This indicated that the β-lactam ring was formed in the biosynthesis of nocardicin A (3) with overall inversion of stereochemistry.

Experimental

M.p.s were determined on a Kofler hot-stage apparatus. I.r. spectra were recorded on Perkin-Elmer 257, 457, and 477 instruments and ^1H n.m.r. spectra on Perkin-Elmer R12 (60 MHz) and R32 (90 MHz) and Bruker WH 360 (^1H, 360 MHz; ^2H, 55.28 MHz) instruments. Specific rotations were determined on a Perkin-Elmer PE241 polarimeter using a 1 dm cell and mass spectra were recorded on Kratos MS25 and MS80 (Sussex) and AEI MS902S and FAB-MS9 (I.C.I.) instruments.

β-*Methyl* L-*Aspartate* (7).—Thionyl chloride (3 ml, 41 mmol) was added dropwise to stirred cold dry methanol (30 ml) at −15 °C. L-Aspartic acid (4 g, 30 mmol) was added and the mixture was allowed to warm to 25 °C during 90 min after which time dissolution was complete. Dry diethyl ether was added slowly to the stirred solution and the resulting solid was recrystallised from methanol–diethyl ether (1 : 7) to give β-methyl L-aspartate hydrochloride (3.57 g, 65%), m.p. 184—186 °C (lit.,[9] 187—190 °C); $[\alpha]_D^{25}$ +9.7° (*c* 0.601, water); *m/z* (bistrimethylsilyl deriv.) 291 (M^+); δ (10% ^2HCl–^2H_2O, referenced to external $SiMe_4$) 3.05 (2 H, d, *J* 5 Hz, β-H_2), 3.63 (3 H, s, OMe), and 4.32 (1 H, t, *J* 5 Hz, α-H).

β-*Methyl* (2S,3R)-[3-$^2H_1$]*aspartate* (7; $H_B = {}^2H$). This was prepared as the hydrochloride from (2*S*,3*R*)-[3-$^2H_1$]aspartic acid (6; $H_B = {}^2H$) [4] using the method described above for the unlabelled compound. Spectra were identical with those of the unlabelled compound except that the mass spectral parent ion of the bistrimethylsilyl derivative, *m/z* 292, was one mass number higher and the ^1H n.m.r. spectrum showed but one β-proton at δ 2.98 (br d, *J* 5.5 Hz) with the α-H signal as a doublet (*J* 5.5 Hz) at δ 4.38.

β-*Methyl* (2S,3S)-[2,3-$^2H_2$]*aspartate* (7; $H_A = {}^2H$). This was prepared as the hydrochloride from (2*S*,3*S*)-[2,3-$^2H_2$]aspartic acid (6; $H_A = {}^2H$) [4] using the method described above for the unlabelled compound. Spectra were identical with those of the unlabelled compound except that the mass spectral parent ion of the bistrimethylsilyl derivative, *m/z* 293, was two mass numbers higher and the ^1H n.m.r. spectrum showed but one β-proton at δ 3.02 and no α-proton.

L-*Asparagine* (8).—β-Methyl L-aspartate hydrochloride (1.1 g, 5.99 mmol) was dissolved in a mixture of methanol (20 ml), tetrahydrofuran (THF) (20 ml) and diethyl ether (20 ml). Ammonia gas was passed through the solution at 0—5 °C for 15 min. The reaction vessel was sealed and left at 25 °C for 4 d. The solvent was removed from the turbid solution and the residue was dissolved in water (50 ml). The solution was passed through a column of Amberlite IR45 weakly basic ion-exchange resin (30 ml) and eluted with water. The first fractions (150 ml) were lyophilized to dryness and the residue was recrystallised from water–ethanol (5 : 2) to give L-asparagine (609 mg, 77%), m.p. 229—231 °C; $[\alpha]_D^{23}$ −10.9° (*c* 0.57, 1M NaOH) [lit.,[12] m.p. 236 °C; lit.,[13] $[\alpha]_D$ −12.4° (1M NaOH)], $[\alpha]_D^{25}$ −9.5° (*c* 0.727, 2M NaOH); *m/z* of tetrakistrimethylsilyl derivative 420 (M^+); δ(10% NaO^2H–H_2O; referenced to ex-

ternal $SiMe_4$) 1.60 (1 H, *A*BX, J_{AB} 14.7, J_{BX} 8.9 Hz, 3-H *pro-S*), 1.81 (1 H, A*B*X, J_{AB} 14.7, J_{BX} 4.8 Hz, 3-H *pro-R*), and 2.74 (1 H, AB*X*, J_{AX} 8.9, J_{BX} 4.8 Hz, 2-H).

(2S,3R)-[3-$^2H_1$]*Asparagine* (8; H_B = ^2H). This was prepared from the hydrochloride of (7; H_B = ^2H) using the method described above for the unlabelled compound, m.p. 229—231 °C; $[\alpha]_D^{25}$ −9.31° (*c* 0.511, 2M NaOH); *m/z* of tetrakistrimethylsilyl derivative 421; δ (10% NaO^2H–^2H_2O; referenced to external $SiMe_4$) 1.57 (1 H, br d, *J* 8.8 Hz, 3-H) and 2.72 (1 H, d, *J* 8.8 Hz, 2-H).

(2S,3S)-[2,3-$^2H_2$]*Asparagine* (8; H_A = ^2H). This was prepared from the hydrochloride of (7; H_A = ^2H) using the method described above for the unlabelled compound, m.p. 230—232 °C; $[\alpha]_D^{25}$ −10.11° (*c* 0.910, 2M NaOH); *m/z* of tetrakistrimethylsilyl derivative 422; δ (10% NaO^2H–^2H_2O; referenced to external $SiMe_4$) 1.78 (s).

(2S)-N-p-*Tolylsulphonylasparagine* (9).—L-Asparagine (3.96 g, 30 mmol) and triethylamine (9.6 g, 95 mmol) were dissolved in a stirred mixture of water (48 ml) and THF (24 ml). Toluene-*p*-sulphonyl chloride (8.7 g, 45 mmol) was added in portions during 30 min to the stirred solution. The mixture was then stirred vigorously for a further 90 min. THF was removed under reduced pressure and the aqueous solution was washed with diethyl ether (2 × 20 ml). The aqueous solution was purged with air to remove the last traces of ether and the pH was adjusted to 1.8 with 6M aqueous hydrochloric acid. The solution was cooled and crystals which deposited during several hours were recrystallised from aqueous sodium hydroxide by the addition of acetic acid (7.92 g; 92%), m.p. 182—183 °C, $[\alpha]_D^{23}$ +5.8° (*c* 0.572, K^+ salt in H_2O at pH 7.5) [lit.,[14] m.p. 191 °C; $[\alpha]_D$ +9.7° (in water containing 1 equiv. KOH)], $[\alpha]_D^{25}$ −68.54° (*c* 0.765, 2M NaOH); $\nu_{max.}$ (Nujol) 3 400, 3 180 (NH, OH), 1 718 (CO_2H), and 1 663 cm^{-1} (amide); δ (10% NaO^2H–^2H_2O, referenced to ^2HOH at δ 4.6) 2.0 (2 H, s, ArCH_3), 2.03 (2 H, m, 3-H), 3.33 (1 H, q, J_{AX} 8, J_{BX} 6 Hz, 2-H), and 6.94 and 7.28 (each 2 H, d, *J* 8 Hz, together ArH).

(2S,3R)-[3-$^2H_1$]-N-p-*Tolylsulphonylasparagine* (9; H_B = ^2H). This was prepared from (2*S*,3*R*)-[3-$^2H_1$]asparagine (8; H_B = ^2H) using the method described above for the unlabelled compound, m.p. 181—182 °C; $[\alpha]_D^{25}$ −67.2° (*c* 0.482, 2M NaOH); $\nu_{max.}$ (Nujol) 3 400, 3 180 (NH, OH), 1 718 (CO_2H), and 1 663 cm^{-1} (amide); δ (10% NaO^2H–^2H_2O; referenced to ^2HOH at δ 4.6) 1.91 (1 H, d, *J* 8 Hz, 3-H), 1.96 (3 H, s, ArCH_3), 3.26 (1 H, d, *J* 8 Hz, 2-H) and 6.9 and 7.22 (each 2 H, d, *J* 8 Hz, together ArH).

(2S,3S)-[2,3-$^2H_2$]-N-p-*Tolylsulphonylasparagine* (9; H_A = ^2H). This was prepared from (2*S*,3*S*)-[2,3-$^2H_2$]asparagine (8; H_A = ^2H) using the method described above for the unlabelled compound, m.p. 182—183 °C, $[\alpha]_D^{25}$ −63.9° (*c* 0.47, 2M NaOH); $\nu_{max.}$ (Nujol) 3 400, 3 180 (NH, OH), 1 718 (CO_2H) and 1 663 cm^{-1} (amide); δ (10% NaO^2H–^2H_2O; referenced to ^2HOH at δ 4.6) 1.95 (3 H s, Ar-CH_3), 1.97 (1 H, s, 3-H), and 6.89 and 7.21 (each 2 H, d, *J* 8 Hz, together ArH).

(2S)-3-*Amino*-2-p-*tolylsulphonamidopropanoic Acid* (10).*—Bromine (3.2 g, 20 mmol) was added slowly at −8 °C to a stirred solution of sodium hydroxide (5.6 g, 140 mmol) in water (45 ml) during 5 min. A solution of *N*-*p*-tolylsulphonyl-L-asparagine (6 g, 20.9 mmol) in 2.5M aqueous sodium hydroxide (17 ml) was added to this solution during *ca.* 1 min, avoiding a rapid rise in temperature. The solution was heated to 85 °C for 22 min and then allowed to cool slowly. 12M Aqueous hydrochloric acid was added to the solution dropwise to pH 6.5 and the resultant crystals were filtered off and washed in turn with ice-cold water and methanol (yield 4.48 g, 83%), m.p. 189—191 °C; $[\alpha]_D^{23}$ −57.7° (*c* 1.05, 1M NaOH) [lit.,[15] m.p. 214—216 °C (decomp.); $[\alpha]_D$ −52.5° (1M NaOH)]; $[\alpha]_D^{25}$ −68.54° (*c* 0.765, 2M NaOH); $\nu_{max.}$ (Nujol) 3 500 and 3 300 (NH) and 1 600br cm^{-1} (CO_2H); δ (10% ^2HCl–^2H_2O; referenced to ^2HOH at δ 5.8) 2.0 (3 H, s, ArCH_3), 2.83 (1 H, *A*BX, J_{AB} 13, J_{AX} 9.3 Hz, 3-H *pro-S*), 3.10 (1 H, A*B*X, J_{AB} 13, J_{BX} 4.8 Hz, 3-H *pro-R*), 3.98 (1 H, AB*X*, J_{AX} 9.3, J_{BX} 4.8 Hz, 2-H), and 7.0 and 7.4 (each 2 H, d, *J* 8 Hz, together ArH).

(2S,3R)-[3-$^2H_1$]-3-*Amino*-2-p-*tolylsulphonamidopropanoic acid* (10; H_B = ^2H). This was prepared from (2*S*,3*R*)-[3-$^2H_1$]-*N*-*p*-tolylsulphonylasparagine (9; H_B = ^2H) using the method described above for the unlabelled compound, m.p. 200—201 °C; $[\alpha]_D^{25}$ −66.73° (*c* 0.486, 2M NaOH); $\nu_{max.}$ (Nujol) 3 500 and 3 300 (NH) and 1 600br cm^{-1} (CO_2H); δ (10% ^2HCl–^2H_2O; referenced to ^2HOH at δ 5.8) 2.0 (3 H, s, ArCH_3), 2.79 (1 H, d, *J* 9.3 Hz, 3-H), 3.94 (1 H, d, *J* 9.3 Hz, 2-H), and 7.0 and 7.4 (each 2 H, d, *J* 8 Hz, together ArH).

(2S,3S)-[2,3-$^2H_2$]-3-*Amino*-2-p-*tolylsulphonamidopropanoic acid* (10; H_A = ^2H). This was prepared from (2*S*,3*S*)-[2,3-$^2H_2$]-*p*-tolylsulphonylasparagine (9; H_A = ^2H) using the method described above for the unlabelled compound, m.p. 191—192 °C; $[\alpha]_D^{25}$ −62.01° (*c* 0.477, 2M NaOH); $\nu_{max.}$ (Nujol) 3 500 and 3 300 (NH) and 1 600br cm^{-1} (CO_2H); δ (10% ^2HCl–^2H_2O; referenced to ^2HOH at δ 5.8) 2.0 (3 H, s, ArCH_3), 3.05 (1 H, s, 3-H), and 7.0 and 7.4 (each 2 H, d, *J* 8 Hz, together ArH).

(3S)-3-p-*Tolylsulphonamidoazetidin*-2-*one* (5).—Triphenylphosphine (80 mg, 0.305 mmol) and 2,2′-dipyridyl disulphide (60 mg, 0.273 mmol) were added to a suspension of 3-amino-2-*p*-tolylsulphonamidopropanoic acid (10) (60 mg, 0.233 mmol) in acetonitrile (80 ml) and water (5 ml). The mixture was heated to reflux for 5.5 h during which time the solution became homogeneous and dark yellow. The solvent was removed under reduced pressure, the residue was dissolved in chloroform (10 ml), and the solution was filtered. The filtrate was reduced to 2 ml volume and preparative t.l.c. (p.l.c.) [silica; $CHCl_3$–MeOH (10 : 1)] followed by further p.l.c. of the more polar fractions gave a solid, R_F 0.23, which crystallised as pale yellow cubes from chloroform–hexane (9 mg, 16%), m.p. 142—143 °C, $[\alpha]_D^{25}$ +9.21° (*c* 0.389, $CHCl_3$); *m/z* (E.I.) 238 ($M^+ - H_2$), 197.0519 ($C_{10}H_{12}N_2O_3S$ − NHCO requires *m/z*, 197.051 04); *m/z* (negative FAB) 239 $(M - H)^-$; *m/z* (DCI, NH_3 carrier gas) 258 ($M^+ + NH_4$); $\nu_{max.}$ (Nujol) 1 750 cm^{-1} (β-lactam); δ (C^2HCl_3) 2.43 (3 H, s, ArCH_3), 3.29 (1 H, *A*BX, J_{AB} 6, J_{AX} 2.5 Hz, 4-H_R), 3.58 (1 H, A*B*X, J_{AB} 6, J_{BX} 5 Hz, 4-H_S), 4.53 (1 H, m, 3-H), 5.21 and 5.67 (each 1 H, NH), and 7.32 and 7.76 (each 2 H, d, *J* 8 Hz, together ArH).

(3S,4R)-[4-$^2H_1$]-3-p-*Tolylsulphonamidoazetidin*-2-*one* (5; 4-H_R = ^2H). This was prepared from the β-amino acid (10; H_B = ^2H) using the method described above for the unlabelled compound, m.p. 140—142 °C; *m/z* (E.I.) 239 ($M^+ - H_2$) and 198 (M^+ − NHCO); $\nu_{max.}$ (Nujol) 1 750 cm^{-1} (β-lactam); δ_H (C^2HCl_3) 2.42 (3 H, s, ArCH_3), 3.55 (1 H, d, *J* 5 Hz, 4-H), 4.52 (1 H, d, *J* 5 Hz, 3-H), 5.45 and 5.85 (each 1 H, NH), and 7.32 and 7.76 (each 2 H, d, *J* 8 Hz, together ArH); $\delta_{^2H}$ ($CHCl_3$) 3.25 (br s, 4-^2H).

(3S,4S)-[3,4-$^2H_2$]-3-p-*Tolylsulphonamidoazetidin*-2-*one* (5; 3-H = 4-H_S = ^2H). This was prepared from the β-amino acid (10; H_A = ^2H) using the method described above for the unlabelled compound, m.p. 140—142 °C; *m/z* (E.I.) 199 (M^+ − NHCO); $\nu_{max.}$ (Nujol) 1 750 cm^{-1} (β-lactam); δ_H (C^2HCl_3) 2.43 (3 H, s, ArCH_3), 3.22 (1 H, s, 4-H), 5.60 and 5.87 (each 1 H, NH), and 7.32 and 7.76 (each 2 H, d, *J* 8 Hz, together ArH); $\delta_{^2H}$ ($CHCl_3$) 3.51 (1 ^2H, br s, 4-^2H_S) and 4.52 (1 ^2H, br s, 3-^2H).

* (2S)-2-*p*-Tolylsulphononamido-β-alanine.

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