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Synthesis, Structure Elucidation and Reactivity of Novel Azetidinone-oxiranes

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Oxidation of 2,3-*cis*-2-sulfonamide-*N*-butenoate-azetidinone **3** and 2,3-*trans*-2-acetoxy-*N*-butenoate-azetidinone **6** led to the formation of azetidinone-oxiranes **5** and **7**. Molecular structure of **5**, including its absolute configuration, was unambiguously determined by X-ray structure analysis. Cleavage of the benzyl ester group using $AlCl_3$ / anisole as well as H_2Pd/C procedures failed. Instead, new methods for the removal of the substituent from the azetidinone nitrogen of compounds **5** and **7** were found. Azetidinone **8** was isolated in the reaction of azetidinone-oxiranes **5** with $AlCl_3$/anisole. During hydrogenation of azetidinone-oxirane **5**, afforded azetidinone β-hydroxy acid sodium salt **9**, which was transformed into azetidinone **8** in acidic media. Compounds **5** and **7** gave the azetidinone-halohydrines **10** and **11** by the oxirane ring opening with HCl.

Key words: azetidinone-*N*-butenoat, azetidinone-oxirane, azetidinone β-hydroxy acid, azetidinone-halohydrine, synthesis, X-ray structure analysis, antibacterial activity.

INTRODUCTION

The unresolved problem of the β-lactamase mediated resistance of clinically important bacteria to β-lactam antibiotics has encouraged the search for more effective inhibitors of these enzymes.

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In last two decades there has been a considerable expansion of efforts devoted to the search for new β-lactamase inhibitors by chemical transformation of natural penicillins. Some of the semi-synthetic inhibitors, like sulbactam, brobactam, tazobactam, cloxacillin sulfoxide and others have been shown to be potent β-lactamase inhibitors.[1]

Here we present the synthesis, structure elucidation and reactivity of some novel β-lactam species derived from cloxacillin.

RESULTS AND DISCUSSION

Cloxacillin **1** was transformed into 2-sulfonamide-azetidinone **3** using our earlier established methodology.[2] In spite of the fact that unsaturated acids react with peracids with great difficulty to form oxirane derivatives,[3] the reaction of **3** with *m*-chloroperbenzoic acid (*m*-CPBA) resulted in generation of 2-sulfonamide-azetidinone-oxirane **5** in good yield, Scheme 1.

X-ray structure analysis was used to determine the molecular structure of azetidinone-oxirane **5** and to define its absolute configuration. The known

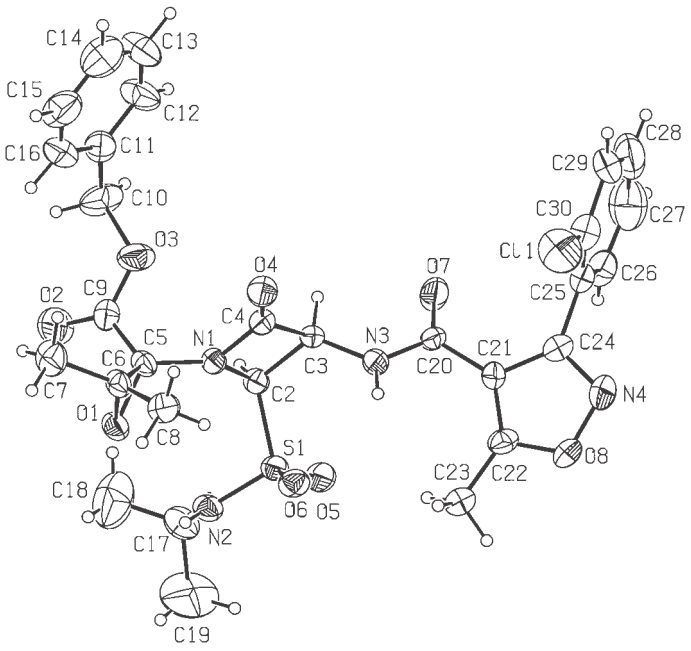
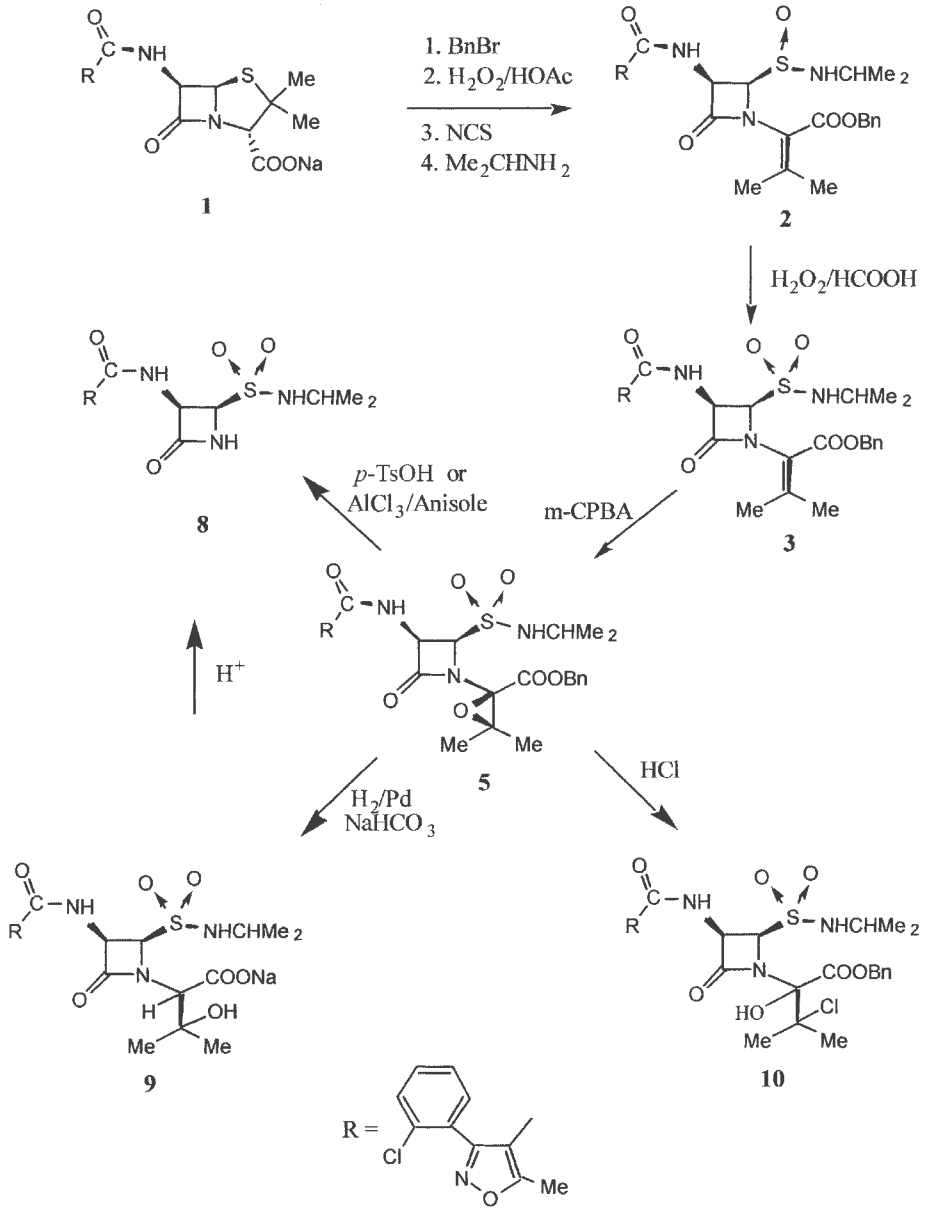


Figure 1. Molecular structure (ORTEP II) of (2*R*,3*R*)-1-[(1*R*)-1'-Benzyloxycarbonyl-2'-methyl-1',2'-epoxyprop-1'-yl]-2-isopropylaminosulphonyl-3-[3'-(*o*-chlorophenyl)-5'-methyl-isoxazol-4'-yl]-carbonylamino-4-oxoazetidine **5**.

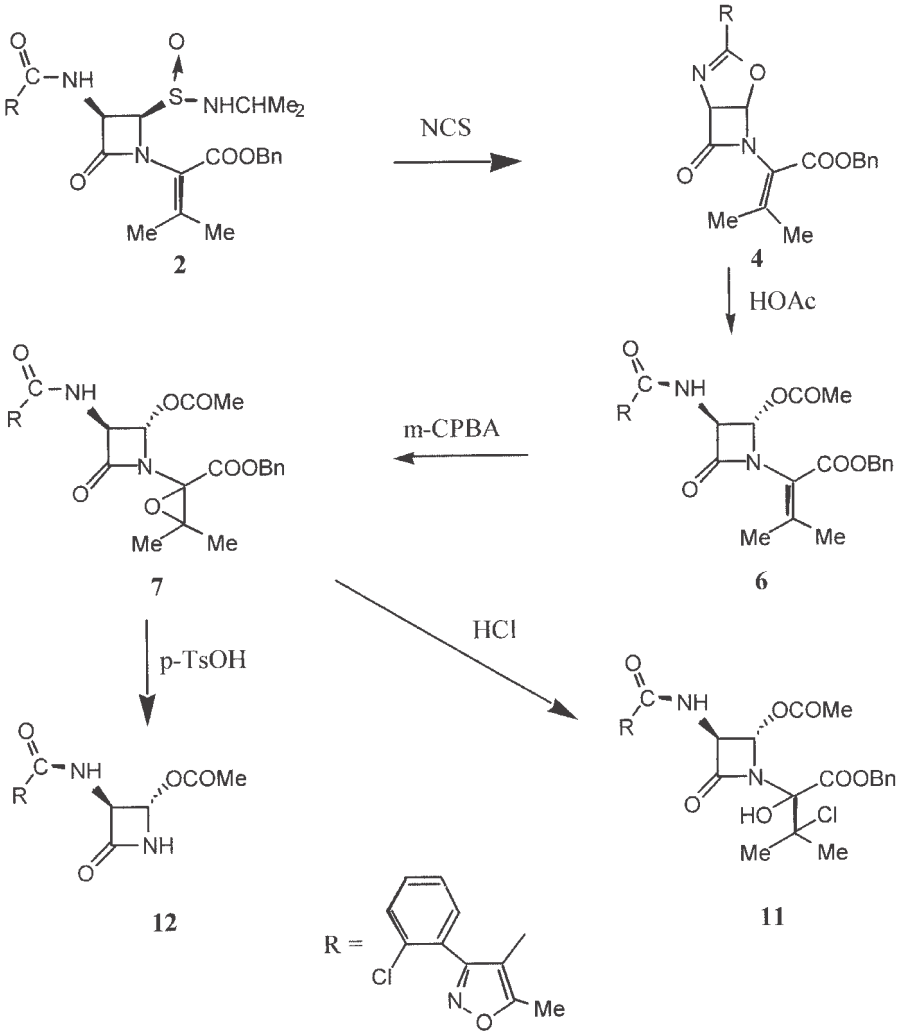
chirality *R* of two carbon atoms of the β-lactam ring were used as the internal standard to determine the configuration of a new chiral centre of the oxirane ring (Figure 1); its configuration proved to be *R*. The anomalous dis-



Scheme 1.

persion values of sulphur and chlorine are used as an alternative approach to determine the absolute configuration of three chiral carbon atoms. The β-lactam ring is planar [the mean torsion angle is 0.8(6)°]. Van der Waals contacts define the crystal packing. Intramolecular hydrogen bond N3–H3···O6 [N3···O6, 2.969(8)Å; N3–H, 0.86(1)Å; and angle N3–H···O6, 118(2)°] stabilize the overall conformation of the folded shape.

Furthermore, 2-acetyloxy-azetidinone-oxirane **7** was also generated by oxidation of **6** with *m*-CPBA, Scheme 2.



Scheme 2.

Formation of azetidinone-oxazoline **4** was carried out by treating 2-sulfinamide-azetidinone **2** with *N*-chlorosuccinimide. The reaction of **4** with acetic acid in the presence of hydrogen chloride[4] affords 2,3-*trans*-2-acetyloxy-azetidinone-butenoat **6**.

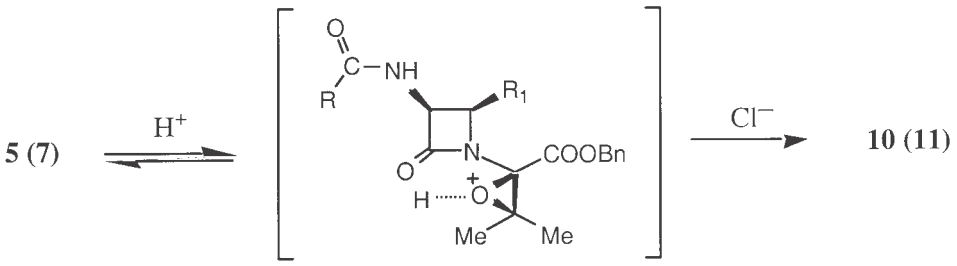
The stereochemical assignment of **6** is readily made by virtue of its H_2-H_3 coupling constant J = 1.2 Hz, diagnostic of their *trans* relative orientation.[5]

New methods for the removal of the substituent from the azetidinone nitrogen of compounds **5** and **7** were found. Attempts to prepare the corresponding carboxylic acid derivatives by the benzyl group cleavage failed. Instead, in the reaction of azetidinone-oxirane **5** with $AlCl_3$/anisole,[6] azetidinone **8** was produced in 80% yield. Furthermore, during hydrogenolysis of azetidinone-oxirane **5** with palladium on charcoal in ethanolic solution in the presence of sodium bicarbonate the azetidinone β-hydroxy acid sodium salt **9** was prepared in 87% yield. Treated with acids, compound **9** was transformed into azetidinone **8**, Scheme 1.

Finally, the removal of the substituent on the azetidinon nitrogen was carried out with a catalytic amount of *p*-TSA; azetidinone-oxirane **5** (**7**) affords azetidinone **8** (**12**), Schemes 1 and 2.

The reactivity of azetidinone-oxiranes **5** and **7** was also studied in reactions with hydrochloric acid. Compounds **5** and **7** gave the azetidinone-halohydrines **10** and **11** by the oxirane ring opening with HCl. The fact that, upon the action of hydrochloric acid, both **5** and **7** gave a product with the same halohydrine side-chain substantiates the identical steric structure of the oxirane rings in these compounds.

The mechanism of the oxirane ring opening in hydrochloric acid media involves primarly addition of a proton to the oxygen and forming of an oxiranium ion **13**. In the next step, the nucleophile (Cl^-) is attached to the car-



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$R_1 = -SO_2NHCHMe_2$ and $-OCOMe$

Scheme 3.

bon atom in position 2' (ß-carbon atom), which is probably a sterically preferable site for generating azetidinone halohydrines **10** or **11**, Scheme 3.

In conclusion, in spite of the fact that unsaturated acids react with peracids with great difficulty to form oxirane derivatives, oxidation of 2,3-*cis*-2-sulfonamide-*N*-butenoate-azetidinone **3** and 2,3-*trans*-2-acetoxy-*N*-butenoate-azetidinone **6** with *m*-CPBA led to the formation of azetidinone-oxiranes **5** and **7**.

Molecular structure of **5**, including its absolute configuration, was unambiguously determined by X-ray structure analysis.

New methods for the removal of the substituent from the azetidinone nitrogen of compounds **5** and **7** were found using $AlCl_3$ / anisole or *p*-TsOH. Opening of the oxirane ring by using H_2/Pd/$NaHCO_3$ gave azetidinone β-hydroxy acid whose reaction in acidic media offers a new possibility of removing the substituent from the azetidinone nitrogen atom.

Finally, azetidinone-oxiranes gave azetidinone-halohydrines by the oxirane ring opening with HCl.

Novel azetidinone derivatives (monobactames) did not show any significant intrinsic antibacterial activity against *S.aureus* B008 and *E.coli* ATCC 35218, nor synergistic activity with amoxycillin.

EXPERIMENTAL

Melting points were determined using a Fischer-Johns apparatus and were uncorrected. IR spectra were recorded using a Perkin-Elmer Model 257G or Nicolet Magna 760 IR Spectrometer. ^1H and ^{13}C NMR were recorded on a Varian Gemini XL-300 spectrometer using TMS as the internal standard. Chemical shifts, δ, were in ppm downfield to Me_4Si and *J* values were given in Hz. Mass spectra were determined using the fast bombardment method with an Auto Spec Q (VG Analytical) mass spectrometer. Tlc was run on Merck Kieselgel HF_{254} plates and spots were visualized under UV light or I_2 vapour adsorption following water flush. Column chromatography was performed on Merck Kieselgel 60 (70–230 mesh ASTM) activated at 105 °C.

*(3*S*,5*R*,6*R*) Benzyl 6-[3'-(o-chlorophenyl)-5'-methyl-4'-isoxazolyl]carbonyl-amino penicillinate*

Cloxacillin sodium (10 g, 22 mmol) was dissolved in *N,N*-dimethylformamide (600 mL) and benzyl bromide (2.9 mL, 24 mmol) was added. After being stirred for 5 hours at room temperature, the reaction mixture was added dropwise into ice-water (600 mL). The resulting mixture was allowed to warm to room temperature, stirred for a further 2 hours and decanted. The crude product was dissolved in dichloromethane (200 mL), the solution was dried (Na_2SO_4) and evaporated under reduced pressure. Purification of the residue by silica gel chromatography in benzene–ethylacetate (5:1) gave the benzyl penicillinate as a white solid (7.18 g, 67.6%): m.p. 60–

62 °C; R_f = 0.54 in benzene–ethylacetate (5:1); IR (film) ν_{max}/cm^{-1}: 3400m, 1785vs, 1740s, 1670vs, 1600m, 1500m, 1440–1150bm, 1295s, 1200m, 1185m, 750m, 700m; ^{1}H NMR (CDCl3) δ/ppm: 1.32 (6H, s, CMe_2), 2.78 (3H, s, Me), 4.33 (1H, s, C_3H), 5.15 (2H, s, CH_2), 5.44 (1H, d, J = 4.3 Hz, C_5H), 5.76 (1H, dd, J = 4.3 and 9.3 Hz, C_6H), 5.97 (1H, d, J = 9.3Hz, CONH), 7.57–7.26 (9H, m, C_6H_4 and C_6H_5).

Anal. Calcd. for $C_{26}H_{24}N_3ClO_5S$ (Mr = 525.99): C 59.37, H 4.60, N 7.98, Cl 6.74, S 6.10%; found C 59.33, H 4.69, N 7.92, Cl 6.73, S 6.06%.

(3S,5R,6R) Benzyl 6-[3'-(o-chlorophenyl)-5'-methyl-4'-isoxazolyl]carbonylamino penicillinate sulphoxide

The benzyl ester of cloxacilline (7.2 g, 14 mmol) was dissolved in 80% acetic acid (30 mL) and 30% hydrogen peroxide (2.5 mL) was added. Reaction mixture was stirred at room temperature for 10 hours and then added dropwise into ice-water (60 mL). The resulting suspension was stirred at room temperature for 5 hours and filtered. The solid was washed with water and dried in air (4.8 g, 66.5%). Crystallization from ethylacetate afforded benzyl penicillinate sulfoxide as a white crystalline solid: m.p. 153–155 °C; R_f = 0.36 in benzene–ethylacetate (5:1); IR (CH_2Cl_2) ν_{max}/cm^{-1}: 3360s, 1790vs, 1755vs, 1675vs, 1605s, 1500vs, 1455m, 1340m, 1295m, 1270m, 1205vs, 1035m, 765m, 755s, 735s, 700m; ^{1}H NMR ($CDCl_3$) δ/ppm: 1.02 and 1.55 (6H, 2s, CMe_2), 2.73(3H, s, Me), 4.53 (1H, s, C_3H), 4.96 (1H, d, J = 4.6Hz, C_5H), 5.14 and 5.26 (2H, 2d, J = 11.7Hz, CH_2), 6.12 (1H, dd, J = 4.6 and 10.3 Hz, C_6H), 6.90 (1H, d, J = 10.3 Hz, CONH), 7.27–7.53 (9H, m, C_6H_5 and C_6H_4).

Anal. Calcd. for $C_{26}H_{24}N_3ClO_6S$ (Mr = 542.00): C 57.61, H 4.46, N 7.75, Cl 6.54; S 5.92%; found C 57.54, H 4.51, N 7.83, Cl 6.90, S 5.79%.

*(2R,3R)-1-(1'-Benzyloxycarbonyl-2'-methylprop-1'-enyl)-2-isopropylaminosulphinyl-3-[3'-(o-chlorophenyl)-5'-methylisoxazol-4'-yl]carbonylamino-4-oxoazetidine (**2**)*

A suspension of toluene (200 mL) and calcium oxide (1.22 g, 22 mmol) was heated in an equipment having a Dean-Stark water trap to remove azeotropically any moisture. To the resulting dried toluene (180 mL), benzyl penicillinate sulfoxide (2.0 g, 3.7 mmol) and *N*-chlorosuccinimide (0.55 g, 4.15 mmol) were added. The mixture was refluxed for 1.5 h, cooled to 15 °C and isopropylamine (0.9 mL, 11 mmol) was added. The reaction mixture was stirred at room temperature for 3 hours and then filtered. The filtrate was washed with water (3 × 30 mL), dried (Na_2SO_4) and evaporated under reduced pressure (foam, 2.06 g, 93%). Crude product (1 g) was purified by silica gel with dichloromethane–ethylacetate (4:1); two epimeric sulfinamides were obtained.

*Compound **2a***. – 221 mg (21%): m.p. 53 °C; R_f = 0.32 in dichloromethane–ethylacetate (4:1): IR (KBr) ν_{max}/cm^{-1}: 3310–3290bm, 1780vs, 1725m, 1675s, 1605m, 1515–1495bm, 1450–1430bm, 1385m, 1365m, 1290m, 1210s, 1110m, 1060m, 970m, 695m; ^{1}H NMR ($CDCl_3$) δ/ppm: 1.04 and 1.06 (6H, 2d, J = 6.3 Hz, $CHMe_2$), 2.09 and 2.22 (6H, 2s, CMe_2), 2.75 (3H, s, Me), 3.31–3.38 (2H, m, S–NH and C*H*Me_2), 4.81 (1H, d, J = 5.4 Hz, C_2H), 5.12 and 5.30 (2H, 2d, J = 12.3 Hz, CH_2), 5.71 (1H, dd, J = 5.1 and 9.9 Hz, C_3H), 6.37 (1H, d, J = 9.9 Hz, CONH), 7.23–54 (9H, m, C_6H_5 and C_6H_4).

Compound ***2b***. – 535 mg (50%): m.p. 65 °C; R_f = 0.22 in dichloromethane–ethylacetate (4:1); IR (KBr) ν_{max}/cm^{-1}: 3410–3220bw, 1780vs, 1720m, 1672s, 1600m, 1580–1495bm, 1385m, 1365m, 1290m, 1215s, 1055s, 970w, 755m, 705w; ^{1}H NMR ($CDCl_3$) δ/ppm : 1.08 and 1.10 (6H, 2d, J = 6.0 Hz, $CHMe_2$), 1.96 and 2.24 (6H, 2s, CMe_2), 2.80 (3H, s, Me), 3.38 (1H, m, C*H*Me_2), 3.86 (1H, d, J = 6.3 Hz, S-NH), 4.66 (1H, d, J = 5.1 Hz, C_2H), 5.14 and 5.25 (2H, 2d, J = 12.2 Hz, CH_2), 5.73 (1H, dd, J = 4.8 and 9.4 Hz, C_3H), 6.96 (1H, d, J = 9.4 Hz, CONH), 7.26–7.55 (9H, m, C_6H_5 and C_6H_4).

*(2*R*,3*R*)-1-(1'-Benzyloxycarbonyl-2'-methylprop-1'-enyl)-2-isopropylaminosulphonyl-3-[3'-(*o*-chlorophenyl)-5'-methylisoxazol-4'-yl]carbonylamino-4-oxoazetidine (**3**)*

Formic acid (13.0 mL) and a 30% aqueous solution of hydrogen peroxide (35 mL) were added to the solution of **2** (4.0 g, 6.5 mmol) in dichloromethane (160 mL). The mixture was stirred for 5 hours at room temperature, whereafter water (80 mL) and dichloromethane (160 mL) were added. The organic layer was separated, washed with water (80 mL), dried (Na_2SO_4) and evaporated. Purification of the residue by silica gel chromatography in dichloromethane–ethylacetate (4:1) gave **3** (3.6 g, 89%) as a white foam: m.p. 75 °C; R_f = 0.80 in dichloromethane–ethylacetate (4:1); IR (CH_2Cl_2) ν_{max}/cm^{-1}: 3400m, 1790vs, 1730s, 1685vs, 1605s, 1520vs, 1440–1425bm, 1390m, 1370m, 1335s, 1290m, 1260m, 1215s, 1060m, 1005m, 895m, 760s, 700m; ^{1}H NMR ($CDCl_3$) δ/ppm: 0.98 and 1.03 (6H, 2d, J = 6.6 Hz, $CHMe_2$), 1.92 and 2.23 (6H, 2s, CMe_2), 2.78 (3H, s, Me), 3.29 (1H, m, C*H*Me_2), 3.83 (1H, d, J = 8.1 Hz, S-NH), 4.96 (1H, d, J = 5.1 Hz, C_2H), 5.07 and 5.30 (2H, 2d, J = 12.1 Hz, CH_2), 5.86 (1H, dd, J = 5.1 and 10.2 Hz, C_3H), 6.30 (1H, d, J = 10.2 Hz, CONH), 7.27–7.55 (9H, m, C_6H_5 and C_6H_4); ^{13}C NMR ($CDCl_3$) δ/ppm: 12.9, 21.9, 23.6, 23.8, 23.9,46.4, 56.6, 66.8, 72.2, 111.0, 118.6, 126.8, 127.4, 128.5, 128.6, 128.7, 130.4, 131.4, 131.7, 133.9, 135.0, 156.0, 158.4, 160.6, 162.4, 164.6, 174.7.

Anal. Calcd. for $C_{29}H_{31}N_4O_7SCl$ (*M*r = 615.09): C 56.62, H 5.08, N 9.11, S 5.21, Cl 5.76%; found C 56.23, H 5.45, N 9.16, S 4.92, Cl 6.75%.

*(1*R*,5*R*)-7-Oxo-3-[3'-(o-chlorophenyl)-5'-methylisoxazol-4'-yl]-4-oxa-2,6-diazabicyclo[3.2.0]hept-2-en-6-(1'-benzyloxycarbonyl-2'-methyl)propen-1'-yl (**4**)*

(2*R*,3*R*)-1-(1'-Benzyloxycarbonyl-2'-methylprop-1'-enyl)-2-isopropylamino-sulphinyl -3-[3'-(o-chlorophenyl)-5'-methylisoxazol-4'-yl]-carbonylamino-4-oxo-azetidine **2** (308 mg, 0.5 mmol) was dissolved in dichloromethane (6 mL) and *N*-chlorosuccinimide (134 mg, 1 mmol) was added. The reaction mixture was stirred at room temperature for 2 hours, then washed with water (6 mL). The organic phase was dried (Na_2SO_4), filtered and evaporated under reduced pressure. The residue was purified by silica gel column chromatography and eluted with dichloromethane–ethylacetate (15:1) to give *cis*-oxazoline-azetidinone **4** as a yellowish foam (150 mg, 62%): R_f = 0.46 in dichloromethane–ethylacetate (15:1); IR (CH_2Cl_2) ν_{max}/cm^{-1}: 1780vs, 1725s, 1665s, 1645m, 1610m, 1500w, 1455mb, 1385m, 1365m, 1285m, 1265–1245mb, 1215vs, 1185m, 1155w, 1105–1075mb, 1030m, 970s, 895m, 880m, 835m, 750sb, 700s, 645w; ^{1}H NMR ($CDCl_3$) δ/ppm : 1.79 and 2.22 (6H, 2s, CMe_2), 2.71 (3H, s, Me), 5.22–5.07 (3H, m, C*H*$_2C_6H_5$ and C_2H), 5.86 (1H, d, J = 3.2 Hz, C_3H), 7.26–7.45 (9H, m, C_6H_5 and C_6H_4); ^{13}C NMR ($CDCl_3$) δ/ppm: 13.0, 21.6, 23.2, 29.4, 66.8, 80.9, 87.0, 106.0, 118.8, 126–131.0, 133.9, 135.2, 155.3, 160.3, 163.0, 165.7, 173.4.

Anal. Calcd. for $C_{26}H_{22}N_3O_5Cl$ (M_r = 491.92): C 63.48, H 4.51, N 8.54, Cl 7.21%; found C 63.15, H 4.36, N 8.33, Cl 7.01%.

*(2*R*,3*R*)-1-[(1*R*)-1'-Benzyloxycarbonyl-2'-methyl-1',2'-epoxyprop-1'-yl]-2-isopropyl-aminosulphonyl-3-[3'-(*o*-chlorophenyl)-5'-methylisoxazol-4'-yl]-carbonylamino-4-oxoazetidine (**5**)*

Procedure (a). – To a solution of sulfonamide **3** (2.0 g, 3.3 mmol) in dichloromethane (100 mL), *m*-chloroperbenzoic acid (0.62 g, 3.57 mmol) in dichloromethane (30 mL) was added. The resulting mixture was stirred for 40 hours at room temperature. Then, it was washed with saturated aqueous sodium hydrogen carbonate (50 mL) and the layers were separated. Evaporation of dry (Na_2SO_4) organic layer under reduced pressure gave a white foam. After purification with silica gel chromatography eluted with dichloromethane–ethylacetate (10:1), it gave epoxide **5** (790 mg, 38.5%). A small sample was recrystallized from isopropanol giving an analytical sample as a white crystalline solid; m.p. 142–143 °C; R_f = 0.47 in dichloromethane–ethylacetate (10:1); IR (CH_2Cl_2) ν_{max}/cm^{-1}: 3400m, 3320s, 1800vs, 1755s, 1735m, 1680vs, 1535–1505bs, 1460m, 1375m, 1335s, 1270–1240mb, 1125s, 1060m, 1005m, 760s, 735s, 700m; ^{1}H NMR ($CDCl_3$) δ/ppm: 0.98 and 1.17 (6H, 2d, J = 6.6 Hz, $CHMe_2$), 1.43 and 1.52 (6H, 2s, CMe_2), 2.76 (3H, s, Me), 3.47–3.54 (1H, m, C*H*Me_2), 4.54 (1H, d, J = 5.4 Hz, C_2H), 4.80 (1H, d, J = 7.7 Hz, S-NH), 5.17 and 5.32 (2H, 2d, J = 11.8 Hz, CH_2), 5.91 (1H, dd, J = 5.4 and 10.5 Hz, C_3H), 6.29 (1H, d, J = 10.2 Hz, CONH), 7.27–7.51 (9H, m, C_6H_5 and C_6H_4); ^{13}C NMR ($CDCl_3$) δ/ppm: 13.0, 18.8, 21.4, 22.9, 24.3, 46.7, 57.9, 68.2,68.7, 69.4, 70.7, 111.0, 126.6, 127.1, 128.6, 128.8, 128.9, 129.0, 129.1, 130.1, 131.4, 133.9, 134.1, 158.6, 160.4, 163.9, 164.3, 174.2; MS m/z: 631 (M 100%).

Anal. Calcd. for $C_{29}H_{31}N_4ClO_8S$ (M_r = 631.11): C 55.19, H 4.95, N 8.88, Cl 5.62, S 5.08%; found C 55.00, H 5.22, N 8.58, Cl 5.95, S 4.86%.

Procedure (b). – The solution of sulfonamide **3** (200 mg, 0.32 mmol) in dichloromethane (10 mL) was cooled to +5 °C, 30% aq. solution of hydrogen peroxide (20 mL) and formic acid (0.6 mL) were added. The mixture was stirred for 35 hours at +5 °C and the layers were separated. The organic phase was washed with water, dried (Na_2SO_4) and evaporated in vacuo. Chromatography on silica gel, using dichloromethane–ethylacetate (10:1) as eluents gave **5** (65 mg, 32.7%); spectral data as in procedure (a).

Procedure (c). – Sulfinamide **2** (200 mg, 0.33 mmol) was dissolved in dichloromethane (20 mL) and *m*-chloroperbenzoic acid (120 mg, 0.70 mmol) in dichloromethane (6 mL) was added. The solution was stirred for 50 hours at room temperature and then worked up according to procedure (a). After the purification of a crude product on a silica gel column by means of the solvent mixture dichloromethane–ethylacetate (10:1), a product identical to the compound described in (a) was isolated (74 mg, 35.6%).

*(2*S*,3*R*)-1-(1'-Benzyloxycarbonyl-2'-methylpropen-1'-yl)-2-acetyloxy-3-[3'-(*o*-chlorophenyl)-5'-methyl-5'-isoxazol-4'-yl]carbonylamino-4-oxoazetidine (**6**)*

To a solution of oxazoline-azetidinone **4** (2 g, 4 mmol) in dichloromethane (20 mL), 80% acetic acid (20 mL) was added. Hydrogen chloride (gaseous) was introduced into

the reaction mixture at room temperature to saturation. The resulting mixture was stirred at room temperature for 20 hours and evaporated in vacuo. The residue was worked up two times with dichloromethane (20 mL), then chromatographed on a silica gel column eluted with dichloromethan–ethylacetate (4:1) to yield **6** (450 mg, 20.4%): R_f = 0.55 in dichloromethane–ethylacetate (4:1); IR (KBr) ν_{max}/cm^{-1}: 3417mb, 1786vs, 1763s, 1718m, 1676s, 1605w, 1522m, 1456w, 1396m, 1371m, 1221vs, 1178m, 1034m, 766w, 698w; ^{1}H NMR ($CDCl_3$) δ/ppm: 1.92 and 2.03 (6H, 2s, CMe_2), 2.20 (3H, s, $OCOCH_3$), 2.73 (3H, s, Me), 4.89 (1H, dd, J = 1.2 and 7.2 Hz, C_3H), 5.18 (2H, d, J = 1.2 Hz, CH_2), 5.66 (1H, d, J = 7.1 Hz, CONH), 5.98 (1H, d, J = 1.2 Hz, C_2H), 7.32–7.52 (9H, m, C_6H_5 and C_6H_4); ^{13}C NMR ($CDCl_3$) δ/ppm: 12.8, 18.7, 20.3, 21.6, 23.1, 29.4, 61.8, 66.8, 82.6, 110.9, 119.2, 126.9–132.1, 133.8, 135.6, 154.3, 158.3, 161.1, 162.8, 170.1, 174.8.

Anal. Calcd. for $C_{28}H_{26}N_3ClO_7$ (M_r = 551.97): C 60.92, H 4.75, N 7.61, Cl 6.42%; found C 60.94, H 4.99, N 7.59, Cl 6.42 %.

*(2*S*,3*R*)-1-(1'-Benzyloxycarbonyl-2'-methyl-1',2'-epoxypropyl)-2-acetyloxy-3-[3'-(*o*-chlorophenyl)-5'-methylisoxazol-4'-yl]carbonylamino-4-oxoazetidine (**7**)*

Compound **6** (160 mg, 0.28 mmol) was dissolved in dichloromethane (5 mL) and *m*-chloroperbenzoic acid (60 mg, 0.34 mmol) was added. The reaction mixture was stirred at room temperature for 44 hours, then washed with saturated aqueous sodium hydrogen carbonate. The organic layer was dried (Na_2SO_4), filtered and evaporated under reduced pressure. The crude product was purified on silica gel chromatography using dichloromethane–ethylacetate (4:1) as eluent and compound **7** was obtained as a white foam (60 mg, 36%): R_f = 0.64 in dichloromethane–ethylacetate (4:1); IR (KBr) ν_{max}/cm^{-1} 3410s, 1801vs, 1753vs, 1676s, 1605w, 1522m, 1387m, 1282w, 1232s, 1184s, 1134m, 1036m, 766m, 752m; ^{1}H NMR ($CDCl_3$) δ/ppm:1.36 and 1.50 (6H, 2s, CMe_2), 2.11 (3H, s, $OCOCH_3$), 2.73 (3H, s, Me), 4.92 (1H, d, J = 7.2 Hz, C_3H), 5.28 (2H, ABq, J = 12.3 and 24 Hz, CH_2), 5.69 (1H, d, J = 7.2 Hz, CONH), 5.79 (1H, s, C_2H), 7.39–7.55 (9H, m, C_6H_5 and C_6H_4).

Anal. Calcd. for $C_{28}H_{26}N_3ClO_8$ (M_r = 567.97): C 59.21, H 4.61, N 7.30, Cl 6.24%; found C 59.02, H 4.81, N 7.39, Cl 6.38 %.

*(2*R*,3*R*)-2-Isopropylaminosulphonyl-3-[3'-(*o*-chlorophenyl)-5'-methylisoxazol-4'-yl]-carbonylamino-4-oxoazetidine (**8**)*

Procedure (a). – The suspension of $AlCl_3$ (1.2 g, 8.3 mmol) in dichloromethane (30 mL) was cooled at 10 °C and a stream of nitrogen was introduced. Then, the solutions of epoxide **5** (870 mg, 1.37 mmol) in dichloromethane (10 mL) and anisole (2 mL, 16.4 mmol) were added. The resulting mixture was stirred at –10 °C for 30 min. and at 0 °C for a further 4 hours. To the reaction mixture, water (10 mL) was added and acidified with HCl 1:1 to pH = 1.0. The layers were separated, the organic layer was washed with water (10 mL), dried (Na_2SO_4), filtered and evaporated in vacuo. The crude product was purified on silica gel, eluted with dichloromethane–methanol (4:1) yielding **8** (470 mg, 80.3%). A small sample was recrystallized from dichloromethane giving an analytical sample as a white crystalline solid: m.p. 178–179 °C; R_f = 0.62 in dichloromethane–methanol (9:1); IR (KBr) ν_{max}/cm^{-1}: 3399s, 3301s, 1797vs, 1787vs, 1650vs, 1597m, 1516m, 1423w, 1327w, 1288w, 1205w, 1159w, 1130w,

1058vw, 1010w, 918vw, 769w; ^{1}H NMR (DMSO-d_6) δ/ppm: 1.09 and 1.121 (6H, 2d, J = 6.2 Hz, $CHMe_2$), 2.68 (3H, s, Me), 3.25–3.53 (1H, m, C*H*Me_2), 4.94 (1H, d J = 4.8 Hz, C_2H), 5.68 (1H, dd, J = 4.8 and 9.3 Hz, C_3H), 7.39–7.59 (4H, m, C_6H_4), 8.18 (1H, d, J = 9.3 Hz, CONH); ^{13}C NMR (DMSO) δ/ppm: 12.6, 24.0, 24.4, 45.6, 58.1, 67.8, 112.8, 127.6, 127.7, 130.0, 131.7, 131.8, 132.9, 160.1, 160.8., 167.7, 171.5.

Anal. Calcd. for $C_{17}H_{19}N_4SO_5Cl$ (M_r = 426.87): C 47.82, H 4.49, N 13.12, S 7.51, Cl 8.39%; found C 47.80, H 4.77, N 12.98, S 7.67, Cl 8.97%.

Procedure (b). – Oxirane **5** (200 mg, 0.32 mmol) was dissolved in absolute ethanol (12 mL) and *p*-toluensulphonic acid was added (30 mg). The rection mixture was heated under reflux for 4.5 hours, cooled and evaporated under reduced pressure. The residue was worked-up with dichloromethane (2 × 10 mL) and purified by silica gel column chromatography eluted with dichloromethane–methanol (9:1) to give azetidinone **8** (28 mg; 21%) of spectroscopic properties identical to those described above.

Procedure (c). – Compound **9** (100 mg, 0.18 mmol) was dissolved in water (4 mL) and HCl 1M was added to pH = 2.0. The resulting suspension was stirred at room temperature for 1 hour, then filtered and dried in air yielding **8** (50 mg; 65.1%). The product was identical to the one described in (a).

*(2*R*,3*R*)-1-(1'-Carboxyl-2'-methyl-2'-hydroxypropyl)-2-isopropylaminosulphonyl-3-[3'-(*o*-chlorophenyl)-5'-methylisoxazol-4'-yl]-4-oxoazetidine sodium salt (**9**)*

Oxirane **5** (260 mg, 0.41 mmol) was dissolved in absolute ethanol (30 mL), saturated aqueous solution $NaHCO_3$ (0.7 mL) and 10% Pd/C (26 mg) were added and treated with hydrogen under pressure (2 Atm.) for 1 hour. The mixture was filtered and the filtrate was evaporated under reduced pressure to a white foam. After purification by silica gel chromatography, the residue was eluted with dichloromethane–methanol (4:1) to give 9 (200 mg, 86.6%): m.p. 162–164 °C; R_f = 0.26 in dichloromethane–methanol (4:1); IR (KBr) ν_{max}/cm^{-1}: 3422vsb, 1786vs, 1671–1594vsb, 1523s, 1414s, 1388m, 1336vs, 1253w, 1215w, 1132s, 1059w, 1014mb, 952w, 896m, 847vw, 763m, 732m, 697vw, 644m, 598m; ^{1}H NMR (DMSO-d_6) δ/ppm: 1.12 and 1.14 (6H, 2s, $CHMe_2$), 1.40 and 1.48 (6H, 2s, CMe_2), 2.66 (3H, s, Me), 3.17 (1H, d, J = 4.7 Hz, C_1'H), 3.33–3.51 (1H, m, C*H*Me_2), 4.15 (1H, d, J = 5.1 Hz, NH-S), 4.98 (1H, d, J = 5.4 Hz, C_2H), 5.88 (1H, dd, J = 5.4 and 9.3 Hz, C_3H), 6.94 (1H, d, J = 4.8 Hz, OH), 7.42–7.58 (4H, m, C_6H_4), 8.72 (1H, d, J = 9.3 Hz, CONH); ^{13}C NMR (DMSO) δ/ppm: 12.5, 19.1, 22.5, 23.6, 24.5, 45.8, 57.7, 63.0, 71.1, 72.8, 98.7, 113.1, 127.5, 127.8, 129.8, 131.7, 132.9, 160.3, 161.0, 165.8, 166.5, 170.9.

*(2*R*,3*R*)-1-(1'-Benzyloxycarbonyl-1'-hidroxy-2'-methyl-2'-chloroprop-1'-yl)-2-isopropylaminosulphonyl-3-[3'-(*o*-chlorophenyl)-5'-methylisoxazol-4'-yl]-carbonylamino-4-oxoazetidine (**10**)*

Oxirane **5** (200 mg, 0.32 mmol) was dissolved in dichloromethane (6 mL). Hydrogen chloride (gaseous) was introduced into the reaction solution at room temperature to saturation. The resulting mixture was stirred at room temperature for 3 hours and evaporated under reduced pressure. The residue, after being worked up with dichloromethane (2 × 10mL), yielded **10** as a yellowish foam (200 mg), which was not purified: R_f = 0.38 in dichloromethane–ethylacetate (15:1); IR (CH_2Cl_2) ν_{max}/cm^{-1}: 3400s, 1765vs, 1730s, 1675vs, 1605s, 1515s, 1505s, 1455–1410s,b, 1390–1370s,b,

1260s, 1220s, 1160s, 1060w, 1000m, 900m, 735s, 700m; ^{1}H NMR ($CDCl_3$) δ/ppm: 1.17 (6H, 2d, J = 6.4 Hz, $CHMe_2$), 1.33 and 1.42 (6H, 2s, CMe_2), 2.79 (3H, s, Me) 3.55–3.62 (1H, m, C*H*Me_2), 4.32 (1H, s, OH), 4.56 (1H, d, J = 6.9 Hz, S-NH), 5.08 (1H, d, J = 5.4 Hz, C_2H), 5.15 and 5.26 (2H, 2d, J = 12 Hz, CH_2) 5.86 (1H, dd, J = 5.4 and 10.2 Hz, C_3H), 6.17 (1H, d, J = 10.2 Hz, CONH), 7.37–7.57 (9H, m, C_6H_4 and C_6H_5).

*(2*S*,3*R*)-1-(1'-Benzyloxycarbonyl-1'-hidroxy-2'-methyl-2'-chloroprop-1'-yl)-2-acetyloxy-3-[3'-(*o*-chlorophenyl)-5'-methylisoxazol-4'-yl]-carbonylamino-4-oxoazetidine (**11**)*

The solution of oxirane **7** (30 mg, 0.06 mmol) in dichloromethane (4 mL) was cooled at 0 °C and hydrogen chloride (gaseous) was introduced to saturation. The reaction mixture was stirred at 0 °C for 30 min. and evaporated *in vacuo*. The residue was worked up with dichloromethane two times (4 mL) to yield **11** as a yellowish foam (30 mg), which was not purified: R_f = 0.62 in dichloromethane–ethylacetate (4:1); IR (CH_2Cl_2) ν_{max}/cm^{-1}: 3386mb, 1758vs, 1670s, 1608m, 1523m, 1452m, 1375m, 1340w, 1261m, 1222m, 1174m, 1132w, 1058w, 1034w, 732w, 698m; ^{1}H NMR ($CDCl_3$) δ/ppm: 1.33 and 1.45 (6H, 2s, CMe_2), 2.05 (3H, s, $OCOCH_3$), 2.73 (3H, s, Me) 4.87 (1H, d, J = 7.7 Hz, C_3H), 5.20–5.29 (3H, m, CH_2 and OH), 5.71 (1H, d, J = 7.7 Hz, CONH), 6.08 (1H, s, C_2H), 7.28–7.53 (9H, m, C_6H_4 and C_6H_5).

*(2*S*,3*R*)-2-Acetyloxy-3-[3'-(*o*-chlorophenyl)-5'-methylisoxazol-4'-yl]carbonyl-amino-4-oxoazetidine (**12**)*

Oxirane **7** (100 mg, 0.17 mmol) was dissolved in toluene (5 mL) and *p*-toluenesulfonic acid (10 mg) was added. The reaction solution was stirred at room temperature for 1.5 hour, then extracted with a saturated aqueous solution of sodium hydrogen carbonate. The layers were separated; the organic layer was dried (Na_2SO_4) and filtered. Evaporation of the organic layer under reduced pressure gave a white foamy solid, which, after purification by silica gel column chromatography, was eluted with dichloromethane–ethylacetate (4:1) to give azetidinone **12** as a white foam: m.p. 174–175 °C (30 mg; 49.7%); R_f = 0.16 in dichloromethane–ethylacetate (4:1); IR (KBr) ν_{max}/cm^{-1}: 3347m, 3269m, 1789vs, 1750vs, 1670vs, 1608m, 1528mb, 1370m, 1234vs, 1154w, 1038s, 773vw; ^{1}H NMR ($CDCl_3$) δ/ppm : 2.11 (3H, s, $OCOCH_3$), 2.76 (3H, s, Me), 4.57 (1H, d, J = 6.9 Hz, C_3H), 5.76 (2H, s, C_2H and NH), 6.69 (1H, s, CONH), 7.45–7.59 (4H, m, C_6H_4).

X-ray Analysis of ***5***

Crystal data and details of data collection and structure determination are listed in Table I.

Supplementary Materials. – Crystallographic data on the structure have been deposited at the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk) and can be obtained on request, free of charge, by quoting the publication citation and the depositon number CCDC 165712.

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TABLE I

Crystallographic data and details of data collection and refinement of **5**

Formula	$C_{29}H_{31}ClN_4O_8S$
M_r	631.1
a/Å	10.261(4)
b/Å	14.834(1)
c/Å	20.051(1)
V/Å^3	3054(1)
Z	4
D_c/g cm^{-3}	1.373
F (000)	330
Crystal system	orthorhombic
Space group	$P2_12_12_1$
Crystal size/mm	0.21 × 0.36 × 0.21
μ (Mo-Kα)/cm^{-1}	2.5
Diffractometer	Enraf-Nonius CAD4
Radiation/Å	λ(Mo-Kα) = 0.71073 graphite monochromator
Temperature/K	295
θ_{min}, θ_{max} /° for cell det.	4.56, 15.95
No. of reflections used for cell det.	23
θ_{min}, θ_{max} /°	2.50, 33.07
(ω/2θ_{scan}) /°	$\Delta\omega$ = 0.64 + 0.71 tan θ
h, k, l limits	0, 15; –22, 0; –30, 0
Independent reflections observed with $I > 4\sigma(I)$	1227
No. of parameters	364
Refinement on:	F^2 [a]
R, wR	0.058, 0.166 [b]
Goodness of fit, S	0.883
Max. shift/error $(\Delta/\sigma)_{max}$	< 0.05
Residual electron density, ($\Delta\rho_{max}$, $\Delta\rho_{min}$)/e Å^{-3}	0.38, –0.31
Data collection and cell refinement	CAD-4 EXPRESS[7]
Data reduction	HELENA[8]
Program used to solve structure	SHELX 86[9]
Program used to refine structure	SHELXL 93[10]

[a] Refinement on F^2 where $P = (F_o^2 + 2F_c^2)/3$.
[b] $R(F) = 0.058$ for $I > 4\sigma(I)$; $wR(F^2) = 0.166$ for all data.

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SAŽETAK

Sinteza, objašnjenje strukture i reaktivnost novih azetidinon-oksirana

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Oksidacijom 2,3-*cis*-2-sulfonamid-*N*-butenoat-azetidininona **3** i 2,3-*trans*-2-acetoksi-*N*-butenoat-azetidinona **6** dobiveni su azetidinon-oksirani **5** i **7**. Molekularna struktura i apsolutna konfiguracija spoja **5** utvrđena je röntgenskom strukturnom analizom. Cijepanje benzilne esterske skupine reakcijom s alumunijevim trikloridom u anisolu kao i hidrogenolizom uz paladij na ugljenu nije dalo očekivane rezultate. Umjesto toga pronađene su nove metode za uklanjanje supstituenata s azetidinonskog dušika kod spojeva **5** i **7**. U reakciji azetidinon-oksirana **5** s aluminijevim trikloridom u anisolu dobiven je spoj **8**. Hidrogenacijom azetidinon-oksirana **5** nastaje natrijeva sol *β*-hidroksi kiseline **9** koja u kiselom mediju daje azetidinon **8**. U reakciji sa klorovodičnom kiselinom spojevi **5** i **7** daju azetidinon-halohidrine **10** i **11**, i to otvaranjem oksiranskog prstena. Novi azetidinonski derivati (monobaktami) nisu pokazali znatniju antibakterijsku aktivnost niti sinergističku aktivnost s amoksicilinom.