Desulfation and Rearrangement of Tigemonam to an Isoxazolidin-5-one and the Synthesis of the Rearrangement Product

Janak Singh*, Thomas P. Kissick, Rita Fox, Octavian Kocy†, and Richard H. Mueller

Squibb Chemical Division, P. O. Box 08540-4000,
Princeton, New Jersey 08540

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The β-lactam antibiotic Tigemonam **2** undergoes desulfation to the *N*-hydroxyazetidinone **4**, which rearranges to the isoxazolidin-5-one **6**. The structure of the rearrangement product **6** was confirmed by synthesis.

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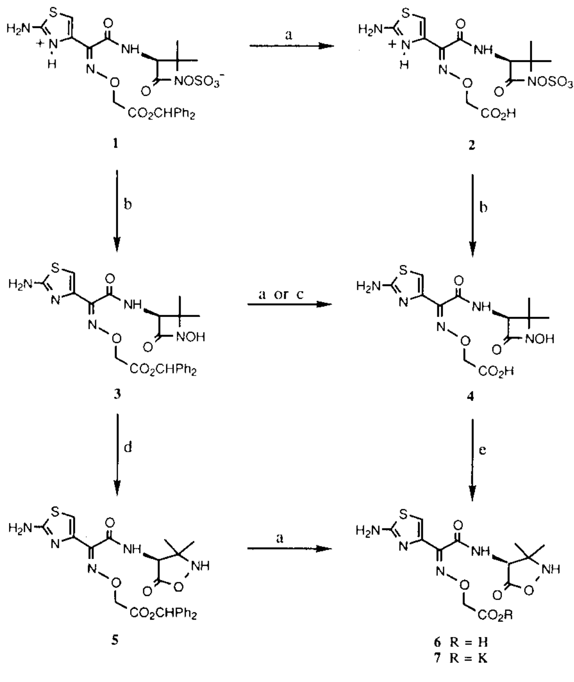
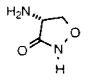
During the past few years there has been intensive activity in the synthesis and chemistry of Monobactams (monocyclic β-lactam antibiotics) [1-13]. Gordon first reported the new *O*-sulfate activating group for *N*-hydroxyazetidinones (which he termed Monosulfactams) [10]. Further synthetic efforts in this area culminated in the preparation of the potent, orally-active antibiotic Tigemonam (**2**), which is being evaluated clinically at Squibb [11,13]. In this communication we describe the desulfation of **1** and **2** to the corresponding *N*-hydroxyazetidinones **3** and **4** and rearrangement of **3** and **4** to the five membered heterocycles isoxazolidin-5-ones **5** and **6**, respectively. The structure of **6** was confirmed by an alternate synthesis. Acid **6** and related compounds are structural analogues of the antibiotic cycloserine (**8**) [14,15].

Scheme I

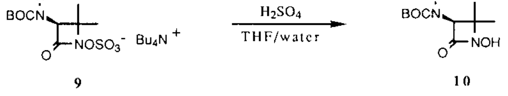


a. trifluoroacetic acid, anisole, dichloromethane; b. tetrahydrofuran, water; c. sodium hydroxide, water; aqueous acid; d. ethyl acetate, triethylamine, reflux; e. 25°



8

During crystallization attempts, Tigemonam (**2**) underwent decomposition in a variety of aqueous solvent mixtures. It was incidentally discovered that a solution of **2** in 5% water in tetrahydrofuran (*p*H ~ 1.0) generated the desulfated product **4**, as its sulfuric acid salt, in 80% yield [tlc: Homogeneity Index (HI) (300 nm) 87%] [16]. Electrophoresis studies showed that as expected the product **4** was a dibasic acid. The material was contaminated with 10-15% of β-lactam cleavage products, which made the purification of **4** quite difficult. However, when the corresponding benzhydryl ester **1** was subjected to these reaction conditions, the desulfation was quite facile and *N*-hydroxyazetidinone **3** was easily isolated (first crop 68% yield; tlc HI (300 nm): 97%; second crop 10% yield, HI 96%). Deprotection of **3** with trifluoroacetic acid (TFA) in the presence of anisole gave the TFA salt of **4** (70% yield; tlc HI (300 nm): 95%). The tetrabutylammonium salt **9** also produced the *N*-hydroxyazetidinone **10** [6,13] (67% yield) under desulfation reaction conditions (tetrahydrofuran/water/sulfuric acid).



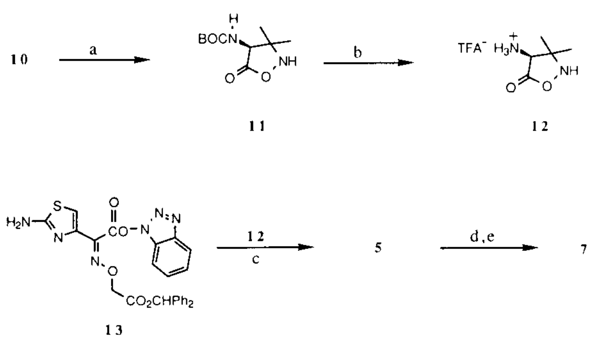
The azetidinone ring system in **10** displayed great stability under aqueous basic conditions. Compound **10** was recovered unchanged (yield 95%) from a solution of aqueous sodium hydroxide in tetrahydrofuran (*p*H 13, 20 hours, ambient temperature). This observation was exploited in the selective base-catalyzed hydrolysis of benzhydryl ester **3** to give **4** by treatment with 1*N* aqueous sodium hydroxide (2.4 molar equivalent) in tetrahydrofuran. Hydroxyazetidinone **4** was obtained after column chromatography over reverse phase adsorbent CHP-20P [17] in 92% yield. This remarkable stability of *N*-hydroxyazetidinones presumably is due to ionization of the hydroxamic acid moiety. The resulting anion electronically retards attack at the carbonyl group and renders cleavage of the C-N bond very difficult.

The trifluoroacetic acid salt of **2** was unstable in the solid state at ambient temperature. It underwent slow decomposition to *N*-hydroxyazetidinone **4** and a more lipophilic product, which was assumed to be the isomeric five membered heterocyclic structure isoxazolidin-5-one **6** (ir (potassium bromide) ~ 1790 cm^{-1}). Rearrangement product **6** was generated in greater amount when **2** was thermolysed at 110° for 1 hour. Purification of acid **6** by crystallization was difficult. In order to establish the identity of **6**, we developed a more efficient and unambiguous synthesis which involved rearrangement at an earlier stage.

There are a few reports concerning the preparation of compounds containing the isoxazolidin-5-one heterocyclic system [18-23]. Recently Baldwin and Hirose independently have described the rearrangement of *N*-hydroxyazetidinones to isoxazolidin-5-ones with catalytic sodium ethanethiolate [22] or by refluxing in ethyl acetate [23]. These reaction conditions [23], however, did not effect efficient conversion of **3** to **5**. It was possible to cause rearrangement of **3** by the use of catalytic triethylamine (0.1 equivalent) in refluxing ethyl acetate to give **5** in 53% yield; however, purification of **5** by crystallization proved difficult. A small amount of material was separated by rapid chromatography on a silica gel analytical plate.

An alternate synthesis of **6** was accomplished (Scheme II) *via* the unambiguously characterized isoxazolidin-5-one **11**. Isomerisation of *N*-hydroxyazetidinone **10** was very slow in refluxing ethyl acetate or in the presence of sodium ethanethiolate in tetrahydrofuran. Thermal rearrangement (160°, 10 minutes) of **10** to **11** was associated with extensive racemisation and some decomposition [24]. Reaction of **10** in the presence of ethanol, water or catalytic *N,N*-dimethylaminopyridine in ethyl acetate did produce **11** after refluxing for 18 to 20 hours, but in all cases the presence of unreacted **10** made the purification difficult. Reaction in the presence of triethylamine (4-fold excess, ethyl acetate, reflux 1 hour) appeared to be complete by tlc. However, on work up an appreciable amount of unreacted **10** was detected. This observation implied that the triethylammonium salt of **10** was strongly bound to the silica gel at the origin and suggested a convenient purification of **11**. The reaction mixture was simply passed through a column of silica gel: elution with ethyl acetate furnished **11** (50% yield, ir (chloroform) 1787 cm^{-1}) and unreacted **10** was recovered in 35% yield [25]. The *t*-butoxy carbonyl group of **11** was removed with trifluoroacetic acid in the presence of anisole to give **12** in 80% yield. The ir (potassium bromide) spectrum of **12** displayed carbonyl absorption at 1793 cm^{-1}, characteristic of the isoxazolidin-5-one moiety [26].

Scheme II



a. triethylamine, ethyl acetate, reflux; b. trifluoroacetic acid, anisole, dichloromethane; c. triethylamine, dichloromethane; d. trifluoroacetic acid, anisole, dichloromethane; e. potassium bicarbonate, column chromatography (CHP-20P) [17]

Coupling of **12** with the active ester **13** derived from α-aminothiazoleiminoxyacetic acid and 1-hydroxybenzotriazole formed **5** in 70% yield. This substance was identical (pmr, ir, tlc) with material obtained from **3** (Scheme I). Ester **5** was further converted to the trifluoroacetic acid salt of **6** by treatment with trifluoroacetic acid in the presence of anisole. This salt was unstable and thus difficult to purify by crystallization. Thus, acid **6** was converted to the potassium salt **7** by reaction with aqueous potassium bicarbonate (*p*H 7.0) and purified by reverse phase column chromatography over CHP-20P [17] (yield 23%).

In summary, we have described the desulfation of Tigemonam **2** to the *N*-hydroxyazetidinone **4** and its ring enlargement to isoxazolidin-5-one **6**. The structure of **6** was confirmed by synthesis. Acid **6** was found to be devoid of antibacterial activity.

EXPERIMENTAL

General.

The nmr spectra were recorded on a JEOL FX 270 and GX 400 spectrometers. The ir spectra were measured on a Perkin-Elmer 983 instrument. The ms spectra were recorded on a VG-ZAB-2F spectrometer. For homogeneity index (HI) [16] values, the developed tlc plates were analyzed by using a Shimadzu CS-920 densitometer.

[4*S*(*Z*)]-[[[1-(2-Amino-4-thiazolyl)-2-[(3,3-dimethyl-5-oxo-4-isoxazolidinyl)amino]-2-oxoethylidene]amino]oxy]acetic Acid, Diphenylmethyl Ester (**3**).

A suspension of **1** (25.0 g, 41.5 mmoles) in tetrahydrofuran and water (3600 ml/200 ml) was stirred at ambient temperature for 45 hours. Sodium bicarbonate (3.5 g) was added and stirred for five minutes. The solvent was evaporated under vacuum at room temperature and the residue was treated with ethyl acetate (600 ml) and water (100 ml). The aqueous layer was separated and extracted with ethyl acetate (100 ml). The organic extracts were combined and washed with brine (100 ml). The solution was dried over magnesium sulphate and filtered. The solvent was removed on a rotary evaporator at ambient temperature to give 22.19 g of crude **3**. The solid was treated with ethyl acetate (500 ml) and filtered. The insoluble product **3** was dried under vacuum at 30° for 2 hours and at room temperature for 18 hours; first crop, 15.31 g (yield 68%), mp 145-147° dec; tlc (silica gel, ethyl acetate, acetone, acetic acid, water, 6:2:1:1, visualized by uv and Rydon's spray): R$_f$ 0.92; HI [16] (300 nm) 97%; ir (potassium bromide): ν C=O, 1759, 1660 cm^{-1}; pmr (dimethyl sulphoxide-d$_6$): δ 1.18 (s, 3, CH$_3$), 1.4 (s, 3, CH$_3$), 4.6 (d, 1, 3-H, J = 8 Hz), 4.87 (s, 2, CH$_2$), 6.83 (s, 1, CHPh$_2$), 6.88 (s, 1, CH), 7.2-7.5 (m, 12, 2Ph and NH$_2$), 9.4 (d, 1, NH, J = 8 Hz), 10.1 ppm (br s, 1, OH); cmr (dimethyl sulphoxide-d$_6$): δ 19.7, 22.7, 60.7, 66.0, 71.0, 76.9, 110.5, 126.6, 127.8, 128.5, 139.3, 140.2, 149.4, 160.1, 161.8, 168.2, 168.9 ppm; ms: (FAB) M+H = 524, M-H = 522; [α]$_D$ = -19.7° (c = 1, acetonitrile), +1.9° (c = 1, 95% ethanol).

Anal. Calcd. for $C_{25}H_{25}N_5O_6S$·0.04 ethyl acetate·0.9 water: C, 55.64; H, 4.99; N, 12.89; S, 5.89. Found: C, 55.53; H, 4.60; N, 12.72; S, 5.59.

The second crop of **3** was obtained as follows: The filtrate was concentrated to 60 ml volume and seeded with **3**. After 1 hour at ambient temperature, the solid was filtered, washed with ethyl acetate (10 ml) and dried under vacuum for 15 hours to furnish 2.22 g (10%) of **3**, mp 144-146° dec; tlc: HI = 96%; total yield 78%.

[4*S*(*Z*)]-[[[1-(2-Amino-4-thiazolyl-2-[(3,3-dimethyl-5-oxo-4-isoxazolidinyl)-amino]-2-oxoethylidene]amino]oxy]acetic Acid (**4**).

Method a.

Anisole (6.0 ml) was added to a suspension of **3** (12.0 g, 22.0 mmoles) in dichloromethane (150 ml, dried over molecular sieves). The mixture was cooled to -20°. Trifluoroacetic acid (45.0 ml) was introduced dropwise over 10 minutes and the reaction mixture was stirred in an ice bath for 2 hours. Ether (500 ml) was added *via* an addition funnel over 10 minutes to the vigorously stirred reaction mixture. The cold bath was removed and the white suspension was stirred for one hour. The product was filtered, washed with ether (100 ml) and dried under vacuum at ~30° for 4.5 hours to give 7.95 g (70%) of the trifluoroacetic acid salt of **4** (light pink solid), mp 115° (softens) and 145° dec; tlc (silica gel): ethyl acetate, ethanol, acetic acid, water, 6:2:1:1, R$_f$ 0.45; HI (300 nm) 97%; ir (potassium bromide): ν C=O, 1671, 1760 cm^{-1}; pmr (dimethyl sulphoxide-d$_6$): δ 1.19 (s, 3, CH$_3$), 1.38 (s, 3, CH$_3$), 4.58 (d, 1, 3-H, J = 8 Hz), 4.65 (d, 2, CH$_2$), 6.9 (s, 1, CH), 9.4 ppm (d, 1, NH, J = 8 Hz); cmr (dimethyl sulphoxide-d$_6$): δ 19.6, 22.7, 60.7, 65.9, 71.0, 110.8, 136.6, 147.5, 159.9, 161.1, 169.5, 170.4 ppm; ms: (FAB) M+H = 358, M-H = 356; [α]$_D$ = -8.2° (c = 1, water).

Anal. Calcd. for $C_{12}H_{15}N_5O_6S$·0.82 trifluoroacetic acid·0.4 ether·2.1 water: C, 35.30; H, 4.63; N, 13.51; S, 6.17; F, 9.02. Found: C, 35.59; H, 4.23; N, 13.10; S, 6.36; F, 8.70.

Method b.

An aqueous solution of sodium hydroxide (6.0 ml, 1*N*) was added dropwise to the solution of **3** (1.36 g, 2.5 mmoles) in tetrahydrofuran (10.0 ml) at ambient temperature. The rate of addition of the base to the reaction mixture was controlled to maintain the *p*H (11-13). After 1.5 hours hydrochloric acid (1.5 ml, 1*N*) was added to bring the medium to *p*H 7.5. The solvent (tetrahydrofuran) was stripped off under vacuum. The material was taken up in water (5 ml) and dichloromethane (10 ml). The aqueous layer was separated and washed with dichloromethane (10 ml). The aqueous solution was further acidified with hydrochloric acid (4.5 ml, 1*N*) to *p*H 3.0. The solution was charged onto a CHP-20P [17] column (37 cm x 2.5 cm). Elution with water (3 liters) removed the uv nonactive contaminants. Further elution was carried out with 20% acetone in water (1 liter) and 30 ml fractions were collected. Fractions 7 to 9 were lyophilized to give 0.61 g of **4** (homogeneous by tlc). An additional 0.21 g of **4** was obtained from fractions 10-14; combined yield = 0.82 g (~92%). The first portion (0.60 g) of **4** obtained above was treated with ether (25 ml), filtered with suction, and washed with ether (15 ml). The substance was dried under vacuum for 0.5 hours to furnish 0.58 g (62%) of **4**, mp 150° (contraction), 172° dec; ir (potassium bromide): ν C=O, 1761, 1664 cm^{-1}; pmr (dimethyl sulphoxide-d$_6$): δ 1.2 (s, 3, CH$_3$), 1.4 (s, 3, CH$_3$), 4.6 (s, 2, CH$_2$), 4.7 (d, 1, H-3, J = 8 Hz), 6.78 (s, 1, CH), 7.22 (s, 2, NH$_2$), 9.28 (d, 1, NH, J = 8 Hz), 10.1 ppm (br s, 1, OH); cmr (dimethyl sulphoxide-d$_6$): δ 19.6, 22.7, 60.6, 65.9, 70.7, 109.9, 142.2, 150.1, 160.2, 162.4, 168.5, 170.7 ppm; ms: (FAB) M+H, 358; M-H, 357; [α]$_D$ = -16.0° (c = 1, water).

Anal. Calcd. for $C_{12}H_{15}N_5O_6S$·1 water: C, 38.40; H, 4.57; N, 18.43; S, 8.41. Found: C, 38.52; H, 4.36; N, 18.67; S, 8.64.

(*S*)-(1-Hydroxy-2,2-dimethyl-4-oxo-3-azetidinyl)carbamic Acid, 1,1-Dimethylethyl Ester (**10**).

To a solution of azetidinone **9** (0.47 g, 0.88 mmole) in 50 ml tetrahydrofuran, 2 ml of 0.5 *N* aqueous sulphuric acid was added and the reaction mixture (*p*H 0.8) was stirred at ambient temperature. After 2 days sodium bicarbonate (0.084 g, 1.0 mmole) was added (*p*H 1.2) and tetrahydrofuran was removed under vacuum. The material was taken up in ethyl acetate (30 ml) and washed with water (3 x 10 ml). The organic phase was dried over magnesium sulphate, filtered and ethyl acetate was removed under vacuum to give 0.174 g of **10** (67%), mp 140-142°, mixture mp with authentic sample (mp 145-146°) was undepressed (143-145° dec). The pmr and cmr spectra of **10** were identical to the corresponding spectra of an authentic sample [6,13].

(*S*)-(3,3-Dimethyl-5-oxo-4-isoxazolidinyl)carbamic Acid, 1,1-Dimethylethyl Ester (**11**).

A solution of **10** (1.15 g, 5.0 mmoles) and triethylamine (2.8 ml, 20.0 mmoles) in ethyl acetate (10.0 ml) was refluxed for one hour. The reaction mixture was concentrated under vacuum to 2.0 ml volume. The material was chromatographed on silica gel (70.0 g) packed in a column (diameter 2.5 cm) and eluted with ethyl acetate. The solvent was removed from fractions 5 to 8 (45 ml each) under vacuum to give 0.57 g (50%) of **11**. The product was dissolved in ethyl acetate (5.0 ml) and concentrated under vacuum to a volume of 2.0 ml. The mixture was cooled in an ice bath for 0.5 hour; a crystalline solid separated. The material was filtered with suction, washed with hexane (5 ml), and dried under vacuum for 16 hours to give 0.365 g of **11** (white crystalline solid), mp 150-152°; ir (chloroform): ν C=O, 1716, 1787 cm^{-1}; pmr (deuteriochloroform): δ 1.15 (s, 3, CH$_3$), 1.4 (s, 3, CH$_3$), 1.48 (s, 9, C (CH$_3$)$_3$), 4.4 (s, 1, H-4), 4.9 (br s, 1, NH), 6.58 ppm (br s, 1, NH); cmr (deuteriochloroform): δ 19.5, 23.3, 28.2, 63.6, 76.5, 81.1, 155.7, 176.8 ppm; ms: (FAB) M+H 231; [α]$_D$ = +18.0° (c = 1, chloroform).

Anal. Calcd. for $C_{10}H_{18}N_2O_4$·0.4 water: C, 50.63; H, 7.93; N, 11.81. Found: C, 50.65; H, 7.81; N, 11.77.

Deprotection of the Carbamate **11** to **12**.

A solution of **11** (0.46 g, 2.0 mmoles) and anisole (0.4 ml) in dichloromethane (10 ml) was treated with trifluoroacetic acid (1.5 ml, added dropwise) at ice bath temperature. The cold bath was removed after 5 minutes and the reaction mixture was stirred for 5 hours. The solvent was removed under vacuum and the residue was triturated with ether (20 ml). The solvent was decanted and the sticky mass was treated with hexane (20 ml) to produce a solid. The solvent was decanted and the product was washed once more with hexane (20 ml). The hexane washings were added to the ether mother liquor to give a solid precipitate. This product was intimately combined with the solid obtained above and dried under vacuum to give 0.9 g of the trifluoroacetic acid salt of **12** (88%); ir (potassium bromide): ν C=O, 1675, 1793 cm^{-1}. The impure material was used in the coupling reaction with active ester **13** without further purification.

(*Z*)-2-Amino-α-[[2-(diphenylmethoxy)-1,1-dimethyl-2-oxoethoxy]imino]-4-thiazoleacetic Acid, 1*H*-Benzotriazol-1-yl Ester (**13**).

A solution of α-aminothiazoleiminoxyacetic acid (6.165 g, 15 mmoles) and *N*-hydroxybenzotriazole (2.115 g, 15 mmoles) in dimethylformamide (30 ml) was cooled to -5°. Dicyclohexylcarbodiimide (3.708 g, 18 mmoles) was added and mixture was stirred under nitrogen for 5 hours. The insoluble solid (dicyclohexylurea) was filtered and washed with ice cold dimethylformamide (10 ml). The filtrate was cooled in an ice bath and diluted with ethyl acetate (150 ml) and washed with ice cold water (5 x 40 ml) containing brine (2 ml) during each washing. The organic phase was dried over magnesium sulphate, filtered, and the solvent was removed under vacuum. The residue was triturated with ether (50 ml). The product was filtered, washed with ether (20 ml), and dried under vacuum to give 6.48 g (79%) of ester **13**, mp ~97°; tlc (silica gel; acetone, dichloromethane, 1:2): R$_f$ 0.71; ir (potassium bromide): ν C=O, 1818, 1740, 1716 cm^{-1}; pmr (dimethyl sulphoxide-d$_6$): δ 5.25 (s, 2, CH$_2$), 6.97 (s, 1, CHPh$_2$), 7.1 to 7.6 (m, 14, 12 aromatic and NH$_2$), 7.7 (t, 1, H, aromatic CH, J = 8

Hz), 7.82 (d, 1, aromatic CH, J = 8 Hz), 8.2 ppm (d, 1, aromatic CH, J = 8 Hz); cmr (dimethyl sulphoxide-d_6): δ 72.1, 77.0, 109.2, 109.8, 119.9, 125.6, 126.4, 127.8, 127.9, 128.4, 130.0, 138.6, 139.4, 142.6, 143.48, 158.5, 167.7, 169.5 ppm; ms: (FAB) M + H = 529, M–H = 527.

Anal. Calcd. for $C_{26}H_{20}N_6O_6S$: C, 58.96; H, 3.95; N, 15.49; S, 5.91. Found: C, 59.36; H, 4.09; N, 15.55; S, 5.84.

[4*S*(*Z*)]-[[[1-(2-Amino-4-thiazolyl)-2-[(3,3-dimethyl-5-oxo-4-isoxazolidinyl)amino]-2-oxoethylidene]amino]oxy]acetic Acid, Diphenylmethyl Ester (**5**).

Compound **5** was prepared by two methods.

Method a. Acylation of **12** with Active Ester **13**.

A suspension of **12** (0.506 g, 2 mmoles) and active ester **13** (1.056 g, 2 mmoles) in dichloromethane (10 ml) was cooled in an ice bath. Triethylamine (0.56 ml, 4 mmoles) was added and the reaction mixture was allowed to warm to ambient temperature with stirring overnight. The reaction mixture was diluted with ethyl acetate (50 ml) and washed with water (2 x 15 ml) and brine (20 ml). The aqueous washings were combined and extracted with ethyl acetate (2 x 10 ml). The organic extracts were combined and dried over magnesium sulphate. The solution was filtered and the solvent was removed under vacuum. The crude product (1.05 g) was treated with ether (30 ml), filtered with suction, and washed with ether (10 ml). The material was dried under vacuum to furnish 0.685 g (64%) of **5**, mp 95° (softens), 110° dec; $[\alpha]_D$ = –17.2° (c = 1, chloroform). The mixture mp with **5** obtained by rearrangement of **3** (method b) was undepressed. The pmr, ir and ms of **5** were also identical to the sample prepared by method b.

Anal. Calcd. for $C_{25}H_{25}N_5O_6S \cdot 0.18$ ether: C, 57.48; H, 4.99; N, 13.04; S, 5.97. Found: C, 57.44; H, 4.98; N, 12.80; S, 5.88.

Method b. Rearrangement of Azetidinone **3** to **5**.

A solution of **3** (0.053 g, 0.1 mmole), triethylamine (0.008 g, 0.01 mmole) and water (0.05 ml) in ethyl acetate (2 ml) was refluxed for ~30 hours. The reaction mixture was cooled, diluted with ethyl acetate (10 ml) and washed with water (2 x 10 ml) and brine. The organic phase was dried (magnesium sulphate) and the solvent was removed under vacuum to give 0.05 g of crude **5**. The material was chromatographed (ethyl acetate) over three analytical silica gel plates. The product (R_f 0.44) was eluted with methanol/ethyl acetate (1:1) and the solvent was evaporated. The residue was treated with 10 ml chloroform and filtered. The filtrate was evaporated and the material was treated with ether and the solvent was removed completely under vacuum to give 0.028 g of **3** (53%), mp 100° (softens) and 115° dec; ir (chloroform): ν C=O, 1685, 1739, 1789 cm^{-1}; pmr (dimethyl sulphoxide-d_6): δ 1.02 (s, 3, CH_3), 1.25 (s, 3, CH_3), 4.84 (s, 2, CH_2), 5.04 (br d, 1, 4-H, J = 8 Hz), 6.8 (s, 1, $CHPh_2$), 6.88 (s, 1, CH), 7.1-7.5 (m, 12, 2Ph and NH_2), 8.2 (s, 1, NH), 9.18 ppm (s, 1, NH, J = 8 Hz); ms: (FAB) M + H = 524, M–H = 522.

[4*S*(*Z*)]-[[[1-(2-Amino-4-thiazolyl)-2-[(3,3-dimethyl-5-oxo-4-isoxazolidinyl)amino]-2-oxoethylidene]amino]oxy]acetic Acid, Potassium Salt (**7**).

Trifluoroacetic acid (1 ml, 12.98 mmoles) was added to a solution of **5** (1.5 g, 2.96 mmoles) and anisole (0.5 ml, 4.6 mmoles) in dichloromethane (5 ml) at ice bath temperature. After 1 hour, a second batch of trifluoroacetic acid (1 ml, 12.98 mmoles) was introduced. After 1 hour the solvent was evaporated under vacuum; the residue was triturated with ether to obtain a solid, which was stirred for 0.5 hours at ambient temperature. The product was filtered, washed with ether, and dried under vacuum to give 1.05 g of **6**. Crude **6** was suspended in water (5 ml) and the *p*H of the medium was adjusted to 7.0 by addition of potassium bicarbonate. The solution was charged on to a Dowex-50 (K^+) column (8 x 2.5 cm). The material was eluted with water and lyophilized to give 0.665 g of **7**. The potassium salt **7** was purified by column chromatography over CHP-20P (8 x 2.5 cm). The column was eluted with water. The tlc homogeneous fractions were combined and lyophilized to furnish 0.286 g of **7** (23%), mp 65-70° dec; tlc (silica gel, ethyl acetate, acetic acid, water, 8:1:1; visualized by uv and Rydon's spray: R_f 0.5; ir (potassium bromide): ν C=O, 1781, 1665, cm^{-1}; pmr (dimethyl sulphoxide-d_6): δ 1.07 (s, 3, CH_3), 1.2 (s, 3, CH_3), 4.2 (AB_q, 2, CH_2, J = 14 Hz), 4.9 (br s, 1, 4-H), 6.8 (s, 1, CH), 7.18 (s, 2, NH_2), 8.16 (s, 1, NH), 11.62 ppm (br s, 1, NH); cmr (dimethyl sulphoxide-d_6): δ 20.5, 22.2, 57.8, 62.9, 73.0, 110.0, 142.8, 151.1, 162.8, 168.2, 172.1, 176.7 ppm; ms: (FAB) M + H 396, M–H 394; $[\alpha]_D$ = –25.7° (c = 1, water).

Anal. Calcd. for $C_{12}H_{14}N_5O_6S \cdot 0.95\ K \cdot 1.7$ water: C, 33.93; H, 4.15; N, 16.49; S, 7.55; K, 8.34; H_2O, 7.4. Found: C, 33.62; H, 3.68; N, 16.22; S, 7.32; K, 8.44; H_2O, 7.36 (KF).

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† Squibb Institute for Medical Research, P. O. Box 08540-4000, Princeton, New Jersey 08540.

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[25] Unisomerised **10** was isolated by elution of the chromatography column with ethyl acetate: $[\alpha]_D = +58^\circ$ (c = 1, chloroform), which is indicative of no racemisation.

[26] The values of carbonyl frequencies of some of the related compounds reported in reference [23] appear low in magnitude (1740-1750 cm^{-1}) for an isoxazolidin-5-one structure.