SQ 26,180, A NOVEL MONOBACTAM. II

ISOLATION, STRUCTURE DETERMINATION AND SYNTHESIS

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A novel monocyclic β-lactam antibiotic SQ 26,180 has been isolated from bacteria and the structure, (*R*)-3-acetylamino-3-methoxy-2-oxo-1-azetidinesulfonic acid was deduced from its spectroscopic properties. Structural confirmation and assignment of absolute configuration were made by synthesis from 6-aminopenicillanic acid.

Beta-lactam antibiotics comprise one of the major chemotherapeutic means for the control of bacterial infections. This group of antibiotics has been the subject of intensive research in recent years, leading to the discovery of several new classes of naturally occurring β-lactams, namely the cephamycins, nocardicins, clavulanic acids and a burgeoning family of carbapenems. We now wish to report the isolation and characterization of a strongly acidic monocyclic β-lactam antibiotic, SQ 26,180, a member of a new class of β-lactam antibiotics, discovered independently by workers at both Takeda[1] and Squibb[2] for which we have suggested the generic term "monobactam". SQ 26,180, produced by fermentations of *Chromobacterium violaceum* SC 11,378, was shown to have structure **1**, (*R*)-3-acetylamino-3-methoxy-2-oxo-1-azetidinesulfonic acid. Isolation of SQ 26,180 was monitored using sensitive strains of *Bacillus licheniformis* and *Pseudomonas aeruginosa*. The characterization of the producing organism and the fermentation are described in the accompanying paper.[3]

SQ 26,180 was isolated from fermentation broths as outlined in Fig. 1.

The antibiotic was extracted into dichloromethane containing a tetraalkylammonium salt, cetyldimethylbenzylammonium chloride being effective for this purpose. Back extraction of SQ 26,180

Fig. 1. Isolation of SQ 26,180.

C. violaceum Broth filtrate

1. Ion-pair extraction into CH_2Cl_2 with cetyldimethylbenzylammonium chloride and back extraction into water with NaI.
2. Chromatography on Sephadex G-10 in MeOH - H_2O, 1 : 1.
3. Ion-exchange chromatography on Whatman DE52, eluting with pH 5 phosphate buffer.
4. Chromatography on Sephadex LH-20 in water.
5. Chromatography on Diaion HP20AG in water.
6. Conversion to K^+ salt with Dowex 50 (K^+).

Crystalline SQ 26,180, K^+ salt.

Table 1. Electrophoresis of SQ 26,180.

Buffer	pH	Mobility[a]
Sodium 0.05 M phosphate	7.0	0.88
Sodium 0.05 M phosphate	5.5	0.86
HOAc - H_2O, 1 : 9	2.2	0.88
HCO_2H - HOAc - H_2O, 1 : 3 : 36	1.8	0.91

[a] On Whatman No. 2 paper, 12 V/cm, 1 hour; mobilities relative to vitamin B_{12} (0) and *p*-nitrobenzenesulfonate anion (1.00).

Table 2. NMR spectra of SQ 26,180.

C/H	Solvent	Assignment[a]						
		2	3	4	5	6	7	8
H (b)	CD_3OD			3.80, 3.92 ($\|J\|$=6.5 Hz)	3.46		2.03	
H (b)	DMSO-d_6			3.55, 3.69 ($\|J\|$=6.6 Hz)	3.30		1.92	9.15
H (c)	D_2O			3.98	3.50		2.09	
C (b, d)	DMSO-d_6	160.5	89.5	53.1	51.8	170.5	22.5	

(a) Chemical shifts are in parts per million downfield from (b) tetramethylsilane or (c) sodium 3-trimethylsilylpropionate-d_4. (d) Multiplicities from a single-frequency off-resonance decoupled spectrum are in accord with structure **1**.

into water was accomplished by transforming the ion-pair into the sodium salt with either sodium iodide or thiocyanate. The resulting aqueous extract was desalted by partition chromatography on Sephadex G-10. Further purification was accomplished by ion-exchange chromatography on either DEAE cellulose or Bio-Rad AG MP-1 resin followed by reverse-phase chromatography on Diaion HP20AG. Conversion of the antibiotic to the potassium salt on Dowex 50W-X2 (K^+) gave a solid that was recrystallized from aqueous methanol, giving analytically pure SQ 26,180.

Electrophoresis of SQ 26,180 (Table 1) indicates the presence of a strongly acidic function.

Elemental analysis of the potassium salt gives an empirical formula, $C_6H_9N_2O_6SK$. The infrared spectrum (KBr) has a strong absorption at 1766 cm^{-1}, indicative of a β-lactam ring. Intense amide I (1673 cm^{-1}) and amide II (1522 cm^{-1}) peaks are evident as are characteristic peaks at 1265, 1051, and 636 cm^{-1}, indicating the presence of an $-SO_3^-$ group, the latter being consistent with the strongly acidic nature of SQ 26,180. The location of the $-SO_3^-$ group was further elucidated by treatment of a solution of SQ 26,180 in 2 N HCl with $BaCl_2$ and $NaNO_2$. Precipitation of $BaSO_4$ indicated that the antibiotic contains a sulfanic acid function (the acid-hydrolysis product of the lactam presumably being the reactive species in this case).[4] The ^{13}C and ^1H NMR spectra (Table 2) led to the assignment of **1** (exclusive of stereochemistry) as the structure of SQ 26,180.

The acetylamino group is apparent from the peaks at around 2 ppm in the ^1H NMR spectrum and at 22.5 ppm in the ^{13}C NMR spectrum. Similarly, a methoxyl group is indicated by peaks at around 3.4 and at 51.8 ppm in the ^1H and ^{13}C NMR spectra, respectively. The peak at 9.15 ppm (^1H spectrum in DMSO-d_6) coincides with the chemical shift of the amide proton in several cephamycin derivatives,[5] supporting the location of the acetyl and sulfo groups as shown in **1** instead of the transposed arrangement. The coupling constant of 6.5 Hz (presumed negative) observed between the geminal protons at C-4 in CD_3OD and DMSO-d_6 is in accord with the coupling of −5.5 Hz reported for the corresponding position in other monocyclic β-lactams.[6]

SQ 26,180 is converted by methanol in the presence of triethylamine to the methyl ester **2**. The chemical shift of the $NHSO_3^-$ proton in **2**, 4.35 ppm, is similar to that found for the analogous proton in sodium cyclamate, 4.00 ppm, in the same solvent (DMSO-d_6) (unpublished observation).

Treatment of **1** with 6 N HCl at 114°C for 15 hours yields an amphoteric compound that is negative to ninhydrin but that gives a yellow color with PAULY's reagent. The ^1H NMR spectrum of the hydrochloride in D_2O [δ 2.66 (s, 3) and 7.86 ppm (s, 1)] and the UV spectra in water (pH 2.85, λ_{max} 215 nm, ε 8900) and in dilute NaOH (pH 12, λ_{max} 238 nm, ε 7300) suggest structure **3** and this was verified by comparison with authentic material.[7,8]

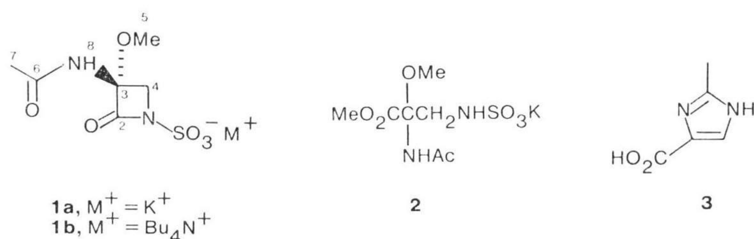
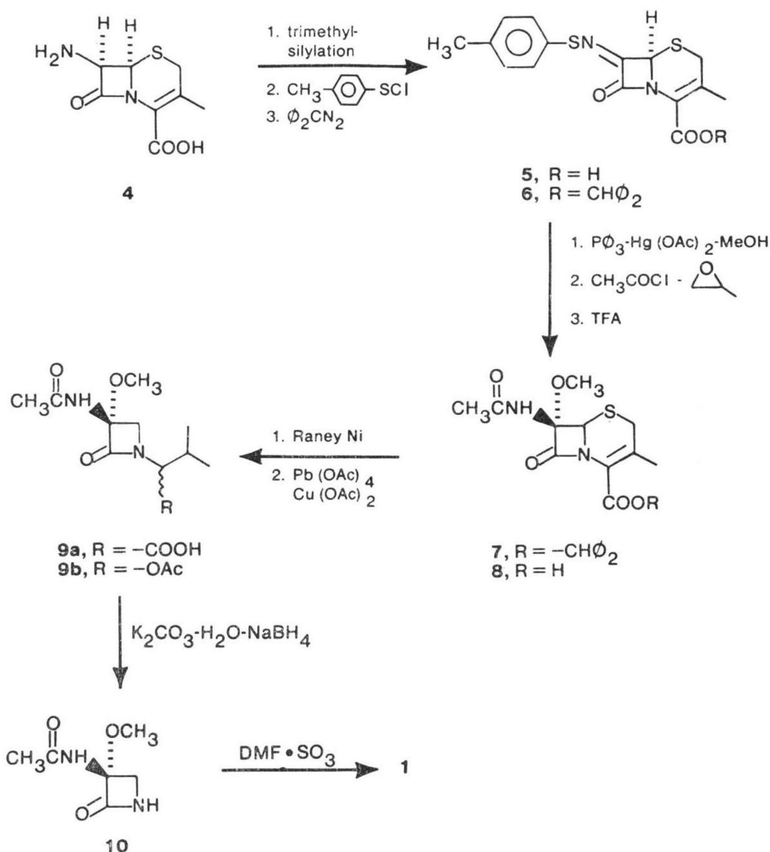


Fig. 2. Synthesis of SQ 26,180.

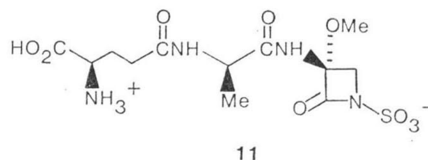


Confirmation of the structural assignment and determination of the C-3 stereochemistry of SQ 26,180 was accomplished by synthesis from a β-lactam antibiotic of known absolute configuration, as shown in Fig. 2. 7-ADCA, **4**, was a convenient source of chiral starting material since it is prepared from 6-APA in bulk *via* the MORIN rearrangement. The stability of the cephem nucleus allowed use of an acid-removable protecting group in the early stages of the synthesis as part of an efficient process for the preparation of *N*-acetyl-7α-methoxy-7-ADCA, utilizing methodology previously reported by workers at Squibb.[9] Using procedures similar to those applied to the degradation of penicillanic acids, the methoxylated cephalosporin was degraded to a monocyclic azetidinone, which upon sulfonation yielded SQ 26,180.

Sulfenylation of silylated 7-ADCA gave thiooxime **5**,[9] which was readily converted to benzhydryl

ester **6**. Solvolytic sulfenyl transfer rearrangement[9] and acetylation *in situ* of the intermediate methoxyamine gave **7** which yielded methoxylated cephalosporin **8** after acidic cleavage of the protecting ester. Desulfurization and reduction of **8** with RANEY nickel formed desthiocepham **9a** as a mixture of diastereomers. Oxidative decarboxylation was accomplished with a lead tetraacetate-cupric acetate mixture[10] yielding isomeric acetates **9b**, that were hydrolytically degraded[11] to (*R*)-3-acetylamino-3-methoxy-2-azetidinone (**10**). Although **10** proved unstable to a variety of sulfonation conditions, a modest yield (23%) of SQ 26,180 was obtained when **10** was treated at 0°C with the highly electrophilic sulfonating agent, DMF·SO_3 complex. The product, isolated as the Bu_4N^+ salt **1b**, was identical to naturally occurring SQ 26,180, thus confirming the structure and assigning the *R*-configuration at the C-3 position.

We have also isolated a related antibiotic, SQ 26,445, having a dipeptide group at the 3-position.[2] The 3-methoxy-2-oxo-1-azetidinesulfonic acid moiety was easily recognized from the NMR and IR spectra. Acid hydrolysis gave D-glutamic acid, D-alanine and ammonia in a ratio of 2: 1: 2. The Glu residue was shown to be *N*-terminal by the SANGER method.[12] Comparison of the ^1H NMR spectrum with data published for γ-Glu-Ala[13,14] showed that the dipeptide side chain is linked in this manner. The structure of SQ 26,445 is thus **11**.



The peculiar ratio of amino acids is presumably due to partial diversion of the hydrolysis to an imidazole analogous to **3**. However, this was not isolated and characterized. Following the completion of our studies, workers at Takeda reported the isolation and structure determination of this antibiotic[1,16] and named it sulfazecin.

Furthermore, a group of five monobactams having *N*-acetylphenylalanyl and related side chains has been isolated by us from cultures of *Agrobacterium radiobacter*;[2] a more complete account of this work is forthcoming.

The diversity of naturally occurring monobactams suggests that they truly constitute a new class of antibiotics that derives activation of the β-lactam ring from the electronegative sulfonic acid residue. All members of this class isolated to date have been produced by bacterial fermentation. An investigation of synthetic monobactams has led to a highly active analog, SQ 26,776, possessing excellent β-lactamase stability and selective activity against Gram-negative organisms, including *Pseudomonas aeruginosa*. This analog is currently being developed for clinical evaluation.

Experimental

NMR spectra were determined on Varian Associates model XL-100-15 and T-60 and on Jeol Ltd. model FX60Q spectrometers; chemical shifts are given in ppm (δ) downfield from internal Me_4Si or $Me_3SiCD_2CD_2CO_2Na$. Infrared spectra were recorded on Perkin-Elmer model 257 and 621 spectrometers. Rotations were measured on a Perkin-Elmer model 141 polarimeter. Melting points were measured with a Thomas-Hoover capillary melting point apparatus and are uncorrected.

Isolation of SQ 26,180 (1)

A culture broth (250 liters) of *Chromobacterium violaceum*[3] was adjusted to pH 5 (H_2SO_4) and filtered using Celite. The filtrate was extracted with two 30-liter portions of 0.005 M cetyldimethylbenzylammonium chloride in CH_2Cl_2 and the combined organic phase extracted with 6 liters of 0.05 M NaI (adjusted to pH 5 with HOAc). The aqueous phase was concentrated *in vacuo* to 400 ml, washed with

butanol, and further concentrated to a residue. This was dissolved as much as possible in methanol and the resulting solution taken to dryness, giving 38.6 g of residue. This was chromatographed on a 2.5× 104 cm column of Sephadex G-10 in methanol - water, 1: 1, giving 5 g of residue from the active fractions. Chromatography on a 5×42 cm column of Whatman DE52 cellulose, eluting with a linear gradient prepared from 4 liters of pH 5 sodium 0.01 M phosphate and 4 liters of pH 5 sodium 0.1 M phosphate buffers, gave 0.46 g of active material after removal of inorganic salts by trituration with methanol and chromatography on a 2.5×98 cm column of Sephadex LH-20 in water. Further chromatography on a 2.5×66 cm column of Diaion HP20AG eluting with water gave 0.24 g of material that was chromatographically homogeneous (TLC on Merck silica gel 60, 2-BuOH - HOAc - H_2O, 3: 1: 1, RYDON-SMITH, Rf 0.40). Conversion to the potassium salt on a 1.5×9 cm column of Dowex 50W-X2 (K^+) gave 0.19 g of a crystalline solid that was recrystallized three times from H_2O - MeOH, 1: 9, to give 96 mg of analytically pure SQ 26,180, **1a**, mp 194°C (dec.), $[\alpha]_D^{21}$ +94° (*c* 1, H_2O).

Anal. Calcd. for $C_6H_9N_2O_6SK$: C 26.08, H 3.28, N 10.14, S 11.60, K 14.15.
Found: C 26.01, H 3.27, N 10.18, S 11.36, K 14.01.

The tetrabutylammonium salt was prepared by extraction of an aqueous solution of the potassium salt and one equivalent of Bu_4NHSO_4 with several portions of CH_2Cl_2. Removal of the solvent *in vacuo* gave **1b**, $[\alpha]_D$ +65° (*c* 1, $CHCl_3$).

Methanolysis of SQ 26,180

A solution of 49 mg of SQ 26,180 in 4 ml of MeOH and 0.04 ml of Et_3N was left at room temperature for 20 hours. The solvents were removed *in vacuo* and the residue lyophilized, giving 50 mg of **2** as a highly deliquescent solid. Upon exposure to air, the lyophilate formed a crystalline monohydrate, mp 156~158°C (dec.): ^1H NMR (DMSO-d_6) δ 1.84 (s, 3), *ca.* 3.1 (m, CH_2), 3.21 (s, 3), 3.58 (s, 3, CO_2CH_3), 4.32 (t, 1, J=6.7 Hz, $NHSO_3^-$) and 9.02 (s, 1, NHAc); IR (KBr) 1732 (ester C=O), 1685 (amide I), 1533 (amide II), 1209 and 1047 cm^{-1} (–SO_3^-).

Sulfenimine 5

The thiooxime was prepared according to the procedure described by GORDON, *et al.* where **5** is isolated as the sodium salt during work-up.[9] The sodium salt of **5** prepared from 26.34 g (123 mmole) of 7-ADCA was suspended in a water-EtOAc mixture and the pH was lowered to 2.3 with 1 N HCl. After extracting several times with EtOAc, the combined extract was dried (Na_2SO_4), filtered, and solvent was removed *in vacuo*. The residue was triturated with ether, yielding **5** as a bright yellow powder (19.62 g) that was used without further purification. Two more crops (3.23 g) were obtained for a total yield of 56%; mp 171~172°C (dec.); ^1H NMR (CD_3COCD_3) δ 2.15 (s, 3, CH_3), 2.35 (s, 3, CH_3), 3.25 and 3.70 (AB quartet, J=18 Hz, 2, CH_2), 5.50 (s, 1, H-6), 7.27 and 7.50 (two d's, J_{ortho}=8 Hz, aromatic); IR (KBr) 1770 (β-lactam C=O) and 1695 cm^{-1} (COOH).

Anal. Calcd. for $C_{15}H_{14}N_2O_3S_2$: C 53.88, H 4.19, N 8.38, S 19.16.
Found: C 53.92, H 4.19, N 8.37, S 19.32.

Sulfenimine Benzhydryl Ester 6

To a solution of acid **5** (19.0 g, 56.9 mmole) in CH_2Cl_2 (250 ml) was added dropwise diphenyldiazomethane (12.0 g, 61.9 mmole) in CH_2Cl_2. After several hours at room temperature the reaction mixture was washed twice with saturated $NaHCO_3$ solution, dried (Na_2SO_4), and solvent was removed *in vacuo*. The residue was triturated several times with ether until all excess diphenyldiazomethane was removed, yielding a yellow powder (27.7 g, 97%): mp 192~193°C; ^1H NMR ($CDCl_3$) δ 2.17 (s, 3, CH_3), 2.37 (s, 3, CH_3), 3.13 and 3.48 (AB quartet, J=17 Hz, 2, CH_2), 5.32 (s, 1, H-6), 6.97 (s, 1, CH) and 7.35 (complex m, 14, aromatic); IR (KBr) 1780 (β-lactam C=O) and 1720 cm^{-1} (ester C=O).

Anal. Calcd. for $C_{28}H_{24}N_2S_2O_3$: C 67.20, H 4.79, N 5.60, S 12.80.
Found: C 66.92, H 4.86, N 5.59, S 12.88.

7β-Acetylamino-7-methoxydesacetoxycephalosporanic Acid Benzhydryl Ester (7)

The procedure described by GORDON *et al.* for direct conversion of a sulfenimine to a 7α-methoxy amide[9] was used to prepare **7** from sulfenimine **6** (4 g, 8 mmole). The crude product was chromatographed on a silica gel column (250 g, Baker 60~200 mesh) eluting with EtOAc - CH_2Cl_2 (1: 9). Impure leading and tailing fractions were combined and purified by preparative thin-layer chromatography

[silica gel, E. Merck 60F, CH_2Cl_2 - EtOAc (2: 1)]. The combined purified product was solidified by precipitation from an ether - pentane mixture (total yield: 1.82 g, 50%); ^{1}H NMR ($CDCl_3$) δ 2.07 and 2.15 (two s's, 6, CH_3's), 3.17 (broad s, 2, CH_2), 3.55 (s, 3, OCH_3), 5.07 (s, 1, H-6), 6.90 (broad s, 2, CH and NH) and 7.32 (m, 10, aromatic); IR (mull) 1770 (β-lactam C=O), 1740 (ester C=O) and 1670 cm^{-1} (amide C=O).

7β-Acetylamino-7-methoxydesacetoxycephalosporanic Acid (**8**)

A solution of cephem ester **7** (1.67 g, 3.69 mmole) in a mixture of anisole (2 ml) and CH_2Cl_2 (20 ml), cooled in an ice bath, was treated with trifluoroacetic acid (4 ml) for 4.5 hours. Solvent was removed *in vacuo* giving a residual oil which, after washing with pentane, was solidified and triturated with an ether-pentane mixture yielding acid **8** as a colorless powder (1.03 g, 97%): mp 183~184°C (dec.); ^{1}H NMR (CD_3OD) δ 2.06 (s, 3, CH_3), 2.16 (s, 3, CH_3), 3.52 (s, 3, OCH_3), and 5.01 (s, 1, H-6); IR (KBr) 1760 (β-lactam C=O), 1740 and 1720 (COOH), 1675 (amide C=O), and 1520 cm^{-1} (amide II).

Anal. Calcd. for $C_{11}H_{14}N_2O_5S$: C 46.14, H 4.93, N 9.79, S 11.20.
Found: C 46.19, H 5.03, N 9.38, S 11.11.

Desthiocepham **9a**

A solution of methoxycephem **8** (650 mg, 2.27 mmole) and $NaHCO_3$ (191 mg, 2.27 mmole) in water was added to a suspension of commercial RANEY nickel (11 ml of slurry, Grace No. 28 washed to neutrality with H_2O) in water (20 ml). After lowering the mixture into an oil bath pre-heated to 170°C, refluxing commenced in 2 minutes and was continued for 15 minutes. The reaction vessel was cooled rapidly in an ice bath, the catalyst was removed by filtration through Celite, and the pH was lowered to 2 with 1 N HCl. After extraction of the acidic solution with four portions of EtOAc, the combined extract was dried (Na_2SO_4). Solvent was removed *in vacuo* and the residue was chromatographed on silica gel (Mallinckrodt SilicAR CC-4, 1×13 cm column). Elution with MeOH - $CHCl_3$ (contains 0.75% EtOH) (1: 49) yielded **9a** as a foam (381 mg, 65%); ^{1}H NMR (CD_3COCD_3) δ 1.03 (broad d, CH_3's), 2.05 (s, $COCH_3$), 3.40 and 3.43 (two s's, diastereomeric OCH_3's), and 3.68~4.20 (complex m, CH and CH_2).

Formation of Acetate **9b** *via* KOCHI Reaction

To a solution of desthiocepham **9a** (514 mg, 1.99 mmole) in dry CH_3CN (15 ml), purged with argon for 15 minutes, was added cupric acetate (397 mg, 1.99 mmole), followed after 1 minute of stirring by lead tetraacetate (882 mg, 1.99 mmole). The mixture was warmed in a pre-heated oil bath at 65~75°C while continuing to purge with a stream of argon. After 15 minutes the reaction was cooled to room temperature, filtered through Celite, and solvent was removed from the filtrate under reduced pressure. The residue was combined with water and extracted with four portions of EtOAc. Drying (Na_2SO_4) of the combined organic extract and removal of solvent *in vacuo* gave **9b** as an oil (394 mg, 73%): ^{1}H NMR ($CDCl_3$) δ 0.97 (broad d, 6, CH_3's), 2.08 (s, 6, CH_3CO's), 3.43 (s, 3, OCH_3), 3.58~3.65 (complex m, 2, CH_2), 5.40 (d, J=8 Hz, 1, –CHO–), and 8.03 (broad s, 1, NH); IR ($CHCl_3$) 1775 (β-lactam C=O), 1740 (sh, $OCOCH_3$), and 1690 cm^{-1} (amide C=O).

(*R*)-3-Acetylamino-3-methoxy-2-azetidinone (**10**)

Acetate **9b** (394 mg, 1.45 mmole), dissolved in a MeOH (10 ml) and water (1 ml) mixture and cooled in an ice-MeOH bath at −15 to −10°C, was treated with solid K_2CO_3 (200 mg, 1.45 mmole) and sodium borohydride (55 mg, 1.45 mmole). After stirring in the cold bath for 2 hours solvent was removed *in vacuo*. The residue was dissolved in water, the pH was lowered to 6 with 1 N HCl, and water was removed under reduced pressure. Extraction of the solid residue with acetone gave the product as an oil, which was purified on silica gel (4 g of Mallinckrodt SilicAR CC-4), eluting with MeOH - CH_2Cl_2 (1: 19). Crystallization of the product with ether gave **10** as a colorless powder (141 mg, 62%), mp 103~113.5°C. Recrystallization from acetone - ether gave analytically pure material, mp 112~113°C: ^{1}H NMR ($CDCl_3$) δ 2.08 (s, 3, CH_3CO), 3.50 (s, 3, CH_3O), 3.72, 3.90 (AB quartet, 2, J=6.5 Hz, CH_2), 5.98 (broad, 1, NH) and 6.79 (broad, 1, NH); IR (KBr) 1760 (β-lactam C=O), 1665 (amide C=O), and 1523 cm^{-1} (amide II).

Anal. Calcd. for $C_6H_{10}N_2O_3$: C 45.56, H 6.37, N 17.72.
Found: C 45.48, H 6.45, N 17.68.

Synthetic SQ 26,180 as the Bu_4N^+ Salt (**1b**)

DMF·SO_3 complex, freshly prepared from trimethylsilyl chlorosulfonate and dry DMF (*cf.* Ref. 15), was used as a 1 M solution in DMF. (*R*)-3-Acetylamino-3-methoxy-2-azetidinone (**10**) (40 mg, 0.254 mmole), placed in a flask cooled in an ice-bath, was treated with the above stock solution (1 ml) and, after 5 minutes, was poured into 0.5 M KH_2PO_4. After washing the aqueous solution with CH_2Cl_2, tetrabutylammonium bisulfate (121 mg, 0.25 mmole) was added and the ion-paired product was extracted with CH_2Cl_2 giving an oil. Chromatography on silica gel (3.5 g, Mallinckrodt SilicAR CC-4), eluting with MeOH - CH_2Cl_2 (3: 97), yielded **1b** as a foam (28 mg, 23%), $[\alpha]_D$ +61.5° (*c* 0.33, $CHCl_3$). The product was identical to the Bu_4N^+ salt of SQ 26,180 from fermentation broths by TLC [silica gel, E. Merck 60F, EtOAc - MeOH (2: 1)], ^1H NMR ($CDCl_3$, 100 MHz), and ^{13}C NMR ($CDCl_3$).

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