Ro 22-5417, A NEW CLAVAM ANTIBIOTIC FROM *STREPTOMYCES CLAVULIGERUS*

III. ABSOLUTE STEREOCHEMISTRY*

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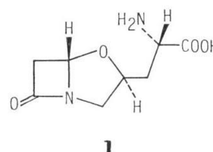
The complete stereostructure of the new antibiotic Ro 22-5417 has been established as 3-[(3*S*,5*S*)-7-oxo-1-aza-4-oxabicyclo[3.2.0]hept-3-yl]-L-alanine. This result together with the synthesis of an (3*R*,5*R*)-L-analog allowed us to postulate that clavams require the *R*-configuration at the ring juncture for β-lactamase inhibitory activity, while the opposite *S*-stereochemistry is essential for antifungal activity.

In two preceding reports from these laboratories, detailed accounts were given of the discovery of Ro 22-5417 from *Streptomyces clavuligerus*[1] and of the establishment of its gross structure.[2] Here, we wish to report that the complete stereostructure of Ro 22-5417 is as depicted in **1**, and hence, the new β-lactam antibiotic is 3-[(3*S*, 5*S*)-7-oxo-1-aza-4-oxabicyclo[3.2.0]hept-3-yl]-L-alanine.



1

This structure assignment is based on chiroptical measurements, the interpretation of the ^{1}H NMR spectrum of Ro 22-5417 and, to a large measure, on comparison with close structural analogs of the metabolite which were obtained by stereorational synthesis. The synthetic work not only served to corroborate spectroscopic inferences, but also to provide more general insights into the stereochemical requirements of clavam antibiotics for biological activity.

The L-configuration of the α-amino acid moiety of Ro 22-5417 was readily ascertained by derivatization with fluorescamine.[3] This reagent (**2**, Scheme 1) reacts with amino acids to afford chromophoric derivatives whose chirality can be unambiguously assigned from the signs of the longer wavelengths COTTON effects in their respective circular dichroism (CD) spectra.[4,5] Thus, the CD-spectrum of the pyrrolinone **3** arising from Ro 22-5417, has a positive COTTON effect at 380 nm ($\theta=+8,000$) and a second, negative one at 316 nm ($\theta=-8,000$) (Fig. 1). This is the spectral pattern generally obtained from fluorescamine derivatives of L-α-amino acids.[4,5]

The stereochemistry at the point of attachment of the alanine side chain to the clavam ring system relative to that at the ring junction is evident from the ^{1}H NMR spectrum of Ro 22-5417. BENTLEY and HUNT[6] recorded the NMR spectral features which allow one to differentiate between 3-substituted 1-aza-4-oxabicyclo[3.2.0]heptan-7-ones of type **4** and **5**. According to their findings the difference in the chemical shift of the two methylene protons at C-2 is about 1~1.4 ppm, when H-3 and H-5 are in an

* We dedicate this paper to the memory of Dr. WILLY LEIMGRUBER (1930~1981), our late director of Chemical Research, with whose encouragement this study was undertaken.

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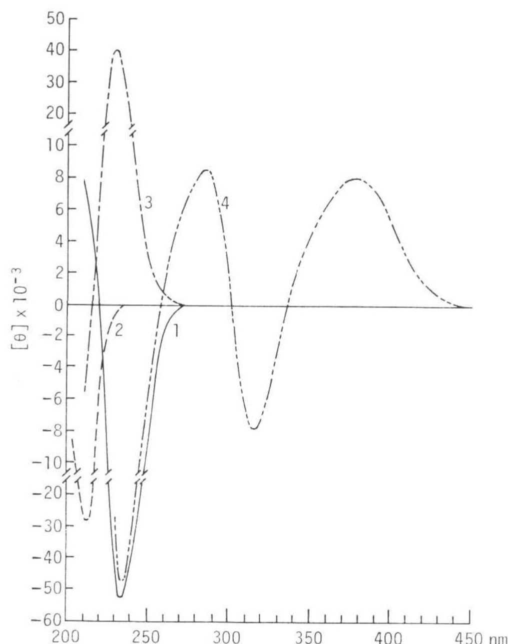
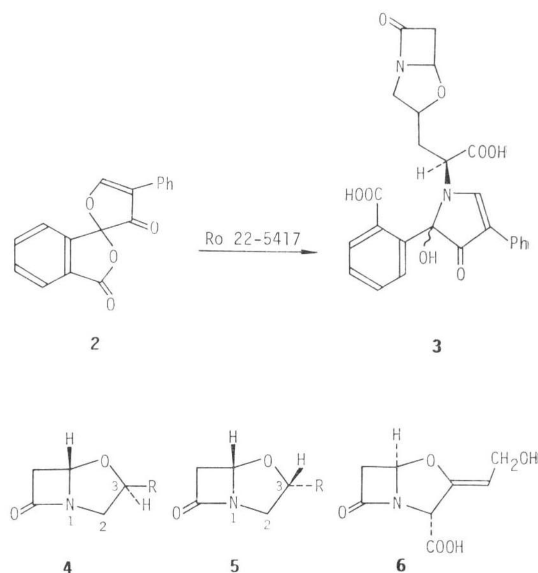
Fig. 1. CD Spectra of **1** (1), **11b** (2) and **14** (3) in methanol, and the CD spectrum of the fluorescamine reaction product **3** measured *in situ* in dioxane/phosphate buffer pH 8 (4).



Scheme 1.



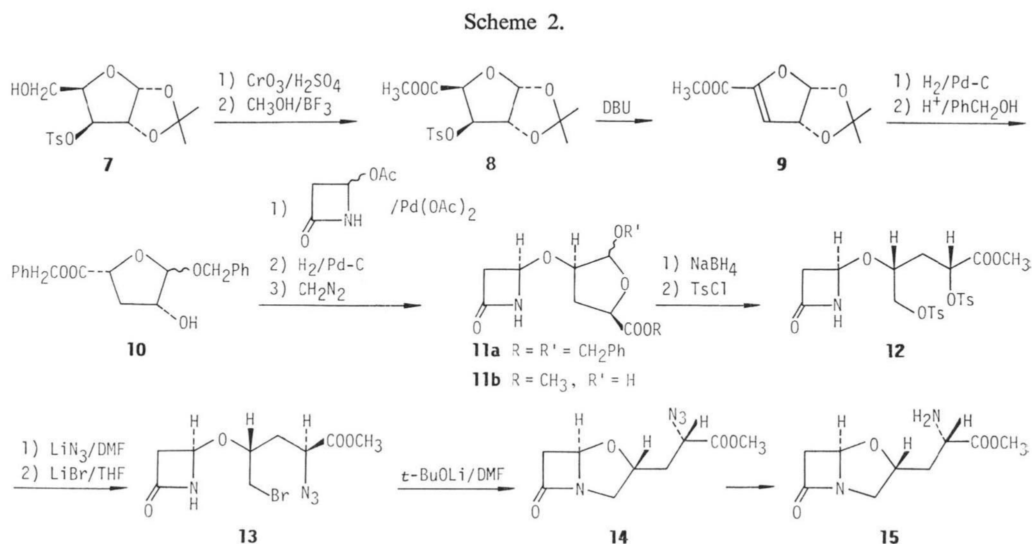
anti-relationship, such as in **4**. When H-3 and H-5 are *syn*, as in **5**, the chemical shifts of the two protons at C-2 differ by only 0.4~0.5 ppm. The difference between the chemical shifts of the two C-2 protons of Ro 22-5417 is 1.28 ppm when measured in D_2O, and 1.26 ppm when recorded in DMSO-d_6.[2] Hence, H-3 (at the site of side chain attachment) and H-5 (at the ring junction) are *anti* with respect to each other in the antibiotic.

The absolute stereochemistry at the ring juncture of the Ro 22-5417 molecule is revealed in the CD-spectrum, which exhibits a strong negative COTTON effect at 235 nm ($\theta = -54{,}000$) (Fig. 1). BUSSON, *et al.*[7] recently measured the CD-spectra of a variety of penicillanates and 5-*epi*-penicillanates. A strong COTTON effect was observed at around 230 nm, which was consistently positive when associated with 5*R*-stereochemistry, and negative in the *epi*-series having 5*S*-configuration. A corresponding interdependence obtains in the carbapenam series,[8] and recently ZEEK *et al.*[9] utilized this effect to assign the absolute configuration of an antibiotic **4** (R=$-CH_2CH_2OH$), which had been isolated from cultures of *Streptomyces antibioticus*. Ostensibly, the sign of this COTTON effect near 230 nm is determined solely by the geometry at the ring juncture of the bicyclic β-lactam, and the phenomenon has been convincingly explained as arising from the dissymmetric n-π^* transition of a lone pair electron of the β-lactam, nitrogen.

Thus, the negative CD-signal at 235 nm in the spectrum of Ro 22-5417 establishes the absolute configuration of the ring juncture as *S*, and consequently (*vide supra*) that of the side chain bearing carbon as *S*, as well.

At this point, it is interesting to remember the remarkable capability of *Streptomyces clavuligerus* to elaborate a plethora of β-lactam antibiotics,[10] including penicillin N, deacetoxycephalosporin C, cephamycin C and clavulanic acid, all of them having *R*-stereochemistry at the ring juncture. The observation that Ro 22-5417 possesses the opposite *S*-configuration at the crucial ring carbon attests further to the synthetic versatility of this organism. It is also relevant to remember, that clavulanic acid (**6**)[11]

Scheme 2.



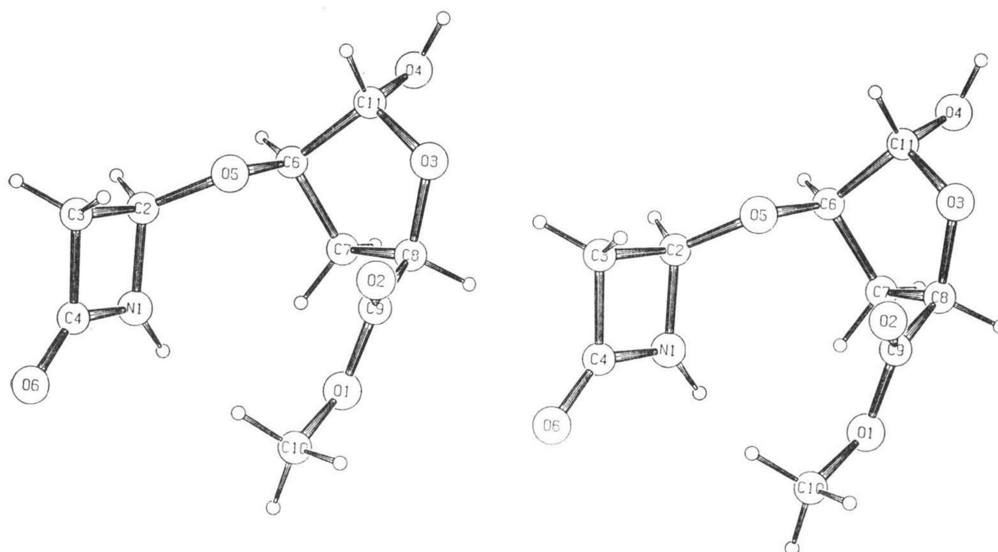
potently inhibits many clinically significant β-lactamases,[10] while Ro 22-5417 is neither an inhibitor nor a substrate of these enzymes.[1] Presumably, this difference in biological properties is largely a function of the profound stereochemical difference. To test this supposition and to further corroborate the stereochemistry assigned to Ro 22-5417, we synthesized the *R*, *R* analogs **14** and **15**. Details of this synthetic work are contained in Scheme 2 and in the Experimental Section.

Starting with D-xylose, a series of standard transformations afforded the L-3-deoxyaraburonic acid derivative **10**. Palladium acetate catalyzed condensation of **10** with 4-acetoxy-2-azetidinone[12] (in benzene) proceeded at room temperature with a good degree of stereoselectivity to give mainly the desired **11a**, along with a minor amount (4 : 1) of the undesired epimer. The dibenzyl derivative **11a** was subsequently converted by hydrogenolysis, followed by reaction with diazomethane to the methyl ester **11b**. The *R*-configuration of the newly generated chiral center in the azetidinone ring of **11b** was confirmed by its CD-spectrum, which had a negative COTTON effect ($\theta=-28{,}000$) at 214 nm, in agreement with the octant rule derived for monocyclic β-lactams by REHLING and JENSEN.[13] The structure of this key intermediate **11b** was further corroborated by a single crystal X-ray analysis. A perspective drawing of the molecule is shown in Fig. 2. Reduction of **11b** with sodium borohydride, followed by reaction of the intermediate diol with *p*-toluenesulfonyl chloride (4-dimethylaminopyridine, methylene chloride) afforded the ditosylate **12**. Reaction of **12** with lithium azide in DMF proceeded with selective replacement of the more reactive tosyl group in α-position to the carboxyl group; substitution of the primary tosyl was subsequently effected with lithium bromide in refluxing tetrahydrofuran, yielding **13**. Cyclization of **13** to the clavam **14** was brought about with lithium *tert*-butoxide in DMF. Hydrogenation of **14** (over platinum oxide) gave the unstable methyl ester **15**, diastereomeric with Ro 22-5417 at the two chiral centers in the ring system. The instability of **15** prevented its further use in subsequent experiments.

As expected, the CD-spectrum of the synthetic clavam **14** exhibited a strong positive COTTON effect at 230 nm ($\theta=+39{,}970$) in agreement with its *R* configuration at the ring juncture (Fig. 1). This contrasts with the spectrum of Ro 22-5417 and lends further support to the structure assignment made for the natural product.

The clavam **14** was tested for antimicrobial activity both in BBL seed agar[1] and Davis minimal agar.

Fig. 2. Stereodrawing of a molecule of **11b**.



The results are summarized in Table 1. Compound **14** showed weak activity only against *Bacillus* sp. in the minimal medium. As with Ro 22-5417, the inhibition was reversed by methionine. While Ro 22-5417 was an antifungal,[1] no such activity was observed with **14**. Most interestingly, contrary to Ro 22-5417, compound **14** showed significant activity as β-lactamase inhibitor in a cell free β-lactamase test (Table 2). In a whole cell test, **14** was weakly active with benzylpenicillin against the β-lactamase producing *Staphylococcus aureus* 1059B.

Thus, it appears that one of the structural requirements of clavams having β-lactamase inhibitory activity is the possession of *R*-configuration at the ring juncture, as is the case with clavulanic acid and compound **14**. *S*-Stereochemistry, as it is present in Ro 22-5417 and in **4** (R=$-CH_2CH_2OH$)[9] is essential for antifungal activity. A series of antifungal substances related to **4** (*e.g.* R=$-CH_2OH$ and $-CH_2$-OCHO) has previously been isolated from *S. clavuligerus*.[14] Although the absolute stereochemistry of

Table 1. Antimicrobial activity.

	Minimal inhibitory concentration* (μg/ml)			
	14		Ro 22-5417	
	BBL seed agar	Davis minimal agar	BBL seed agar	Davis minimal agar
Bacillus subtilis NRRL 558	>500	111	>1,000	0.25
Bacillus sp. ATCC 27860	>500	111	>1,000	0.03
Paecilomyces varioti ATCC 26820	>500	>1,000**	250	15.7**
Candida albicans NRRL 477	>500	>1,000***	>1,000	500***
Saccharomyces cerevisiae ATCC 4226	>500	>1,000**	>1,000	125**

* Lowest concentration still showing zone of inhibition by the agar-diffusion well method previously described.[3]

** Biotin and pantothenate added to Davis minimal agar at 1 μg/ml.

*** Biotin added to Davis minimal agar at 1 μg/ml.

Table 2. β-Lactamase inhibition.

Compound	I_{50}* (μg/ml)			
	Staphylococcus aureus	TEM-1	*Enterobacter cloacae*	*Proteus vulgaris*
14	4.0	33	>100	3
Sodium clavulanate Ro 13-1037/001	0.015	0.010	35	0.012
CP 45,899[16]	0.67	0.32	2.8	0.04
Ro 22-5417	>100	>100	>100	>100

* I_{50} is the concentration necessary to inhibit the rate of hydrolysis of the chromogenic cephalosporin, nitrocefin, by 50%. The test compound is preincubated with enzyme for 20 minutes at 30°C and pH 7. Nitrocefin is added and its initial rate of hydrolysis is recorded spectrophotometrically. Four crude enzyme preparations were employed: a) the inducible extracellular penicillinase from *Staphylococcus aureus* 1059B, b) the constitutive broad-spectrum TEM-1 β-lactamase from *Escherichia coli* 1269B, containing plasmid R-1, c) the type Ia cephalosporinase from *Enterobacter cloacae* purchased from Miles Laboratories, and d) the type Ic cephalosporinase from *Proteus vulgaris* 1028β-C.

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these substances has not been reported, it can now be safely inferred from their biological activity to be *S, S*. In contrast, the subsequently synthesized racemic modifications of these compounds had β-lactamase inhibitory activity as well,[6] undoubtedly owing to the presence of the "unnatural" *R,R*-isomer.

Experimental

General

Melting points were taken on a Kofler hot stage melting point apparatus (Reichert) and are uncorrected. Infrared (IR) and ultraviolet (UV) spectra were recorded on Digilab FTS 14 and Cary Model 14 spectrophotometers, respectively. ^{1}H NMR spectra were obtained on Varian XL-100 and XL-200 instruments. Chemical shifts are reported in parts per million downfield from internal tetramethylsilane. Mass spectra were obtained on a CEC-110 mass spectrometer. Rotations were measured on a Perkin-Elmer 141 polarimeter. CD spectra were recorded on a Durrum-Jasco Spectropolarimeter, Model ORD/CD/UV-5. Elemental analyses were carried out under the supervision of Dr. F. SCHEIDL (of our Microanalytical laboratory).

1,2-*O*-Isopropylidene-3-*O*-tosyl-D-xylofuranose (7)

A stirred solution of 200 g (1.05 mole) of 1,2-*O*-isopropylidene-D-xylofuranose in 1,500 ml of methylene chloride and 1,000 ml of pyridine was cooled under argon to −10°C and treated dropwise with a solution of 82.8 g of acetyl chloride in 200 ml of methylene chloride. The mixture was stirred for 15 hours at room temperature then cooled to 0°C and treated with 2.4 g (0.019 mole) of 4-(*N,N*-dimethylamino)pyridine and 300 g (1.57 mole) of *p*-toluenesulfonyl chloride. The reaction mixture was stirred at room temperature for 3 days. The solvents were evaporated *in vacuo*, and the residue taken up in methylene chloride and washed with 3 N HCl to pH 4. The water phase was back-extracted with methylene chloride and the combined organic phases were washed with brine and dried over magnesium sulfate. Evaporation under vacuum left a residue which was crystallized from ether and hexane to afford 310 g (76.5%) of pure 1,2-*O*-isopropylidene-3-*O*-tosyl-5-acetyl-D-xylofuranose; mp 107~108°C. The obtained material was suspended in 1,500 ml of dry methanol and treated under argon with 100 ml (0.1 mole) of 1 N sodium methoxide in methanol. After 10 minutes of stirring a clear solution was formed and the reaction was quenched after 60 minutes with 10 ml of acetic acid. The solvent was evaporated to dryness and the residue taken up in methylene chloride and washed with brine. The organic phase was evaporated at 45°C and the crude residue was crystallized from ether and hexane to afford 252 g (91.5%) of pure **7**. An analytical sample was recrystallized from ether: mp 87~88°C; $[\alpha]_D$ −22.6° (*c* 1,

EtOH); IR (KBr) 3515, 1596, 1497, 1369, 1178, 1095, 1069, 1043, 999, 850, 822 cm^{-1}; Mass 344 (M^+), 329, 313, 192, 155, 127, 113, 91, 85; ^1H NMR ($CDCl_3$) 7.84 (2H), 7.39 (2H), 5.90 (1H, d, J=4 Hz), 4.92 (1H, d, J=3 Hz), 4.67 (1H, d, J=4 Hz), 4.35 (1H, dt, J=3 Hz, J=8 Hz), 3.71 (3H, dd, J=4 Hz, J=6 Hz), 2.48 (3H, s), 2.23 (1H, s exchangeable), 1.48 (3H, s), 1.28 (3H, s).

Anal. Calcd. for $C_{15}H_{20}O_7S$: 344.38; C 52.32, H 5.85, S 9.31.
Found: C 52.13, H 5.82, S 9.13.

1,2-*O*-Isopropylidene-3-*O*-*p*-tosyl-*α*-D-xylonic Acid Methyl Ester (**8**)

A solution of 120 g (0.349 mole) of **7** in 2,750 ml of acetone was stirred at *ca.* 0°C under argon and 575 ml of a 2.67 M Jones reagent solution were added over a period of 3 hours. The reaction mixture was stirred at 0°C for an additional 5 hours and then 120 ml of 2-propanol were added to destroy the excess Jones reagent. The mixture was kept stirring at 0°C overnight. The precipitated chromium salts were filtered and washed with acetone. The solvent was evaporated at 30°C *in vacuo* and the residue was partitioned between ethyl acetate and water. The phases were separated and the water phase back-extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over magnesium sulfate, and evaporated *in vacuo*. The resulting solid residue was recrystallized from methylene chloride and methanol to afford 89 g (71%) of pure 1,2-*O*-isopropylidene-3-*O*-*p*-tosyl-*α*-D-xylonic acid; mp 188°C. Fifty grams (0.139 mole) of this acid was suspended in 80 ml of methanol and treated with 1.1 ml of boron trifluoride etherate. The xylonic acid derivative rapidly dissolves and the mixture was stirred overnight at room temperature. The formed precipitate was filtered off. The filtrate was treated with potassium carbonate and 10 ml of water to neutral pH. The solid was filtered off and on concentrating *in vacuo* another 13.5 g of ester precipitated. The combined crystals were washed with a minimum of cold methanol and dried to afford 46.1 g (89%) of pure **8**: mp 94~95°C; $[\alpha]_D$ −20.05° (*c* 1.12, MeOH); IR (KBr) 1768, 1375, 1180, 1045, 795 cm^{-1}; Mass 372 (M^+), 343, 172, 155, 113, 91; ^1H NMR ($CDCl_3$) 7.75 (2H), 7.37 (2H), 6.05 (1H, d, J=4 Hz), 5.11 (1H, d, J=3 Hz), 4.84 (1H, d, J=4 Hz), 4.76 (1H, d, J=3 Hz), 3.56 (3H, s), 2.45 (3H, s), 1.47 (3H, s), 1.30 (3H, s).

Anal. Calcd. for $C_{16}H_{20}O_8S$: 372.328; C 51.61, H 5.41, S 8.61.
Found: C 51.51, H 5.42, S 8.48.

1,2-*O*-Isopropylidene-3-desoxy-*α*-D-*glycero*-pent-3-ene Furanuronic Acid Methyl Ester (**9**)[16]

A solution of 42.2 g (0.113 mole) of **8** in 325 ml of chloroform under argon was cooled to −10°C and treated with 20.7 ml (0.136 mole) of 1,8-diazabicyclo[5.4.0]undec-7-ene. The reaction mixture was stirred at −10°C for 1 hour and at room temperature overnight. The solution was then washed with 4 portions of 100 ml of 5% $KHSO_4$, 100 ml of water and 100 ml of brine. The organic phase was dried over magnesium sulfate and evaporated *in vacuo* at 20°C to afford 23.7 g of **9** as an oil which rapidly polymerized upon standing. Material isolated from this elimination step was of sufficient quality so that purification for subsequent hydrogenation was not warranted. ^1H NMR ($CDCl_3$) 6.12 (1H, d, J=5 Hz), 6.06 (1H, d, J=3 Hz), 5.72 (2H, dd, J=3 Hz, J=5 Hz), 3.90 (3H, s), 1.45 (6H, s).

[2*R*-(2*α*,4*α*,5*α* and *β*)]-Tetrahydro-4-hydroxy-5-(phenylmethoxy)-2-furancarboxylic Acid Benzyl Ester (**10**)

A solution of 23.7 g (0.113 mole) of crude **9** in 450 ml of absolute ethanol was hydrogenated at atmospheric pressure in the presence of 0.75 g of 10% palladium on charcoal. The uptake of hydrogen was completed within 95 minutes. The catalyst was filtered off and the solvent evaporated *in vacuo*. The colorless oily residue was dissolved at room temperature in 250 ml of freshly prepared solution of benzyl alcohol containing 5% of HCl gas. The mixture was stirred under argon at room temperature overnight. The hydrochloric acid was pumped off *in vacuo* (10 mm of Hg) at 40°C and the benzyl alcohol removed at 95~110°C (0.5 mm of Hg) by Kugelrohr distillation. The oily residue was chromatographed over silica gel and the desired product eluted with a mixture of cyclohexane - ethyl acetate, 3:2 to afford 27 g (73%) of pure **10**. Liquid chromatography analysis showed that **10** consisted of a mixture of 80.8% of *β* anomer and 19.2% of *α* anomer: IR($CHCl_3$) 3450, 1735, 1200, 1070, 1025, 699 cm^{-1}; Mass 328 (M^+); ^1H NMR ($CDCl_3$) of the pure *β* anomer, 7.36 (5H, aromatic), 7.32 (5H, aromatic), 5.23 (3H, 1s, 1d, J=2 Hz), 4.72 (1H, d, J=11 Hz), 4.68 (1H, d, J=6 Hz), 4.43 (1H, d, J=11 Hz), 4.22 (1H, dd, J=2 Hz, J=6 Hz), 3.00 (1H, exchangeable, J=6 Hz), 2.64 (1H, m), 2.02 (1H, dd, J=1 Hz, J=6 Hz).

Anal. Calcd. for $C_{19}H_{20}O_5$: 328.36; C 69.50, H 6.14.
Found: C 69.40, H 6.18.

[2*R*-(2β-4β(*R*))]-Tetrahydro-4-[(4-oxo-2-azetidinyl)oxy]-5-(phenylmethoxy)-2-furancarboxylic Acid Benzyl Ester (**11a**)

A stirred solution of 24 g (0.073 mole) of **10** and of 1 g (0.0045 mole) of palladium acetate in 350 ml of benzene was treated under argon with a solution of 9.4 g (0.073 mole) of 4-acetoxy-2-azetidinone[12] and 7.35 g (0.073 mole) of triethylamine in 125 ml of benzene. After 6 hours a second portion of 4.7 g (0.036 mole) of 4-acetoxy-2-azetidinone and 3.8 g (0.037 mole) of triethylamine in 50 ml of benzene was added. After 24 hours of stirring the brown residue was filtered and washed with some ethyl acetate. The organic solution was washed with water, brine, dried over magnesium sulfate, and evaporated to dryness. The yellow oil was chromatographed over silica gel and elution with a mixture of ethyl acetate and cyclohexane (3 : 2) gave 20.7 g (71%) of pure **11a**: $[\alpha]_D^{25}$ −40.98° (*c* 1.02, MeOH); IR (film) 3260, 1767, 1748, 1105 cm^{-1}; Mass 397 (M^+), 220, 178, 160, 91; ^1H NMR ($CDCl_3$) 7.30 (5H, s, aromatic), 7.27 (5H, s, aromatic), 6.58 (1H, s, large exchangeable), 5.28 (1H, d, *J*=3 Hz), 5.20 (2H, s), 4.95 (1H, m), 4.74 (1H, d, *J*=11 Hz), 4.64 (1H, d, *J*=7 Hz), 4.50 (1H, d, *J*=11 Hz), 4.03 (1H, m), 3.05~2.10 (4H, m).

Anal. Calcd. for $C_{22}H_{23}NO_6$: 397.43; C 66.49, H 5.83, N 3.52.
Found: C 66.43, H 5.89, N 3.59.

[2*R*-(2β-4β(*R*),5α)]-Tetrahydro-5-hydroxy-4-[(4-oxo-2-azetidinyl)oxy]-2-furancarboxylic Acid Methyl Ester (**11b**)

A mixture of 20 g (0.050 mole) of **11a** and 4 g of 10% palladium over charcoal in 500 ml of absolute ethanol was pressurized at 3.5 kg/cm^2 under a hydrogen atmosphere and shaken for 36 hours. The catalyst was filtered, and washed. The filtrate was evaporated *in vacuo* and the residue dissolved in 100 ml of methanol and treated with a diazomethane solution until completion. The residue obtained after evaporation of the solvent was chromatographed over silica gel. Elution with a mixture of methylene chloride and acetone 1 : 1 gave 10.1 g of **11b**. Crystallization from ether and methylene chloride afforded 8.75 g (75%) of **11b**: mp 103~105°C; $[\alpha]_D^{25}$ −28.18°→+8.13° (*c* 1, MeOH); IR (KBr) 3330, 3285, 1758, 1745 cm^{-1}; Mass 231 (M^+); ^1H NMR (DMSO-d_6) 8.60 (1H, s, large exchangeable), 6.59 (1H, d, *J*=5 Hz, exchangeable), 5.28 (1H, d, *J*=4 Hz), 5.08 (1H, d, *J*=3 Hz), 4.54 (1H, dd, *J*=4 Hz, *J*=6 Hz), 3.89 (1H, dd, *J*=2 Hz, *J*=6 Hz), 3.64 (3H, s), 3.04 (1H, dt, *J*=14 Hz, *J*=3 Hz), 2.6~2.5 (2H, 2m), 2.01 (1H, m).

Anal. Calcd. for $C_9H_{13}NO_6$: 231.20; C 46.75, H 5.67, N 6.06.
Found: C 46.84, H 5.58, N 6.03.

Methyl 4-(2-Oxo-4-azetidinyloxy)-2,5-bis(*p*-tosyloxy)pentanoate (**12**)

A stirred solution of 6.95 g (0.03 mole) of **11b** in 300 ml of absolute methanol was cooled to 0°C and under argon atmosphere was treated with 415 mg (0.011 mole) of sodium borohydride for 75 minutes. The reaction mixture was neutralized with 3% HCl in methanol and evaporated to dryness. The crude was taken up in a minimum amount of acetone and chromatographed over silica gel. Elution with a mixture of chloroform and acetone (1 : 2) afforded 5.8 g of pure diol (83%).

A suspension of 5.75 g (24.6 mmole) of diol in 200 ml of methylene chloride and 50 ml of acetone was treated with 6.0 g (49 mmole) of 4-(*N*,*N*-dimethylamino)pyridine and cooled to 0°C. To the stirred solution was added 10 g (52.5 mmole) of freshly recrystallized *p*-toluenesulfonyl chloride. The mixture was stirred overnight at room temperature and acidified to pH 4. Washing with water, extraction with methylene chloride, and evaporation afforded 10 g of crude. Chromatographic separation over silica gel yielded 5.90 g (44%) of ditosylate **12** which was recrystallized from ether: mp 107~108°C; $[\alpha]_D$ +5.68° (*c* 0.986, $CHCl_3$); IR(KBr) 3400, 3365, 3345, 1763, 1744, 1357, 1188, 1178 cm^{-1}; Mass 541 (M^+), 524, 499, 498, 486; ^1H NMR 7.81 (4H, 2d, A, B), 7.38 (4H, AB), 6.80 (1H, s, large exchangeable), 5.16 (1H, d, *J*=3 Hz), 5.13 (1H, d, *J*=4 Hz), 4.01 (2H, m), 3.97 (1H, m), 3.58 (3H, s), 3.08 (1H, dt, *J*=14 Hz, *J*=3 Hz), 2.75 (1H, d, *J*=14 Hz), 2.47 (6H, s), 2.04~1.80 (2H, m).

Anal. Calcd. for $C_{23}H_{27}NO_{10}S_2$: 541.59; C 51.01, H 5.03, N 2.59, S 11.84.
Found: C 51.08, H 5.05, N 2.67, S 12.05.

Methyl 4-(2-Oxo-4-azetidinyloxy)-2-azido-5-bromopentanoate (**13**)

A solution of 1.15 g (2.12 mmole) of ditosylate **12** in 25 ml of *N*,*N*-dimethylformamide was treated at room temperature with 110 mg (2.2 mmole) of lithium azide for 6 hours. After evaporation of the solvent under reduced pressure the residue was taken up in ethyl acetate, washed with water, and dried. Chromatography over silica gel (elution with methylene chloride - acetone, 95:5) afforded 810 mg (93%) of secondary azide which was dissolved in 25 ml of dry tetrahydrofuran and treated with 190 mg (2.2 mmole) of lithium bromide. The reaction mixture was heated at reflux for 6 hours. After evaporation of the solvent the residue was taken into ethyl acetate and washed with water. Drying over magnesium sulfate and evaporation gave an oil which was purified by chromatography to afford 560 mg (82%) of pure **13**; IR($CHCl_3$) 3415, 2115, 1775, 1745 cm^{-1}; Mass 322, 320 (M^+); ^1H NMR 6.47 (1H, s, large exchangeable), 5.06 (1H, d, J=4 Hz), 4.16 (1H, t, J=6 Hz), 4.05 (1H, m), 3.79 (3H, s), 3.55 (2H, m), 3.12 (1H, dt, J=15.5 Hz, J=4 Hz), 2.74 (1H, d, J=15.5 Hz), 1.98 (2H, m).

3-(7-Oxo-1-aza-4-oxabicyclo[3.2.0]hept-3-yl)-2-azidopropanoic Acid Methyl Ester (**14**)

A solution of 560 mg (1.74 mmole) of **13** in 15 ml of dry *N*,*N*-dimethylformamide was treated at −20°C with 135 mg (1.68 mmole) of lithium *tert*-butoxide for 60 minutes. The mixture was poured into 100 ml of ethyl acetate and washed with water. The organic phase was dried and evaporated. The residue was chromatographed over silica gel (elution with methylene chloride - acetone, 98:2) to afford 28.5 mg of pure **14**; IR($CHCl_3$) 2115, 1783, 1748, 1430, 1095, 1035 cm^{-1}; Mass 240 (M^+); ^1H NMR ($CDCl_3$) 5.29 (1H, d, J=3 Hz), 4.43 (1H, m), 4.12 (1H, dd, J=6 Hz, J=11 Hz), 3.83 (3H, s), 3.30 (1H, dt, J=16 Hz, J=3 Hz), 2.82 (1H, d, J=16 Hz), 2.70 (1H, dd, J=6 Hz, J=11 Hz), 2.10 (2H, m).

3-(7-Oxo-1-aza-4-oxabicyclo[3.2.0]hept-3-yl)alanine Methyl Ester (**15**)

A solution of 5 mg of **14** in 10 ml of ethyl acetate was hydrogenated over 2 mg of platinum oxide for 45 minutes. The catalyst was removed by filtration. TLC showed the formation of a more polar product. Evaporation of the solvent was already accompanied by some decomposition. ^1H NMR ($CDCl_3$): 5.28 (1H, m), 4.72 (1H, m), 4.44 (1H, m), 4.04 (1H, m), 3.78 (3H, s), 3.30 (1H, dd), 2.84 (1H, dd), 2.66 (1H, m), 2.10 (2H, m), 1.90 (2H, D_2O-exchangeable).

Crystallography

Crystals of **11b** are monoclinic, space group $P2_1$, a=7.603(1), b=12.295(2), c=5.646(1)Å, β=94.86(1)°, and D_{calcd}=1.460 g cm^{-3} for Z=2 ($C_9H_{13}NO_6$, M=231.20). The intensity data were measured on a Hilger-Watts diffractometer (Ni-filtered CuKα radiation, 0-2θ scans, pulse height discrimination). The size of the crystal used for data collection was approximately 0.30×0.65×0.8 mm. Of the 749 independent reflections for θ<57°, 747 were considered to be observed [I>2.5σ(I)]. The structure was solved by a multiple solution procedure and was refined by full matrix least squares. Nine reflections which were strongly affected by extinction were excluded from the final refinement and difference map. In the final refinement, anisotropic thermal parameters were used for the non-hydrogen atoms and isotropic temperature factors were used for the hydrogen atoms. The hydrogen atoms were included in the structure factor calculations but their parameters were not refined. The final discrepancy indices are R=0.038 and wR=0.054 for the remaining 738 observed reflections. The final difference map has no peaks greater than ±0.2 e A^{-3}.

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