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Studies on the Syntheses of Heterocyclic Compounds. 800.[1] A Formal Total Synthesis of (±)-Thienamycin and a (±)-Decysteaminylthienamycin Derivative

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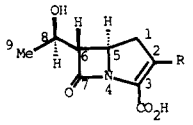
Abstract: The synthesis of a key intermediate for the preparation of (±)-thienamycin (**1**) and its derivatives has been developed. By 1,3-dipolar cycloaddition, the nitrile oxide derived from 3-nitropropanal dimethyl acetal (**3**) was added to methyl crotonate to give selectively *trans*-4-methoxycarbonyl-3-(2′,2′-dimethoxyethyl)-5-methylisoxazoline (**5**). Catalytic reduction of **5** yielded a stereoisomeric mixture of the amino esters **7**, hydrolysis of which followed by treatment with dicyclohexylcarbodiimide gave mainly two trans azetidinones **11** and **12**, together with a small amount of the cis isomer. On the other hand, reaction of **7** with methylmagnesium iodide yielded the desired trans azetidinone **11** along with a trace of the cis isomer **15**. The stereochemistry of the 8*S**-trans isomer **12** was confirmed by X-ray analysis of its derivative **21**. The 8*R**-trans compound **11** was protected with the *p*-nitrobenzyloxycarbonyl group and then converted to the alcohol **17** and to the thioacetal **19**, which had already been correlated to (±)-thienamycin. After protection of **12** with the *o*-nitrobenzyloxycarbonyl group, the acetal **24** was converted to the (±)-8*S**-decysteaminylthienamycin derivative **27** in several steps, involving an intramolecular Wittig reaction.

Thienamycin (**1**) was isolated from fermentation broths of the soil microorganism *Streptomyces cattleya* by a Merck research group.[2,3] It is a β-lactam antibiotic with the carbapenem structure having highly desirable antibacterial activity; activity is relatively high against Gram-positive bacteria and extends over the full range of Gram-negative bacteria, including *Pseudomonas aeruginosa*. Four epithienamycins were recently found in broths of *S. flavogriseus*.[4,5] It has also been found that decysteaminylthienamycin (**2**), derived from



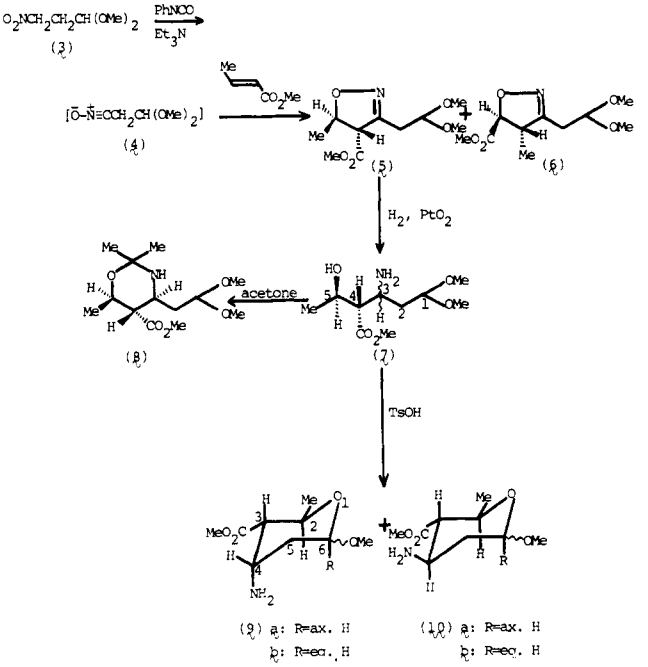
(1) R=SCH$_2$CH$_2$NH$_2$
(2) R=H

thienamycin, has antibacterial activity as potent as that of thienamycin itself.[6] (±)-Thienamycin (**1**)[7] and the derivative **2**[8] have been totally synthesized through the same intermediate by the Merck group. We here report a facile synthesis of the key synthetic intermediate to (±)-thienamycin and the decysteaminylthienamycin derivative, through a trans isoxazoline derivative **5**.

Formal Total Synthesis of (±)-Thienamycin

1,3-Dipolar cycloaddition of the nitrile oxide derived from 3-nitropropanal dimethyl acetal (**3**)[9] and methyl crotonate was carried out in benzene solution[10] as described in the Experimental Section. The product consisted mainly of *trans*-4-methoxycarbonyl-3-(2′,2′-dimethoxyethyl)-5-methylisoxazoline (**5**) together with a small amount of the regioisomer **6**, and these were easily separated by distillation followed by column chromatography. Preferential formation of **5** from 1,3-dipolar cycloaddition of the nitrile oxide **4** and methyl crotonate was expected from Huisgen's report[11] and from application of Houk's molecular orbital perturbation treatment.[12] Because the stereochemical relationship between the C_6 and C_8 positions of thienamycin is already set up in the isoxazoline **5**, it should be possible to synthesize thienamycin with the correct stereochemistry from **5**, if the intervening reaction proceeds with retention of the relative configuration.

Reduction of **5** using Adams' catalyst in acetic acid under 4.5–6 atoms of hydrogen yielded quantitatively a stereoisomeric mixture of the two amino esters **7** in a ratio of 3:2, which



(9) a: R=ax. H b: R=eq. H
(10) a: R=ax. H b: R=eq. H

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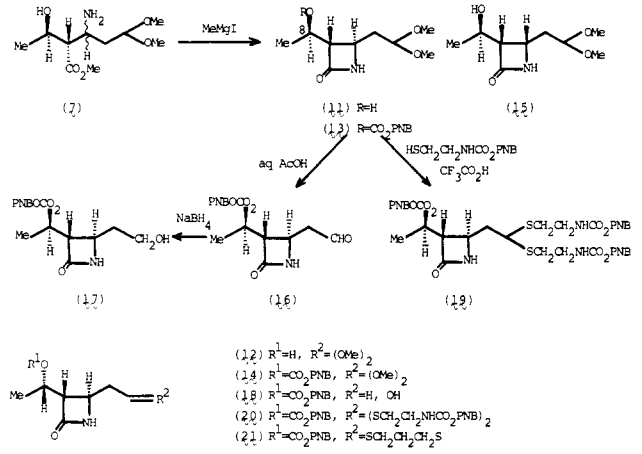
were separable by preparative TLC. The stereochemistries of the products were determined from the following two reactions. On standing **7** in acetone at room temperature, only the less polar isomer reacted quickly with acetone to give the oxazine **8,** while the more polar one reacted very slowly. The all-trans configuration **8** was indicated by the NMR spectral data, namely, a triplet with J = 10 Hz due to the C_5 proton with a double quartet, J = 6 and 10 Hz, attributed to the C_6 proton. Further, treatment of the amino esters **7** with *p*-toluenesulfonic acid in methylene chloride at room temperature afforded, in excellent yield, a mixture of the four cyclic acetals **9** and **10** which was partially separated. In the NMR spectra, both epimers **9a** and **9b** showed C_2 and C_3 methine proton resonances as double quartets (J = 6 and 10 Hz) and double doublets (J = 3 and 10 Hz), respectively, while the epimeric mixture **10,** which could not be separated, displayed these protons as double quartets (J = 6 and 10 Hz) and triplets (J = 10 Hz), respectively. The above results indicated that the stereochemical relationship between the C_4 and C_5 positions of **7** was unchanged from that in **5** as a result of catalytic reduction. The major and less polar isomer of **7** was converted to **8** and **9** by the above reactions.

The epimeric mixture of **7** was hydrolyzed with 1 equiv of sodium hydroxide in refluxing aqueous methanol for 4.5 h. After neutralization with sulfuric acid, the resulting free amino acid was heated with dicyclohexylcarbodiimide[13] in aqueous dioxane for 12 h. Silica gel column chromatography of the product afforded two azetidinone fractions. The minor fraction, obtained in 0.8% yield from the isoxazoline **5,** was assumed to be the cis-substituted azetidinone, while the major one, obtained in 44.7% yield from **5,** was composed of the two trans ones **11** and **12,** which were not separable by ordinary chromatographic techniques. The stereostructures of **11** and **12** were determined by 200-MHz NMR spectroscopy (see Experimental Section) after conversion of the mixture of the alcohols **17** and **18** and to the thioacetals **19** and **20.** These derivatives were prepared as follows. The hydroxyl groups of the mixture of **11** and **12** were protected with the *p*-nitrobenzyloxycarbonyl group by reaction with the corresponding chloride in the presence of pyridine or 4-*N*,*N*-dimethylaminopyridine. Deacetalization of the resulting acetals **13** and **14** (obtained in 85% yield) with aqueous acetic acid at 60 °C, followed by reduction with sodium borohydride, furnished the alcohols **17** and **18** in 90% yield. Reaction of the mixture of **13** and **14** with excess *N*-*p*-nitrobenzyloxycarbonylcysteamine in dry trifluoroacetic acid[14] at room temperature yielded the thioacetals **19** and **20** in 86% yield.



The structure of the so-called 8*S**-trans azetidinone[18] **12**[15] was established by conversion to the propanedithioacetal **21,** followed by X-ray analysis. Crystals of **21** were orthorhombic with four molecules in a unit cell of dimensions a = 17.712, b = 19.944, c = 5.793 Å, space group $P2_12_12_1$. The stereoview of **21** at the R index 0.037 is shown in Figure 1.

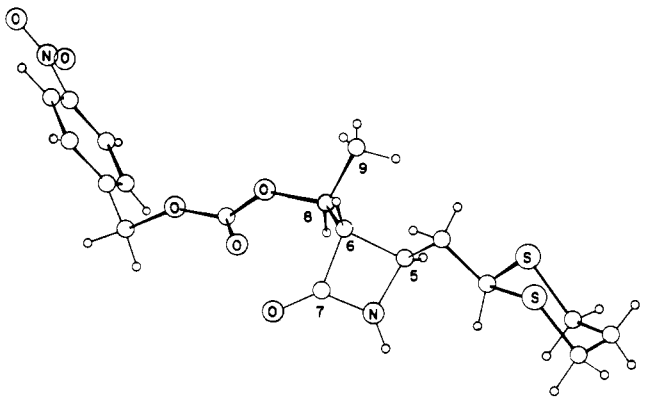
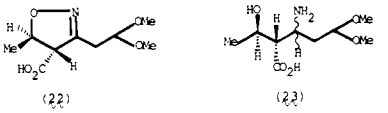


Figure 1. Structure of **21** as shown by X-ray analysis.

The ratio of the trans azetidinones (the desired **11** and its epimer **12**) produced in the above hydrolysis and DCC cyclization sequence from amino esters **7** varied according to the reaction conditions. Under the conditions already described the 8*S** isomer **12** was by far the main product. However, by using milder conditions in both steps, namely, hydrolysis of **7** at room temperature for 6 h and DCC cyclization at 100 °C for 5 h, the proportion of the desired 8*R**-trans azetidinone **11** increased with the ratio **11:12** being approximately 1:2.5.

Hydrolysis of isoxazoline **5** with sodium hydroxide in aqueous methanol at room temperature, followed by catalytic hydrogenation of the resulting acid **22** with Adams' catalyst in acetic acid, gave the epimeric mixture of the amino acids **23.**



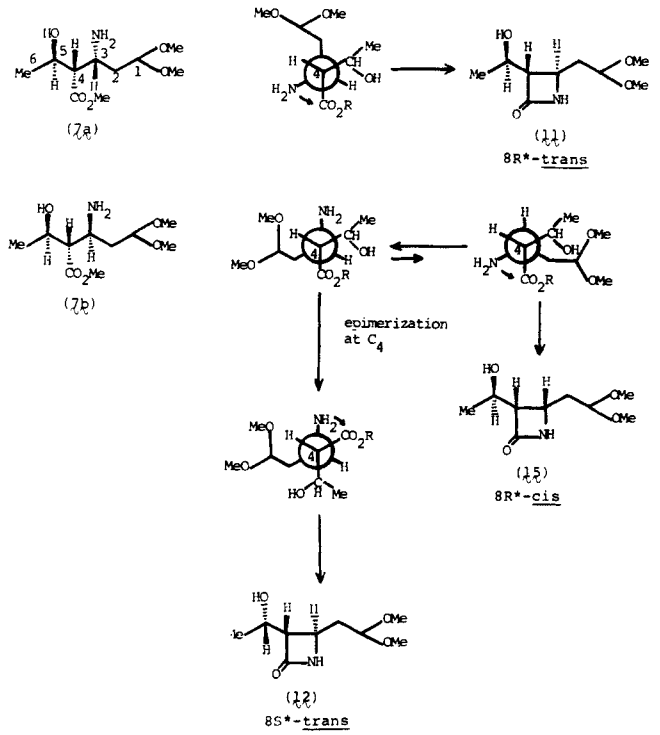
(22) (23)

Cyclization with dicyclohexylcarbodiimide in hot aqueous dioxane afforded a mixture of the trans azetidinones **11** and **12** in almost the same ratio as above, along with a trace amount of the cis isomers. From the above results it is considered that the abominable epimerization occurred during both procedures, the hydrolysis of **7** at high temperature and β-lactam formation using dicyclohexylcarbodiimide. Namely, the polar amino ester **7a** should be converted to the desired 8*R**-trans azetidinone **11,** if epimerization did not occur. On the other hand, cyclization of the epimer **7b** to the cis isomer **15** would be difficult because of the steric effect. With epimerization at the C_4 position, **7b** gives rise to the 8*S**-trans one **12.**

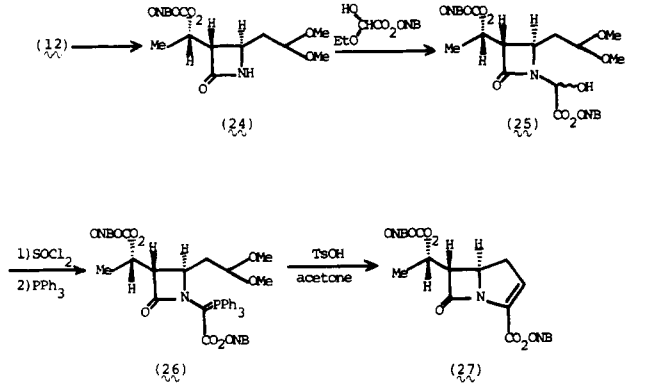
Ring closure of the amino ester **7** was examined by different methods which excluded both the aforementioned procedures. Reaction of the mixture of **7** with methylmagnesium iodide[16] in a mixture of ether and tetrahydrofuran at room temperature for 16 h yielded the desired trans azetidinone **11,** together with a trace amount of the cis isomer **15,** whose spectral data and chromatographic behavior were identical with those of the product formed using dicyclohexylcarbodiimide. The trans azetidinone **11** was converted, according to the same procedure as above, to the alcohol **17,** the NMR spectrum of which was identical with that of an authentic sample provided by Dr. Christensen (Merck Sharp and Dohme Research Laboratories). Furthermore, **11** was transformed, as above, to the thioacetal **19,** an epimeric mixture of which has already been correlated to (±)-thienamycin.[7] Separation problems as encountered in the DCC route to **19** are thus avoided by the adoption of this modification.

Synthesis of Decysteaminylthienamycin Derivative

The Merck group converted a derivative of alcohol **17,** which was protected with the *o*-nitrobenzyloxycarbonyl group instead



of the *p*-nitrobenzyloxycarbonyl group, to (±)-decysteaminylthienamycin (**2**) by several steps involving an intramolecular Wittig reaction.[8] Our synthetic intermediate has some advantage over **17** in that the β-lactam **11** has a protected aldehyde group. From both chemical and pharmaceutical interest, transformation of the 8*S** trans azetidinone **12** to the decysteaminylthienamycin derivative was investigated. Reaction of **12** with *o*-nitrobenzyl chloroformate in the presence of 4-*N*,*N*-dimethylaminopyridine in methylene chloride at −5 to 0 °C gave the protected acetal **24** in 84% yield. Condensation of **24** with *o*-nitrobenzyl glyoxylate ethyl hemiacetal,[8] using



activated molecular sieves (3 Å)[17] in dimethylformamide and toluene at room temperature for 24 h, yielded an epimeric mixture of the alcohols **25** in 69% yield. On reaction with thionyl chloride and 2,6-lutidine in tetrahydrofuran at −20 °C, the alcohols **25** gave the corresponding chloro compound, which without purification was converted to the phosphorane **26** in 74% yield after purification by silica gel column chromatography. Deacetalization was carried out using *p*-toluenesulfonic acid in acetone at room temperature for 2 h, and on evaporation of the solvent and neutralization with saturated aqueous sodium hydrogen carbonate spontaneous intramolecular Wittig reaction occurred to give *o*-nitrobenzyl 6-((8*S**)-*o*-nitrobenzyloxycarbonyloxyethyl)-1-carba-2-penem-3-carboxylate (**27**), which is a synthetic precursor of (±)-(8*S**)-decysteaminylthienamycin.[8] Pharmaceutical evaluation of this intermediate **27** is being carried out.

Experimental Section

Melting points were determined on a Yanaco micro melting point apparatus and are uncorrected. IR spectra were obtained with a Hitachi 215 spectrometer, 60-MHz NMR spectra with a JNM-PS-60, 200-MHz NMR with a Varian XL-200 spectrometer (tetramethylsilane as internal reference), and mass spectra with Hitachi M-52G and JMS-01SG-2 spectrometers. Diffraction data were collected on a Phillips four-circle diffractometer.

***trans*-4-Methoxycarbonyl-3-(2′,2′-dimethoxyethyl)-5-methylisoxazoline (5) and *trans*-5-Methoxycarbonyl-3-(2′,2′-dimethoxyethyl)-4-methylisoxazoline (6).** A mixture of 6 g (40.3 mmol) of 3-nitropropanal dimethyl acetal (**3**),[9] 6.1 g (60.1 mmol) of methyl crotonate, 9.6 g (80.6 mmol) of phenyl isocyanate, and several drops of Et_3N in 100 mL of dry benzene was stirred at room temperature for 24 h and refluxed for 5 h under a current of nitrogen. After filtration of the reaction mixture, the filtrate was evaporated under reduced pressure to give a brownish liquid. Distillation of the liquid (bp 80–120 °C (0.025 mm)) afforded a reddish liquid which was further purified by silica gel column chromatography using benzene–ether (9:1 v/v) as eluent. From the first fraction 5 g (53.8%) of isoxazoline **5** was obtained as a pale reddish liquid: IR ν_{max} ($CHCl_3$) 1735 cm^{-1} (C=O); NMR (CCl_4) δ 1.36 (3 H, d, J = 6 Hz, C_5 Me), 2.72 (2 H, d, J = 5.5 Hz, >CHCH_2C=N), 3.30, 3.36 (each 3 H, each s, 2 OMe), 3.76 (3 H, s, CO_2Me), 4.60 [1 H, t, J = 5.5 Hz, CH($OMe)_2$], 4.80 (1 H, m, C_5 H); MS *m/e* 246 (M^+ + 15), 232 (M^+ + 1). Anal. Calcd for $C_{10}H_{17}NO_5$: C, 51.94; H, 7.41; N, 6.06. Found: C, 51.90; H, 7.39; N, 6.26.

Further elution afforded 2 g (21.5%) of isoxazoline **6**: IR ν_{max} ($CHCl_3$) 1740 cm^{-1} (C=O); NMR (CCl_4) δ 1.29 (3 H, d, J = 6 Hz, C_4 Me), 2.42, 2.73 (each 1 H, each dd, J = 15 and 5.5 Hz, >CHCH_2C=N), 3.33 (6 H, s, 2 OMe), 3.77 (3 H, s, CO_2Me), 4.44 (1 H, d, J = 6 Hz, C_5H), 4.57 [1 H, t, J = 5.5 Hz, CH($OMe)_2$]; MS *m/e* 246 (M^+ + 15), 232 (M^+ + 1). Anal. Calcd for $C_{10}H_{17}NO_5$: C, 51.94; H, 7.41; N, 6.06. Found: C, 52.10; H, 7.48; N, 5.99.

Methyl 3-Amino-5-hydroxy-1,1-dimethoxyhexane-4-carboxylate (7). A mixture of 5 g (21.6 mmol) of the isoxazoline **5** and 500 mg of PtO_2 in 150 mL of AcOH was stirred at room temperature under a current of hydrogen (4.5 atm) for 3 days. After filtration and washing of the solid with AcOH, evaporation of the combined filtrates gave a pale yellowish syrup which was dissolved in $CHCl_3$. The $CHCl_3$ solution was washed with 10% NH_4OH and brine, dried over Na_2SO_4, and evaporated to afford 5.08 g of the two stereoisomers of the amino ester **7** as a pale yellowish syrup: IR ν_{max} ($CHCl_3$) 1720 cm^{-1} (C=O); NMR ($CDCl_3$) δ 2.83 (2 H, bs, NH_2), 3.33 (6 H, s, 2 OMe), 3.70 (3 H, s, CO_2Me); MS *m/e* 236 (M^+ + 1). Anal. Calcd for $C_{10}H_{21}NO_5$: C, 51.05; H, 9.00; N, 5.95. Found: C, 51.30; H, 9.13; N, 6.13.

(±)-3,4,5,6-Tetrahydro-5α-methoxycarbonyl-4β-(2′,2′-dimethoxyethyl)-2,2,6β-trimethyloxazine (8). A solution of 521 mg (2.21 mmol) of the amino ester **7** in 20 mL of acetone was stirred at room temperature for 1 h. The acetone was evaporated and the oily residue was chromatographed on 20 g of silica gel, using benzene–acetone (15:1 v/v) as eluent, to give 367 mg (60%) of the acetonide **8** as a colorless oil: IR ν_{max} ($CHCl_3$) 1725 cm^{-1} (C=O); NMR ($CDCl_3$) δ 1.08 (3 H, d, J = 6 Hz, Me), 1.33 (3 H, s, Me), 1.40 (3 H, s, Me), 1.81 (1 H, t, J = 10 Hz, C_5 H), 3.27 (6 H, s, 2 OMe), 3.67 (3 H, s, CO_2Me), 4.00 (1 H, dq, J = 6 and 10 Hz, C_6 H), 4.67 [1 H, dd, J = 4 and 8 Hz, CH$(OMe)_2$]; MS *m/e* 276 (M^+ − 1).

Reaction of Methyl 3-Amino-5-hydroxy-1,1-dimethoxyhexane-4-carboxylate (7) with *p*-Toluenesulfonic Acid. A mixture of 1.14 g of the amino ester **7** and 1.0 g of *p*-toluenesulfonic acid in 50 mL of CH_2Cl_2 was stirred for 2 h at room temperature. After addition of a saturated $NaHCO_3$ solution under cooling with ice, the separated organic layer was washed with brine, dried over Na_2SO_4, and evaporated. The resulting residue was chromatographed on silica gel. Elution with AcOEt–benzene–MeOH (30:20:1 v/v) gave 220 mg of the cyclic acetal **9a** as a colorless syrup: NMR ($CDCl_3$) δ 1.27 (3 H, d, J = 6 Hz, Me), 2.42 (1 H, dd, J = 3 and 10 Hz, C_3 H), 3.47 (3 H, s, OMe), 3.68 (3 H, s, CO_2Me), 4.18 (1 H, dq, J = 6 and 10 Hz, C_2 H), 4.77 (1 H, dd, J = 3 and 10 Hz, C_6 H); MS *m/e* 204 (M^+ + 1).

Further elution with AcOEt–benzene–MeOH (30:20:1 v/v) gave 150 mg of **9b** as a colorless syrup: NMR ($CDCl_3$) δ 1.23 (3 H, d, J = 6 Hz, Me), 2.47 (1 H, dd, J = 3 and 10 Hz, C_3 H), 3.35 (3 H, s, OMe), 3.70 (3 H, s, CO_2Me), 4.20 (1 H, dq, J = 6 and 10 Hz, C_2 H), 4.75 (1 H, t, J = 3 Hz, C_6 H); MS *m/e* 204 (M^+ + 1).

Further elution with AcOEt–benzene–MeOH (60:40:3 v/v) gave

250 mg of a mixture of **10a** and **10b** as a colorless syrup: NMR ($CDCl_3$) δ 2.05 (1 H, t, J = 10 Hz, C_3 H); MS m/e 204 ($M^+ + 1$).

***trans*-3-(2',2'-Dimethoxyethyl)-5-methylisoxazoline-4-carboxylic Acid (22).** A mixture of 1.155 g (5 mmol) of the isoxazoline **5,** 115 mg (5 mmol) of Na, and several drops of H_2O in 20 mL of MeOH was stirred for 2 h at room temperature. After evaporation of the solvent the residue was dissolved in H_2O and washed with ether. The H_2O layer was acidified to pH 3 with dilute H_2SO_4 and extracted with $CHCl_3$. The $CHCl_3$ layer was washed with brine, dried over Na_2SO_4, and evaporated to afford 1.085 g of the carboxylic acid **22** as a syrup: IR ν_{max} ($CHCl_3$) 1720 cm^{-1} (C=O); NMR ($CDCl_3$) δ 1.43 (3 H, d, J = 6 Hz, C_5 Me), 2.87 (2 H, bd, J = 5.5 Hz, $CHCH_2C$=N), 3.36 (3 H, s, OMe), 3.39 (3 H, s, OMe), 3.78 (1 H, d, J = 7.5 Hz, C_4 H), 4.68 [1 H, t, J = 5.5 Hz, $CH(OMe)_2$], 4.96 (1 H, m, C_5 H), 7.33 (1 H, bs, CO_2H); MS m/e 232 ($M^+ + 15$), 218 ($M^+ + 1$). This carboxylic acid **22** proved to be unstable and was used in the next reaction without further purification.

***trans*- and *cis*-3-(1'-Hydroxyethyl)-4-(2',2'-dimethoxyethyl)-2-azetidinones (11, 12, and 15). A.** A mixture of 2.35 g (0.01 mol) of the above amino ester **7,** 0.23 g (0.01 mol) of Na, and several drops of H_2O in 15 mL of MeOH was refluxed for 4.5 h. After evaporation of the solvents the residue was dissolved in H_2O and washed with ether. The H_2O layer was neutralized with dilute H_2SO_4 and evaporated to give a solid which was extracted with EtOH. After evaporation of the solvent the resulting residue was dissolved in 60 mL of dioxane and 30 mL of H_2O, and 2.06 g (0.01 mol) of *N,N'*-dicyclohexylcarbodiimide added. The reaction mixture was stirred under reflux for 12 h. After evaporation of the solvent the residue was dissolved in H_2O, washed with ether, and evaporated to yield a syrup. Purification by silica gel column chromatography using benzene-MeOH (97:3 v/v) as eluent gave 16 mg (0.8%) of the cis azetidinone **15** as a colorless syrup [IR ν_{max} ($CHCl_3$) 3450 (NH), 1758 cm^{-1} (C=O); NMR ($CDCl_3$) δ 1.42 (3 H, d, J = 6.5 Hz, $C_{1'}$ Me), 3.21 (1 H, dd, J = 10 and 4.5 Hz, C_3 H), 3.42 (6 H, s, 2 OMe), 6.12 (1 H, bs, NH); MS m/e 204 ($M^+ + 1$)] and 908 mg (44.7%) of a mixture of trans azetidinones **11** and **12** [IR ν_{max} ($CHCl_3$) 3450 (NH), 1758 cm^{-1} (C=O); NMR ($CDCl_3$) δ 1.33 (3 H, d, J = 6.5 Hz, $C_{1'}$ Me), 1.98 [2 H, dd, J = 5 and 7 Hz, $CH_2CH(OMe)_2$], 2.92 (1 H, dd, J = 6 and 2 Hz, C_3 H), 3.35 (6 H, s, 2 OMe), 3.66 (1 H, td, J = 7 and 2 Hz, C_4 H), 4.15 (1 H, qd, J = 6.5 and 6 Hz, CHOH), 4.51 [1 H, t, J = 5 Hz, $CH(OMe)_2$], 6.90 (1 H, bs, NH); MS m/e 204 ($M^+ + 1$); m/e 204.1213 (calcd for $C_9H_{18}NO_4$ ($M^+ + 1$), m/e 204.1235)].

B. A mixture of 2.35 g (0.01 mol) of the amino ester **7,** 0.23 g (0.01 mol) of Na, and several drops of H_2O in 15 mL of MeOH was stirred for 6 h at room temperature. The same workup as above afforded a syrup which was dissolved in 60 mL of dioxane and 30 mL of H_2O, and 2.06 g (0.01 mol) of *N,N'*-dicyclohexylcarbodiimide added. The reaction mixture was refluxed for 5 h. The same workup and purification procedures as above gave 457 mg of the trans azetidinones **11** and **12** and 16 mg of the cis azetidinone **15.**

C. A mixture of 1.085 g (5 mmol) of the carboxylic acid **22** and 100 mg of PtO_2 in 50 mL of AcOH was stirred at room temperature under a current of hydrogen (4.5 atm) for 2 days. After filtration and washing of the solid with AcOH, evaporation of the combined filtrates gave a pale yellowish syrup which was dissolved in $CHCl_3$ and basified with 10% NH_4OH. After evaporation of the solvent at 100 °C under reduced pressure the residue was dissolved in 40 mL of dioxane and 20 mL of H_2O, and 1.236 g (6 mmol) of *N,N'*-dicyclohexylcarbodiimide added. The reaction mixture was stirred for 24 h at room temperature. The same workup and purification procedures as above gave 228 mg of the trans azetidinones **11** and **12** and 8 mg of the cis azetidinone.

***trans*-4-(2',2'-Dimethoxyethyl)-3-(1'-*p*-nitrobenzyloxycarbonyloxyethyl)-2-azetidinone (13 and 14).** To a mixture of 203 mg (1 mmol) of the azetidinones **11** and **12** and 244 mg (2 mmol) of 4-*N,N*-dimethylaminopyridine in 1 mL of dry CH_2Cl_2 at 0 °C was added a solution of 238 mg (1.1 mmol) of *p*-nitrobenzyl chloroformate in 0.5 mL of dry CH_2Cl_2 under a current of nitrogen. The resulting reaction mixture was stirred at 0 °C for 1 h. The mixture was poured into 50 mL of CH_2Cl_2, washed with H_2O, dried over Na_2SO_4, and evaporated to afford a brownish syrup which was purified by silica gel column chromatography using benzene-MeOH (99:1 v/v) as eluent to give 325 mg (85%) of the azetidinones **13** and **14:** IR ν_{max} ($CHCl_3$) 3450 (NH), 1760, 1750 (C=O), 1345 cm^{-1} (NO_2); NMR ($CDCl_3$) δ 1.47 (3 H, d, J = 6.5 Hz, $C_{1'}$ Me), 1.97 [2 H, dd, J = 5 and 7 Hz, $CH_2CH(OMe)_2$], 3.18 (1 H, dd, J = 6 and 2 Hz, C_3 H), 3.36 (6 H, s, 2 OMe), 3.67 (1 H, td, J = 7 and 2 Hz, C_4 H), 4.50 [1 H, t, J = 5 Hz, $CH(OMe)_2$], 5.04-5.53 (1 H, m, CHOH), 5.33 (2 H, s, CH_2Ar), 6.26 (1 H, bs, NH), 7.66 (2 H, d, J = 9 Hz, 2 ArH), 8.34 (2 H, d, J = 9 Hz, 2 ArH); MS m/e 383 ($M^+ + 1$); m/e 383.1482 (calcd for $C_{17}H_{23}N_2O_8$ ($M^+ + 1$), m/e 383.1454).

***trans*-4-(2'-Hydroxyethyl)-3-(1'-*p*-nitrobenzyloxycarbonyloxyethyl)-2-azetidinones (17 and 18). A.** A mixture of 38 mg (0.1 mmol) of the acetal azetidinones **13** and **14,** derived from the trans azetidinones **11** and **12** prepared by method A, in 0.4 mL of AcOH and 0.1 mL of H_2O was heated at 60 °C for 5 h. After evaporation of the solvent the residue was dissolved in 1 mL of MeOH and 5 mg of $NaBH_4$ was added under cooling. The mixture was stirred for 5 min at 5 °C. After 1 drop of AcOH had been added the solvent was evaporated under reduced pressure. The residue was dissolved in $CHCl_3$, washed with H_2O, and evaporated to give a pale yellowish oil which was purified by silica gel column chromatography using benzene-MeOH (98:2 v/v) as eluent to afford 30.5 mg (90%) of the alcohols **17** and **18:** IR ν_{max} ($CHCl_3$) 3450 (NH), 1758 (C=O), 1340 cm^{-1} (NO_2); NMR (acetone-d_6) δ 1.43 (3 H, d, J = 6.5 Hz, $C_{1'}$ Me), 2.82 (1 H, bs, OH), 3.19 (1 H, dd, J = 6 and 2 Hz, C_3 H), 5.40 (2 H, s, CH_2Ar), 7.23 (1 H, bs, NH), 7.78 (2 H, d, J = 9 Hz, 2 ArH), 8.39 (2 H, d, J = 9 Hz, 2 ArH); MS m/e 339 ($M^+ + 1$); m/e 339.1168 (calcd for $C_{15}H_{19}N_2O_7$ ($M^+ + 1$), m/e 339.1192); 200-MHz NMR ($CDCl_3$) δ 3.08 ($^1/_{10}$ H, dd, J = 2 and 8 Hz, C_3 H of **17**), 3.19 ($^9/_{10}$ H, dd, J = 2 and 4.5 Hz, C_3 H of **18**).

B. A mixture of 38 mg (0.1 mmol) of the acetal azetidinones **13** and **14,** derived from the trans azetidinones **11** and **12** prepared by methods B and C, in 0.4 mL of AcOH and 0.1 mL of H_2O was heated at 60 °C for 5 h. The same workup as above afforded a syrup which was dissolved in 1 mL of MeOH and 5 mg of $NaBH_4$ added under cooling. The same workup and purification procedures as above gave 30.5 mg of the alcohols **17** and **18:** 200-MHz NMR ($CDCl_3$) δ 3.08 ($^2/_7$ H, dd, J = 2 and 8 Hz, C_3 H of **17**), 3.19 ($^5/_7$ H, dd, J = 2 and 4.5 Hz, C_3 H of **18**).

***trans*-4-[2',2'-Bis(2''-*p*-nitrobenzyloxycarbonylaminoethylthio)-ethyl]-3-(1-*p*-nitrobenzyloxycarbonyloxyethyl)-2-azetidinones (19 and 20). A.** A mixture of 38 mg (0.1 mmol) of the acetal azetidinones **13** and **14,** derived from the trans azetidinones **11** and **12** prepared by method A, and 256 mg (1 mmol) of *N*-*p*-nitrobenzyloxycarbonylcysteamine in 0.5 mL of CF_3CO_2H was stirred at room temperature for 16 h under a current of nitrogen. After evaporation of the solvent the residue was dissolved in $CHCl_3$, washed with a saturated $NaHCO_3$ solution, dried over Na_2SO_4, and evaporated to afford a caramel-like material which was purified by silica gel column chromatography using benzene-MeOH (99:1 v/v) as eluent to give 70 mg (86%) of the thioacetals **19** and **20:** IR ν_{max} ($CHCl_3$) 3480, 3450 (NH), 1760, 1720 (C=O), 1340 cm^{-1} (NO_2); NMR ($CDCl_3$) δ 1.43 (3 H, d, J = 6.5 Hz, $C_{1'}$ Me), 2.10 (2 H, dd, J = 5 and 7 Hz, CH_2CHS_2), 6.77 (1 H, bs, NH), 7.59 (6 H, d, J = 9 Hz, 6 ArH), 8.27 (6 H, d, J = 9 Hz, 6 ArH); MS m/e 831 ($M^+ + 1$), determined by field desorption mass spectrometry; 200-MHz NMR (acetone-d_6) δ 3.10 ($^1/_{10}$ H, dd, J = 2 and 7.5 Hz, C_3 H of **19**), 3.18 ($^9/_{10}$ H, dd, J = 2 and 4.5 Hz, C_3 H of **20**).

B. A mixture of 38 mg (0.1 mmol) of the acetal azetidinones **13** and **14,** derived from the trans azetidinones **11** and **12** prepared by method B, and 256 mg (1 mmol) of *N*-*p*-nitrobenzyloxycarbonylcysteamine in 0.5 mL of CF_3CO_2H was stirred at room temperature for 16 h under a current of nitrogen. The same workup and purification procedures as above afforded 70 mg of the thioacetals **19** and **20:** 200-MHz NMR (acetone-d_6) δ 3.10 ($^2/_7$ H, dd, J = 2 and 7.5 Hz, C_3 H of **19**), 3.18 ($^5/_7$ H, dd, J = 2 and 4.5 Hz, C_3 H of **20**).

(±)-3α-((1'*R)-Hydroxyethyl)-4β-(2',2'-dimethoxyethyl)-2-azetidinone (11).** To a solution of methylmagnesium iodide (prepared from 17.46 g of methyl iodide and 3.49 g of magnesium) in 100 mL of dry ether was added dropwise a solution of 5.7 g (24.2 mmol) of the amino ester **7** in 100 mL of dry THF under ice cooling. The resulting mixture was stirred for 18 h at room temperature and excess saturated NH_4Cl solution was then added under ice cooling. The separated aqueous layer was further extracted with $CHCl_3$. The combined organic layers were dried over Na_2SO_4 and evaporated to give a brown syrup which was purified by silica gel column chromatography to give 23 mg (0.4%) of the cis azetidinone **15** (the IR and NMR spectra and TLC behavior of which were identical with those of the product obtained by the above methods) and 498 mg (10.1%) of the trans azetidinone **11:** NMR ($CDCl_3$) δ 1.26 (3 H, d, J = 6.5 Hz, $C_{1'}$ Me), 1.97 [2 H, dd, J = 5 and 7 Hz, $CH_2CH(OMe)_2$], 2.86 (1 H, dd, J = 6 and 2 Hz,

C_3 H), 3.34 (6 H, s, 2 OMe), 3.77 (1 H, td, J = 7 and 2 Hz, C_4 H), 4.17 (1 H, qd, J = 6.5 and 6 Hz, >C*H*OH), 4.52 [1 H, t, J = 5 Hz, CH(OMe)$_2$], 6.80 (1 H, bs, NH).

(±)-4β-(2′,2′-Dimethoxyethyl)-3α-((1′*R)-*p*-nitrobenzyloxycarbonyloxyethyl)-2-azetidinone (13).** To a stirred mixture of 37 mg of the above compound **11** and 89 mg of 4-*N*,*N*-dimethylaminopyridine in 3 mL of CH_2Cl_2 was added 79 mg of *p*-nitrobenzyl chloroformate under ice cooling. The mixture was stirred for 2 h at 0 °C and then workup as before gave 57 mg (83%) of the azetidinone **13** as a yellowish syrup: NMR ($CDCl_3$) δ 1.47 (3 H, d, J = 6.5 Hz, $C_{1'}$ Me), 1.97 [2 H, dd, J = 5 and 7 Hz, C*H*$_2$CH(OMe)$_2$], 3.08 (1 H, dd, J = 7 and 2 Hz, C_3 H), 3.37 (6 H, s, 2 OMe), 3.78 (1 H, td, J = 7 and 2 Hz, C_4 H), 4.51 [1 H, t, J = 5 Hz, CH(OMe)$_2$], 5.07–5.47 (1 H, m, >C*H*OH), 5.33 (2 H, s, C*H*$_2$Ar), 6.33 (1 H, bs, NH), 7.64 (2 H, d, J = 9 Hz, 2 ArH), 8.34 (2 H, d, J = 9 Hz, 2 ArH).

(±)-4β-(2′-Hydroxyethyl)-3α-((1′*R)-*p*-nitrobenzyloxycarbonyloxyethyl)-2-azetidinone (17).** A mixture of 28 mg of the above azetidinone **13** in 0.4 mL of AcOH and 0.1 mL of H_2O was heated at 60 °C for 4.5 h. The same workup as before afforded a syrup which was dissolved in 1 mL of MeOH and 5.6 mg of $NaBH_4$ added under cooling. The same workup and purification procedure as before gave 22.5 mg of the alcohol **17**, the NMR spectrum ($CDCl_3$) of which was identical with that of an authentic sample donated by Dr. B. G. Christensen.

(±)-4β-[2′,2′-Bis(2″-*p*-nitrobenzyloxyaminoethylthio)ethyl]-3α-((1′-*R)-*p*-nitrobenzyloxycarbonyloxyethyl)-2-azetidinone (19).** A mixture of 34 mg of the azetidinone **13** and 222 mg of *N*-*p*-nitrobenzyloxycarbonylcysteamine in 0.5 mL of CF_3CO_2H was stirred at room temperature for 16 h under a current of nitrogen. The same workup and purification procedures as before afforded 59 mg of the thioacetal **19:** 200-MHz NMR (acetone-d_6) 1.40 (3 H, d, J = 6.5 Hz, $C_{1'}$ Me), 3.10 (1 H, dd, J = 2 and 7.5 Hz, C_3 H), 3.90 (1 H, m, C_4 H), 4.13 (1 H, t, J = 7 Hz, -CHS$_2$), 5.06 (1 H, m, $C_{1'}$ H), 5.24 (4 H, s, 2 C*H*$_2$Ar), 5.34 (2 H, s, C*H*$_2$Ar).

(±)-4β-(2′,2′-Trimethylenedithioethyl)-3α-((1′*S)-*p*-nitrobenzyloxycarbonyloxyethyl)-2-azetidinone (21).** A solution of 177 mg of the dimethyl acetals **13** and **14,** derived from the trans azetidinones **11** and **12** prepared by method A and 300 mg of 1,3-propanedithiol in 1 mL of trifluoroacetic acid, was stirred at room temperature for 6 h under nitrogen. The reaction mixture was dissolved in benzene, basified with a saturated $NaHCO_3$ solution, washed with brine, and dried over Na_2SO_4. The benzene was evaporated to yield 477 mg of an oil which was chromatographed on 25 g of silica gel using benzene–acetone (15:1 v/v) as eluent, to give 147 mg (74.6%) of a syrup. Recrystallization from 99% ethanol afforded the thioacetal **21** as colorless needles: mp 132–133 °C; IR ν_{max} ($CHCl_3$) 3445 (NH), 1760, 1750 (C=O), 1345 cm^{-1} (NO_2); NMR ($CDCl_3$) δ 1.46 (3 H, d, J = 6.5 Hz, $C_{1'}$ Me), 1.77–2.30 (4 H, m, 2 CH_2), 2.70–3.03 (4 H, m, 2 CH_2), 3.13 (1 H, dd, J = 6 and 2 Hz, C_3 H), 3.67 (1 H, td, J = 6 and 2 Hz, C_4 H), 4.10 (1 H, t, J = 7 Hz, -CHS$_2$), 4.93–5.50 (1 H, m, $C_{1'}$ H), 5.27 (2 H, s, C*H*$_2$Ar), 6.47 (1 H, bs, NH), 7.58 (2 H, d, J = 9 Hz, 2 ArH), 8.26 (2 H, d, J = 9 Hz, 2 ArH); MS *m/e* 426 (M$^+$) *m/e* 426.0902 (calcd for $C_{18}H_{22}N_2O_6S_2$, *m/e* 426.0917 (M$^+$)).

***trans*-4-(2′,2′-Dimethoxyethyl)-3-(1′-*o*-nitrobenzyloxycarbonyloxyethyl)-2-azetidinone (24).** To a mixture of 253 mg (1.25 mmol) of the azetidinone **12,** prepared by method A, and 305 mg (2.5 mmol) of 4-*N*,*N*-dimethylaminopyridine in 1.5 mL of dry CH_2Cl_2 at 0 °C was added a solution of 298 mg (1.38 mmol) of *o*-nitrobenzyl chloroformate in 1.5 mL of dry CH_2Cl_2 under a current of nitrogen. The reaction mixture was stirred at 0 °C for 1 h. The same workup as in the case of compounds **13** and **14** afforded 400 mg (84%) of the azetidinone **24:** IR ν_{max} ($CHCl_3$) 3450 (NH), 1760, 1750 (C=O), 1345 cm^{-1} (NO_2); NMR ($CDCl_3$) δ 1.46 (3 H, d, J = 6.5 Hz, $C_{1'}$ Me), 1.95 [2 H, dd, J = 5 and 7 Hz, C*H*$_2$CH(OMe)$_2$], 3.15 (1 H, dd, J = 6 and 2 Hz, C_3 H), 3.34 (6 H, s, 2 OMe), 3.65 (1 H, td, J = 7 and 2 Hz, C_4 H), 4.45 (1 H, t, J = 5 Hz, C*H*(OMe)$_2$], 4.90–5.38 (1 H, m, C*H*OH), 5.58 (2 H, s, C*H*$_2$Ar), 6.61 (1 H, bs, NH), 7.55–8.30 (4 H, m, 4 ArH); MS *m/e* 383 (M$^+$ + 1).

***o*-Nitrobenzyl 6-((8*S*)-*o*-Nitrobenzyloxycarbonyloxyethyl)-1-carba-2-penem-3-carboxylate (27).** A solution of 310 mg (0.81 mmol) of the above azetidinone **24** and 660 mg (2.59 mmol) of the *o*-nitrobenzyl glyoxylate ethyl hemiacetal in 3 mL of DMF and 17 mL of toluene was stirred at room temperature in the presence of molecular sieves (3 Å; activated at 250 °C (2 mmHg)) under a current of nitrogen for 24 h. After filtration of the reaction mixture, the filtrate was evaporated under reduced pressure. The residue was triturated with ether–pentane to remove excess glyoxylate. The residual material was purified by silica gel column chromatography using benzene–AcOEt (9:1 ~ 8:2 v/v) as eluent to afford 329 mg (69%) of two epimers of the alcohol **25:** IR ν_{max} ($CHCl_3$) 1760 cm^{-1} (C=O); NMR ($CDCl_3$) δ 3.33 (6 H, s, 2 OMe).

To a mixture of 210 mg (0.355 mmol) of the above alcohol **25** and 80 mg (0.746 mmol) of 2,6-lutidine in 4 mL of dry THF at −20 °C was added dropwise a solution of 84.5 mg (0.71 mmol) of $SOCl_2$ in 1.5 mL of dry THF under a current of nitrogen, and the reaction mixture was stirred for 30 min. After filtration, the filtrate was evaporated under reduced pressure. The residue was dissolved in 5 mL of dry dioxane, and 76 mg (0.71 mmol) of 2,6-lutidine and 186 mg (0.71 mmol) of triphenylphosphine were added. The reaction mixture was stirred overnight at room temperature under a current of nitrogen. After filtration, the filtrate was evaporated to give a yellowish caramel which was purified by silica gel column chromatography using benzene–AcOEt (8:2 v/v) as eluent to afford 220 mg (74%) of the phosphorane **26** as a caramel: IR ν_{max} ($CHCl_3$) 1750, 1620 (C=O), 1340 cm^{-1} (NO_2); NMR ($CDCl_3$) δ 1.40 (3 H, d, J = 6.5 Hz, $C_{1'}$ Me), 3.23 (6 H, s, 2 OMe).

A solution of 301 mg (0.36 mmol) of the phosphorane **26** and 167 mg (0.10 mmol) of *p*-toluenesulfonic acid in 7 mL of acetone was stirred at room temperature for 2 h. After evaporation of the solvent the residue was dissolved in CH_2Cl_2 and neutralized with a saturated $NaHCO_3$ solution under cooling. The CH_2Cl_2 extract was dried over Na_2SO_4 and evaporated. Purification of the resulting pale yellowish oil by preparative TLC on silica gel using benzene–acetone (4:1 v/v) gave 101 mg (55.4%) of the (±)-(8*S**)-decysteaminylthienamycin derivative **27:** R_f 0.6; IR ν_{max} ($CHCl_3$) 1778, 1740, 1720 cm^{-1} (C=O); NMR ($CDCl_3$) δ 1.55 (3 H, d, J = 6.5 Hz, CH*Me*), 2.90 (2 H, m, C_1 H), 3.46 (1 H, dd, J = 4.5 and 2 Hz, C_6 H), 4.30 (1 H, dt, J = 7 and 2 Hz, C_5 H), 5.20 (1 H, m, MeC*H*), 5.60 (2 H, s, C*H*$_2$Ar), 5.70 (2 H, AB q, C*H*$_2$Ar), 6.60 (1 H, t, J = 2 Hz, C_2 H), 7.3–8.3 (8 H, m, ArH).

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Conformation and Structure of *cyclo*-(Dibenzylglycyl-L-proline) and *cyclo*-(Di-L-prolyl-D-proline) in the Crystalline State

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Abstract: The molecular and crystal structures of two cyclic tripeptides containing prolyl residues were studied by X-ray analysis. *cyclo*-(Dibenzylglycyl-L-proline) and *cyclo*-(di-L-prolyl-D-proline) have a backbone chain of nine members with cis peptide bonds. Both peptides crystallize in the orthorhombic space group $P2_12_12_1$ with lattice constants a = 10.348 (6) Å, b = 8.856 (4) Å, c = 23.235 (13) Å, Z = 4, and a = 8.742 (5) Å, b = 15.423 (10) Å, c = 21.987 (15) Å, Z = 8, respectively. Intensity data were collected on a four-circle diffractometer using Mo Kα radiation. Both structures were solved by direct methods and refined to $R(F)$ = 0.104 and 0.107. Weighted values $R_w(F)$ = 0.064 and 0.045. *cyclo*-(Bzl·Gly$_2$-L-Pro) adopts a crown conformation, while a boat conformation was found for the two independent molecules of *cyclo*-(L-Pro$_2$-D-Pro). NMR measurements in solution revealed an equilibrium between boat and crown conformation for Bzl·Gly$_2$-L-Pro, while only the boat was found for the triproline. Two of the peptide bonds in the boat show significant deviations from planarity (ω = −12 and −17°). The conformation of the prolyl ring is discussed and compared to other prolyl residues.

Introduction

The relationship between the conformation of a peptide in solution and in the solid state is of considerable interest. Small cyclic peptides are especially suited for such a comparison because of their lack of flexibility in solution. Cyclic tripeptides contain three cis peptide bonds in a nine-membered ring. Amino acids suitable for cyclization are Pro, Hyp, Sar, and Bzl·Gly, which are all substituted at the N atom.

X-ray analyses of *cyclo*-(L-Pro$_3$)[1] and *cyclo*-(L-Hyp-L-Pro$_2$)[2] are reported in the literature. In both structures the backbone adopts a crown configuration with the three C_α atoms on one side of the plane defined by the N atoms. A similar conformation was ascribed to the molecule of *cyclo*-(L-Pro$_3$) in solution by NMR measurements.[3] ^{1}H and ^{13}C NMR experiments revealed that tripeptides of the general formula *cyclo*-(L-Pro$_x$-Bzl·Gly$_{3-x}$), with x = 0, 1, 2 and Bzl·Gly = *N*-benzylglycine, exhibit equilibria between a crown and a flexible boat conformation.[4] The latter form is characterized by one C_α atom lying on the opposite side of the two others relative to the plane of the N atoms.

A relation between the chirality of the amino acids and the conformation of the backbone has been proposed recently.[5] Three amino acids with the same chirality should adopt the crown form in the cyclic backbone; the boat should dominate if one of the residues differs in chirality.

Boat and crown forms may coexist for tripeptides with achiral residues. ^{1}H NMR results of *cyclo*-(L-Pro$_2$-D-Pro) indicated the boat form. The present X-ray study reveals this conformation also in the solid state. A brief account of both studies has been published elsewhere.[6] The structure determination of *cyclo*-(Bzl·Gly$_2$-L-Pro) was undertaken to determine which of the conformations found in the solution is most stable in the solid state. Both tripeptides contain proline and therefore the mutual influence of proline and peptide conformation may be studied.

Experimental Section

***cyclo*-(Dibenzylglycyl-L-proline).** The compound was synthesized by Krämer.[7] Rather large single crystals were obtained by recrystallization from methanol that contained traces of water. After a few days in air they showed cracks and became opaque. The crystal used for the data collection was sealed in a glass capillary to prevent decomposition. Data collection was performed on a Syntex $P2_1$ diffractometer with Nb-filtered Mo Kα radiation. The cell dimensions (Table I) were derived by a least-squares fit from the setting angles of 15 well-centered reflections with $10° \leq 2\theta \leq 20°$. Data were collected in two octants of reciprocal space (hkl and $hk\bar{l}$) up to 2θ = 40 and 45°, respectively, with a $\theta/2\theta$ scan. The reflections showed broad profiles due to poor crystal quality. A minimum scan range of 3.4° was therefore required. The scan speed was 5.9° min^{-1}. Background corrections were made by profile analysis.[8] Three standard reflections observed after every 65 reflections showed gradual changes up to 10% over the period of data collection. This was attributed to structural changes caused by the X-ray exposure. Since not all reflections drifted in the same direction, no correction for this effect was attempted.

The equivalent reflections were averaged with appropriate weights. The weight of an individual reflection was taken as:

$$w(I) = \{\sigma^2(I)_{\text{counting}} + (0.03I)^2\}^{-1}$$

The weight of an averaged intensity was taken as the sum of the weights of the individual reflections. The internal consistency expressed by the R factor for equivalent reflections was $R(I) = \Sigma|I - \langle I\rangle|/\Sigma I$ = 0.06.

***cyclo*-(Di-L-prolyl-D-proline).** The synthesis of the compound has been described by Maestle and Rothe.[9] Recrystallization from ether resulted in single crystals suitable for the measurements. A specimen was sealed in a glass capillary. Data were collected in three octants of reciprocal space (hkl, $hk\bar{l}$, and $\bar{h}k\bar{l}$) up to 2θ = 45, 43, and 43°, respectively, as described above. The crystal was found to be of good

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