Total Synthesis of Tetranormethyl-calcimycin

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The title compound **2** was synthesized. Asymmetric epoxidation of **9** followed by reductive cleavage of the epoxide **10** and acidification gave a symmetrical spiroketal (**3**), to which pyrrole and benzoxazole moieties were introduced in an efficient manner by the previously developed method. Since the synthetic material was less stable than natural calcimycin under basic conditions, cleavage of the methyl ester was achieved with LiH-pyridine.

A divalent cation ionophore, calcimycin (A23187) **1**, was isolated from a culture of *Streptomyces chartreusensis*.¹⁾ The unique structure²⁾ of this antibiotic as well as its significant role in biochemical studies has drawn much attention from synthetic chemists. Evans,³⁾ Grieco's⁴⁾ and our groups⁵⁾ have accomplished the total synthesis of **1** in the independently developed manner of stereo-control. Several reports have also appeared describing the preparations of calcimycin analogs which might be helpful, not only to elucidate the ionophoric action in biological systems, but also to develop new chemotherapeutic agents. Among them, chemical modifications of natural calcimycin have been achieved principally at the pyrrole and/or benzoxazole ring in the molecule through either halogenation^{6a)} or semisynthesis.^{6b,c)} We would like to clarify the role played by methyl groups attached to the carbon framework of **1**, which might either contribute to the structural stability of the dioxaspiro ring system or enhance the permeability across biological membranes. From this viewpoint, we started our project on synthetic studies of unnatural calcimycin analogs, and now describe a total synthesis of the tetranormethyl analog **2**, lacking four methyl groups at C-11, 15, 17 and 19.

We have already shown a successful strategy for the synthesis of this type of complex molecule, in which construction of the chiral di-

oxaspiro ring system was accomplished prior to the attachment of pyrrole and benzoxazole rings. The symmetrical structure of the dioxaspiro framework in the target compound **2** indicated the advantage of an approach to this molecule through the intermediacy of the spiroketal intermediate **3**. Pyrrole and benzoxazole moieties would be successively introduced to the spiro compound **3** through the appropriate chemical conversion of each side chain in the molecule. A plausible precursor of the spiroketal **3** was (3*S*,11*S*)-7-oxotridecan-1,3,11,13-tetraol (**4**) that was expected to give the desired "anomeric" configuration(s) at the spiro center by thermodynamically controlled ring closure.⁷⁾ The chiral ketotetraol would be prepared by using the asymmetric epoxidation and reduction procedure developed by Sharpless *et al.*^{8,9)} In this manner, our synthesis was realized as shown in scheme 3.

Methyl acetoacetate (**5**) was alkylated twice with 4-bromo-1-butene in separate steps to give **6**, which was converted to the ketal **7** in a

SCHEME 1.

SCHEME 2.

SCHEME 3.

moderate yield *via* saponification, decarboxylation and ketalization. Ozonolysis of 7 followed by a reductive workup gave a dialdehyde which, upon a Wittig reaction with the stable phosphorane (Ph₃P=CHCOOEt), afforded the diester 8 in a 73% overall yield. The desired *E,E*-isomer was readily separated from the minor (<6%) *E,Z*-isomer by column chromatography on silica gel. Reduction of 8

with DIBAL gave the corresponding diol 9, which was used as the substrate for asymmetric epoxidation.

According to the reported procedure,⁸⁾ the dienediol 9 was treated with titanium tetraisopropoxide and *t*-butyl hydroperoxide using (+)-diethyl tartrate. The reaction took place slowly at -20°C, and the diepoxide 10 was obtained in a 50~58% yield after 3 days. It

was then submitted to regioselective epoxide opening by reducing with Red-al. Without isolating the resultant tetraol, the reaction mixture was heated with a large excess of 10% phosphoric acid, resulting in hydrolysis of the ketal and concomitant cyclization to the spiroketal **3**. The isolated product (67.5%) was a single isomer indicated by seven signals for its ^{13}C -NMR spectrum, and had a specific rotation of $+97.7^\circ$.¹⁰⁾

In order to make the introduction of a pyrrole ring selective to the symmetrical molecule, one of the hydroxyl groups had to be masked during the process. Selective mono-protection was not effected even with a bulky silylating agent (*t*-BuPh₂SiCl/imidazole) and the yield of the desired mono-silyl ether **11** was moderate (40~46%). The accompanying bis-silyl ether (25~30%), however, could be quantitatively returned to the starting diol **3** by treating with tetra-*n*-butylammonium fluoride. The mono-protected compound **11** was oxidized with 8N Jones' reagent to the corresponding carboxylic acid, which, without purification, was converted to the 2-pyridylthiolester¹¹⁾ **12** in a 67% yield. According to the previously developed procedure by our group,¹²⁾ regioselective acylation of the pyrrole with **12** was effected in an 86% yield with pyrrolylmagnesium bromide in the presence of cuprous iodide.

The next task, construction of the characteristic benzoxazole system, was done as follows. Removal of the silyl protecting group with the fluoride ion gave **14**, which was oxidized with 8N Jones' reagent at -20°C to the carboxylic acid **15** in a 77% overall yield. Condensation of **15** with an aminophenol (**19**)⁵⁾ was achieved with the help of benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP)¹³⁾ to give an amide (**16**), which was heated with a catalytic amount of pyridinium *p*-toluenesulfonate¹⁴⁾ in dichloroethane to produce the benzoxazole derivative **17** in a reasonable yield. Completion of the synthesis required deprotecting the amino and carboxyl groups. The conventional method for deprotection under

basic conditions, including those reported for calcimycin and its analogs (*e.g.*, KOH/EtOH, $\text{K}_2\text{CO}_3/\text{aq. MeOH}$ and $\text{LiSPr}^n/\text{DMF}$), however, resulted in extensive decomposition. Although none of the decomposed products was characterized, it can be presumed that this molecule was more alkaline-labile than the natural material due to the absence of four methyl groups in the framework. Careful screening of the conditions gave us a reasonable solution to this problem. The trifluoroacetyl group was removed under a mildly basic condition (LiOH , H_2O_2 , THF, 0°C , 3 hr) and then the methyl ester was cleaved with lithium iodide in boiling pyridine. After acidification with dil. HCl, the product was extracted at pH 3~4 and purified on a column packed with citric acid-impregnated silica gel.^{6b)} In this manner, the desired tetranormethyl calcimycin **2** was obtained in a 77% yield. The physical data (NMR, MS and $[\alpha]_D$) of the sample confirmed the assigned structure **2**, and its chromatographic behavior was found to be very similar to the natural material **1**.

Having synthesized the target analog **2** in an unambiguous way, this compound **2** was submitted to biological evaluation. Compound **2**, however, exhibited no activity comparable with natural calcimycin **1** in our study of the contractile response in smooth muscle from guinea pig ileum.¹⁵⁾ Since we observed a high affinity of this normethyl compound **2** toward the calcium ion in the course of the experiments, its loss of biological activity may be explained in terms of the decreased lipophilicity of this synthetic analog, which would have prevented the facile transport of the calcium ion through the cell membrane. In conclusion, our synthetic studies could demonstrate the crucial role played by the methyl groups of calcimycin in order to exhibit its biological activity.

EXPERIMENTAL

All boiling points (bp) and melting points (mp) are uncorrected. ^1H -NMR and ^{13}C -NMR spectra were measured with JEOL JNMFX90Q and GX 400 spectrometers. IR spectra were recorded on a Shimadzu IR-430

spectrophotometer, and mass spectra were obtained with a Hitachi M-80 mass spectrometer. Optical rotations were measured with a Perkin-Elmer 141 polarimeter.

5-Carbomethoxy-6-oxo-1,10-undecadiene (6). A dianion solution of methyl acetoacetate (12.5 ml, 116.3 mmol) was prepared with a 60% NaH (5.0 g, 125.1 mmol) and 1.36 N *n*-BuLi hexane solution, (92.0 ml, 125.1 mmol) in dry THF (200 ml), according to the literature.¹⁶⁾ To the solution was added 4-bromo-1-butene (15.0 ml, 147.8 mmol) with stirring at 0°C under Ar, stirring being continued for 2 hr. AcOH was then added to the mixture to destroy any excess base, and the mixture was concentrated *in vacuo*. The residue was diluted with water and extracted with ether. The extract was washed with water and brine, and then dried (MgSO₄). Removal of the solvent gave a crude material (18.2 g), which was used for the next reaction without purification. A mixture of this product and 4-bromo-1-butene (21.7 ml, 214 mmol) in CHCl₃ (80 ml) was added to a mixture of *n*-Bu₄NHSO₃ (36.4 g, 107 mmol) and NaOH (8.56 g, 214 mmol) in water (80 ml) with stirring in an ice-cooled water bath. The mixture was then stirred at room temperature for 6 hr, before the CHCl₃ layer was separated and concentrated *in vacuo*. The residue was diluted with ether, washed with water and brine, and dried (MgSO₄). After removing the solvent, the residue was distilled under reduced pressure to give **6** (10.7 g, 41.2%). bp 117~122°C/3 mmHg. IR $\nu_{\max}$ (neat) 3100, 1745, 1720, 1640 cm⁻¹. ¹H-NMR (CDCl₃) δ 1.5~2.7 (11H, m), 3.72 (3H, s), 4.8~5.2 (4H, m), 5.5~6.0 (2H, m). Anal. Found: C, 69.45; H, 8.96. Calcd. for C₁₃H₂₀O₃: C, 69.61; H, 8.99%.

6-Ethylenedioxy-1,10-undecadiene (7). A mixture of **6** (40 g, 178 mmol), Ba(OH)₂ (119 g), 95% EtOH (140 ml) and water (374 ml) was heated under reflux for 19 hr. After cooling, the mixture was poured into a dilute HCl solution (12 N HCl, 60 ml; water, 800 ml), stirred for 1 hr at room temperature, and then extracted with ether. The extract was successively washed with water, satd. NaHCO₃ and brine, and then dried (MgSO₄). Removal of the solvent gave an oil (26.7 g), which was heated with ethyleneglycol (19.9 g, 320 mmol) and *p*-TsOH (0.2 g) in benzene (300 ml) under reflux in a Dean-Stark water separator for 3 hr. The mixture was washed well with aqueous NaHCO₃ and brine. After removing the solvent, the residue was distilled under reduced pressure to give **7** (23.5 g, 62.5%). bp 80~82°C/2 mmHg. IR $\nu_{\max}$ (neat) 3100, 1640 cm⁻¹. ¹H-NMR (CDCl₃) δ 1.20~1.75 (8H, m), 1.90~2.20 (4H, m), 3.92 (4H, s), 4.82~5.12 (4H, m), 5.55~6.02 (2H, m). ¹³C-NMR 23.1, 33.9, 36.6, 64.9, 111.7, 114.5, 138.6.

Diethyl 7-ethylenedioxy-2,11-tridecenedioate (8). Ozonized oxygen was bubbled through a cold solution of **7** (11.0 g, 52.3 mmol) in CH₂Cl₂ (800 ml) in a dry ice-cooled acetone bath until the blue color of ozone resulted. The excess oxidant was then removed by bubbling of N₂.

Aqueous AcOH (AcOH, 66 ml; water, 6.6 ml) was added to the mixture, and Zn-dust (55 g) was then added portionwise to the vigorously stirred mixture. After completing the addition, the bath was removed and stirring was continued for 1.5 hr at room temperature. The insoluble material was filtered off, before the filtrate was carefully washed with a mixture of satd. NaHCO₃ and satd. NaCl (1:1), and finally dried (Na₂SO₄). After removing the solvent, the crude dialdehyde (11.3 g) obtained was stirred with ethyl triphenylphosphoranylideneacetate (28.3 g, 114 mmol) in dry benzene (200 ml) at room temperature. The mixture was concentrated *in vacuo* to remove benzene and the residue was extracted with *n*-hexane. The insoluble material was removed by filtration, and a *n*-hexane solution was concentrated *in vacuo*. The crude product was chromatographed on silica gel (1 kg) in *n*-hexane-EtOAc (7:3) to afford **8** (13.5 g, 73.1%) and its *E,Z*-isomer (1.0 g) as the less polar fraction. IR $\nu_{\max}$ (neat), 1715, 1650 cm⁻¹. ¹H-NMR (CDCl₃) δ 1.28 (6H, t, *J*=7.3 Hz), 1.44~1.70 (8H, m), 2.08~2.35 (4H, m), 3.92 (4H, s), 4.18 (4H, q, *J*=7.3 Hz), 5.82 (2H, dm, *J*=15.6 Hz), 6.95 (2H, dt, *J*=15.6, 6.8 Hz). ¹³C-NMR (CDCl₃) 14.3, 22.3, 32.2, 36.7, 60.1, 65.0, 111.2, 121.6, 148.6, 166.6. *E,Z*-isomer: ¹H-NMR (CDCl₃) δ 1.28 (6H, t, *J*=7.3 Hz), 1.40~1.70 (8H, m), 2.08~2.80 (4H, m), 3.92 (4H, s), 4.16 (2H, q, *J*=7.0 Hz), 4.18 (2H, q, *J*=7.3 Hz), 5.76 (1H, dm, *J*=11.7 Hz), 5.81 (1H, dm, *J*=15.8 Hz), 6.20 (1H, dt, *J*=6.5 Hz), 6.95 (1H, dt, *J*=15.8, 6.8 Hz).

7-Ethylenedioxy-2,11-tridecene-1,13-diol (9). To a solution of **8** (4.9 g, 13.8 mmol) in dry CH₂Cl₂ (100 ml) was added a 1.76 N diisobutylaluminum hydride *n*-hexane solution (DIBAL, 31.3 ml, 55.1 mmol) with stirring under Ar in an ice-cooled water bath. After stirring for 1 hr at 0°C, the mixture was diluted with ether (300 ml). The excess reducing agent was then destroyed by carefully adding water, and to the mixture was then added a satd. potassium sodium tartrate solution (100 ml). The mixture was stirred at room temperature for 20 min, before the insoluble material was removed by filtration through celite. The organic layer separated was washed with satd. NaHCO₃ and brine, dried (Na₂SO₄), and evaporated *in vacuo*. The crude product was purified by column chromatography on silica gel (450 g) in CHCl₃-MeOH (95:5) to give **9** (3.3 g, 88.6%) as an oil. ¹H-NMR (CDCl₃) δ 1.20~1.72 (8H, m), 1.9~2.1 (4H, m), 2.20 (2H, br. s), 3.92 (4H, s), 4.02~4.10 (4H, m), 5.5~5.7 (4H, m). ¹³C-NMR (CDCl₃) 23.4, 32.2, 36.7, 63.5, 64.9, 111.7, 129.5, 132.6.

[2R,3S,11S,12R]-2,3-,11,12-Diepoxy-7-ethylenedioxytridecane-1,13-diol (10). According to the literature,⁸⁾ titanium tetraisopropoxide (1.29 ml, 4.44 mmol) and L-(+)-diethyl tartrate (0.78 ml, 4.44 mmol) were dissolved in dry CH₂Cl₂ (200 ml) under N₂ with stirring in a dry ice-cooled CCl₄ bath. The mixture was stirred for 30 min. The substrate **9** (2.0 g, 7.4 mmol) and then a 3.9 M *t*-butylhydroperoxide/CH₂Cl₂ solution (19 ml, 74 mmol) were

added to the mixture. The resulting mixture was stored for 4 days in a freezer at about -20°C . The mixture was diluted with ether (200 ml), stirred for 15 min with diethyleneglycol (0.94 g, 8.88 mmol) and a satd. Na_2SO_4 solution (1.0 ml), and then filtered through celite. The filtrate was concentrated *in vacuo* and the residue was co-evaporated with toluene to remove the water. The crude product was purified by flash-chromatography on silica gel (500 g) with EtOAc–acetone (4:1) containing pyridine (1%) to give **10** (1.31 g, 58.4%). $[\alpha]_{\text{D}}^{27} - 33.8^{\circ}$ ($c=0.49$, CHCl_3). $^1\text{H-NMR}$ (CDCl_3) δ 1.4–1.7 (12H, m), 2.7 (2H, br. s), 2.8–3.0 (4H, m), 3.6–3.9 (4H, m), 3.92 (4H, s). *Anal.* Found: C, 59.84; H, 8.79. Calcd. for $\text{C}_{15}\text{H}_{26}\text{O}_6$: C, 59.58; H, 8.67%.

[2*S*,6*S*,8*S*]-2-*Bis*-(2-hydroxyethyl)-1,7-dioxaspiro[5,5]-undecane (**3**). To a solution of **10** (385 mg, 1.27 mmol) in dry THF (5 ml) was added a solution of sodium bis(2-methoxyethoxy)aluminum hydride (3.4 M Red-al/toluene, 1.2 ml, 4.1 mmol) in dry THF (10 ml) in a dry ice-cooled acetone bath. The mixture was stirred for 2.5 hr at -78° and then gradually warmed to ambient temperature after removing the bath. Stirring was continued for 2.5 hr. To the mixture was carefully added 10% phosphoric acid (40 ml), before the mixture was heated under reflux for 5 hr. After cooling, the resultant mixture was concentrated *in vacuo* to remove most of the THF and then extracted with EtOAc–ether (1:1). The extract was washed with water and brine, and dried (Na_2SO_4). Removal of the solvent *in vacuo* gave a solid, which was recrystallized from *n*-hexane–EtOAc to give **3** (178 mg). The mother liquor was concentrated and chromatographed on silica gel (10 g) with EtOAc–acetone (9:1) to afford an additional amount (32 mg) of **3** (total yield, 67.5%). mp $118\sim 119^{\circ}\text{C}$. $[\alpha]_{\text{D}}^{27} + 97.7^{\circ}$ ($c=0.44$, CHCl_3). $^1\text{H-NMR}$ (CDCl_3) δ 1.1–1.9 (16H, m), 3.2 (2H, br. s), 3.6–4.1 (6H, m). $^{13}\text{C-NMR}$ (CDCl_3) 19.1, 31.2, 35.3, 38.5, 60.3, 67.8, 96.4. *Anal.* Found: C, 64.04; H, 9.91. Calcd. for $\text{C}_{13}\text{H}_{24}\text{O}_4$: C, 63.91; H, 9.91%.

[2*S*,6*S*,8*S*]-2-(2-*t*-Butyldiphenylsilyloxyethyl)-8-(2-hydroxyethyl)-1,7-dioxaspiro[5,5]undecane (**11**). A mixture of **3** (890 mg, 3.64 mmol), imidazole (496 mg, 7.29 mmol) and *t*-BuPh₂SiCl (1 ml, 3.83 mmol) in dry DMF (20 ml) was stirred for 2 hr in an ice-cooled water bath. The mixture was diluted with ether (300 ml) and successively washed with water and brine. The ethereal extract was dried (Na_2SO_4) and concentrated *in vacuo*. The resultant product was chromatographed on silica gel (100 g) with *n*-hexane–EtOAc (7:3) to give a bis-silylated compound (871 mg, 33.2%) and **11** (720 mg, 40.9%). Finally, the column was eluted with EtOAc–acetone (9:1) to afford unreacted **3** (178 mg, 20.1%). $[\alpha]_{\text{D}}^{27} + 33.9^{\circ}$ ($c=1.4$, CHCl_3). $^1\text{H-NMR}$ (CDCl_3) δ 1.05 (9H, s), 1.90–1.75 (16H, m), 2.95 (1H, br. s), 3.73–3.84 (6H, m), 7.33–7.44 (6H, m), 7.61–7.73 (4H, m). *Anal.* Found: C, 72.46; H, 8.87. Calcd. for $\text{C}_{29}\text{H}_{42}\text{O}_4\text{Si}$: C, 72.16; H, 8.77%. The bis-silylated compound was treated with *n*-Bu₄NF in THF at

room temperature to quantitatively regenerate the starting diol **3**.

[2*S*,6*S*,8*S*]-2-(2-*t*-Butyldiphenylsilyloxyethyl)-8-[2-oxo-2-(pyrid-2-ylthio)ethyl]-1,7-dioxaspiro[5,5]undecane (**12**). A solution of **11** (276 mg, 0.57 mmol) in acetone (70 ml) was cooled in an ice-cooled MeOH bath. Jones' reagent (8 N, 0.7 ml) was added dropwise to the solution with stirring. After stirring for 20 min, the excess oxidant was destroyed by adding MeOH. The mixture was stirred for a further 5 min and then diluted with water (40 ml). After adjusting to pH 4.0 by adding a satd. NaHCO_3 solution, the mixture was concentrated to remove most of the acetone. The resulting aqueous layer was extracted with EtOAc–ether (1:1). The extract was washed with water and brine, and then dried (MgSO_4). Removal of the solvent gave a crude carboxylic acid (274 mg), which was treated with 2,2'-dipyridyldisulfide (201 mg, 0.91 mmol) and triphenylphosphine (239 mg, 0.91 mmol) in dry CH_2Cl_2 (20 ml) at room temperature for 3 hr. The mixture was concentrated *in vacuo* and the residue was chromatographed on silica gel (125 g) with *n*-hexane–EtOAc (7:3) to afford **12** (226 mg, 66.9%). $[\alpha]_{\text{D}}^{27} + 23.8^{\circ}$ ($c=0.6$, CHCl_3). IR (neat) ν_{max} 1730, 1700 cm^{-1} . $^1\text{H-NMR}$ (CDCl_3) δ 1.03 (9H, s), 1.17–2.03 (14H, m), 2.70–2.79 (2H, m), 3.76–4.15 (4H, m), 7.22–7.38 (7H, m), 7.55–7.72 (6H, m), 8.50–8.62 (1H, m).

[2*S*,6*S*,8*S*]-2-(2-*t*-Butyldiphenylsilyloxyethyl)-8-[2-oxo-2-(1*H*-pyrrol-2-yl)ethyl]-1,7-dioxaspiro[5,5]undecane (**13**). A solution of pyrrolylmagnesium bromide (0.5 N, 2.98 ml, 1.49 mmol) in ether–THF (1:1) was added to a suspension of CuI (141 mg, 0.74 mmol) in dry THF (0.9 ml) with stirring in an ice-cooled water bath, under Ar. After stirring for 30 min, a solution of **12** (293 mg, 0.49 mmol) in dry THF (4 ml) was added to the mixture, stirring being continued for 1.5 hr at 0°C . The mixture was then diluted with ether (30 ml) and stirred with a satd. NH_4Cl aqueous solution (9 ml) for 15 min. The insoluble material was filtered off through celite, before the filtrate was washed with water and brine, dried (MgSO_4) and finally evaporated. Chromatography of the residue on silica gel (35 g) in *n*-hexane–EtOAc (9:1) afforded **13** (233 mg, 85.9%). $[\alpha]_{\text{D}}^{21} + 15.5^{\circ}$ ($c=0.8$, CHCl_3). IR (neat) ν_{max} 1730 cm^{-1} . $^1\text{H-NMR}$ (CDCl_3) δ 1.03 (9H, s), 1.11–1.80 (14H, m), 2.74 (1H, dd, $J=5.5$, 14.3 Hz), 2.89 (1H, dd, $J=7.6$, 14.3 Hz), 3.55 (1H, m), 3.68–3.81 (2H, m), 4.02 (1H, m), 6.14 (1H, m), 6.84 (1H, m), 6.91 (1H, m), 7.35–7.43 (6H, m), 7.66–7.69 (4H, m), 9.31 (1H, br. s). *Anal.* Found: C, 72.46; H, 7.95; N, 2.50. Calcd. for $\text{C}_{33}\text{H}_{43}\text{NO}_4\text{Si}$: C, 72.62; H, 7.94; N, 2.57%.

[2*S*,6*S*,8*S*]-2-(2-hydroxyethyl)-8-[2-oxo-2-(1*H*-pyrrol-2-yl)ethyl]-1,7-dioxaspiro[5,5]undecane (**14**). To a solution of **13** (212 mg, 0.39 mmol) in THF (3 ml) was added a 1 N *n*-Bu₄NF–THF solution (3 ml). After stirring for 50 min at room temperature, the mixture was diluted with ether

(80 ml), washed with water and brine, and dried (Na_2SO_4). Removal of the solvent and subsequent chromatography on silica gel (25 g) with *n*-hexane–EtOAc (7:3) gave **14** (110 mg, 92%), $[\alpha]_D^{25} + 71.4^\circ$ ($c = 0.73$, CHCl_3). IR (neat) ν_{max} 3300, 1730 cm^{-1} . $^1\text{H-NMR}$ (CDCl_3) δ 1.15–2.17 (15H, m), 2.63 (1H, dd, $J = 3.4, 15.3$ Hz), 3.12 (1H, dd, $J = 9.5, 15.3$ Hz), 3.73 (1H, m), 3.84 (1H, m), 3.96 (1H, m), 4.29 (1H, m), 6.28 (1H, m), 6.98 (1H, m), 7.04 (1H, m), 9.49 (1H, br. s). *Anal.* Found: C, 66.36; H, 8.20; N, 4.56. Calcd. for $\text{C}_{17}\text{H}_{25}\text{NO}_4$: C, 66.43; H, 8.20; N, 4.56%.

[2*S*,6*S*,8*S*]-2-*Carboxymethyl*-8-[2-*oxo*-2-(1*H*-pyrrol-2-yl)ethyl]-1,7-dioxaspiro[5,5]undecane (**15**). Jones' reagent (8 N, 4.4 ml) was added dropwise to a stirred solution of **14** (740 mg, 2.4 mmol) in acetone (200 ml) in an ice-cooled MeOH bath. After stirring for 20 min, the excess oxidant was destroyed by adding MeOH. The mixture was then diluted with water, and satd. NaHCO_3 solution was added to adjust the pH to 4.0. Most of acetone was removed by evaporation. The resulting aqueous layer was extracted with EtOAc–ether (1:1). The extract was washed with brine, dried (MgSO_4), and evaporated. Chromatography of the residue on silica gel (70 g) in EtOAc–iso-PrOH (95:5) containing AcOH (3%) afforded **15** (645 mg, 83.4%) as crystals. mp 130–131°C (recrystallized from *n*-hexane–iso-PrOH). $[\alpha]_D^{27} + 32.1^\circ$ ($c = 0.56$, CHCl_3). IR (KBr) ν_{max} 3300, 1730, 1660 cm^{-1} . $^1\text{H-NMR}$ (CDCl_3) δ 1.10–1.90 (12H, m), 2.45 (2H, m), 2.79 (1H, dd, $J = 4.7, 14.1$ Hz), 3.03 (1H, dd, $J = 7.7, 14.1$ Hz), 3.99 (1H, m), 4.32 (1H, m), 6.26 (1H, m), 7.02 (1H, br. s), 7.11 (1H, br. s). *Anal.* Found: C, 63.61; H, 7.31; N, 4.36. Calcd. for $\text{C}_{17}\text{H}_{23}\text{NO}_5$: C, 63.54; H, 7.21; N, 4.36%.

[2*S*,6*S*,8*S*]-2-[6-*Hydroxy*-5-(*N*-methyltrifluoroacetamido)-2-methoxycarbonylphenyl]carbamoymethyl-8-[2-*oxo*-2-(1*H*-pyrrol-2-yl)ethyl]-1,7-dioxaspiro[5,5]undecane (**16**). A mixture of **15** (117 mg, 0.364 mmol) and **19** (107 mg, 0.366 mmol) was coevaporated with dry toluene (10 ml) to remove the moisture and then dissolved in dry DMF (2 ml). Benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP, 170 mg, 0.384 mmol) and triethylamine (102 μl) were added to this mixture, before heating with stirring at 60°C for 6 hr in an atmosphere of argon. After cooling, the mixture was diluted with EtOAc–ether (1:1), washed with water and brine, dried (Na_2SO_4), and evaporated. Chromatography of the residue on silica gel in toluene–EtOAc (7:3) afforded **16** (182 mg, 83.9%). $[\alpha]_D^{22} - 12.0^\circ$ ($c = 3.9$, CHCl_3). IR (neat) ν_{max} 3300, 1730, 1695, 1640 cm^{-1} . $^1\text{H-NMR}$ (CDCl_3) δ 3.25 (3H, br. s), 3.90 (3H, s), 4.0–4.4 (2H, m), 6.25 (1H, m), 6.96 (1H, m), 7.0–7.2 (3H, m), 9.07 (1H, br.). *Anal.* Found: C, 56.16; H, 5.36; N, 6.92. Calcd. for $\text{C}_{28}\text{H}_{32}\text{N}_3\text{O}_8\text{F}_3$: C, 56.47; H, 5.42; N, 7.06%.

[2*S*,6*S*,8*S*]-8-[2-*Oxo*-2-(1*H*-pyrrol-2-yl)ethyl]-2-[5-(*N*-methyltrifluoroacetamido)-4-methoxycarbonyl-benzoxazol-2-yl]methyl-1,7-dioxaspiro[5,5]undecane (**17**). A mixture of

16 (164 mg, 0.275 mmol) and pyridinium *p*-toluenesulfonate (PPTS, 44 mg, 0.175 mmol) in dichloroethane (18 ml) was heated with stirring at 80–90°C for 9 hr. After cooling, the mixture was diluted with CHCl_3 , successively washed with satd. NaHCO_3 , water and brine, dried (Na_2SO_4), and then evaporated. Chromatography of the residue on silica gel (20 g) in toluene–EtOAc (65:35 ~ 1:1) afforded **17** (64 mg) and unreacted **16** (67 mg). The latter was again submitted to heating with PPTS and an additional amount (38 mg) of **17** was obtained (total, 64%). $[\alpha]_D^{24} + 46.2^\circ$ ($c = 1.8$, CHCl_3). $^1\text{H-NMR}$ (CDCl_3) δ : 3.36 (3H, s), 4.00 (3H, s), 4.34 (1H, m), 4.45 (1H, m), 6.30 (1H, m), 6.96 (1H, m), 7.05 (1H, m), 7.25 (1H, d, $J = 8.5$ Hz), 7.66 (1H, d, $J = 8.5$ Hz). MS. Found: 559.19276. Calcd. for $\text{C}_{28}\text{H}_{28}\text{F}_3\text{N}_3\text{O}_6$ (**17**– H_2O): 559.19298.

Tetranormethyl-calcimycin (**2**). A solution of **17** (21 mg, 0.036 mmol) in THF (4 ml) was cooled in an ice-cooled water bath. To the solution were added, with stirring, H_2O_2 (30%, 0.5 ml) and aqueous LiOH (1.25 N, 0.5 ml). After stirring for 3 hr at 0°C, the mixture was diluted with ether (30 ml). The ethereal solution was successively washed with water, aqueous $\text{Na}_2\text{S}_2\text{O}_3$, water and brine, and then dried (Na_2SO_4). Removal of the solvent gave a crude **18** (about 20 mg), which was heated under reflux with LiI (50 mg, 0.374 mmol) in pyridine (4.5 ml) for 2 hr in an atmosphere of nitrogen. After cooling, the mixture was poured into ice-cooled water and acidified with diluted HCl (1 N) to adjust the pH to 3–4. The mixture was then extracted with ether. The ethereal extract was washed with water and brine, dried (Na_2SO_4), and evaporated *in vacuo*. The residue was chromatographed on a column of citric acid-impregnated silica gel^(6b) (5 g) in *n*-hexane–EtOAc (1:1). The fraction corresponding to **2** [R_f 0.41, on a silica gel HPTLC plate sprayed with citric acid, *n*-hexane–EtOAc (1:1)] was dissolved in ether. The ethereal solution was washed with water to remove the contaminating citric acid, and then dried (Na_2SO_4). Removal of the solvent afforded **2** (13 mg, 76.5%). $[\alpha]_D^{23} + 43.0^\circ$ ($c = 0.8$, CHCl_3). $^1\text{H-NMR}$ (CDCl_3) δ 2.97 (3H, d, $J = 5.1$ Hz), 4.37 (1H, m), 6.26 (1H, m), 6.64 (1H, d, $J = 9.0$ Hz), 6.90 (1H, m), 7.03 (1H, m), 7.52 (1H, d, $J = 9.0$ Hz), 8.10 (1H, m), 9.42 (1H, br.). MS. Found: 467.20449. Calcd. for $\text{C}_{25}\text{H}_{29}\text{N}_3\text{O}_6$: 467.20563.

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REFERENCES AND NOTES

- 1) R. L. Hamill, M. Gorman, R. M. Gale, C. E.

- Higgins and M. M. Hoehn, Abstracts of Papers, 12th Interscience Conference on Antimicrobial Agents and Chemotherapy, Atlantic City, N. J., Sept. 26~29, 1972, p. 65.
- 2) M. O. Chaney, P. V. Demarco, N. D. Jones and J. L. Occolowitz, *J. Am. Chem. Soc.*, **96**, 1932 (1974).
 - 3) D. A. Evans, C. E. Sacks, W. A. Kleschick and T. R. Taber, *J. Am. Chem. Soc.*, **101**, 6789 (1979).
 - 4) a) P. A. Grieco, E. Williams, H. Tanaka and S. Gilman, *J. Org. Chem.*, **45**, 3537 (1980); b) G. R. Martinez, P. A. Grieco, E. Williams, K. Kanai and C. V. Srinivasan, *J. Am. Chem. Soc.*, **104**, 1436 (1982).
 - 5) Y. Nakahara, A. Fujita and T. Ogawa, *J. Carbohydr. Chem.*, **3**, 487 (1984).
 - 6) a) M. Debono, R. M. Molloy, D. E. Dorman, J. W. Paschal, D. F. Babcock, C. M. Deber and D. R. Pfeiffer, *Biochemistry*, **20**, 6865 (1981); b) M. Prudhomme and G. Jeminet, *Experientia*, **39**, 256 (1983); c) M. Prudhomme, G. Dauphin, J. Guyot and G. Jeminet, *J. Antibiot.*, **37**, 627 (1984).
 - 7) Relative stability between the stereoisomers of 1,7-dioxaspiro[5,5]undecane was discussed: P. Deslongchamps, D. D. Rowan, N. Pothier, T. Sauvé and J. K. Saunders, *Can. J. Chem.*, **59**, 1105 (1981).
 - 8) T. Katsuki and K. B. Sharpless, *J. Am. Chem. Soc.*, **102**, 5974 (1980).
 - 9) P. Ma, V. S. Martin, S. Masamune, K. B. Sharpless and S. M. Viti, *J. Org. Chem.*, **47**, 1378 (1982).
 - 10) Racemic **3** has been reported: D. A. Evans, C. E. Sacks, R. A. Whitney, N. G. Mandel, *Tetrahedron Lett.*, **1978**, 727.
 - 11) M. Araki, S. Sakata, H. Takei and T. Mukaiyama, *Bull. Chem. Soc. Jpn.*, **47**, 1777 (1974).
 - 12) Y. Nakahara, A. Fujita and T. Ogawa, *Agric. Biol. Chem.*, **49**, 1491 (1985).
 - 13) B. Castro, J. R. Dormoy, B. Dourtoglou, G. Evin, C. Selve and J. C. Ziegler, *Synthesis*, **1976**, 751.
 - 14) M. Miyashita, A. Yoshikoshi and P. A. Grieco, *J. Org. Chem.*, **42**, 3772 (1977).
 - 15) K. Kubota "Yakurigaku Jisshu," II-6, Kodansha, 1977, p. 106.
 - 16) S. N. Huckin and L. Weiler, *J. Am. Chem. Soc.*, **96**, 1082 (1974).
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