ANTIBIOTIC A 19009

STRUCTURAL INVESTIGATION AND SYNTHESIS

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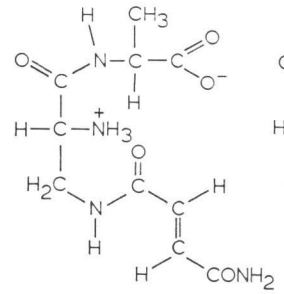
The structure of fermentation product A 19009 was reinvestigated by ^{13}C and ^{1}H NMR spectroscopy and established by independent synthesis to be N^{2}-L-alanyl-N^{3}-fumaramoyl-L-2,3-diaminopropanoic acid (**2**), *i.e.* a structure isomeric with the originally proposed structure **1**. In contrast to **1** which also was synthesized, **2** has a very low activity against *Trichomonas vaginalis*.

The antibiotic A 19009, isolated from a strain of *Streptomyces collinus* Lindenbein has been assigned the structure *N*-(N^{3}-fumaramoyl-L-2,3-diaminopropanoyl)-L-alanine (**1**)[1], a simple derivative of the non-protein amino acid L-2,3-diaminopropanoic acid (L-DAP) which occurs in several other biologically active metabolites of vegetable and microbial origin. The antibiotic is of interest, not only because of its significant activity against *Salmonella gallinarum* and *Trichomonas vaginalis*, but also because it contains L-DAP as a straightforward building block. We were interested in this aspect because it was expected to permit a direct investigation of the biosynthesis of L-DAP in a microorganism; such unambiguous result could not be obtained for L-DAP in malonomicin due to further metabolic transformations[2]. However, before starting our biosynthetic studies, we had to be absolutely sure about the structure assignment of A 19009. The original paper[1] gave reliable information on the three constituents only; their connectivity was proposed without supporting evidence. Furthermore, biosynthetic studies required a complete interpretation of the ^{1}H and ^{13}C NMR spectra.



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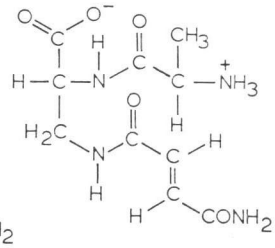

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In this communication we wish to report a spectral and synthetic investigation of the fermentation product A 19009 showing it to be **2**, a structure which is isomeric with the originally proposed structure **1**.

Structural Investigation

A 19009 was obtained both as a gift of Eli Lilly and Company and by isolation from a culture of *S. collinus* Lindenbein (obtained from Eli Lilly and Company, and from Centraal Bureau Schimmelcultures, Baarn (NRRL 5332 and CBS 718.19)).

The three samples were identical according to ^{1}H NMR, ^{13}C NMR, IR and TLC, but exhibited slightly different physicochemical properties as compared to the published data[1].

Thus, recrystallization from a 1:1 mixture of water and methanol as described was not satisfactory and a 1:4 mixture had to be used instead, indicating differing solubilities. The melting point of the

recrystallized compounds was 292~294°C (dec.) and differed significantly from the published mp 275~280°C (dec.). Most surprisingly, however, the biological activity of all three samples against *T. vaginalis* was very low so that the identity of the metabolite as compared to the original claim was questionable and a reinvestigation of its structure was required.

The ^{13}C NMR spectrum of A 19009 at pH 5 showed the presence of ten C-atoms with chemical shift and multiplicity values consistent with the proposed structure **1** (Fig. 1). The ^{1}H NMR spectrum (in D_2O at pH 5) (Fig. 2) was fully compatible with the exclusive presence of a fumaramoyl-, an alanyl-, and a 2,3-diaminopropanoyl moiety in the metabolite as originally established. Thus, a doublet at δ 1.32 (3H) and a corresponding quartet at δ 3.84 (1H) is assigned to the alanyl moiety; a singlet at δ 6.66 (2H) is attributed to the almost equivalent double bond protons of the fumaramoyl group; the presence of the CH_2CH-group of the DAP moiety is indicated by an ABX spin system at δ 3.38 (dd, 1H), 3.59 (dd, 1H) and 4.16 (dd, 1H) (J_{AB}=14 Hz, J_{AX}=4.2 Hz, J_{BX}=7.8 Hz).

However, measurement of the pH-dependence of the ^{1}H NMR chemical shifts gave rise to the suspicion that the assembly of the component parts of A 19009 was different from the presentation in **1**, so

Fig. 1. Natural-abundance proton noise-decoupled ^{13}C NMR spectrum (22.63 MHz) of A 19009 in H_2O - D_2O (10: 1) at pH 5 (dioxane, δ 67.7 ppm, as internal standard).
Spectral width 6,000 Hz; pulse delay 4 s; transients 50,000; data points 4 K after Fourier transform.

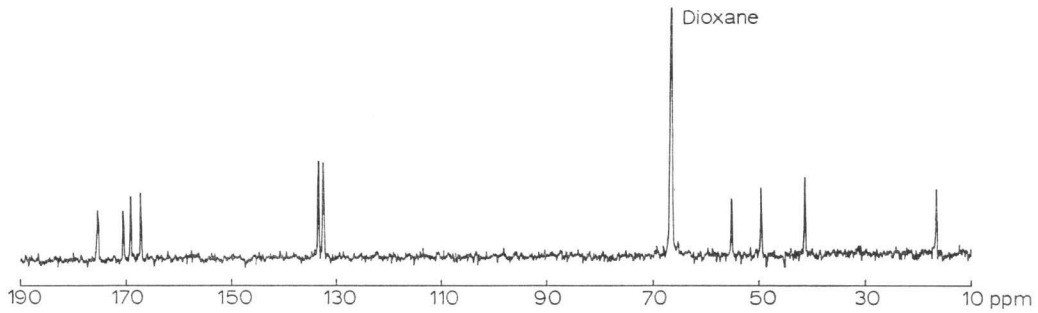


Fig. 2. ^{1}H NMR spectrum (90 MHz) of A 19009 in D_2O at pH 5 (DMSO, δ 2.50 ppm, as internal standard).

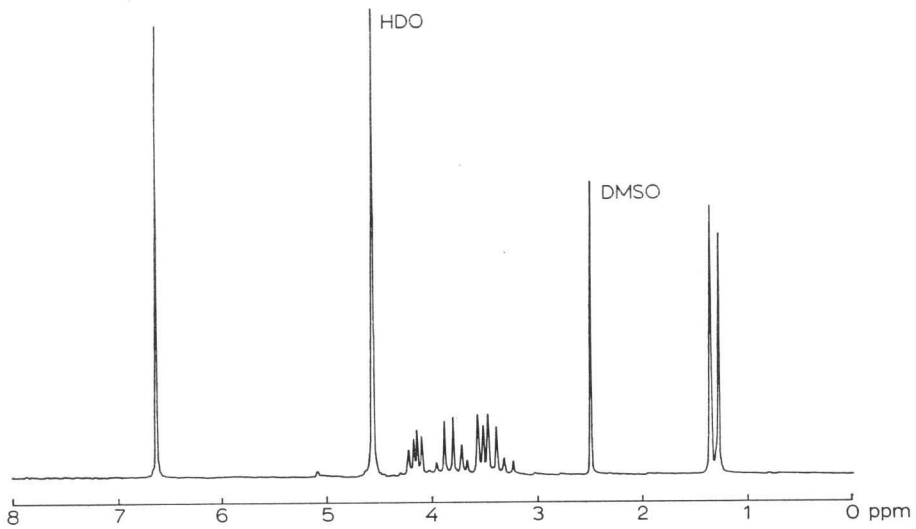
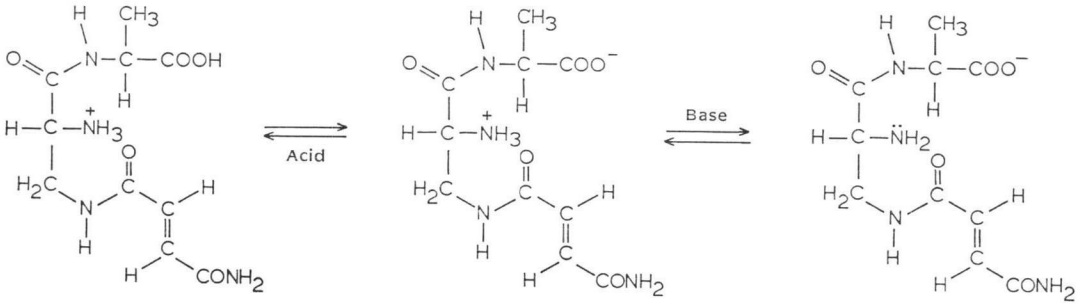


Fig. 3. Structure of **1** in acidic, neutral and basic solution.



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that in fact a structural isomer of **1** had been obtained. For, if **1** were the correct structure of A 19009, changing the pH of a solution in D_2O from neutral to acidic (Fig. 3) would be expected to cause a significant shift of the α-proton of the alanyl part only (downfield by protonation of the carboxylate anion); the chemical shifts of the other carbon bonded protons should change only very slightly.

In basic solution, on the other hand, removal of the positive charge on the free amino group would result in a considerable upfield shift of the α-proton of DAP and a smaller but significant upfield shift of the β-protons of DAP, whereas the remainder of the ^1H NMR spectrum should be largely unchanged.

Table 1a. ^1H NMR spectral data of A 19009 (**2**) in acidic, neutral and basic solution.

A 19009 (**2**)	pH≤1	pH 5	pH≥13
Ala-CH_β	1.32 (d, 3H, 7 Hz)	1.32 (d, 3H, 7 Hz)	1.01 (d, 3H, 7 Hz)
Ala-CH	3.90 (q, 1H, 7 Hz)	3.84 (q, 1H, 7 Hz)	3.26 (q, 1H, 7 Hz)
DAP-CH_AH_B	3.49 and 3.64 (2×dd, 2×1H) (J_{AB}=14 Hz)	3.38 and 3.59 (2×dd, 2×1H) (J_{AB}=14 Hz)	3.36 and 3.52 (2×dd, 2×1H) (J_{AB}=14 Hz)
DAP-CH_X	4.47 (dd, 1H) (J_{AX}=4.3 Hz; J_{BX}=7.5 Hz)	4.16 (dd, 1H) (J_{AX}=4.2 Hz; J_{BX}=7.8 Hz)	4.18 (dd, 1H) (J_{AX}=4.5 Hz; J_{BX}=7.5 Hz)
Fumaroyl-CH=CH–	6.66 (s, 2H)	6.66 (s, 2H)	6.67 (s, 2H)

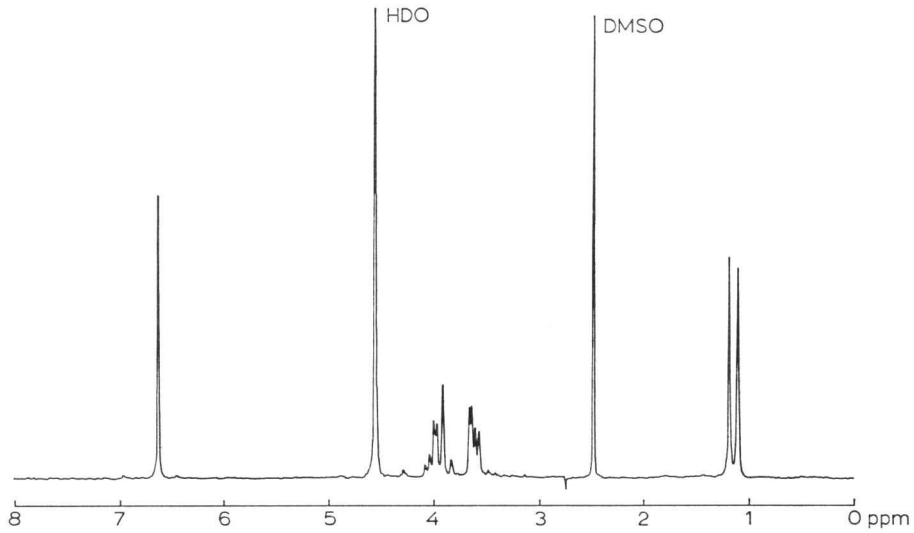
Chemical shifts of D_2O-solution (at 90 MHz) in ppm downfield from Me_4Si (δ=0) calculated from internal DMSO (δ=2.50 ppm): multiplicities (s, d, t, q and m), number of protons, and coupling constants in parentheses.

Table 1b. ^1H NMR spectral data of **1** in acidic, neutral and basic solution.

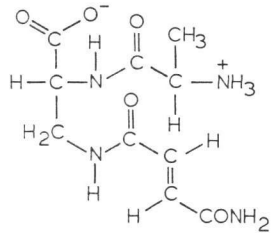
1	pH≤1	pH 5	pH≥13
Ala-CH_β	1.23 (d, 3H, 7.3 Hz)	1.17 (d, 3H, 7.3 Hz)	1.12 (d, 3H, 7.3 Hz)
Ala-CH	4.23 (q, 1H, 7.3 Hz)	3.97 (q, 1H, 7.3 Hz)	3.92 (q, 1H, 7.3 Hz)
DAP-CH_AH_B	3.56 and 3.66 (2×dd, 2×1H) (J_{AB}=14.5 Hz)	3.55 and 3.65 (2×dd, 2×1H) (J_{AB}=14.5 Hz)	ABC spin system max. *ca.* 3.32 (m, 3H)
DAP-CH_X	4.04 (dd, 1H) (J_{AX}=4.5 Hz; J_{BX}=7.5 Hz)	3.99 (dd, 1H) (J_{AX}=4.5 Hz; J_{BX}=7.5 Hz)	
Fumaroyl-CH=CH–	6.67 (d, 2H, 1.2 Hz)	6.66 (broad s, 2H)	6.65 (s, 2H)

See footnote of Table 1a.

Fig. 4. ^{1}H NMR spectrum (90 MHz) of **1** in D_2O at pH 5 (DMSO, δ 2.50 ppm, as internal standard).



However, exactly the opposite phenomena were observed. Thus, acidification of a neutral solution of A 19009 in D_2O gave a noticeable downfield shift of the α-proton of DAP only, whereas in basic solution a large upfield shift of the α-proton of the alanyl moiety and a smaller upfield shift of its β-protons was observed (Table 1a). It was inferred, therefore, that it is not the α-amino group of the DAP moiety in A 19009 which is free as in **1**, but the amino group of Ala such as in **2**, *i.e.* a N^2,N^3-disubstituted derivative of L-2,3-diaminopropanoic acid. This conclusion was fully corroborated by total synthesis of both **1** and **2** (*vide infra*). It was established that the synthetic compound **2** was identical in all respects to the compound obtained from Eli Lilly and Company, and by independent fermentation. Synthetic compound **1** indeed had the predicted ^{1}H NMR chemical shift dependence on pH (Table 1b) and, significantly, displayed a distinct activity (MIC 3.12 μg/ml) against *T. vaginalis* which fits the published value (MIC 3.9 μg/ml).



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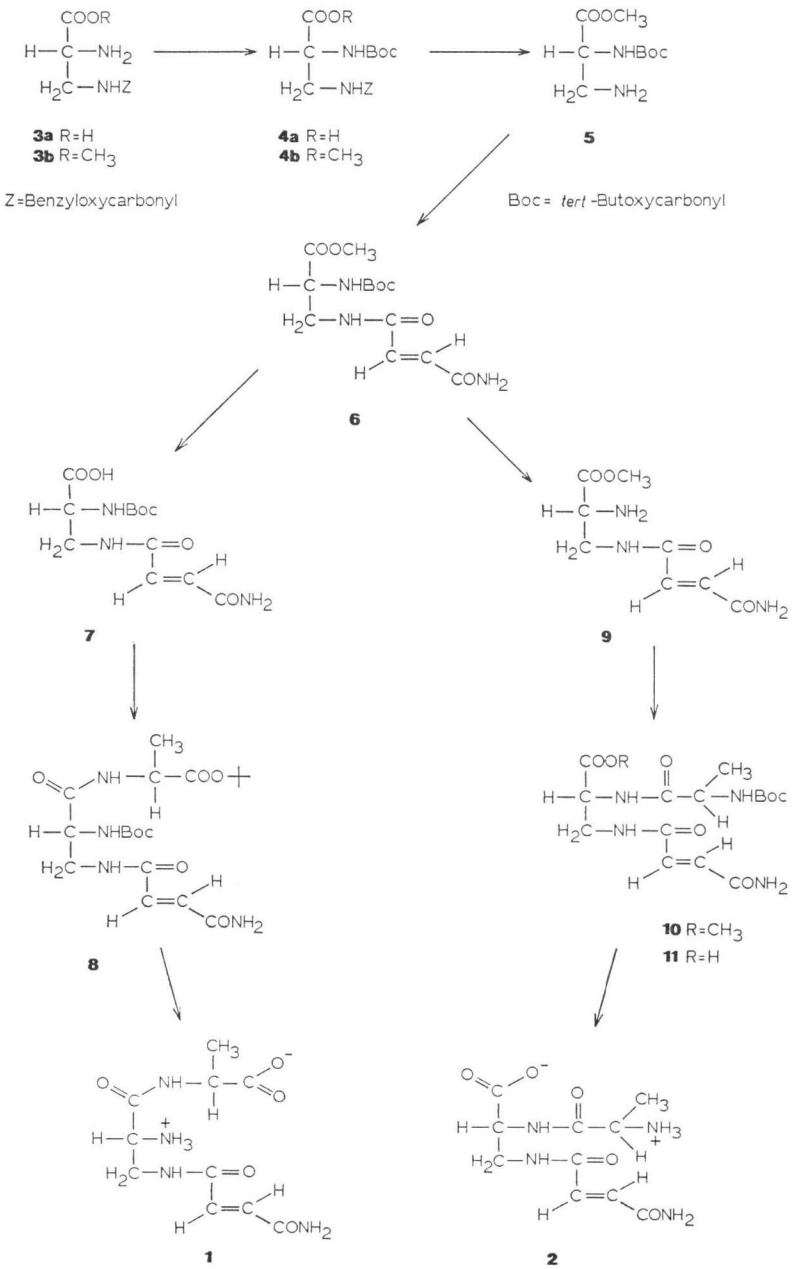
As compounds **1** and **2** are reasonably stable, in the solid state as well as in solution, and do not show any tendency to interconvert, the discrepancy between the data of the original publication and our own findings, in our view, can be explained only if the microorganism in the course of time has lost its ability to produce the active compound **1** and now forms the inactive isomer **2** instead. In fact, it seems very likely that this has unobservedly passed during the earlier structure determination study[1] as the published rotation value ($[\alpha]_{810}$ +107°) obviously refers to the inactive compound **2** (see Experimental). With respect to the planned investigation into the biosynthetic origin of L-DAP, it is immaterial in principle whether it is **1** or **2** which is produced by the microorganism. Actually, it is profitable that **2** is now obtained by fermentation because the chemical shift difference between the β-protons of L-DAP is much more pronounced in **2** than in **1** (Table 1a, b) facilitating a stereochemical investigation with ^{2}H labelled precursors and highfield ^{2}H NMR spectroscopy[3].

Synthesis

As **1** and **2** are structural isomers which differ only in the place and type of junction of the alanyl moiety to the diaminopropanoyl backbone, it is obviously efficient to design a common central intermediate derived from N^3-fumaramoyl-L-2,3-diaminopropanoic acid, from which both **1** and **2** can be synthesized.

Compound **6** (Scheme 1) is such an intermediate which on the one hand by hydrolysis of the methyl ester followed by coupling with a suitably protected alanyl derivative could give access to **1**, and, on the

Scheme 1. Synthesis of **1** and **2**.



other hand, by substitution of the protecting Boc-group* by alanine should be convertible to **2**.

The synthesis of central intermediate **6** can be accomplished in a straightforward way from the known[4,5] L-DAP derivatives **3a** and **3b** (Scheme 1). Reaction of **3a** with *tert*-butyl azidoformate and triethylamine in dioxane - water[6] gave N^2-*tert*-butoxycarbonyl-N^3-benzyloxycarbonyl-L-2,3-diaminopropanoic acid (**4a**) which was converted quantitatively with diazomethane into the corresponding methyl ester **4b**. Alternatively, **4b** could be prepared directly in 87% yield by reaction of the free amino acid ester **3b** with *tert*-butyl azidoformate in pyridine. Deprotection of the 3-amino group of **4b** was accomplished by Pd/C-catalyzed hydrogenolysis in methanol. The free amino acid ester **5** was obtained quantitatively and coupled with fumaramic acid[7] in anhydrous *N*,*N*-dimethylformamide (DMF) by means of 1-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ)[5] to give methyl N^2-*tert*-butoxycarbonyl-N^3-fumaramoyl-L-2,3-diaminopropanoate, the central intermediate **6**, in 54% yield.

Starting from **6**, the total synthesis of the bioactive isomer **1** can be achieved readily. Carefully controlled hydrolysis of **6** in DMF - H_2O at pH 12~13 with 0.5 N KOH furnished carboxylic acid **7** (86% yield) which was reacted with alanine *tert*-butyl ester[8] and EEDQ in dry DMF. The coupling product **8** (85% yield) was deprotected in one step by treatment with CF_3COOH during 2 hours at room temperature, yielding crude **1** (97% yield; almost pure according to TLC) which was recrystallized from MeOH - H_2O, 1: 1, to give the antibiotic **1** in 75% yield.

For the synthesis of **2**, first the protecting Boc-group of intermediate **6** was removed by treatment with CF_3COOH at 0°C. The resulting free amino acid ester **9** was coupled with *tert*-butoxycarbonyl-L-alanine by means of EEDQ to give **10** in 72% yield. Finally, the carboxylic acid group of the DAP moiety was generated by controlled hydrolysis with 0.5 N KOH in DMF - H_2O, and the protecting Boc-group was removed by the action of CF_3COOH at 0°C. The obtained product (98% yield) was recrystallized from MeOH - H_2O, 4: 1, and proved to be fully identical with the fermentation product **2**.

As far as we know, the original microorganism which was able to produce **1** does not exist anymore. Therefore, total synthesis seems at present to be the only way to prepare the antibiotic **1**.

Experimental

NMR spectra were determined on a Bruker model WH90 spectrometer; chemical shifts are given in ppm (δ) downfield from Me_4Si ($\delta=0$ ppm) with dioxane as an internal reference ($\delta_{TMS}=\delta_{dioxane}-67.7$) in the case of ^{13}C NMR spectra and with DMSO as an internal reference in the case of ^1H NMR spectra ($\delta_{TMS}=\delta_{DMSO}-2.50$); multiplicities are indicated with s, d, t, q, and m. Optical rotations were measured on a Perkin Elmer model 241MC polarimeter. Melting points were measured with a Kofler hot stage apparatus under a Reichert microscope and are uncorrected.

Isolation of A 19009 (**2**)

A strain of *Streptomyces collinus* Lindenbein, CBS 718.79 (NRRL 5332), maintained on oatmeal agar, was inoculated into nine 500-ml baffled conical flasks with wadding closure, each containing 125 ml of sterilized medium (adjusted to pH 8.0 with 2 N NaOH) of the following composition: malt extract (Oxoid L39; 15 g), peptone (Oxoid L34; 10 g), NaCl (5 g), and tap water (1 liter). Incubations were performed at 28°C on a rotary shaker (300 rpm; 3.8 cm eccentricity). After 48 hours, mycelium was centrifuged off (10 minutes at 11,000 rpm) and the filtered supernatant (*ca.* 1 liter) (pH *ca.* 6.5~7) was adsorbed on a column (22.5×5 cm) of carbon beads (BDH; 0.25~0.60 mm) in H_2O. After washing

* The choice of the Boc-group as a protection for the α-amino group of L-DAP is not trivial as use of other investigated protecting groups such as the tosyl and trichloroethoxycarbonyl group lead to inferior yields of **1** and **2**.

with water, elution with acetone - H_2O, 1: 2, and evaporation *in vacuo* at 40°C gave *ca.* 9 g of a yellow-brown foam.

This product was dissolved in MeOH - H_2O, 1: 1, and adsorbed on a column of aluminum oxide, acidic (act. I) (*ca.* 200 g; column 26×3 cm) in MeOH - H_2O, 1: 1. After washing with MeOH - H_2O, 1: 1, the metabolite was eluted with a linear gradient of MeOH - H_2O, 1: 1,→H_2O (1 liter; flow rate 60 ml/hour). The collected fractions (10 ml each) containing the metabolite (identified by TLC (SiO_2; EtOH - 4 N NH_4OH, 9: 1)) appeared after *ca.* 10 hours and were combined (*ca.* 300 ml) to yield a light-yellow foam (450 mg) after evaporation to dryness *in vacuo* at 40°C.

The crude product was dissolved in H_2O (*ca.* 25 ml) and, after adjustment of the pH to 8.5, adsorbed on DEAE-Sephadex A-25 (column 26×2 cm; pH adjusted to 8.5 with 4 N NH_4OH). After washing with water, A 19009 (**2**) was eluted with a linear gradient of H_2O→0.2 N AcOH. The fractions containing (**2**) according to TLC were combined and evaporated to dryness *in vacuo* yielding 69 mg of a white solid pure according to TLC (SiO_2; EtOH - 4 N NH_4OH, 9: 1, Rf 0.7; violet with ninhydrin) and ^1H NMR. Recrystallization from H_2O - MeOH, 1: 4, gave **2** as white needles, mp 293~294°C (dec.); $[\alpha]_D^{20}$ +3.7° (*c* 0.99, H_2O), $[\alpha]_{365}^{20}$ +30.0° (*c* 0.99, H_2O), $[\alpha]_{310}^{20}$ +96.3° (*c* 0.25, H_2O); ^1H NMR, see Fig. 2 and Table 1a; ^{13}C NMR (H_2O - D_2O, 10: 1) δ 16.6 (Ala-CH_3), 41.2 (DAP-CH_2), 49.5 (Ala-*C*H), 55.2 (DAP-*C*H), 132.5, 133.4 (fumaroyl-*C*H=*CH*-), 167.3, 169.2, 170.6 and 175.5 (4×*C*=O) (Fig. 1).

Methyl N^2-*tert*-Butoxycarbonyl-N^3-benzyloxycarbonyl-L-2,3-diaminopropanoate (**4b**)

Methyl N^3-benzyloxycarbonyl-L-2,3-diaminopropanoate (**3b**)[5] (4.2 g, 16.5 mmol) was dissolved in anhydrous pyridine (15 ml) and stirred with *tert*-butyl azidoformate (4.8 g, 33 mmol) at room temperature during 18 hours. After evaporation to dryness *in vacuo*, the oily residue was dissolved in diethyl ether and washed with 1 N citric acid, water and $NaHCO_3$-solution, respectively. Drying (Na_2SO_4) and evaporation to dryness *in vacuo* yielded **4b** (5.1 g, 87% yield) as a light-yellow oil which crystallized slowly after standing at −20°C, mp *ca.* 50°C; ^1H NMR ($CDCl_3$) δ 1.46 (s, 9H, *tert*-butyl), 3.50~3.75 (m, 2H, DAP-CH_2), 3.76 (s, 3H, CH_3O), 4.21~4.55 (m, 1H, DAP-CH), 5.13 (s, 2H, ϕCH_2O), 5.1~5.6 (2×s, very broad, 2H, 2×NH).

In an alternate procedure, N^3-benzyloxycarbonyl-L-2,3-diaminopropanoic acid (**3a**)[4] (1.9 g, 8 mmol) was dissolved in H_2O (12 ml) and Et_3N (3.36 ml). *tert*-Butyl azidoformate (1.36 ml) in dioxane (12 ml) was added and the mixture stirred until a clear solution was obtained (*ca.* 4 hours)[6]. After evaporation to dryness *in vacuo* (t≤50°C), the residue was dissolved in diethyl ether - H_2O and filtered to remove unreacted **3a**. The water layer was separated, acidified with 2 N citric acid, extracted with EtOAc (3×), and the organic phase washed with water (2×). Drying (Na_2SO_4) and evaporation *in vacuo* yielded **4a** (2.1 g, 78% yield) as a very viscous pale yellow oil; ^1H NMR ($CDCl_3$) δ 1.39 (s, 9H, *tert*-butyl), 3.35~3.75 (m, 2H, DAP-CH_2), 4.15~4.6 (m, 1H, DAP-CH), 5.09 (s, 2H, ϕCH_2O), 5.5~6.3 (broad s, 2H, 2×NH), 7.3 (broad s, 5H, ϕ), 10.8 (broad s, 1H, COOH).

Crude **4a** was dissolved in the minimum amount of tetrahydrofurane and treated at 0°C with a slight excess of diazomethane in ether. Evaporation *in vacuo* yielded an oil, which solidified upon standing, mp *ca.* 50°C; according to ^1H NMR fully identical with **4b** as obtained above.

Methyl N^2-*tert*-Butoxycarbonyl-N^3-fumaramoyl-L-2,3-diaminopropanoate (**6**)

A solution of **4b** (8.28 g, 23.5 mmol) in methanol (80 ml) was stirred vigorously with Pd/C 10% (800 mg) in a H^2-atmosphere during 3 hours. Filtration and evaporation to dryness *in vacuo* yielded **5** (5.13 g, ~100%) as a yellow oil (homogeneous according to TLC) which was immediately used without further purification.

To a solution of **5** (5.13 g, 23.5 mmol) and fumaramic acid[7] (3.0 g, 26 mmol) in dry DMF (65 ml) at −15°C, EEDQ[8] (6.4 g, 26 mmol) was added. After stirring overnight (in the course of which the temperature was slowly raised to room temperature) and subsequently for 8 hours at room temperature, the colorless solution was evaporated to dryness at 1 mm Hg (t≤40°C). The semi-solid residue was triturated with EtOAc to yield after suction filtration a white solid (6.5 g) which was suspended in H_2O, treated with 4 N NH_4OH (pH 8) and stirred during ½ hour. Suction filtration, washing with ice-cold water and drying *in vacuo* over P_2O_5 yielded **6** (4.0 g, 54% yield) which was pure according to TLC (SiO_2; $CHCl_3$ - CH_3COCH_3 - EtOH, 6: 4: 1). Recrystallization from acetone gave analytically pure material,

mp 190~192°C; ^{1}H NMR (DMSO-d_6) δ 1.38 (s, 9H, *tert*-butyl), 3.22~3.79 (m, 2H, DAP-CH_2), 3.62 (s, 3H, CH_3O), 3.89~4.32 (m, 1H, DAP-CH), 6.77 (s, 2H, –HC=CH–), 7.16 (broad d, 1H, NH), 7.30 and 7.75 (2×broad s, 2×1H, NH_2), 8.47 (broad t, 1H, NH).

Anal. Calcd. for $C_{13}H_{21}N_3O_6$: C 49.51, H 6.71, N 13.33.
Found: C 49.54, H 6.82, N 13.08.

N-(*N*^{2}-*tert*-Butoxycarbonyl-*N*^{3}-fumaramoyl-L-2,3-diaminopropanoyl)-L-alanine *tert*-Butyl Ester (**8**)

A solution of **6** (315 mg, 1 mmol) in DMF (5 ml) and H_2O (2 ml) was treated dropwise with 0.5 N KOH under stirring in a nitrogen atmosphere, the pH being kept between 12 and 13 (pH-meter). After addition of the theoretical amount of base and stirring until pH 11 was attained, the mixture was cooled to 0°C and neutralized carefully with 1 N HCl (pH 3). Evaporation to dryness *in vacuo* (t≤30°C), dissolution of the residue in the minimum amount of dry DMF and centrifugation to remove KCl, gave a clear solution which was evaporated to dryness at 1 mm Hg (t≤30°C) during several hours to yield **7** (314 mg, 86% yield); ^{1}H NMR (DMSO-d_6) δ 1.37 (s, 9H, *tert*-butyl), 3.18~3.66 (m, 2H, DAP-CH_2), 3.87~4.23 (m, 1H, DAP-CH), 6.78 (s, 2H, –CH=CH–), 6.97 (broad d, 1H, NH), 7.26 and 7.74 (2× broad s, 2×1H, NH_2), 8.46 (broad t, 1H, NH).

Crude **7** (311 mg, 1.03 mmol) was reacted with L-alanine *tert*-butyl ester[9] (200 mg, 1.37 mmol) and EEDQ[5] (340 mg, 1.37 mmol) in dry DMF (7 ml) under stirring overnight at room temperature. Evaporation *in vacuo* and trituration with EtOAc yielded **8** (375 mg, 85% yield), practically pure according to TLC (SiO_2; CH_3COCH_3 - $CHCl_3$, 3: 1), mp 178~180°C (dec.); ^{1}H NMR (DMSO-d_6) δ 1.24 (d, 3H, Ala-CH_3), 1.38 (s, 9H, *tert*-butyl), 3.14~3.62 (m, 2H, DAP-CH_2), 3.86~4.39 (m, 2×1H, DAP-CH and Ala-CH), 6.79 (s, 2H, –HC=CH–), 6.81 (broad d, 1H, NH), 7.26 and 7.74 (2×broad s, 2×1H, NH_2), 8.12 (broad d, 1H, NH), 8.32 (broad t, 1H, NH).

N-(*N*^{3}-Fumaramoyl-L-2,3-diaminopropanoyl)-L-alanine (**1**)

Protected dipeptide **8** (575 mg, 1.34 mmol) was stirred during 2 hours with CF_3COOH (5.8 ml) at room temperature. Evaporation to dryness *in vacuo* and trituration with dry EtOH gave crude **1** as a colorless solid (355 mg, 97% yield), pure according to TLC (SiO_2; EtOH - 4 N NH_4OH, 9: 1; Rf 0.75; brownish color with ninhydrin). Recrystallization was effected by dissolving 300 mg in H_2O (1 ml) and addition of MeOH (1 ml) to yield a clear solution which deposited **1** as colorless needles (225 mg) after cooling at 0°C; mp 290~293°C (dec.); $[\alpha]_D^{20}$ −6.9° (*c* 1.1, H_2O), $[\alpha]_{365}^{20}$ −15.1° (*c* 1.1, H_2O), $[\alpha]_{310}^{20}$ −8.2° (*c* 1.1, H_2O); ^{1}H NMR, see Fig. 4 and Table 1b. ^{13}C NMR (H_2O - D_2O, 10: 1) δ 17.3 (Ala-*C*H_3), 40.4 (DAP-*C*H_2), 51.6 (Ala-*C*H), 53.2 (DAP-*C*H), 132.9 and 133.1 (–*C*H=*C*H–), 167.2, 167.7, 168.9 and 179.5 (4×*C*=O).

Methyl *N*^{2}-(*tert*-Butoxycarbonyl-L-alanyl)-*N*^{3}-fumaramoyl-L-2,3-diaminopropanoate (**10**)

A solution of **6** (500 mg, 1.59 mmol) in CF_3COOH (5 ml) was stirred at 0°C during 2 hours. The residue obtained after evaporation *in vacuo* (t≤25°C) was dissolved in H_2O and treated dropwise with Et_3N until pH 8.5 was obtained. Evaporation to dryness *in vacuo* and careful washing with a little EtOAc gave **9** as a colorless solid (320 mg, 94%) which, apart from a minor quantity of $CF_3COOH\cdot Et_3N$, was pure and could be used directly in the next step; ^{1}H NMR of **9** (DMSO-d_6) δ 3.37~3.68 (m, 2H, DAP-CH_2), 3.70 (s, 3H, CH_3O), 3.78~4.03 (m, 1H, DAP-CH), 6.82 (s, 2H, –HC=CH–), 7.37 and 7.82 (2×broad s, 2×1H, NH_2), 8.61 (t, 1H, NH).

To an ice-cold solution of **9** (242 mg, 1.13 mmol) and *tert*-butoxycarbonyl-L-alanine[6] (195 mg, 1.24 mmol) in dry DMF (4.5 ml), EEDQ[5] (306 mg, 1.24 mmol) was added. After stirring overnight at room temperature, EtOAc (15 ml) was added and the precipitate was centrifuged off, washed with EtOAc (5 ml) and dried *in vacuo*, yielding **10** (313 mg, 72% yield) as a colorless solid, homogeneous according to TLC (SiO_2; $CHCl_3$ - CH_3COCH_3 - EtOH, 4: 4: 1); ^{1}H NMR (DMSO-d_6) δ 1.18 (d, 3H, Ala-CH_3), 3.32~3.79 (m, 2H, DAP-CH_2), 3.62 (s, 3H, CH_3O), 3.81~4.18 (m, 1H, DAP-CH), 4.27~4.58 (m, 1H, Ala-CH), 6.80 (s, 2H, –HC=CH–), 6.87 (broad d, 1H, NH), 7.32 and 7.79 (2×broad s, 2×1H, NH_2), 8.14 (broad d, 1H, NH), 8.46 (broad t, 1H, NH).

A sample for elemental analysis was recrystallized from methanol - ether, mp 167°C, solidifying again at *ca.* 200°C, and melting with decomposition at *ca.* 280°C.

Anal. Calcd. for $C_{16}H_{26}N_4O_7$: C 49.73, H 6.78, N 14.50.
Found: C 49.25, H 6.97, N 14.28.

N^2-(*tert*-Butoxycarbonyl-L-alanyl)-N^3-fumaramoyl-L-2,3-diaminopropanoic Acid (**11**)

Methyl ester **10** (280 mg, 0.73 mmol) was hydrolyzed with 0.5 N KOH in DMF - H_2O as described for **7**, yielding quantitatively amorphous **11** (270 mg), practically pure according to TLC (SiO_2; CH_3COCH_3 - EtOH - 4 N NH_4OH, 6: 3: 1); ^1H NMR (DMSO-d_6) δ 1.17 (d, 3H, Ala-CH_3), 3.28~3.70 (m, 2H, DAP-CH_2), 3.78~4.17 (m, 1H, DAP-CH), 4.20~4.53 (m, 1H, Ala-CH), 6.80 (s, 2H, –HC=CH–), 6.90 (broad d, 1H, NH), 7.33 and 7.80 (2×broad s, 2×1H, NH_2), 8.02 (broad d, 1H, NH), 8.45 (broad t, 1H, NH).

N^2-L-Alanyl-N^3-fumaramoyl-L-2,3-diaminopropanoic Acid (**2**)

11 (658 mg, 1.7 mmol) was stirred during 2 hours at 0°C in CF_3COOH (6.6 ml) in a nitrogen atmosphere. After evaporation to dryness *in vacuo* ($t \leq 25$°C), dissolution of the residue in H_2O and evaporation to dryness again, the obtained solid was dissolved in H_2O and neutralized with Et_3N (pH 5). After evaporation to dryness *in vacuo* and trituration with acetone, the product was collected by suction filtration and rinsed thoroughly with acetone to yield **2** (470 mg, 98%) as a colorless solid which was crystallized by dissolving in the minimum quantity of H_2O and adding four volumes of methanol; mp 293~294°C, $[\alpha]_D^{20}$ +3.5° (*c* 0.25, H_2O), $[\alpha]_{365}^{20}$ +26.7° (*c* 0.25, H_2O), $[\alpha]_{310}^{20}$ +88.4° (*c* 0.25, H_2O). This product was indistinguishable from natural **2** by (mixed) melting point, TLC, ^1H NMR (pH-dependent) and ^{13}C NMR (*vide supra*).

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References

1) MOLLOY, B. B.; D. H. LIVELY, R. M. GALE, M. GORMAN, L. D. BOECK, C. E. HIGGENS, R. E. KASTNER, L. L. HUCKSTEP & N. NEUSS: A new dipeptide antibiotic from *Streptomyces collinus*, Lindenbein. J. Antibiotics 25: 137~140, 1972
2) SCHIPPER, D.: Biosynthesis of malonomicin. Dissertation, Vrije Universiteit, Amsterdam, 1980
3) VAN DER BAAN, J. L.; J. W. F. K. BARNICK, D. SCHIPPER & F. BICKELHAUPT: Forthcoming publication
4) GRZYBOWSKA, J.; R. ANDRUSZKIEWICZ & H. WOJCIECHOWSKA: Unambiguous synthesis of N^3-benzyloxycarbonyl-(*S*)-2,3-diaminopropionic acid. Pol. J. Chem. 53: 935~936, 1979
5) SCHNEIDER, F.: Überführung von L(−)-Asparagin in L(−)-Serin. Liebigs Ann. Chem. 529: 1~10, 1937
6) GRZONKA, Z. & B. LAMMEK: A simple method of preparation of *t*-butoxycarbonyl amino acids. Synthesis 1974: 661~662, 1974
7) TALLEY, A. E.; TH. J. FITZPATRICK & W. L. PORTER: Formation of fumaramic acid from asparagin in phosphate buffer. J. Am. Chem. Soc. 81: 174~175, 1959
8) BELLEAU, B. & G. MALEK: A new convenient reagent for peptide syntheses. J. Am. Chem. Soc. 90: 1651~1652, 1968
9) ROESKE, R. W.: Preparation of *t*-butyl esters of free amino acids. J. Org. Chem. 28: 1251~1253, 1963