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THE TOTAL SYNTHESIS OF THE ANTIBIOTIC MALONOMICIN (K16)

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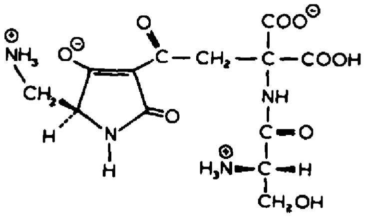
Abstract—The total synthesis of the antibiotic malonomicin has been accomplished by a convergent synthesis in which the crucial step is a very mild coupling of the dipeptide sidechain to the 3-position of the pyrrolidin-2,4-dione ring.

Some years ago,[1] malonomicin (**1**) (formerly known as K16) was shown to be a representative of the continuously growing group of metabolites which all have in common a pyrrolidin-2,4-dione (tetramic acid) nucleus, acylated at the 3-position.

The first and still most simple member of this series is tenuazonic acid, the structure of which was determined as 3 - acetyl - 5 - *sec*-butyl - 4 - hydroxy - Δ^3 - pyrrolin - 2 - one (**2**) by Stickings[2] (1959). In historical order, the other metabolites which have been shown to belong to this group are: streptolydigin (1963),[3] erythroskyrin (1964),[4] cyclopiazonic acid (1968),[5] tirandamycin (1971),[6] ikarugamycin (1972),[7] lipomycin (1973),[8] oleficin (1973),[9] magnesidin (1974),[10] and very recently equisitin (1976).[11]



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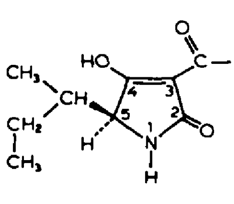

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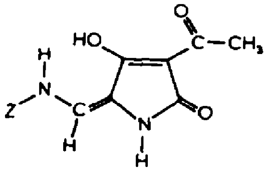
The substituents at C-3 and C-5 of the common tetramic acid nucleus of these antibiotics are structurally very divergent and their biosynthetic origins largely unknown.[12] The metabolic source of the *sec*-Bu group of tenuazonic acid (**2**) together with C-4, C-5 and N-1 has been shown to be L-isoleucin.[12a] Likewise, the results of preliminary biosynthetic investigations indicate that the aminomethyl group at C-5 of malonomicin has its origin in L-2,3-diaminopropanoic acid.[13] A more conspicuous feature of the structure of **1** is the presence of the unique aminomalonic acid moiety,[14] incorporated into the acyl substituent at C-3; this part of the molecule is of paramount importance for the biological activity of malonomicin as all activity is lost on decarboxylation.[1] The biosynthetic origin of this moiety is still unknown but is the object of active investigation in this laboratory.

As far as we know up till now, only the total synthesis of tenuazonic acid[15] and magnesidin,[10] the more simple and stable representatives of this group of antibiotics, have been realized. Attempts to synthesize the tryptophane derived cyclopiazonic acid[16] are on the way. Not only as a keystone of the structure determination but also to explore the possibilities of synthesis of structural analogues, we undertook the total synthesis of malonomicin, expecting the construction of the sensitive aminomalonic acid part to constitute the main obstacle on the way.

RESULTS AND DISCUSSION

At the outset, an obvious starting-point for the synthetic endeavour seemed to be the readily available 3 - acetyl - 5 - *N* - benzyloxycarbonylaminomethyl - 4 - hydroxy - Δ^3 - pyrrolin - 2 - one (**5a**) (Scheme 1) which had served as an intermediate for the preparation of the valuable model compound **5b** in the structure elucidation of malonomicin.[1] Proper functionalization of the acetyl group, followed by coupling with the anion of a suitable protected aminomalonic acid derivative, would then furnish a product which was expected to be easily transformed into a direct precursor of malonomicin. Furthermore, achievement of this goal would have provided considerable flexibility in attaching a variety of substituents leading to structural variations of malonomicin. However, all attempts to accomplish a selective transformation at the acetyl group of **5a** were unsuccessful. E.g. bromination with *N*-bromosuccinimide, bromine-dioxane complex, pyridinium hydrobromide perbromide, pyrrolidone hydrotribromide, or elementary bromine, merely yielded starting material or intractable mixtures of products. Only with $CuBr_2$ in $CHCl_3/EtOAc$[17] a single product was obtained in moderate yield, tentatively assigned structure **8** according to ^1H NMR and elemental analysis, most probably the result of bromination at C-5 followed by elimination of HBr.



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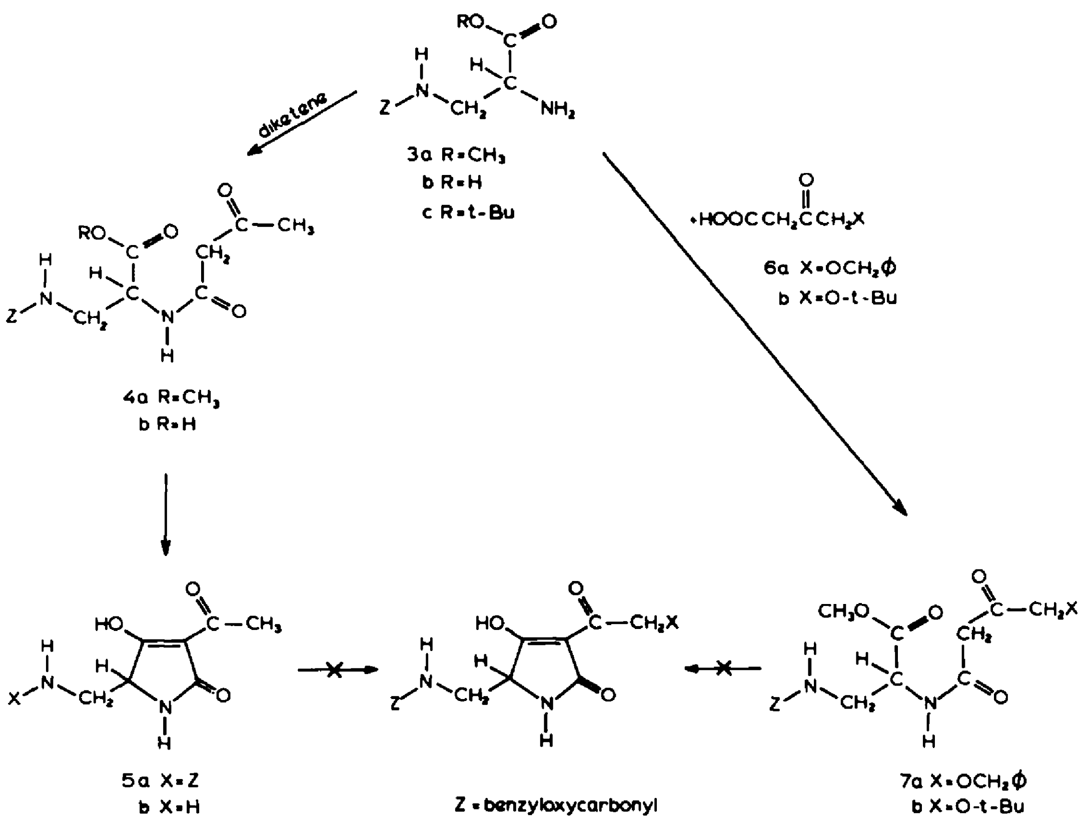
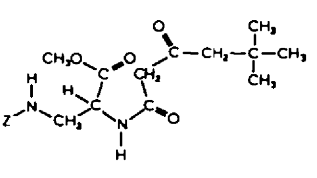


Scheme 1.

As the idea of a simply functionalized acetyltetramic acid derivative as a starting-point for the total synthesis was still appealing, we tried to cyclize **7a** and **7b**, the benzyloxy- and t-butoxy-substituted α-N-acetoacetyl derivatives of methyl N^3 - benzyloxycarbonyl - 2,3 - diaminopropanoate,[18] prepared from **3a** and benzyloxy- or t-butoxyacetoacetic acid, **6a** and **6b**, respectively (Scheme 1). However, neither under the original conditions of Lacey[19] nor with other combinations of bases and solvents, this deceptively simple looking cyclization (analogous to the successful reaction **4a** ⟶ **5a**) could be realized satisfactorily. Presumably, electronic effects of the alkoxy group are responsible for this failure; steric effects can be excluded, as cyclization of **9**, prepared from **3a** and γ-t-butylacetoacetic acid, was accomplished smoothly in the standard way,[19] yielding **10** in good yield.



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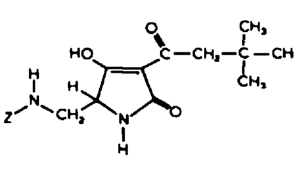

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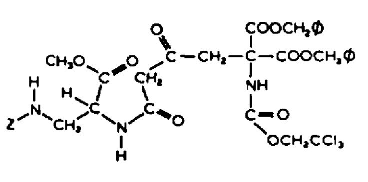
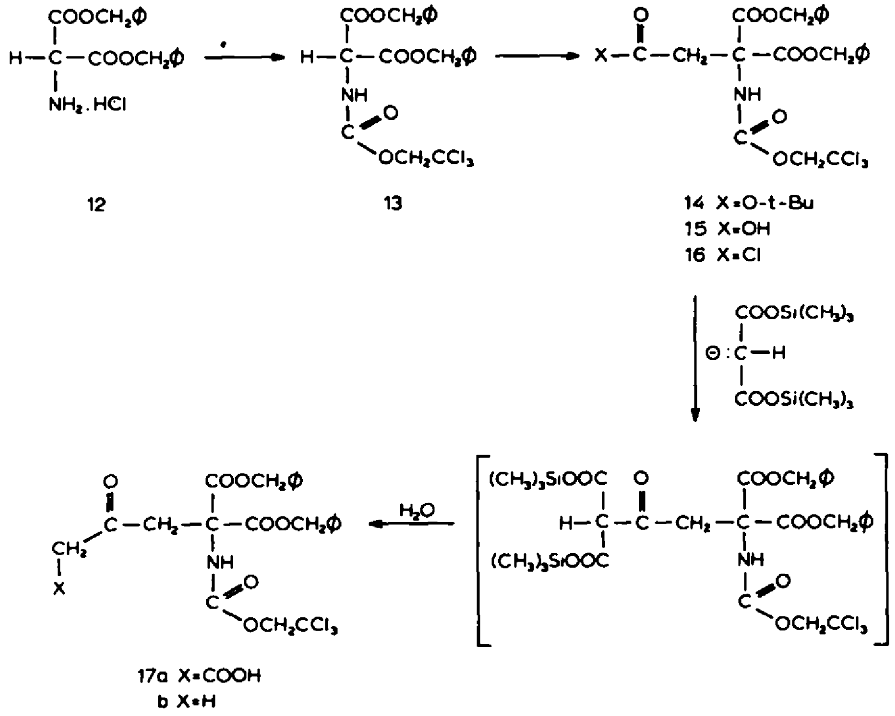


11

The conversion of **9** to **10** illustrated that in principle the cyclization of derivatives of **4a** carrying bulky substituents could be achieved, even, it was hoped, if provided with the appropriate functionality to elaborate the dipeptide side-chain. We therefore envisaged the synthesis of **11**, in which, besides the diaminopropanoic and acetoacetic acid residues, also the aminomalonic acid moiety is present, substituted with independently removable protecting groups for both the carboxy and amino functions.

The obvious approach to the synthesis of **11** seemed to be the coupling of **3a** with the β-ketoacid **17a**, which in its turn could be easily synthesized as shown in Scheme 2. Dibenzyl aminomalonate hydrochloride **12**[20] was protected by means of trichloroethyl chloroformate[21] in sodium bicarbonate solution, and alkylated with t-butyl chloroacetate in DMF to afford **14** in 78% overall yield. Boiling **14** for 4 min with a catalytic amount of p-toluenesulfonic acid in glacial acetic acid effected the removal of the t-Bu group. Treatment of the resulting carboxylic acid **15** with a tenfold excess of thionyl chloride in chloroform solution at room temperature furnished the acid chloride **16** in excellent yield.

As conventional methods for synthesis of β-ketoacids, e.g. *via* (selective) hydrolysis of the corresponding alkyl β-keto-esters (Experimental, preparation of **6a** and **6b**) were expected to be troublesome in this particular case, a very mild and simple new method of general utility was developed, in which practically neutral conditions were preserved during the conversion of **16** into **17a**. Thus, **16**



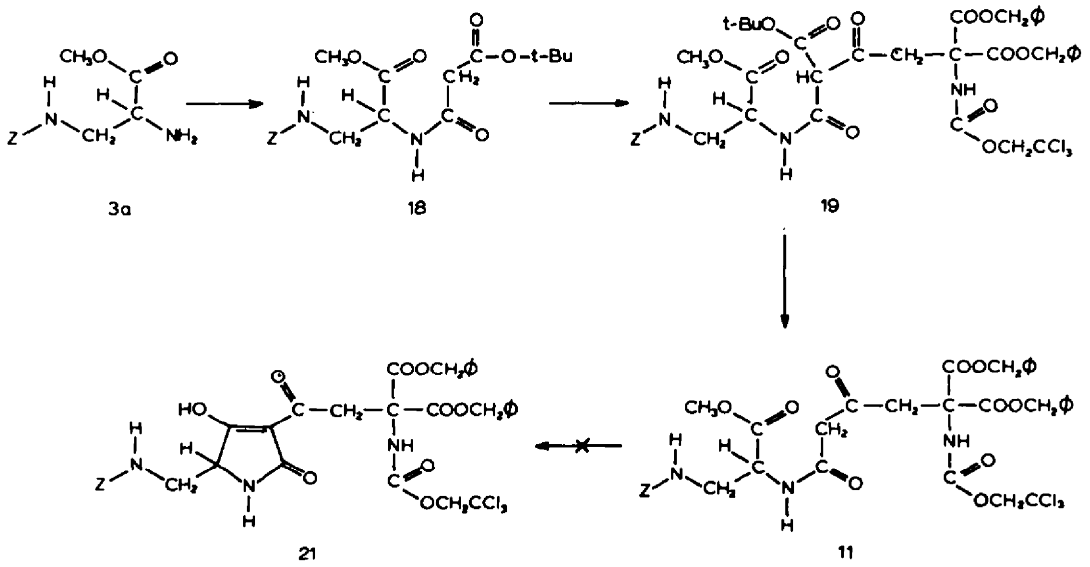
Scheme 2.

was quenched into a 100% excess of the mono-anion of bis(trimethylsilyl) malonate, followed by a short treatment with H_2O in which the intermediate triacyl compound was both hydrolyzed and decarboxylated to yield **17a** in almost quantitative yield.[22]

In numerous attempts with various coupling reagents, the condensation of **3a** with **17a** gave almost no yield of the desired **11**, but instead the decarboxylation product **17b**, the structure of which was proven by independent synthesis from **13** and bromoacetone. Successful coupling was only achieved *via* the benztriazolyl ester, formed *in situ* from **17a** by the action of 1-hydroxybenztriazole (HOBT) and dicyclohexylcarbodiimide (DCC).[23]

In a second approach, the synthesis of **11** could also be accomplished as shown in Scheme 3. Methyl N^3 - benzyloxycarbonyl - 2,3 - diaminopropanoate **3a** was first coupled with t-butyl hydrogen malonate, followed by low temperature acylation with acid chloride **16** to give **19**. Heating with *p*-toluenesulfonic acid in glacial acetic acid resulted in removal of the t-Bu group and simultaneous decarboxylation, yielding **11** in *ca.* 30% overall yield from **3a**.

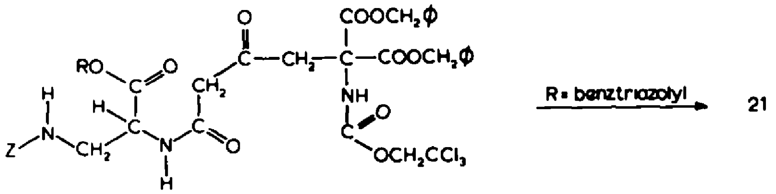
However, cyclization of **11** to **21** could not be effected. Neither under the standard conditions of Lacey[19] (*cf.* conversion of **9** into **10**) nor by employing other strong bases and solvents, such as potassium t-butoxide in



Scheme 3.

benzene or t-butanol, butyllithium in THF at low temperature, sodium hydride in DMF or benzene, or phase transfer agents and potassium carbonate in benzene/water system, any success was met. In most cases, not even a trace of starting material **11** could be recovered, probably due to the high base-sensitivity of the compounds involved. Obviously, base-induced decomposition of **11** and analogous compounds could only be prevented by application of a *weak* base; this, in turn, would presumably lead to a successful cyclization only if a very reactive ester instead of the methyl ester was present as the reaction partner.

Acid **20b** was considered to be a suitable starting material for the preparation of such an active ester. Its synthesis was realized by coupling t-butyl N^3 - benzyloxycarbonyl - 2,3 - diaminopropanoate **3c** with β-keto-acid **17**, followed by acid catalyzed removal of the protecting group. After some unsuccessful attempts, e.g. with mixed anhydrides (**20c**), the very reactive benztriazolyl ester **20d**, prepared *in situ* from **20b**, HOBT and DCC in anhydrous THF at 0°, was treated with a slight excess of triethylamine. The UV spectrum of the crude reaction mixture indicated the presence of the typical 3-acyltetramic acid chromophore (240 and 280 nm; the 240 nm band disappears on acidification[1,3]). Working up was facilitated by the fact that the acidic cyclization product **21** (pK$_A$ is expected to be *ca.* 3.5) was not extracted from ethyl acetate into sodium bicarbonate solution, so that most of the byproducts (HOBT, DCU) could be removed easily. Preparative TLC afforded **21** in a modest 18% yield, which presumably may be raised somewhat by proper choice of reaction conditions. Under the same conditions (but with 2 equivs of Et$_3$N) **4b**, prepared by direct condensation of **3b** and diketene,[15,19,24] afforded **5a** in 41% yield.



20 a R = t-Bu

b R = H

c R = ethoxycarbonyl

d R = benztriazolyl

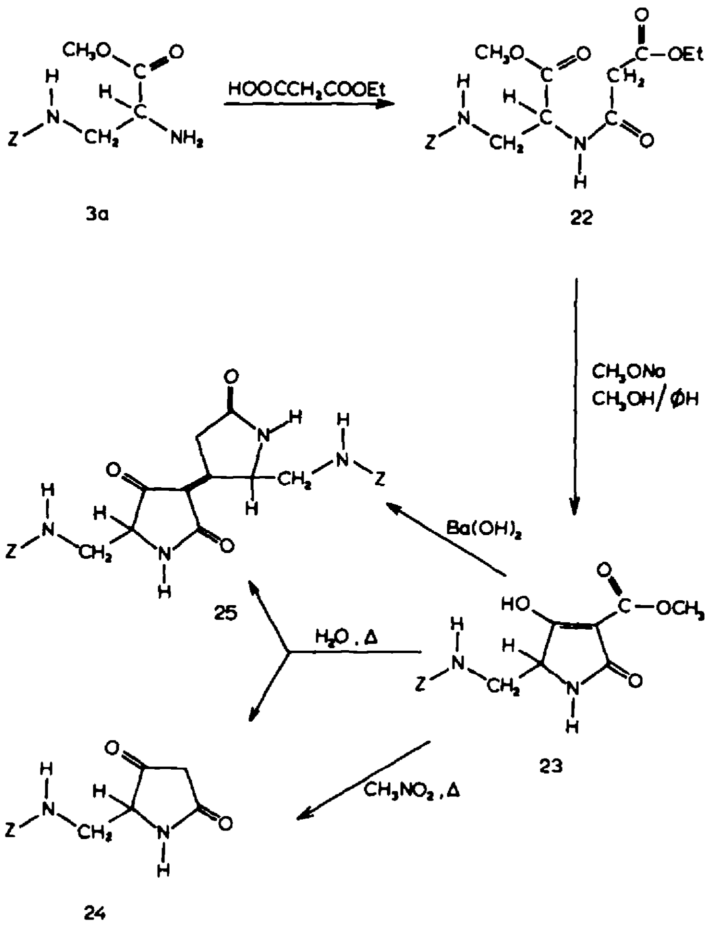
Scheme 4.

The newly developed method for C-acylation is expected to be more generally applicable; in particular, it will be useful in those cases where, due to the presence of sensitive groups in the reactants, the usual acylation methods (e.g. those involving acid halides or anhydrides) fail (cf. also Scheme 7).

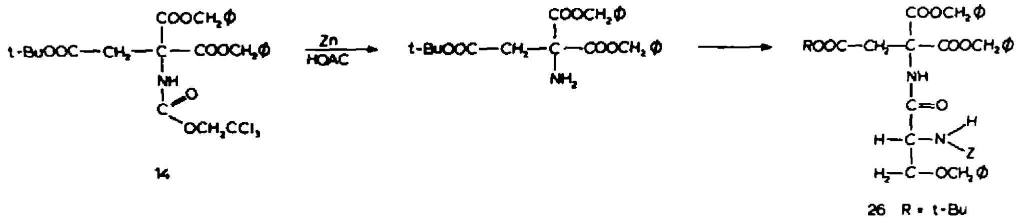
Removal of the protecting trichloroethoxycarbonyl group from **21** was easily accomplished with zinc in 90% acetic acid.[21] However, coupling of the resulting amino ester with protected serine derivatives under a variety of conditions, including agents such as EEDQ,[25] DCC (with and without additives)[26] and DPPA (diphenylphosphorylazide)[27] had little success; hydrogenolysis of the crude reaction mixtures afforded only traces of malonomicin as indicated by TLC. An effective solution of this unexpected problem might have been found in the attachment of a seryl moiety to the aminomalonic acid part in an earlier stage of the synthesis, for instance by development of a β-ketoacid analogous to **17a**, but with a (protected) seryl residue instead of the amino-protecting trichloroethoxycarbonyl group of **20a**. Preliminary efforts in this direction were temporized when progress was achieved in a different approach to the total synthesis, i.e. the direct acylation of the tetramic acid nucleus.

Acyltetronic acids, the oxygen analogues of acyltetramic acids, have been made accessible in recent years by Bloomer *et al.* through acid-catalyzed acylation of the appropriate tetronic acids.[28] In contrast, synthetic work in the area of acyltetramic acids and derivatives still mostly follows lines described by Lacey as early as 1954, i.e. base-catalyzed cyclization of *N*-acetoacetylaminoester precursors.[19] Only magnesidin has been prepared by TiCl$_4$-catalyzed acylation of a preformed pyrrolidinedione nucleus with a simple straight-chain aliphatic acyl component.[10] But as far as we are aware no *base-induced* acylations of pyrrolidinediones have been carried out successfully, very probably due to a strong tendency of these compounds to dimerize under even mildly basic conditions. This became already evident in the preparation of **24** (Scheme 5), the requisite pyrrolidinedione precursor of malonomicin. Hydrolysis with simultaneous decarboxylation of **23** by Ba(OH)$_2$ according to the method of Ohta[29] led to self-condensation of the intermediate **24**, giving **25** almost exclusively. Careful hydrolysis of **23** by boiling in water for 6 min yielded **24** in moderate yield; prolonged heating lowered the yield considerably because of conversion to **25**. Highest yields of **24** (up to 90%) were obtained by heating **23** in nitromethane or acetonitrile containing traces of water.[30,31]

As already mentioned, replacement of the trichloroethoxycarbonyl group in **21** by serine was not very successful. Therefore, it seemed advisible to perform the acylation of **24** with acid **27** or active derivatives thereof, in which serine is already present as an integral part. Acid **27** could be easily prepared (Scheme 6), starting from t-butyl ester **14**. Deprotection of **14** with zinc in 90% acetic acid, followed by coupling with *N*-benzyloxycarbonyl-*O*-benzylserine[32] afforded the oily t-butyl ester **26** (homogeneous



Scheme 5.

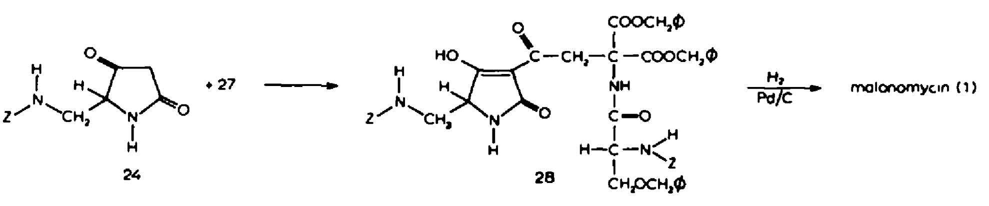


Scheme 6.

in TLC) which was treated with a catalytic amount of *p*-toluenesulfonic acid in boiling glacial acetic acid during 4 min to remove the t-Bu group. The resulting product was purified by preparative TLC to provide **27** in 45% overall yield from **14**. As was to be feared, all attempts to accomplish an acylation of **24** at C-3 under normal, strongly basic conditions failed, mostly because of formation of dimer **25**, but also because solubility problems prevented the use of solvents which were expected to stimulate C-acylation. Likewise, acylation under catalysis of Lewis acids such as $TiCl_4$ or BF_3 was unsuccessful.[10,28]

At the same time, the cyclization of **20b** to give acyltetramic acid **21** (Scheme 4) had demonstrated that active methylene compounds could be acylated under very mild conditions, i.e. in the presence of weak base (Et_3N) and at moderate temperature (25° or below) *if* benztriazolyl esters were used as the acylating agent. Application of this principle to the particular problem of tetramic acid acylation seemed promising because under these conditions dimerization of **24** to **25** was expected to be very slow. Thus, carboxylic acid **27** was converted *in situ* into the corresponding benztriazolyl ester by treatment with HOBT and DCC in acetonitrile. Addition to the active methylene compound **24**, followed by an equivalent of triethyl amine, afforded a reaction mixture from which after 48 hr at room temperature the C-acylated product **28** could be isolated by means of preparative TLC in *ca.* 40% yield (Scheme 7). Although the reaction product was pure by analytical TLC, the NMR spectrum was very difficult to interpret because of extensive line-broadening. The UV spectrum, showing the typical absorptions of the 3-acyltetramic acid chromophore, however, proved beyond all doubt that **28** indeed had been obtained.

As all protective groups of **28** are of the same type, i.e. benzyl and benzyloxycarbonyl groups, they can be removed in one step. After several attempts, this was



Scheme 7.

accomplished by hydrogenolysis at atmospheric pressure in methanol/water solution with 10% Pd on charcoal as a catalyst, activated by the presence of a little hydrochloric acid in order to remove even the stubbornly resistant benzyl group protecting the hydroxyl function of the seryl residue. The deprotection was performed at 0° to suppress decarboxylation as much as possible. The resulting product was purified by adsorption at pH 8.0 on a weakly basic anion-exchange column and elution with a gradient water/1N acetic acid. The active fractions were concentrated *in vacuo* leaving a white solid which according to IR, UV, ^{13}C-NMR and chromatographic properties was indistinguishable from the natural antibiotic. The same was true of simple derivatives obtained by decarboxylation and/or dinitrophenylation.[1]

The biological activity of the synthetic product, tested in mice infected with *Trypanosoma congolense*, was 25–50% of that of the microbiologically prepared product, owing to the fact that racemic derivatives of serine and 2,3-diaminopropanoic acid were used in the total synthesis. Due to the rather large margin of error of the biological test (mainly as a consequence of the availability of a too small quantity of synthetic material) it cannot be decided whether or not both asymmetric centers of malonomicin are required for full biological activity. As a matter of fact, it is not even certain whether the natural antibiotic is optically pure at the rather labile C-5 of the tetramic acid nucleus. However, the biological activity of the synthetic product must be considered as an extremely discriminative test of its structural identity, because even closely related derivatives such as the decarboxylation product of malonomicin lack this activity entirely.[1]

Experiments leading to optically active precursors and therefrom to synthetic malonomicin with partial and total natural stereochemistry are on the way.

EXPERIMENTAL

All m.ps were determined on a Kofler hot stage apparatus under a Reichert microscope and are uncorrected. Unless otherwise stated, IR spectra were recorded in $CHCl_3$ with a Perkin–Elmer 237 spectrophotometer, UV spectra in 96% EtOH with a Perkin–Elmer 137 spectrophotometer, ^{1}H NMR spectra in $CDCl_3$ with a Varian A-60A spectrometer and ^{13}C NMR spectra with a Varian XL-100 instrument (chemical shifts relative to TMS as an internal standard, $\delta = 0$ ppm). All reactions were performed with purified reagents in a N_2 atmosphere.

t-Butyl N^3 - *benzyloxycarbonyl - 2,3 - diaminopropanoate* (**3c**)

A soln of **3b**,[18] (952 mg, 4 mmole), t-butyl acetate (60 ml) and perchloric acid (8.8 mmole) was kept at room temp. during 4 days.[33] After cooling in ice the mixture was extracted with 2N citric acid (4 × 5 ml) and the combined extracts immediately neutralized with solid $NaHCO_3$. Extraction with EtOAc, drying and evaporation *in vacuo* yielded **3c** as a yellow oil (647 mg, 55%) which according to TLC (SiO_2; $CHCl_3$/acetone 3:1) was pure enough for conversion to **19**; NMR: δ 1.42 (s, 9H), 1.95 (t, 3H), 3.0–3.6 (m, 3H), 5.03 (s, 2H), 6.05 (t, 1H) and 7.25 ppm (s, 5H).

Methyl N^2 - *acetoacetyl* - N^3 - *benzyloxycarbonyl - 2,3 - diaminopropanoate* (**4a**)

A mixture of **3a**, hydrochloride (3.77 g, 13.1 mmole), freshly distilled diketene (1.4 g, 25% excess) and $NaHCO_3$ (1.4 g) in 50% EtOH/H_2O (25 ml) was stirred for 2 hr at 0° and then another 2 hr at room temp.[19] Extraction with $CHCl_3$ (3x), washing with brine, drying and evaporation *in vacuo*, yielded **4a** as a colourless oil (4.53 g, 100%), ninhydrin-negative and pure by TLC (SiO_2; $CHCl_3$/acetone 4:1); NMR: δ 2.18 (s, 3H), 3.38 (s, 2H), 3.4–3.6 (m, 2H), 3.68 (s, 3H), 4.45–4.85 (m, 1H), 5.07 (s, 2H), 5.68 (t, 1H), 7.32 (s, 5H) and 7.61 ppm (d, 1H).

N^2 - *Acetoacetyl* - N^3 - *benzyloxycarbonyl - 2,3 - diaminopropanoic acid* (**4b**)

To **3b**[18] (595 mg, 2.5 mmole) dissolved in such a quantity of 2N NaOH, that basic conditions were sustained during the reaction, freshly distilled diketene (270 mg, 30% excess) was added at 0° with vigorous stirring. After 2 hr at 0° and another 3 hr at room temp., the mixture was extracted with ether and the aqueous layer acidified with 2N HCl. After extraction with EtOAc and concentration *in vacuo*, **4b** (810 mg) was obtained as a yellow oil (pure by TLC) which was used as such in the synthesis of **5a**.

3 - *Acetyl - 5 - N - benzyloxycarbonylaminomethyl - 4 - hydroxy* - Δ^3 - *pyrrolin - 2 - one* (**5a**)[1]

(a) *By the method of Lacey.*[19] A soln of **4a** (772 mg, 2.3 mmole) in dry EtOH (1.4 ml) was added to Na (59 mg, 2.6 mmole) in dry EtOH (0.35 ml). After refluxing during 18 hr the mixture was cooled, poured on ice, and acidified with 2N HCl. The ppt was collected, washed with ice-water and dried over P_2O_5 *in vacuo*, yielding **5a** (562 mg, 80%), m.p. 151–156°, almost pure by TLC (SiO_2; acetone/EtOH/4N NH_4OH 3:2:1). Crystallization from EtOH gave 460 mg of white crystals, m.p. 156–158°, identical by TLC, UV, IR and NMR with the material described below.

(b) *By the* DCC/HOBT/Et_3N-*method.* Crude **4b** (810 mg, was dissolved in dry THF (10 ml) and then there were added at 0° sequentially HOBT (528 mg, 3.9 mmole), Et_3N (500 mg, 5 mmole) and DCC (575 mg, 2.8 mmole) in dry THF (2 ml). After standing for 1 hr at 0° and for 24 hr at room temp., the mixture was filtered and concentrated *in vacuo*. The residue was dissolved in $NaHCO_3$aq, washed with EtOAc, acidified to pH 1 and extracted again with EtOAc. After drying and evaporation, crystallization of the residue from MeOH furnished **5a** (315 mg, 41%), m.p. 157–158.5°; positive $FeCl_3$-spot test; NMR (D_6-DMSO): δ 2.35 (s, 3H), 3.05–3.55 (m, 2H), 3.8–4.1 (m, 1H), 5.03 (s, 2H), 7.22 (t, 1H), 7.33 (s, 5H), 8.65 (s, 1H) and 11.17 ppm (s, 1H).((Found: C, 59.37; H, 5.32; N, 9.30. Calc. for $C_{15}H_{16}N_2O_5$: C, 59.20; H, 5.30; N, 9.21%).

3 - *Acetyl - 5 - aminomethyl - 4 - hydroxy* - Δ^3 - *pyrrolin - 2 - one* (**5b**)[1]

A partial suspension of **5a** (2.55 g, 8.4 mmole) and 5% Pd-C (735 mg) in MeOH (70 ml) and glacial AcOH (1.75 ml) was stirred in a H_2 atmosphere, first at room temp. and then for 30 min at 50°. Cooling and filtration yielded a filter cake which was extracted with a small volume of 2N HCl. The filtered acidic soln was adjusted to pH 5 with Et_3N and the collected ppt of **5b** dried over P_2O_5 *in vacuo* (1.1 g, 77%), m.p. > 300°; UV (max): 239 and 278 nm; NMR (D_2O + NaOD, pH 12): δ 2.33 (s, 3H), 2.89 (d, 2H, J = 4.2 cps) and 3.77 ppm (t, 1H, J = 4.2 cps); pK_A (H_2O): 1.93 (UV method).

4 - *Benzyloxy - 3 - oxobutanoic acid* (**6a**)

To a stirred suspension of NaH (24 g, 1 mole) in dry THF

(400 ml) was added dropwise (within 30 min at 10°) anhyd benzyl alcohol (250 ml), followed by a soln of ethyl 4 - bromo - 3 - oxobutanoate[34] (41.8 g, 0.2 mole) in dry benzyl alcohol (100 ml). After stirring overnight at room temp. the mixture was neutralized with glacial AcOH (60 ml) under ice-cooling. Water was added and the mixture extracted with ether. The combined organic layers were washed with $NaHCO_3$ aq, dried, concentrated *in vacuo* and distilled to yield ethyl 4-benzyloxy - 3 - oxo - butanoate (20.5 g, 43%), b.p. 120–125°/10^{-2} mm; NMR: δ 1.20 (t, 3H), 3.48 (s, 2H), 4.10 (s, 2H), 4.13 (q, 2H), 4.55 (s, 2H) and 7.31 ppm (s, 5H).

This ethyl ester (11.8 g, 0.05 mole) was stirred with 1N NaOH (55 ml) and H_2O (85 ml) during 20 hr. The resulting soln was washed with ether (5×), cooled in ice and acidified with 1N H_2SO_4. Saturation with NaCl, extraction with ether (5×), washing with brine, drying and evaporation (temp. $\leq$ 25°C) furnished **6a** (7.9 g, 76%) as a low-melting white solid, stable during months at −25°; IR (max): 3600–2400 (COOH), 1730 and 1710 cm^{-1} (C=O); NMR: δ 3.49 (s, 2H), 4.11 (s, 2H), 4.52 (s, 2H) and 7.28 ppm (s, 5H).

4-t-Butoxy-3-oxobutanoic acid (**6b**)

This compound was prepared analogously to **6a** from ethyl 4 - bromo - 3 - oxobutanoate[34] and t-BuOH via ethyl 4 - t - butoxy - 3 - oxobutanoate (44% yield; b.p. 72–76°/1 mm; NMR: δ 1.21 (s, 9H), 1.28 (t, 3H), 3.54 (s, 2H), 4.02 (s, 2H) and 4.20 ppm (q, 2H), and saponification with NaOH (**6b**; 84% yield as a yellow oil; NMR: δ 1.22 (s, 9H), 3.60 (s, 2H), 4.07 (s, 2H) and 11.02 ppm (s, 1H)).

3 - Acetyl - 5 - N - benzyloxycarbonylaminomethylidene - 4 - hydroxy - Δ^3 - pyrrolin - 2 - one (**8**)

Compound **5a** (365 mg, 1.2 mmole) and $CuBr_2$ (530 mg, 2.37 mmole) were suspended in $CHCl_3$ (3 ml) and EtOAc (6 ml), and boiled for 1 hr.[17] After cooling to room temp. the ppt was removed by suction filtration and the filtrate evaporated to dryness *in vacuo*. The solid residue was treated with a small volume of acetone, leaving 52 mg of yellow crystals. The filter cake was extracted also with acetone and the extract evaporated to a small volume yielding another 70 mg of crystalline material, identical in all respects with the first crop. Total yield 122 mg of **8** (34%), m.p. 203–205°; UV (max): 281 and 340 nm; NMR (D_6-DMSO): δ 2.40 (s, 3H), 5.22 (s, 2H), 6.80 (d, 1H), 7.39 (s, 5H), 9.22 (s, 1H), 9.60 (d, 1H) and 10.56 ppm (s, 1H). (Found: C, 59.31; H, 4.60; N, 9.11. Calc. for $C_{15}H_{14}N_2O_5$: C, 59.60; H, 4.67; N, 9.27%).

Methyl N^3 *- benzyloxycarbonzyl -* N^2 *- (5,5 - dimethyl - 3 - oxohexanoyl) - 2,3 - diaminopropanoate* (**9**)

To a stirred soln of 5,5 - dimethyl - 3 - oxo - hexanoic acid (395 mg, 2.5 mmole) in dry DMF (5 ml) was added a soln of **3a** (723 mg, 2.5 mmole) and Et_3N (250 mg, 2.5 mmole) in dry DMF (10 ml) under cooling in ice, followed by diphenylphosphorylazide[27] (690 mg, 2.5 mmole). After stirring overnight the mixture was diluted with H_2O (150 ml) and extracted by EtOAc. Washing with 1 N HCl, H_2O, $NaHCO_3$ aq and brine, drying and concentration *in vacuo* yielded **9** (830 mg, 85%) as a thick oil, reasonably pure by TLC (SiO_2; $CHCl_3$/ether 4:1) and NMR: δ 0.99 (s, 9H), 2.38 (s, 2H), 3.33 (s, 2H), 3.3–3.7 (m, 3H), 3.67 (s, 3H), 4.3–4.8 (m, 1H), 5.08 (s, 2H), 5.75 (t, 1H), 7.32 (s, 5H) and 7.65 ppm (d, 1H).

5 - N - benzyloxycarbonylaminomethyl - 3 - (3,3 - dimethylbutanoyl) - 4 - hydroxy - Δ^3 - pyrrolin - 2 - one (**10**)

A solution of **9** (240 mg, 0.61 mmole) in dry benzene (2 ml) was added to a stirred soln of NaH (55% in oil; 30 mg, 0.7 mmole) in dry MeOH (0.4 ml), and refluxed for 5 hr. Water was added under cooling and the aqueous phase extracted with ether. Acidification, extraction with EtOAc, drying and evaporation *in vacuo*, yielded **10** (174 mg, 73%). Recystallization from benzene gave white crystals, m.p. 126–130°; UV (max): 242 and 283 nm; NMR (D_6-DMSO): δ 0.99 (s, 9H), 2.68 (s, 2H), 3.1–3.5 (m, 2H), 3.8–4.1 (m, 1H), 5.03 (s, 2H), 7.22 (t, 1H), 7.34 (s, 5H) and 8.5–9.2 ppm (m, 2H). (Found: C, 63.42; H, 6.70; N, 7.65. Calc. for $C_{19}H_{24}N_2O_5$: C, 63.32; H, 6.71; N, 7.79%).

Coupling product (**11**)

(a) *By coupling of* **3a** *with* **17a**. Compound **3a** (941 mg, 3.74 mmole; free amino-ester prepared from the hydrochloride by treatment with cold $NaHCO_3$aq and extraction with EtOAc, drying and evaporation), HOBT (764 mg, 5.66 mmole) and **17a** (2.16 g, 3.74 mmole) were dissolved in dry THF (11 ml) at 0°. Then DCC (854 mg, 4.14 mmole) in dry THF (3 ml) was added with stirring.[23] After 1 hr at 0° and 4 hr at room temp., the precipitated dicyclohexylurea was filtered off and the filtrate concentrated *in vacuo*. The residue was dissolved in EtOAc and washed with $NaHCO_3$aq, 2N citric acid, $NaHCO_3$aq again, and brine. Drying and evaporation *in vacuo* gave 3.07 g of an oily product which was purified by preparative TLC (SiO_2; $CHCl_3$/ether 3:1) yielding **11** (1.72 g, 56%) and **17b** (399 mg, 20%), both fully identical with the independently synthesized products.

(b) *By hydrolysis/decarboxylation of t-butyl ester* **19**. Compound **19** (145 mg, 0.16 mmole) and *p*-toluenesulfonic acid (5 mg) in glacial AcOH (1 ml) were boiled for 4 min. Concentration *in vacuo*, redissolving in EtOAc, washing with $NaHCO_3$aq, 0.5 N HCl and brine, drying and evaporation *in vacuo* yielded **11** (117 mg, 90%) as a viscous oil, identical in all respects with the product obtained under (a); NMR: δ 3.32 (s, 2H), 3.4–3.65 (m, 2H), 3.70 (s, 3H), 3.80 (s, 2H), 4.45–4.85 (m, 1H), 4.61 (s, 2H), 5.0–5.2 (m, 3×2H), 5.40 (t, 1H), 6.70 (s, 1H), and 7.1–7.5 ppm (m, 3×5H + 1H).

Dibenzyl N - trichloroethoxycarbonylaminomalonate (**13**)

To **12**[20] (2.68 g, 8 mmole) in sat. $NaHCO_3$aq (22 ml) there was added dropwise in about 20 min trichloroethyl chloroformate[21a] (1.91 g, 9 mmole) with vigorous stirring. After 3 hr the white ppt was collected, washed with water and dried *in vacuo* over P_2O_5 to yield **13** (3.71 g, 98%), m.p. 91–93° (from EtOH); NMR: δ 4.68 (s, 2H), 5.13 (d, 1H), 5.13 (s, 2×2H), 6.28 (d, 1H) and 7.28 ppm (s, 2×5H). (Found: C, 50.43; H, 3.94; Cl, 22.42; N, 2.82. Calc. for $C_{20}H_{18}Cl_3NO_6$: C, 50.60; H, 3.82; Cl, 22.40; N, 2.95%).

O^1 *- Benzyl -* O^4 *- t - butyl 2 - benzyloxycarbonyl - N - trichloroethoxycarbonylaspartate* (**14**)

Compound **13** (2.37 g, 5 mmole) dissolved in dry DMF (15 ml), was added to a stirred suspension of NaH (120 mg, 5 mmole) in dry DMF (25 ml). After 1.5 hr t-butyl chloroacetate (2.5 ml) was added and stirring continued for 18 hr. The mixture was then poured into dil. $NaHCO_3$ aq (200 ml) and the white ppt collected and dried over P_2O_5 *in vacuo*, yielding **14** (2.31 g, 79%), m.p. 123–124° (after recrystallization from EtOH); NMR: δ 1.36 (s, 9H), 3.43 (s, 2H), 3.64 (s, 2H), 5.14 (s, 2×2H), 6.61 (s, 1H) and 7.27 ppm (s, 2×5H). (Found: C, 52.95; H. 4.92; Cl, 18.13; N, 2.27. Calc. for $C_{26}H_{28}Cl_3NO_8$: C, 53.03; H, 4.79; Cl, 18.06; N, 2.38%).

O^1 *- Benzyl -* O^4 *- hydrogen 2 - benzyloxycarbonyl - N - trichloroethoxycarbonylaspartate* (**15**)

Compound **14** (1.175 g, 2 mmole) in glacial AcOH (12 ml) was boiled for 4 min with *p*-toluenesulfonic acid (60 mg). Concentration *in vacuo* and recrystallization from EtOH furnished **15** (900 mg, 85%), m.p. 142–144°; NMR: δ 3.58 (s, 2H), 4.62 (s, 2H), 5.13 (s, 2×2H), 6.60 (s, 1H), 7.27 (s, 2×5H) and 8.73 ppm (s, 1H). (Found: C, 49.69; H, 3.91; Cl, 20.10; N, 2.51. Calc. for $C_{22}H_{20}Cl_3NO_8$: C, 49.59; H, 3.78; Cl, 19.96; N, 2.63%).

Acid chloride **16**

A soln of **15** (532.5 mg, 1 mmole) and $SOCl_2$ (720 μl, 10 mmole) in dry $CHCl_3$ (3 ml) was kept at room temp. for 4 days. Evaporation *in vacuo* left a colourless oil (550 mg, 100%) which solidified on standing, m.p. 78–86°. A sample for analysis was recrystallized from benzene/n-hexane, m.p. 86–88°; IR: 1797 cm^{-1} (COCl); NMR: δ 4.15 (s, 2 H), 4.62 (s, 2H), 5.12 (s, 2×2H), 6.67 (s, 1H) and 7.28 ppm (s, 2×5H).

β-Keto-acid **17a**

A soln of bis(trimethylsilyl) malonate[22] (447 mg, 1.8 mmole) in dry ether (4 ml) at −60° was treated with n-BuLi (0.9 ml, 2N in hexane). After warming to 0°, **16** (497 mg, 0.9 mmole) in dry ether (2 ml) was added in one lot. After stirring for 10 min at 0° the mixture was shaken with water and extracted with ether. Drying and evaporation yielded a yellow oil, which was taken up in a small volume of CHCl$_3$ and diluted with hexane to give **17** (480 mg, 92%) as white crystals, m.p. 105–115° (dec). An analytical sample was prepared by repeated crystallization from CHCl$_3$/hexane, m.p. 114–117°; NMR: δ 3.43 (s, 2H), 3.87 (s, 2H), 4.62 (s, 2H), 5.15 (s, 2×2H),' 6.68 (s, 1H), and 7.30 ppm (s, 2×5H). (Found: C, 49.82; H, 4.09; Cl, 19.05; N, 2.30. Calc. for C$_{24}$H$_{22}$Cl$_3$NO$_9$: C, 50.15; H, 3.86; Cl, 18.51; N, 2.44%).

Acetonyl compound 17b

A soln of **13** (1.19 g, 2.5 mmole) in dry DMF (10 ml) was added to a stirred suspension of NaH (62 mg, 2.6 mmole) in dry DMF (5 ml). When the H$_2$ evolution had ceased (*ca.* 1 hr) bromoacetone (514 mg, 50% excess) was added and stirring continued overnight. The clear mixture was diluted with NaHCO$_3$ aq (100 ml) and extracted with ether. Washing with brine, drying and evaporation *in vacuo* gave 1.28 g of a yellow oil which solidified on standing. Recrystallization from EtOH yielded **17b** (1.08 g, 80%), m.p. 75–78°; NMR: δ 1.99 (s, 3H), 3.70 (s, 2H), 4.63 (s, 2H), 5.11 (s, 2×2H), 6.74 (s, 1H) and 7.25 ppm (s, 2×5H). (Found: C, 51.87; H, 4.16: Cl, 20.18; N, 2.50. Calc. for C$_{23}$H$_{22}$Cl$_3$NO$_7$: C, 52.05; H, 4.18; Cl, 20.04; N, 2.64%).

Methyl N^3 *- benzyloxycarbonyl -* N^2 *- (O^3 - t - butyl malonyl) - 2,3 - diaminopropanoate* (18)

t-Butyl hydrogen malonate (544 mg, 3.4 mmole) was added to a stirred soln of **3a** (free aminoester; 852 mg, 3.4 mmole) and EEDQ[25] (900 mg, 3.6 mmole) in dry THF (25 ml). After 20 hr at room temp. the mixture was concentrated *in vacuo* and the residue dissolved in ether, washed with ice-cold 2N citric acid, H$_2$O, NaHCO$_3$ aq and brine, respectively. Drying and evaporation *in vacuo* gave **18** (1.12 g, 84%) as a thick oil, homogeneous in TLC; NMR: δ 1.43 (s, 9H), 3.20 (s, 2H), 3.4–3.7 (m, 2H), 3.65 (s, 3H), 4.4–4.85 (m, 1H), 5.07 (s, 2H), 5.85 (t, 1H), 7.32 (s, 5H) and 7.77 ppm (d, 1H).

Acylation product 19

To a stirred soln of **18** (392 mg, 1 mmole) in dry ether (2 ml) at −60°, BuLi (0.5 ml of a 2N soln in hexane) was added. After 15 min, **16** (552 mg, 1 mmole) in dry ether (1 ml) was quickly added at 0° and stirring continued for 1 hr at 0° and 1 hr at room temp. Acidification with 1N HOAc (4 ml), extraction with ether, washing with NaHCO$_3$ aq and brine, drying and evaporation *in vacuo* yielded a glassy product (840 mg) which was purified by TLC (SiO$_2$; CHCl$_3$) to furnish **19** (362 mg, 40%) as a colourless glass; NMR: δ 1.50 (s, 9H), 3.4–3.65 (m, 2H), 3.72 (s, 3H), 4.15 (s, 2H), 4.45–4.85 (m, 1H), 4.63 (s, 2H), 5.0–5.2 (m, 3×2H), 5.25 (t, 1H), 6.63 (s, 1H), 7.15–7.5 (m, 3×5H+1H) and 9.75 ppm (m, *ca.* 1H).

Coupling product 20a

This product was prepared from **3c** and **17a** analogous to the synthesis of **11** (reaction time 20 hr at room temp.), yield (after preparative TLC) 64%; NMR: δ 1.43 (s, 9H), 3.34 (s, 2H), 3.4–3.8 (m, 2H), 3.85 (s, 2H), 4.3–4.8 (m, 1H), 4.63 (s, 2H), 5.13 (m, 3×2H), 5.70 (t, 1H), 6.79 (s, 1H) and 7.1–7.5 ppm (m, 3×5H+1H).

Acid 20b

This product was prepared from **20a** analogous to the synthesis of **11** (method b), yield 83%, homogeneous in TLC (SiO$_2$; EtOAc/EtOH 4:1); IR: 3700–2700 cm^{-1} (COOH); NMR: no t-Bu group.

Cyclization product 21

A stirred soln of **20b** (769 mg, 0.96 mmole) in dry THF (4 ml) was treated at 0° with HOBT (185 mg, 1.42 mmole) and DCC (220 mg, 1.1 mmole). After 1 hr at 0° and 2 hr at room temp., Et$_3$N (100 mg, 1 mmole) was added and the mixture was stirred during 2 days. After filtration and dilution with EtOAc, the mixture was washed with NaHCO$_3$ aq, H$_2$O, 1N citric acid and brine, respectively. Drying and concentration *in vacuo* gave 730 mg of a viscous oil which was purified by TLC (SiO$_2$; CHCl$_3$/acetone 1:1), yielding **21** (131 mg, 18.4%) as an amorphous powder (dec. at 130–135°); positive FeCl$_3$-spot test; UV (max): 240 (ε 9600) *and* 280 *nm* (11.250).

Methyl N^3 *- benzyloxycarbonyl -* N^2 *- (O^3 - ethyl malonyl) - 2,3 - diaminopropanoate* (22)

To **3a** (2.21 g, 7.7 mmole) and Et$_3$N (774 mg, 7.7 mmole) in CH$_2$Cl$_2$ (15 ml) was added at 0° a soln of ethyl hydrogen malonate (1.01) g, 7.6 mmole) in CH$_2$Cl$_2$ (6 ml) and then EEDQ (2.02 g, 8.1 mmole). After stirring for 6 hr the mixture was washed with 1N HCl, NaHCO$_3$ aq and H$_2$O. Upon concentration *in vacuo*, **22** (2.49 g, 89%) was obtained as an oil, almost pure according to TLC and NMR: δ 1.23 (t, 3H), 3.28 (s, 2H), 3.68 (s, 3H), 3.45–3.75 (m, 2H), 4.15 (q, 2H), 4.45–4.85 (m, 1H), 5.07 (s, 2H), 5.68 (t, 1H), 7.30 (s, 5H) and 7.73 ppm (d, 1H).

5 - N *- Benzyloxycarbonylaminomethyl - 4 - hydroxy - 3 - methoxycarbonyl - Δ^3 - pyrrolin - 2 - one* (23)

A soln of **22** (8.82 g, 0.024 mole) and NaH (1.22 g of a 57% suspension in oil, 0.03 mole) in dry MeOH (16 ml) and dry benzene (40 ml) was stirred and boiled for 3 hr. After cooling, H$_2$O (50 ml) was added and the organic layer separated. The aqueous layer was acidified to pH1 and the ppt collected and recrystallized from MeOH to yield **23** (4.36 g, 57%), m.p. 145–148°; positive FeCl$_3$-spot test; NMR (D$_6$-DMSO): δ 2.9–3.85 (m, 2H), 3.67 (s, 3H), 3.85–4.20 (m, 1H), 5.06 (s, 2H), 7.22 (t, 1H), 7.33 (s, 5H) and 10.08 ppm (s, 1H). (Found: C, 56.15; H, 5.28; N, 8.91. Calc. for C$_{15}$H$_{16}$N$_2$O$_6$: C, 56.24; H, 5.03; N, 8.75%).

5 - N *- Benzyloxycarbonylaminomethylpyrrolidin - 2,4 - dione* (24)

A soln of **23** (1.63 g, 5.1 mmole) in nitromethane (32 ml) containing H$_2$O (180 μl, 10 mmole) was boiled for 30 min. The residue obtained after evaporation of the solvent *in vacuo* was recrystallized from CHCl$_3$, yielding **24** (1.17 g, 88%), m.p. 131–135°; NMR (CD$_3$CN): δ 2.88 (2×s, 2H), 3.25–3.5 (m, 2H), 3.8–4.25 (m, 1H), 5.07 (s, 2H), 5.83 (t, 1H), 6.90 (d, 1H) and 7.33 ppm (s, 5H). (Found: C, 59.39; H, 5.49: N, 10.63. Calc. for C$_{13}$H$_{14}$N$_2$O$_4$: C, 59.54; H, 5.38; N, 10.68%).

O^1 *- Benzyl* O^4 *- t - butyl* N *- (O^3 - benzyl -* N *- benzyloxycarbonylseryl) - 2 - benzyloxycarbonylaspartate* (26)

To a stirred soln of **14** (1.2 g, 2.04 mmole) in 90% AcOH (60 ml) there were added over a period of 15 min three portions of 1.5 g each of Zn powder at 0°. After stirring for 1 hr at 0° and for 1.5 hr at room temp., the mixture was filtered, the filtrate concentrated *in vacuo* and the residue dissolved in EtOAc and H$_2$O. The organic layer was washed with 1N NH$_4$OH and evaporated *in vacuo* to dryness, yielding the deprotected product O^1-benzyl O^4 - t - butyl 2 - benzyloxycarbonylaspartate (843 mg, 100%); (NMR: δ 1.38 (s, 9H), 2.55 (s, 2H), 3.09 (s, 2H), 5.18 (s, 2×2H) and 7.34 ppm (s, 2×5H)). This compound (843 mg, 2.04 mmole) and *N*-benzyloxycarbonyl - *O* - benzylserine[32] (671 mg, 2.04 mmole) were dissolved in dry THF (9 ml) and a soln of DCC (430 mg, 2.09 mmole) in dry THF (1 ml) was added at about −10°. After stirring for 1 hr at −10°, the mixture was kept for 2 days at 0°.[35] Filtration and concentration *in vacuo* gave an oil which was redissolved in EtOAc and washed with cold 1N HCl and sat. NaHCO$_3$aq. Drying and evaporation *in vacuo* yielded **26** (1.51 g, 100%) as a viscous oil, almost pure by TLC (SiO$_2$; CHCl$_3$/CCl$_4$ 5:2); NMR: δ 1.32 (s, 9H), 3.48 (s, 2H), 3.4–3.9 (m, 2H), 4.45 (s, 2H), 4.1–4.6 (m, 1H), 5.10 (m, 3×2H), 5.64 (d, 1H), 7.1–7.4 (m, 4×5H) and 8.17 ppm (s, 1H).

O^1 *- Benzyl* O^4 *- hydrogen* N *- (O^3 - benzyl -* N *- benzyloxycarbonylseryl) - 2 - benzyloxycarbonylaspartate* (27)

This compound was synthesized from **26** analogous to the preparation of **11** (method b), yield 93%, purification (with substantial loss) by preparative TLC (SiO$_2$; CHCl$_3$/acetone 4:1); NMR: δ 3.2–3.9 (m, 4H), 4.15–4.6 (m, 1H), 4.37 (s, 2H), 5.08 (m, 3×2H), 5.87 (d, 1H), 7.1–7.4 (m, 4×5H), 8.18 (s, 1H) and 9.82 ppm (s, 1H).

3 - [O^1 - *Benzyl* N - (O^3 - *benzyl* - N - *benzyloxycarbonylseryl*) - 2 - *benzyloxycarbonyl* - 4 - *aspartyl*] - 5 - N - *benzyloxycarbonylaminomethyl* - 4 - *hydroxy* - Δ^3 - *pyrrolin* - 2 - *one* (**28**)

To **27** (890 mg, 1.33 mmole) and HOBT (196 mg, 1.45 mmole) in dry acetonitrile (30 ml) was added at 0° DCC (299 mg, 1.45 mmole) in dry acetonitrile (15 ml). After stirring for 45 min at 0° and for 1 hr at room temp., the mixture was added to a soln of **24** (349 mg, 1.33 mmole) and Et_3N (145 mg, 1.45 mmole) in dry acetonitrile (225 ml). After 3 days at room temp., the mixture was filtered and the filtrate concentrated *in vacuo* to dryness. The residue was dissolved in EtOAc, washed with $NaHCO_3$ aq, 2N citric acid and H_2O, sequentially, dried and concentrated *in vacuo* yielding 1.07 g of a viscous oil. Further purification by preparative TLC (SiO_2; $CHCl_3$/EtOH/acetone/25% NH_4OH 9:1:1:0.1) gave **28** (486 mg, 40%); UV (max)(CH_3OH): 243 and 284 nm; positive $FeCl_3$-spot test.

5 - *Aminomethyl* - 3 - (2 - *carboxy* - N - *seryl* - 4 - *aspartyl*) - 4 - *hydroxy* - Δ^3 - *pyrrolin* - 2 - *one* (*malonomicin*) (1)

A soln of purified **28** (138 mg, 0.15 mmole) in MeOH (3.5 ml) containing a few drops of 6 N HCl was vigorously stirred for 18 hr at 0° with 10% Pd–C (25 mg) in a H_2 atmosphere. After filtration and concentration *in vacuo*, the residue was dissolved in 10 mM NH_4OAc-soln (100 ml) and adsorbed on DEAE-Sephadex A-25 (column of 35 × 1.5 cm; adsorbens swollen in 0.5 M NH_4OAc and equilibrated with 10 mM NH_4OAc-soln). After washing with H_2O (pH 7), the product was eluted with a linear gradient H_2O/1N HOAc (total of 600 ml; flow rate 30 ml/hr. The fractions containing the product were collected and evaporated *in vacuo* at 25°, yielding racemic 1 (48 mg, 86%) (contaminated with a very small amount of its decarboxylation product), fully identical with the natural product[1] by TLC (SiO_2; EtOH/1N NH_4OH 3:2), UV, IR and ^{13}C-NMR (D_2O + NaOD, pD 8.5): δ 40.4, 44.5, 55.6, 56.6, 62.3, 67.6, 102.9, 169.2, 175.3, 178.5, 193.5 and 194.4 ppm.

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