Tetramic Acid Chemistry. Part 1. Reinvestigation of Racemisation during the Synthesis of Tetramic Acids *via* Dieckmann Cyclisation

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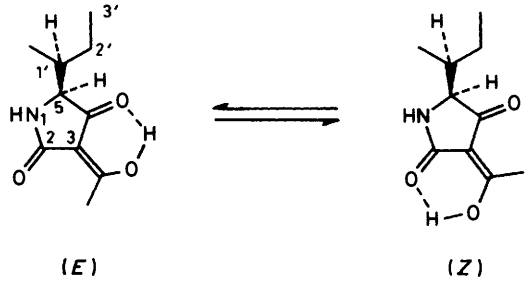
Epimerisation during the synthesis of tetramic acids by Dieckmann cyclisation of the corresponding chiral *N*-acyl-α-amino esters was investigated. In the case of the isoleucine derivative, the extent of epimerisation was directly evaluated by ^{1}H NMR analysis of the 3-acylated tetramic acids (**3a,b**) and (**5a**). A chemical correlation was carried out in the case of the 5-benzyl derivative (**8h**). The moderate overall yield and the partial epimerisation at position C-5 limit the usefulness of this approach for tetramic acid preparation.

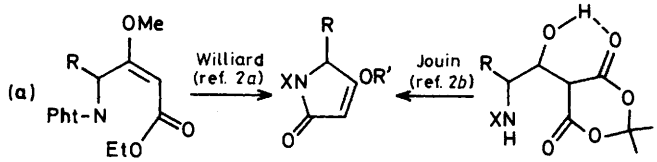
Tenuazonic acid (**3a**), a phytotoxin produced by several fungi, is structurally related to the 3-acylpyrrolidine-2,4-dione (tetramic acid) family, which is also found in numerous antibiotics.[1]

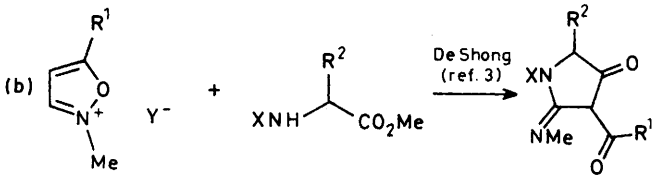


(*E*) (*Z*)

Figure. Tenuazonic acid (**3a**).

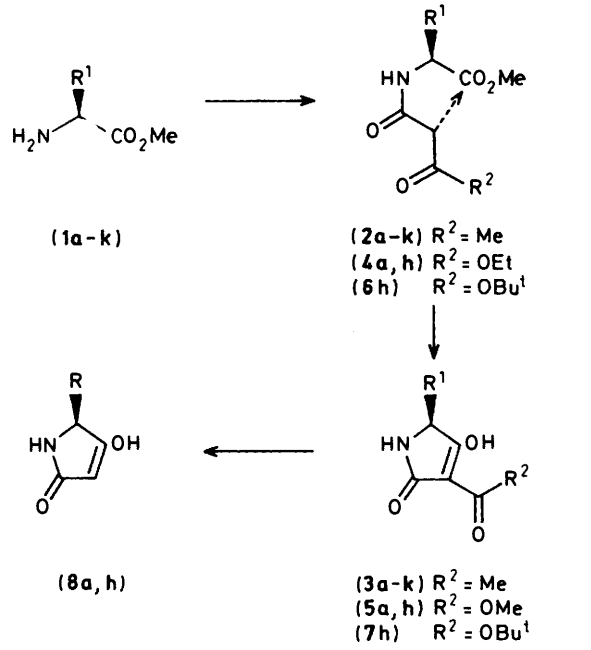
Although there are several other possible approaches, including cyclisation by *N*-acylation of a *C*-acyl amino acid (Scheme 1a)[2] or tentative isoxazolone rearrangement (Scheme 1b),[3] the general method for synthesizing tetramic acid is Dieckmann





Scheme 1. Preparations of tetramic acids. (a) *N*-acylation, (b) Isoxazolone rearrangement.

intramolecular *C*-acylation of *N*-acyl amino esters, which produces 3-acetyl or 3-alkoxycarbonyl *N*-free tetramic acids (Scheme 2).[4] In the earliest reports, DL-amino acids were used as the starting material in this procedure.[4a] To our knowledge, the problem of epimerisation has never really been studied even



R^1	
a; CH(Me)CH$_2$Me (5*S*,6*S*)	**g**; Bu^i
b; CH(Me)CH$_2$Me (5*R*,6*S*)	**h**; CH$_2$Ph
c; Me	**i**; CH(Me)OH
d; Et	**j**; CH$_2$CH$_2$CO$_2$Bu^t
e; Pr	**k**; CH$_2$[CH$_2$]$_3$NHCO$_2$CH$_2$Ph
f; Pr^i	

Scheme 2. Dieckmann intramolecular synthesis of tetramic acids.

in more recent works. A racemic starting material was often employed,[4b-f] but when chiral compounds were used neither the optical rotation,[4g,h] nor the enantiomeric excess, was measured.[4i,j] Moreover, Hofheinz and Oberhansli noted complete racemisation during neutralisation of the sodium salt of 3-ethoxycarbonyltetramic acid derived from valine.[4k] Likewise, while preparing statine from L-leucine, Katsuki and Yamaguchi also noted racemisation of the unsaturated cyclic intermediate.[4l] We found it of interest to evaluate epimerisation in this synthetic process, which includes both Dieckmann cyclisation and a decarboxylation step.

Table 1. Tenuazonic acid synthesis. Ratio L-AT (**3a**)/D-*allo*-AT (**3b**).

Time (h)	CH_3ONa (1 mol equiv.)	CH_3ONa (2 mol equiv.)
2.5	90/10	58/42
4	77/23	50/50
6	78/22	50/50

Results and Discussion

Dieckmann Cyclisation of N-*Acyl Amino Acids.*—Dieckmann condensation leads to either 3-acetyl or 3-carboxyl derivatives, depending on the starting *N*-acyl compound. We studied the Dieckmann reaction while preparing the 3-acetyl derivatives (**3a–k**) and the 3-carboxyl derivatives (**5a,h**) and (**7h**). The *N*-acyl compounds (**2a**) and (**4a**) were prepared from methyl L-isoleucinate (**1a**) ($C^{\alpha}S,C^{\beta}S$) according to the known procedure* (Scheme 2).[4g,5] These compounds were then cyclised into the corresponding 3-acetyl- and 3-carboxy-tetramic salts under basic conditions (MeO^-/MeOH), and were neutralised by acidic work-up giving the L-tenuazonic acid (L-AT) (**3a**) and the carboxylic compound (**5a**) respectively. We noted that non-reproducible optical rotations were obtained if special care was not taken during the process. Taking advantage of the presence of a second asymmetric centre on the side-chain of isoleucine, we used ^1H NMR spectroscopy to measure the extent of epimerisation at position C-5. A triplet at 0.76 ppm belonging to the D-*allo*-AT (**3b**) isomer, alternatively obtained from D-*allo*-isoleucine, was observed together with a triplet at 0.90 ppm belonging to the L-AT (**3a**) derivative. As showed in Table 1, the ratio between L-AT and D-*allo*-AT was related to the base concentration and reaction time. This drawback was partially avoided by rigorously controlling the stoicheiometry of the reaction, using 0.9 mol equiv. of base. Even so, in the case of tenuazonic acid (**3a**), the diastereoisomeric excess was found to be only 89%. We performed the same study starting from D-*allo*-isoleucine ($C^{\alpha}R,C^{\beta}S$), to verify that the presence of an asymmetric centre at position C-6 was not critical for C-5 epimerisation.[6] Indeed, compound (**3b**) (5*R*,6*S*) was also obtained with a diastereoisomeric excess of 89%. In each case, the major epimer presented the same configuration as its precursor. This consistent finding implies that epimerisation during the Dieckmann cyclisation is not related to thermodynamic equilibrium between the two epimers. Otherwise, starting from D-*allo*-isoleucine, it could be expected that the 5*S*,6*S* isomer would be formed as a major compound if the diastereoisomeric excess were dependent on the racemic intermediate at position C-5. The same analysis was performed on the 3-carboxy derivative (**5a**). The ^1H NMR spectrum shows two separate signals: one at 1.01 ppm for the 1′-Me protons in the 5*S* isomer and another one at 0.77 ppm for the 5*R* isomer. The chemical shifts of the 5-H proton are also distinct (4.10 ppm/5*S*; 4.17 ppm/5*R*). Integration of these signals gave the ratio 5*S*/5*R* as 91:9 (diastereoisomeric excess 82%). The major epimer 5*S* was purified by recrystallisation from hexane–ethyl acetate and had a specific optical rotation of −49°.†

Similarly, the 3-acetyltetramic acid derivatives (**3a–k**) were synthesized from different L- or D-amino acids (Table 2). In the case of methyl L-phenylalaninate (**1h**) the salts of the acids (**3h**), (**5h**), and (**7h**) were isolated. The first two compounds showed optical rotations of −218° and −99° respectively. This former high value is consistent with the results of Schmidlin and Tamm (−210° to −216°, measured in ethanol).[4j]

Although the extent of epimerisation was not directly accessible to ^1H NMR measurements in the case of compounds (**3h**), (**5h**), and (**7h**),‡ partial racemisation was expected and this is corroborated in the following study.

Synthesis of Tetramic Acids.—Tetramic acids are synthesized by decarboxylation or deacylation at position C-3 of the cyclic precursor obtained after Dieckmann cyclisation. ^1H NMR analysis showed that tetramic acid (**8a**), generated from the pure 3-carboxylated tetramic acid (**5a**) by nitromethane–water reflux, was obtained in an enantiomerically pure form. This observation prompted us to evaluate the enantiomeric purity of tetramic acid (**8h**) obtained from compounds (**3h**), (**5h**), and (**7h**) under three different conditions. Decarboxylation of compound (**5h**) was best accomplished by short reflux (15 min) in 25mM-sulphuric acid; different conditions [reflux in acetic acid, trifluoroacetic acid (TFA)–acetic acid, or nitromethane] produced tetramic acid (**8h**) in lower yields, together with a product identified as the dimer. This dimer has previously been isolated by Mulholland *et al.*[4c] Similar conditions but with longer reflux times (3 h) led to the deacylation of compound (**3h**), with a lower yield. To improve the efficiency of this route we synthesized compound (**8h**) starting from the t-butyl derivative (**7h**). The last compound can be easily decarboxylated by trifluoroacetic acid (TFA) treatment at room temperature. In this case the cyclisation step was carefully performed using potassium t-butoxide as a base (10 min; 20 °C). Unfortunately, the expected salt (**7h**) was accompanied by the potassium salt of the starting compound (**6h**) which could not be removed (14%, evaluated by ^1H NMR); the t-butyl ester (**7h**) was not further purified but was directly transformed into tetramic acid (**8h**).

Tetramic acid (**8h**) could not be completely purified. We chose to evaluate the optical purity of the compound by chemical correlation with the reduced compound (**9**) (α_D −44°), alternatively obtained from the enantiomerically pure derivative (**11**).[2b]

The crude product (**8h**) obtained from compounds (**3h**), (**5h**), and (**7h**), respectively, was reduced by sodium borohydride. Both epimeric alcohols at position C-4 (**9**) and (**10**) were obtained as shown in Scheme 3, and were separated. As there was no observable racemisation during reduction of tetramic acid (**8h**) to alcohols (**9**) and (**10**),§ the enantiomeric excess (ee) of alcohol (**9**), prepared from compound (**8h**), indicated the degree of epimerisation during the cyclisation and deacylation (*e.g.* decarboxylation) of compounds (**2h**), (**4h**), and (**6h**). The results in Table 3 confirm that access to tetramic acid (**8h**) *via* compounds (**2h**), (**4h**), and (**6h**) was not enantioselective.

Although the Dieckmann cyclisation strategy is a convenient way of preparing tetramic acids, it is not completely suitable for the synthesis of compounds of high enantiomeric purity, mainly because it is accompanied by non-negligible epimerisation at position C-5 (10–30%). Furthermore, the total yields of non-acylated compounds are moderate. As expected, in the case of t-butyl ester (**7h**) the yield from decarboxylation was higher than for the methyl ester (**5h**) but this advantage was counterbalanced by greater epimerisation. However, in a recent report on the preparation of the polyene acyltetramic acid fuligorubin A, using potassium t-butoxide in t-butyl alcohol, Ley *et al.* mentioned no observable racemisation of the chiral centre.[7] Thus, in this conflicting context, tetramic acids are best prepared by intramolecular *N*-acylation of α-amino acyl

* In the NMR spectrum of compound (**2a**), the signal corresponding to the $C^{\alpha}H$ of the epimer $C^{\alpha}R,C^{\beta}S$ was not observed; no epimerisation occurred during the reaction.

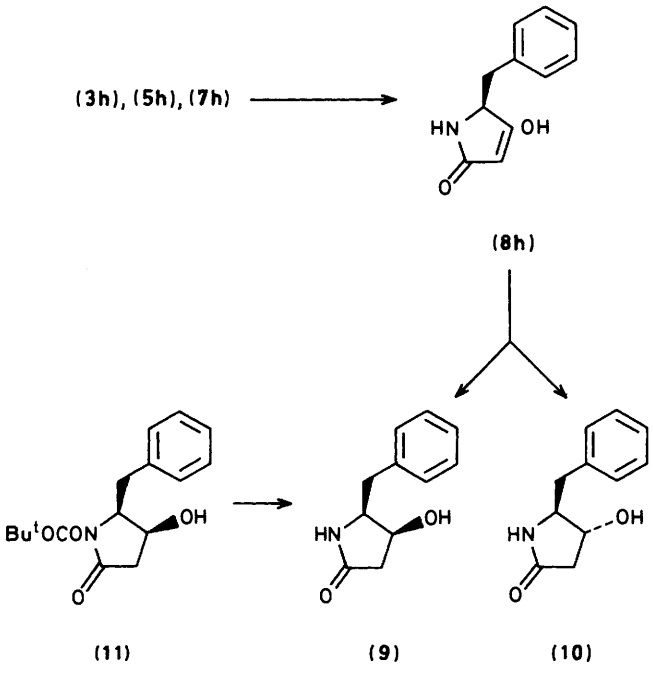
† All the optical rotations mentioned were measured at 589 nm in methanol (*c* 1, 20 °C).

‡ Attempts at evaluation using chiral chemical shift reagents failed.

§ This is demonstrated in Part 2, to be published subsequently.

Table 2. Analytical and spectroscopic data for 3-acetyl 5-substituted tetramic acids (**3a–k**).

Compd.	Yield (%)	M.p./ °C	Formula	Found (%) (Calc.) C	H	N	MS (%)	UV (λ_{max}/nm) (ε)	^{1}H NMR (δ_H, solvent)
(**3a**)	78	oil	$C_{10}H_{15}NO_3$				198 (MH^+) (100), 141(40)	280 (14 450) 240 (11 480)	(CD_3OD) 0.9 (3 H, t, *J* 8 Hz), 1.0 (3 H, d, *J* 7 Hz), 1.25 (2 H, m), 1.90 (1 H, m), 2.45 (3 H, s), 3.84 (1 H, d, *J* 3 Hz)
(**3b**)	83	oil	$C_{10}H_{15}NO_3$				198 (MH^+) (100), 141 (13)	280 (13 720) 240 (10 820)	(CD_3OD) 0.76 (3 H, d, *J* 7 Hz), 0.95 (3 H, t, *J* 6.5 Hz), 1.30 (2 H. m), 1.90 (1 H, m), 2.42 (3 H, s), 3.92 (1 H, d, *J* 3 Hz)
(**3c**)	72	115–117	$C_7H_9NO_3$	54.3 (54.19)	5.6 (5.85)	9.0 (9.03)	156 (MH^+) (89), 84(48)	280 (14 160) 240 (11 100)	($CDCl_3$) 1.38 (3 H, d, *J* 7 Hz), 2.45 (3 H, s), 3.90 (1 H, m), 6.70 (1 H, m)
(**3d**)	60	87–87	$C_8H_{11}NO_3$	56.5 (56.80)	6.45 (6.55)	8.0 (8.28)	170 (MH^+) (100), 141(49)	280 (14 480) 240 (12 000)	($CDCl_3$) 0.95 (3 H, t, *J* 8 Hz), 1.70 (2 H, m), 2.45 (3 H, s), 3.80 (1 H, t, *J* 6 Hz), 7.30 (1 H, m)
(**3e**)	66	87–89	$C_9H_{13}NO_3$	59.15 (59.0)	7.1 (7.15)	7.85 (7.65)	184 (MH^+) (100), 141(27)	280 (14 750) 240 (11 600)	($CDCl_3$) 0.95 (3 H, t, *J* 7 Hz), 1.45 (2 H, m), 1.65 (2 H, m), 2.45 (3 H, s), 3.80 (1 H, m), 7.35 (1 H, m)
(**3f**)	66	oil	$C_9H_{13}NO_3$				184 (MH^+) (20), 141(100)	280 (13 780) 240 (10 870)	(CD_3OD) 0.80 (3 H, d, *J* 8 Hz), 1.0 (3 H, d, *J* 8 Hz), 2.15 (1 H, m), 2.40 (3 H, s), 3.65 (1 H, d, *J* 6 Hz)
(**3g**)	68	105–107	$C_{10}H_{15}NO_3$	60.95 (60.90)	7.85 (7.67)	7.6 (7.10)	198 (MH^+) (100), 141(18)	280 (14 250) 240 (11 240)	($CDCl_3$) 1.0 (6 H, d, *J* 8 Hz), 1.45 (1 H, m), 1.75 (2 H, m), 2.45 (3 H, s), 3.85 (1 H, m), 7.30 (1 H, m)
(**3h**)	68	85–87	$C_{13}H_{13}NO_3$	67.4 (67.52)	5.5 (7.67)	6.2 (6.06)	232 (MH^+) (100), 141(63)	280 (14 900) 240 (11 700)	($CDCl_3$) 2.45 (3 H, s), 2.75 (1 H, q), 3.30 (1 H, q), 4.05 (1 H, q), 6.70 (1 H, m), 7.20 (5 H, m)
(**3i**)	50	148–149	$C_8H_{11}NO_4$	50.9 (51.89)	6.2 (5.99)	7.2 (7.56)	186 (MH^+) (100), 141(21)	277 (11 825) 240 (10 190)	(CD_3OD) 1.22 (3 H, d, *J* 8 Hz), 2.35 (3 H, s), 3.65 (1 H, d, *J* 6 Hz), 4.0 (1 H, m)
(**3j**)	60	decomp.	$C_{13}H_{19}NO_5$				270 (MH^+) (25), 214(100)	280 (8 580) 240 (10 420)	(CD_3OD) 1.35 (9 H, s), 1.80 (4 H, m), 2.35 (3 H, s), 3.75 (1 H, m)
(**3k**)	32	104–106	$C_{18}H_{22}N_2O_5$	60.5 (62.42)	6.8 (6.40)	8.4 (8.09)	347 (MH^+) (100), 141(8)	278 (9 240) 240 (10 400)	($CDCl_3$) 1.5 (6 H, m), 2.40 (3 H, s), 3.20 (2 H, m), 3.80 (1 H, m), 5.10 (2 H, s), 6.90 (1 H, m), 7.35 (5 H, m)



Scheme 3. Reduction of tetramic acid (**8h**).

malonate, previously described by us, in the synthesis of γ-amino acids of *syn*-configuration.[2b]

Experimental *

M.p.s are uncorrected and were determined using a Buchi melting-point apparatus. NMR data were obtained at 360 MHz or 250 MHz for ^{1}H and at 90 MHz for ^{13}C, using Bruker WM-360 or AM-250 instruments. Specific optical rotations were measured on a Schmidt and Haensch Polartronic D apparatus or a Perkin-Elmer Model 241 polarimeter and are correct to ±1°. Elemental analyses were performed at the 'Service de microanalyses du C.N.R.S.' Mass spectral data were obtained on a Ribermag R-10-10 spectrometer (chemical ionisation with NH_3). UV spectra were recorded on a Perkin-Elmer Lambda 5 UV–visible spectrometer. Analytical TLC and HPTLC were performed on silica gel F254 aluminium sheets (0.2 mm thick; Merck). Column chromatography was performed using silica gel (70–200 μm, Amicon). BOP reagent [benzotriazolyloxy-tris(dimethylamino)phosphonium hexafluorophosphate] was obtained from Sempa-Chimie. Amino acid derivatives were of the L-form and were purchased from Bachem or Fluka.

Methyl N-*Acetoacetyl*-L-*isoleucinate* (**2a**).—To a cooled suspension (0 °C) of methyl L-isoleucinate hydrochloride (2.16 g, 12 mmol) in chloroform (50 ml) were successively added dropwise, triethylamine (1.75 ml, 12.5 mmol) and diketene (2.5 ml, 12.5 mmol; 50% in acetone). The mixture was then stirred at room temperature for 16 h. The solvent was evaporated off under reduced pressure, and the residue was dissolved in ethyl acetate (50 ml). The organic phase was washed successively

* All pyrrolidines named herein have been given the tetramic acid numbering scheme as shown in the Figure.

Table 3. Enantiomeric puritives of compound (**9**).

Initial compound	Optical rotation/° (*c* 1, MeOH)	Enantiomeric excess (%)	Chemical yield (%)
(**11**)	−44	100	90
(**3h**)	0	0	16
(**5h**)	−31	70	53
(**7h**)	−27	61	62

with 1M-HCl and saturated aqueous sodium hydrogen carbonate, dried over sodium sulphate, filtered, and concentrated to dryness under reduced pressure. The yellow oily residue was chromatographed on silica gel (250 g; ethyl acetate–hexane 50:50) to yield the title compound (**2a**) as an oil (2.13 g, 78%), R_f 0.70 ($CHCl_3$–MeOH 90:10); $[\alpha]_D^{20}$ − 17° (*c* 1 in MeOH); δ_H ($CDCl_3$) 0.93 (6 H, m, β- and γ-Me), 1.32 (2 H, m, γ-H_2), 1.90 (1 H, m, β-H), 2.27 (3 H, s, Me_3CO), 3.46 (2 H, s, CH_2CO), 3.73 (3 H, s, OMe), 4.56 (1 H, dd, J_1 8, J_2 5 Hz, α-H) and 7.45 (1 H, m, NH); *m/z* 230 (MH^+, 2%), 170(27) and 86(100).

The following methyl-*N*-(acetoacetyl)-L-amino esters (**2**) were prepared by the same procedure.

Methyl N-*acetoacetyl*-D-allo-*isoleucinate* (**2b**). An oil [89% from (**1b**)], R_f 0.72 ($CHCl_3$–MeOH 90:10); δ_H($CDCl_3$) 0.82 (3 H, t, *J* 7 Hz, β-Me), 0.88 (3 H, t, *J* 7 Hz, γ-Me), 1.30 (2 H, m, γ-H_2), 1.90 (1 H, m, β-H), 2.25 (3 H, s, MeCO), 3.45 (2 H, s, CH_2CO), 3.75 (3 H, s, OMe), 4.15 (1 H, dd, J_1 9, J_2 5 Hz, α-H), and 7.45 (1 H, m, NH); *m/z* 230 (MH^+, 3%) and 170(71).

Methyl N-*acetoacetyl*-L-*alaninate* (**2c**). An oil [88% from (**1c**)], R_f 0.55 ($CHCl_3$–MeOH 90:10); δ_H($CDCl_3$) 1.35 (3 H, d, *J* 7.5 Hz, α-Me), 2.28 (3 H, s, MeCO), 3.50 (2 H, s, CH_2CO), 3.80 (3 H, s, OMe), 4.40 (1 H, m, α-H), and 7.45 (1 H, m, NH).

(S)-*Methyl* N-*acetoacetyl*-2-*aminobutanoate* (**2d**). An oil [63% from (**1d**)], R_f 0.58 ($CHCl_3$–MeOH 90:10); δ_H($CDCl_3$) 0.90 (3 H, t, *J* 7 Hz, β-Me), 1.60 (2 H, m, β-H_2), 2.20 (3 H, s, MeCO), 3.40 (2 H, s, CH_2CO), 3.60 (3 H, s, OMe), 4.30 (1 H, m, α-H), and 7.60 (1 H, m, NH).

Methyl N-*acetoacetyl*-L-*norvalinate* (**2e**). An oil [83% from (**1e**)], R_f 0.70 ($CHCl_3$–MeOH 90:10); δ_H($CDCl_3$) 1.0 (3 H, t, *J* 7 Hz, γ-Me), 1.50 (4 H, m, β- and γ-H_2), 2.28 (3 H, s, MeCO), 3.50 (2 H, s, CH_2CO), 3.75 (3 H, s, OMe), 4.50 (1 H, m, α-H), and 7.55 (1 H, m, NH).

Methyl N-*acetoacetyl*-L-*valinate* (**2f**). An oil [93% from (**1f**)], R_f 0.70 ($CHCl_3$–MeOH 90:10); δ_H($CDCl_3$) 0.90 (3 H, t, *J* 6.5 Hz, β-Me), 0.98 (3 H, d, *J* 6.5 Hz, β-Me), 2.10 (1 H, m, β-H), 2.25 (3 H, s, MeCO), 3.45 (2 H, s, CH_2CO), 3.85 (3 H, s, OMe), 4.50 (1 H, dd, J_1 9, J_2 6 Hz, α-H), and 7.35 (1 H, m, NH).

Methyl N-*acetoacetyl*-L-*leucinate* (**2g**). An oil [75% from (**1g**)], R_f 0.75 ($CHCl_3$–MeOH 90:10); δ_H($CDCl_3$) 0.95 (6 H, d, *J* 6 Hz, γ-Me_2), 1.40 (H, m, γ-H), 1.80 (2 H, m, β-H_2), 2.28 (3 H, s, MeCO), 3.47 (2 H, s, CH_2CO), 3.75 (3 H, s, OMe), 4.65 (1 H, m, α-H), and 7.35 (1 H, m, NH).

Methyl N-*acetoacetyl*-L-*phenylalaninate* (**2h**). A yellow oil [80% from (**1h**)], R_f 0.68 ($CHCl_3$–MeOH 90:10); δ_H($CDCl_3$) 2.15 (3 H, s, MeCO), 3.05 (2 H, m, β-H_2) 3.30 (2 H, s, CH_2CO), 3.70 (3 H, s, OMe), 4.7 (1 H, dd, J_1 12, J_2 6 Hz, α-H), 7.30 (1 H, m, NH), and 7.7 (5 H, m, Ph); *m/z* 263 (MH^+, 100%) and 183(6).

Methyl N-*acetoacetyl*-L-*threoninate* (**2i**). Yellow crystals [50% from (**1i**)], m.p. 79 °C; R_f 0.65 ($CHCl_3$–MeOH 90:10); δ_H($CDCl_3$) 1.20 (3 H, d, *J* 8 Hz, β-Me), 2.30 (3 H, s, MeCO), 3.50 (2 H, s, CH_2CO), 3.75 (3 H, s, OMe), 4.35 (1 H, m, α-H), 4.60 (1 H, m, β-H), and 7.35 (1 H, m, NH); *m/z* 218 (MH^+, 100%) and 173(8.3).

α-*Methyl* γ-*butyl* N-*acetoacetyl*-L-*glutamate* (**2j**). A yellow oil [88% from (**1j**)], R_f 0.55 ($CHCl_3$–MeOH 90:10); δ_H($CDCl_3$) 1.45 (9 H, s, Bu^t), 2.0 (4 H, m, β- and γ-H_2), 2.30 (3 H, s, MeCO), 3.45 (2 H, s, CH_2CO), 3.70 (3 H, s, OMe), 4.50 (1 H, m, α-H), and 7.30 (1 H, m, NH); *m/z* 302 (MH^+, 43%), 246(100), and 186(15).

Methyl N^{α}-*acetoacetyl* N^{ε}-*benzyloxycarbonyl*-L-*lysinate* (**2k**). Crystals from diethyl ether [90% from (**1k**)], m.p. 74 °C; R_f 0.42 ($CHCl_3$–MeOH 90:10); δ_H($CDCl_3$) 1.50 (6 H, m, β-, γ-, and δ-H_2), 2.20 (3 H, s, MeCO), 3.20 (2 H, m, ε-H_2), 3.40 (2 H, s, CH_2CO), 3.70 (3 H, s, OMe), 4.20 (1 H, m, α-H), 4.90 (1 H, m, N^{ε}-H), 5.08 (2 H, s, CO_2CH_2), and 7.32 (6 H, m, N^{α}-H and Ph) (Found: C, 60.2; H, 7.0; N, 7.6. Calc. for $C_{19}H_{26}N_2O_6$: C, 60.31, H, 6.87; N, 7.40%); *m/z* 379 (MH^+, 100%), 271(16), and 218(5).

(5S,6S)-3-*Acetyl*-2,4-*dioxo*-5-*s*-*butylpyrrolidine* (**3a**).—A solution of compound (**2a**) (5.46 g, 23.8 mmol) in methanol (12 ml) was added to sodium methoxide [from sodium (0.545 g) and methanol (7 ml)]. The mixture was refluxed for 2 h. The solvent was removed under reduced pressure and water (12 ml) was added. The aqueous solution was carefully acidified to pH 2 with 3M-HCl and extracted with diethyl ether. After drying (Na_2SO_4) and evaporation of the solvent, the resulting yellow oil was triturated three times with pentane (20 ml). Removal of the solvent gave an oil (4.54 g, 97%) which was a mixture of the two 5-epimers (**3a**) (5*S*,6*S*) and (**3b**) (5*R*,6*S*) (94.5:5.5; ^{1}H NMR evaluation), R_f 0.20 ($CHCl_3$–MeOH 90:10); $[\alpha]_D^{20}$ −120° (*c* 1, MeOH) {lit.,[8] $[\alpha]_{546}^{20}$ −136° (*c* 0.2 in $CHCl_3$)}; δ_H(CD_3OD) 0.90 (3 H, t, *J* 7 Hz CH_2Me), 0.98 (3 H, d, *J* 7 Hz, $CHMe$), 1.15–1.50 (2 H, m, CH_2), 1.90 (1 H, m, $CHMe$), 2.43 (3 H, s, MeCO), and 3.84 (1 H, d, *J* 3 Hz, 5-H); δ_C($CDCl_3$) 11.77 (q, CH_2CH_3), 15.85 (q, $CHCH_3$), 19.52 (t, CH_2Me), 23.70 (q, *Me*CO), 37.05 (d, *C*HMe), 67.32 (d, C-5), 124.63 (s, C-3), 175.8 (s, C-2), 184.38 (s, *C*OMe), and 195.76 (s, C-4).

The other 3-acetyltetramic acid analogues [except for (**3h**); Table 2] were synthesized by the same procedure.

(5S)-3-*Acetyl*-5-*benzyl*-2,4-*dioxopyrrolidine* (**3h**).—To a refluxing solution of compound (**2h**) (4.66 g, 17.7 mmol) in anhydrous methanol (50 ml) under an atmosphere of argon was added, during 10 min, a 2.19M solution of sodium methoxide in methanol (7 ml, 15.3 mmol). The reflux was continued for a further 1 h. After cooling, the mixture was concentrated to 15 ml under reduced pressure. The sodium salt was precipitated as a white powder by dropwise addition of diethyl ether (100 ml), collected, washed twice with diethyl ether, and dried *in vacuo* (3.46 g, 77%), $[\alpha]_D^{20}$ −192° (*c* 1 in MeOH); δ_H[$(CD_3)_2SO$] 2.13 (3 H, s, MeCO), 2.55 (1 H, dd, J_1 13.7, J_2 7.9 Hz, 2.99 (1 H, dd, J_1 13.7, J_2 3.8 Hz, CH_2Ar), 3.55–3.60 (1 H, m, 5-H), 6.30 (1 H, s, NH), and 7.12–7.25 (5 H, m, Ph); δ_C[$(CD_3)_2SO$] 27.93 (q, CH_3CO), 38.55 (t, CH_2Ar), 60.06 (d, C-5), 100.76 (s, C-3), 125.76, 127.88, 129.17 (3 d, Ar), 138.68 (s, Ar), 176.05 (s, C-2), 190.42 (s, C-4), and 194.30 (s, *C*OMe).

To a vigorously stirred solution of the salt of compound (**3h**) (507 mg, 2 mmol) in water (2 ml) was added 1M-HCl (10 ml). Compound (**3h**) precipitated out and was collected, washed twice with water, and dried under reduced pressure over phosphorus pentaoxide (390 mg, 84%), m.p. 133–134 °C (decomp.) (lit.,[4j] 133–134 °C); R_f 0.50 ($CHCl_3$–MeOH 90:10); $[\alpha]_D^{20}$ −218° (*c* 1 in MeOH) {lit.,[4j] $[\alpha]_D^{23}$ −216° (*c* 1 in EtOH)}; δ_H[$(CD_3)_2SO$] 2.30 (3 H, s, MeCO), 2.89 (1 H, dd, J_1 14, J_2 5.7 Hz) and 2.98 (1 H, dd, J_1 14, J_2 4.6 Hz, CH_2Ar), 4.15 (1 H, m, 5-H), 7.14–7.27 (5 H, m, Ph), 8.82 (1 H, s, NH), and 11.26 (1 H, s, OH); δ_C[$(CD_3)_2SO$] 19.66 (q, CH_3CO), 36.56 (t, CH_2Ar), 62.01 (d, C-5), 101.10 (s, C-3), 126.39, 127.93, 129.49 (3 d, Ar), 135.86 (s, Ar), 179.10 (s, C-2), 184.70 (s, C-4), and 194.80 (s, *C*OMe) (Found: C, 67.6; H, 5.7; N, 6.0. Calc. for $C_{13}H_{13}NO_3$: C, 67.52; H, 5.67; N, 6.06%).

Methyl N-(*Ethoxycarbonylacetyl*)-L-*isoleucinate* (**4a**).—To a cooled (0 °C) solution of ethyl hydrogen malonate (1.32 g, 10 mmol) in dichloromethane (20 ml) were successively added

dropwise solutions of dicyclohexylcarbodi-imide (2.44 g, 11.8 mmol) in dichloromethane (12 ml) and methyl L-isoleucinate (1.43 g, 10 mmol) in dichloromethane (2 ml). The mixture was stirred under reflux for 20 h, cooled to room temperature, and filtered. The filtrate was dried over sodium sulphate and concentrated under reduced pressure to yield an oily residue, which was purified by chromatography (hexane–ethyl acetate) to give diester (**4a**) as an oil (1.60 g, 63%), R_f 0.60 ($CHCl_3$–MeOH 90:10); $[\alpha]_D^{20}$ −20° (*c* 1 in MeOH); $\delta_H(CDCl_3)$ 0.95 (6 H, m, β- and γ-Me), 1.30 (3 H, t, *J* 7 Hz, OCH_2Me), 1.35 (2 H, m, γ-H_2), 1.90 (1 H, m, β-H), 3.35 (2 H, s, CH_2CO), 3.73 (3 H, s, OMe), 4.22 (2 H, q, *J* 7 Hz, CH_2O), 4.60 (1 H, dd, J_1 9, J_2 5 Hz, α-H), and 7.60 (1 H, n, NH) (Found: C, 55.9; H, 8.15; N, 5.1. Calc. for $C_{12}H_{21}NO_5$: C, 55.58; H, 8.16; N, 5.40%).

Methyl N-(*Ethoxycarbonylacetyl*)-L-*phenylalaninate* (**4h**).—To a cooled (0 °C) and stirred suspension of methyl L-phenylalaninate hydrochloride (1.16 g, 5 mmol) in dichloromethane (25 ml) were successively added, dropwise, triethylamine (1.6 ml, 11.5 mmol), and freshly distilled ethylmalonyl chloride (0.77 ml, 6 mmol). The mixture was stirred for 16 h at room temperature. The organic phase was washed successively with 1M-HCl and saturated aqueous hydrogencarbonate, dried over sodium sulphate, filtered, and concentrated under reduced pressure to produce an oily residue, which crystallised in diethyl ether at −30 °C (1.13 g, 73%), m.p. 59 °C; R_f 0.50 (ethyl acetate–hexane 50:50); $[\alpha]_D^{20}$ +25° (*c* 1 in MeOH); $\delta_H[(CD_3)_2SO]$ 1.16 (3 H, t, *J* 7.1 Hz, OCH_2Me), 2.92 (1 H, dd, J_1 13.8, J_2 8.6 Hz) and 3.03 (1 H, dd, J_1 13.8, J_2 5.7 Hz, β-H_2), 3.60 (3 H, s, OMe), 4.05 (2 H, q, *J* 7.1 Hz, CH_2O), 4.5 (1 H, m, α-H), 7.18–7.31 (5 H, m, Ph), and 8.55 (1 H, d, *J* 7.7 Hz, NH) (Found: C, 61.35; H, 6.6; N, 4.9. Calc. for $C_{15}H_{19}NO_5$: C, 61.42; H, 6.53; N, 4.78%).

(5S,6S)-3-*Methoxycarbonyl*-2,4-*dioxo*-5-*s*-*butylpyrrolidine* (**5a**).—A solution of compound (**4a**) (1.43 g, 5.56 mmol) in methanol (2.2 ml) was added to dry methanolic sodium methoxide [from sodium (0.128 g) and methanol (1.8 ml)]. The mixture was refluxed for 2 h. After cooling, a white precipitate appeared, which was collected, rapidly washed with methanol, and acidified with 3M-HCl to pH 2. The aqueous phase was extracted three times with chloroform (20 ml). The organic phase was then dried (Na_2SO_4), filtered, and concentrated under reduced pressure to yield a crude product, which was a mixture of the two 5-epimers (1.00 g, 85%), $[\alpha]_D^{20}$ −38° (*c* 1, MeOH) in the ratio 5*S*/5*R* 91:9 (^{1}H NMR evaluation). Crystallisation from hexane–ethyl acetate (50:50) gave the pure isomer (**5a**) (5*S*,6*S*) (0.80 g, 68%), m.p. 104 °C; $[\alpha]_D^{20}$ −49° (*c* 1 in MeOH); $\delta_H(CD_3OD)$ 0.92 (3 H, t, *J* 8 Hz, 3′-H_3), 1.01 (3 H, d, *J* 7 Hz, 4′-H_3), 1.15–1.47 (2 H, m, 2′-H_2), 1.95 (1 H, m, 1′-H), 3.81 (3 H, s, MeO), 4.10 (1 H, d, *J* 3.5 Hz, 5-H) [isomer 5*R*,6*S* exhibited the same resonances except for 5-H: 4.17 (d, *J* 3 Hz), and 4′-H_3: 0.77 (d, *J* 7 Hz)] (Found: C, 56.0; H, 7.2; N, 6.15. Calc. for $C_{10}H_{15}NO_4$: C, 56.33; H, 7.09; N, 6.57%).

(5S)-5-*Benzyl*-3-*methoxycarbonyl*-2,4-*dioxopyrrolidine* (**5h**).— To a stirred solution of compound (**4h**) (2.93 g, 10 mmol) in anhydrous methanol (50 ml) under argon was added, during 10 min and at room temperature, a 1.0M solution of sodium methoxide in methanol (9 ml). After being stirred for 3 h, the mixture was concentrated to 15 ml under reduced pressure, and the salt of (**5h**) was precipitated as a white powder by dropwise addition of diethyl ether (100 ml). The sodium salt was collected, washed twice with diethyl ether and dried *in vacuo* (1.91 g, 71%), $[\alpha]_D^{20}$ −126° (*c* 1 in MeOH); $\delta_H[(CD_3)_2SO]$ 2.54 (1 H, dd, J_1 13.7, J_2 7.8 Hz) and 2.95 (1 H, dd, J_1 13.7, J_2 3.9 Hz, CH_2Ar), 3.46 (3 H, s, MeO), 3.50 (1 H, m, 5-H), 6.09 (1 H, s, NH), and 7.12–7.25 (5 H, m, Ph); $\delta_C[(CD_3)_2SO]$ 38.51 (t, CH_2Ar), 48.89 (q, CH_3O), 60.13 (d, C-5), 87.97 (s, C-3), 125.79, 127.91, and 129.26 (3 d, Ar), 138.77 (s, Ar), 166.21 (s, COMe), 176.15 (s, C-2), and 192.74 (s, C-4).

A solution of the sodium salt of (**5h**) (283 mg, 1.05 mmol) in water (2 ml) was added to 1M-HCl (2 ml). The precipitate formed was collected, washed twice with water, and dried under reduced atmosphere over phosphorus pentaoxide (190 mg, 73%), m.p. 148–149 °C (decomp.); $[\alpha]_D^{20}$ −99° (*c* 1 in MeOH); $\delta_H[(CD_3)_2SO]$ 2.88 (1 H, dd, J_1 13.9, J_2 5.6 Hz) and 3.02 (1 H, dd, J_1 13.9, J_2 4.7 Hz, CH_2Ar), 3.59 (3 H, s, MeO), 4.25 (1 H, m, 5-H), 7.16–7.27 (5 H, m, Ph), and 7.86 (s, NH); $\delta_C[(CD_3)_2SO]$ 36.56 (t, CH_2Ar), 50.39 q, CH_3O), 57.25 (d, C-5), 88.85 (s, C-3), 126.43, 127.88, 129.51 (d, Ar), 135.72 (s, Ar), 163.30 (s, C=O), 170.00 (s, C-2), and 183.51 (s, C-4) (Found: C, 62.8; H, 5.4; N, 5.8. Calc. for $C_{13}H_{13}NO_4$: C, 63.15; H, 5.30; N, 5.66%).

Methyl N-(*t*-*Butoxycarbonylacetyl*)-L-*phenylalaninate* (**6h**).—To a solution of t-butyl hydrogen malonate (1.60 g, 10 mmol) and methyl L-phenylalaninate hydrochloride (2.16 g, 10 mmol) in dichloromethane (30 ml) were added triethylamine (3 ml, 21 mmol) and BOP (4.86 g, 11 mmol). The mixture was stirred for 2 h at room temperature. The solvent was evaporated off under reduced pressure and the residue was dissolved in ethyl acetate (50 ml). The organic phase was washed successively with 1M-HCl and saturated aqueous sodium hydrogencarbonate, dried over sodium sulphate, filtered, and concentrated under reduced pressure. The oily residue was chromatographed on silica gel (120 g; ethyl acetate–hexane 30:70) to produce the title compound (**6h**) as an oil, which solidified upon being kept at −30 °C (2.89 g, 90%), m.p. 55 °C; R_f 0.75 (ethyl acetate–hexane 50:50); $[\alpha]_D^{20}$ +210° (*c* 1 in MeOH); $\delta_H[(CD_3)_2SO]$ 1.37 (9 H, s, Bu^t), 2.91 (1 H, dd, J_1 13.8, J_2 8.6 Hz) and 3.03 (1 H, dd, J_1 13.6, J_2 5.7 Hz, β-H_2), 3.60 (3 H, s, MeO), 4.44 (1 H, m, α-H), 7.16–7.32 (5 H, m, Ph), and 8.49 (1 H, d, *J* 7.5 Hz, NH) (Found: C, 63.5; H, 7.2; N, 4.4. Calc. for $C_{17}H_{23}NO_5$: C, 63.54; H, 7.21; N, 4.36%).

(5S)-*Potassium* 5-*Benzyl*-3-*butyloxycarbonyl*-2,4-*dioxopyrrolidine* (**7h**).—To a solution of compound (**6h**) (1.20 g, 3.7 mmol) in t-butyl alcohol (5 ml) was added, at room temperature, potassium t-butoxide (330 mg, 3 mmol). After being stirred for 10 min the white precipitate was filtered off, washed with t-butyl alcohol (3 ml) and dried *in vacuo* (0.91 g). The ^{1}H NMR spectrum [$(CD_3)_2SO$] showed that the solid was a mixture of the potassium salt of the expected product (**7h**) and the potassium salt of methyl *N*-(t-butoxycarbonylacetyl)-L-phenylalaninate (**6h**) in the ratio 86:14. Thus, this product was used without further purification for the next step.

(5S,6S)-5-*s*-*Butyl*-2,4-*dioxopyrrolidine* (**8a**).—A solution of compound (**5a**) (193 mg, 0.9 mmol) in a mixture of nitromethane (3.3 ml) and water (33 ml) was refluxed for 1 h, and then concentrated under reduced pressure. The solid residue was recrystallised from hexane–ethyl acetate (50:50) to give compound (**8a**) as white crystals (91 mg, 65%), m.p. 115 °C (lit.,[5] 117.5–119 °C); R_f 0.50 ($CHCl_3$–MeOH 80:20); $[\alpha]_D^{20}$ −40° (*c* 1 in MeOH); $\delta_H(CDCl_3)$ 0.91 (3 H, t, *J* 7 Hz, 3′-H_3), 1.03 (3 H, d, *J* 7 Hz, 4′-H_3), 1.35 (2 H, m, 2′-H_2), 1.90 (1 H, m, 1′-H), 3.00 (2 H, s, 3′-H_2), and 3.93 (1 H, d, *J* 4 Hz, 5-H) (Found: C, 61.5; H, 8.3; N, 9.0. Calc. for $C_8H_{13}NO_2$: C, 61.91; H, 8.44; N, 9.03%).

(4S,5S)-5-*Benzyl*-2-*oxo*-4-*hydroxypyrrolidine* (**9**).—(4*S*,5*S*)-5-Benzyl-1-butoxycarbonyl-4-hydroxy-2-oxopyrrolidine (**11**) (291 mg, 1.00 mmol) was treated with TFA (2 ml) for 20 min at room temperature. The solvent was removed under reduced pressure and the residue was recrystallised from di-isopropyl

ether to give compound (**9**) as white crystals (172 mg, 90%), m.p. 134–135 °C; R_f 0.64 (CH_2Cl_2–MeOH 90:10, HPTLC); $[\alpha]_D^{20}$ −44° (*c* 1 in MeOH); $\delta_H[(CD_3)_2SO]$ 1.99 (1 H, dd, J_1 16.5, J_2 2.7 Hz) and 2.39 (1 H, dd, J_1 16.5, J_2 5.9 Hz, 3-H_2), 2.66 (1 H, dd, J_1 13.5, J_2 7.4 Hz) and 2.97 (1 H dd, J_1 13.5, J_2 7.8 Hz, CH_2Ar), 3.66–3.73 (1 H, m, 5-H), 4.1 (1 H, m, 4-H), 5.13 (1 H, d, *J* 4.8 Hz, OH), 7.16–7.32 (5 H, m, Ph), and 7.51 (s, NH) (Found: C, 69.0; H, 6.8; N, 7.55. Calc. for $C_{11}H_{13}NO_2$: C, 69.09; H, 6.85; N, 7.32%).

Synthesis of Compound (**9**) *from the* 3-*Acyl Pyrrolidines* (**3h**), (**5h**), *and* (**7h**).—*From compound* (**5h**). A solution of compound (**5h**) (500 mg, 2.02 mmol) in 0.025M-H_2SO_4 (pH 1.6) (8 ml) was refluxed for 10 min. The solution was rapidly cooled in an ice-bath and extracted three times with ethyl acetate (10 ml). The organic phase was dried over sodium sulphate, filtered, and concentrated under reduced pressure. The resulting unpurified compound (**8h**) was dissolved in acetic acid–dichloromethane (10:90; 10 ml), cooled to 0 °C, and $NaBH_4$ (80 mg, 2.1 mmol) was added in three batches. Treatment as described above produced compound (**9**) (205 mg, 53%), $[\alpha]_D^{20}$ −31°, and (4*R*,5*S*)-5-benzyl-4-hydroxy-2-oxopyrrolidine (**10**) (45 mg, 12%), R_f 0.60 (CH_2Cl_2–MeOH 90:10), m.p. 141–142 °C (from di-isopropyl ether); $\delta_H[(CD_3)_2SO]$ 1.83 (1 H, dd, J_1 16.9, J_2 2.5 Hz) and 2.28 (1 H, dd, J_1 16.8, J_2 6.2 Hz, 3H_2), 2.65–2.75 (2 H, m, CH_2Ar), 3.49–3.54 (1 H, m, 5-H), 3.93–3.98 (1 H, m, 4-H), 5.09 (1 H, d, *J* 4.1 Hz, OH), 7.19–7.33 (5 H, m, Ph), and 7.67 (1 H, s, NH) (Found: C, 69.0; H, 6.75; N, 7.4. Calc. for $C_{11}H_{13}NO_2$: C, 69.09; H, 6.85; N, 7.32%).

From compound (**3h**). A solution of compound (**3h**) (500 mg, 2.16 mmol) in 0.05M-H_2SO_4 (10 ml) was refluxed for 4 h to give compound (**8h**). Using the procedure described above, compounds (**9**) (67 mg, 16%), $[\alpha]_D^{20}$ 0° and (**10**) (14 mg, 4%) were isolated.

From compound (**7h**). A solution of the potassium salt of (**7h**) (500 mg, 1.52 mmol) in TFA was stirred at room temperature for 2 h to give compound (**8h**). The solvent was evaporated off under reduced pressure and the residue was subjected to the usual reduction procedure to produce compounds (**9**) (180 mg, 62%), $[\alpha]_D^{20}$ −44° and (**10**) (43 mg, 15%).

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