PREPARATION OF (*Z*)-3,4-DIDEHYDRONORVALINE, (*E*)- AND (*Z*)-3,4-DIDEHYDROORNITHINE AND (*E*)- AND (*Z*)-3,4-DIDEHYDRO-2,6-DIAMINOPIMELIC ACID

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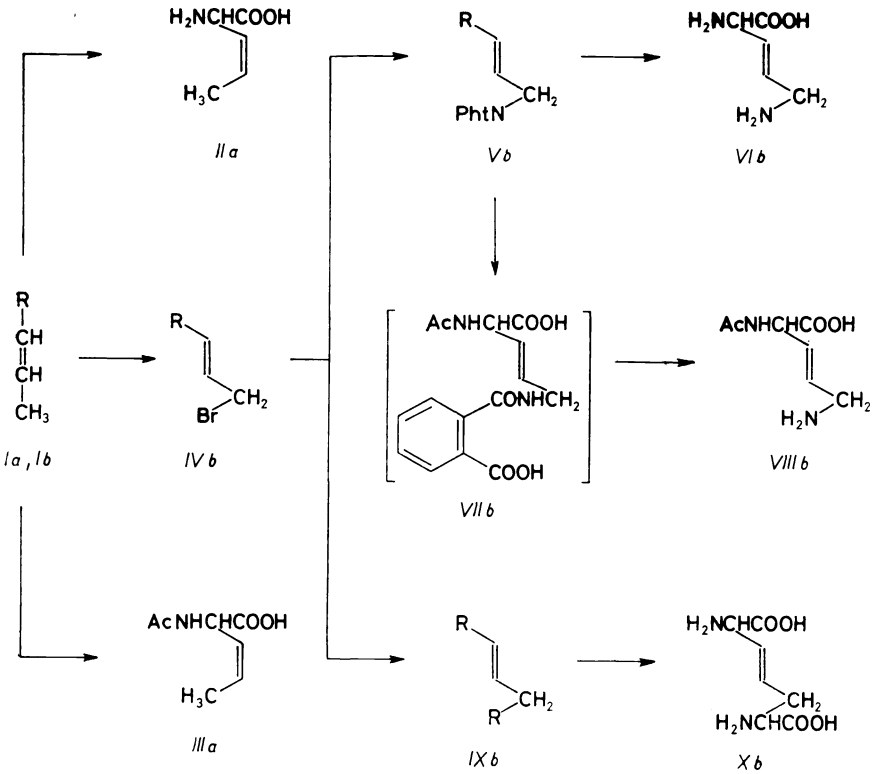
(*Z*)-3,4-Didehydronorvaline (*IIa*), (*E*)- and (*Z*)-didehydroornithine (*VIb* and *VIa*, respectively) and (*E*)- and (*Z*)-3,4-didehydro-2,6-diaminopimelic acid (*Xb* and *Xa*, respectively) were prepared by addition of Grignard reagents to diethyl acetiminomalonate and subsequent reactions. The mechanism of cyclization of 2-acetamido-2-alkynylmalonate to the oxazoline derivative in a basic medium was studied by ^{1}H NMR spectroscopy.

The growing interest in the synthesis of 3,4-didehydro-2-amino acids[1-4] reflects the fact that these compounds exhibit interesting biological properties[5-7] and are suitable precursors for the preparation of tritium-labelled amino acids[8] and peptides[9]. Syntheses of the following compounds of this type have already been described: (i) (*Z*)-3,4-didehydronorvaline[10] and the corresponding (*E* + *Z*)-isomer mixture[11], (ii) (*E*)-3,4-didehydro-2,6-diaminopimelic acid[7], and (iii) (*E*)-3,4-didehydroornithine[8,12-14]. The synthesis of 3,4-didehydro-2-amino acids is often complicated by rearrangement of the double bond into the position 2,3 (refs[15,16]). Kober and coworkers[17,18] prepared 2-vinyl and 2-ethynyl derivatives of diethyl 2-benzoylaminomalonate by addition of vinyl or ethynyl Grignard reagent to diethyl benzoyliminomalonate. Analogously, we added 1-propenylmagnesium bromide to diethyl acetyliminomalonate and obtained 2-acetylamino-2-ethoxycarbonyl-3-pentenoate (*I*) as a 3 : 7 mixture of *E*- and *Z*-isomers. The pure *Z*-isomer was obtained on crystallization. In accord with the literature[17], we isolated the dimer of acyliminomalonate, diethyl 2,3-bis(acetamido)-2,3-diethoxycarbonylsuccinate, in 5% yield. Acid hydrolysis of isomer *Ia* (Scheme 1) afforded (*Z*)-3,4-didehydronorvaline (*IIa*) whereas the alkaline hydrolysis gave its N-acetyl derivative *IIIa* which is suitable substrate for enzymatic resolution with acylase *I*.

The intermediate *XVI*, arising in the alkaline hydrolysis of *Ia* (Scheme 2), undergoes spontaneous decarboxylation at pH 7–8 and room temperature. The facile decarboxylation of *XVI* can be explained by stabilization of the reaction inter-

mediate by double bond conjugation, in accord with the generally accepted cyclic mechanism of decarboxylation of malonic acid derivatives[19].

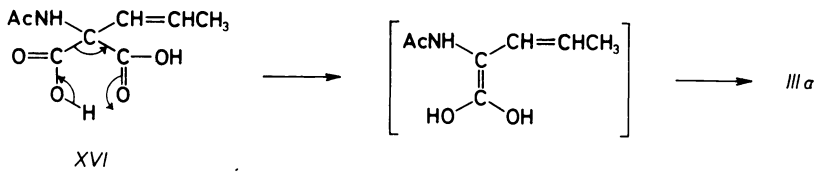


In formulae *I*–*X*: R = $C(COOC_2H_5)_2$ (NHAc); *a*, *Z*-isomer; *b*, *E*-isomer

SCHEME 1

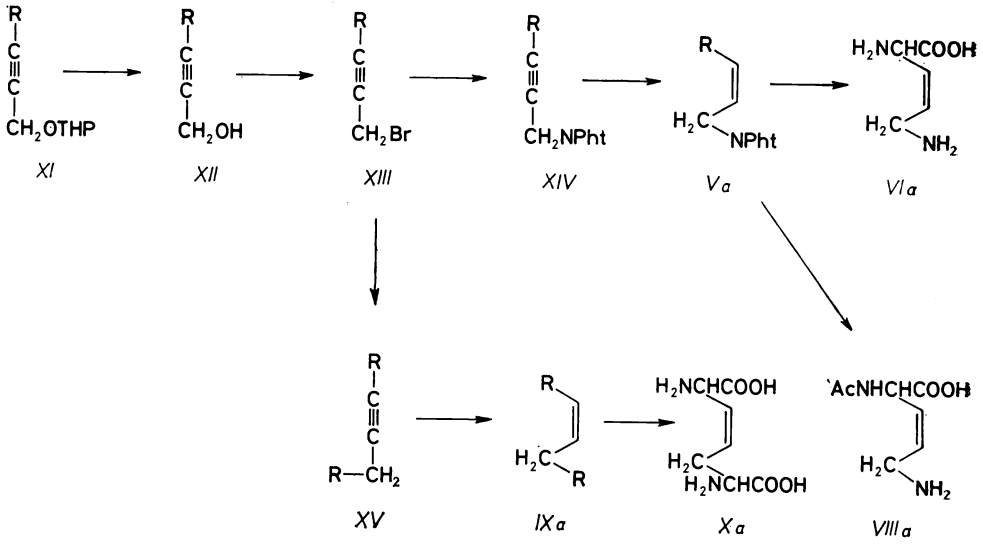
The Wohl–Ziegler bromination of malonate *I* (Scheme 1) afforded the bromo derivative *IVb*. Its yield, as well as the amount of the undesired product of bromine addition, are very sensitive to the reaction conditions. As found by ^{1}H NMR spectroscopy, the bromination of both the pure *Z*-isomer and the 7 : 3 mixture of *Z* and *E* isomers of *I* led only to the *E*-isomer *IVb*. The mentioned bromination reaction is generally assumed[20] to proceed via the radical $^{\bullet}CH_2$—CH=CHR. Accordingly, the exclusive formation of the isomer *E* may be explained by excitation of the double bond electrons in the energetically rich radical and free rotation about the C—C

bond. The molecule then returns into the ground state, in which the thermodynamically more stable isomer *E* with bulky groups in the *trans*-relation is favoured.



SCHEME 2

Gabriel reaction of the bromo compound *IVb* afforded the N-alkyl phthalimide derivative *Vb* which was converted into racemic (*E*)-3,4-didehydroornithine (*VIb*) by acid hydrolysis. The conversion of *Vb* into *VIIIb* could be accomplished thanks to the unusually facile acid cleavage of the phthalamide intermediate *VIIb* obtained by alkaline hydrolysis of *Vb*. The phthalic acid was removed by treatment with 0·6M hydrochloric acid at room temperature; under these conditions the N^{α}-acety group remained intact. This lability of the CONH bond in the phthalamide inter mediate *VIIb* agrees with the reported[21] extreme sensitivity of N-propargylphthal amide towards acidolysis (at pH 4 and 25°C already after 24 h). The bromo deriva-



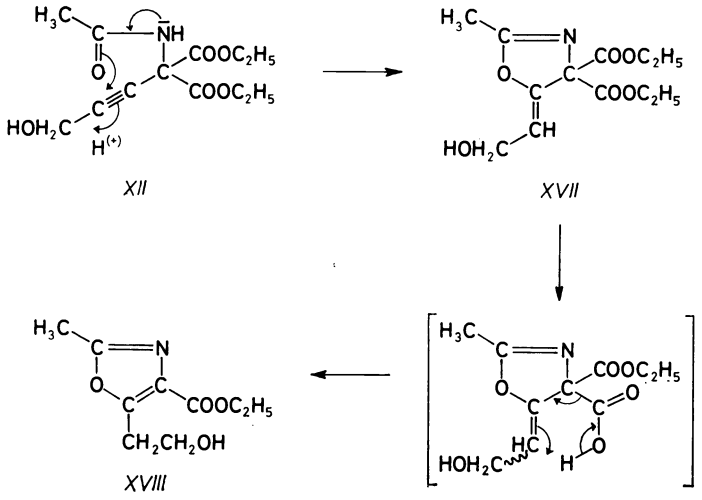
R as in Scheme 1

SCHEME 3

tive *IVb* was used in the alkylation of acetamidomalonate. Acid hydrolysis of the obtained compound *IXb* gave (*E*)-3,4-didehydro-2,6-diaminopimelic acid (*Xb*).

For the preparation of *Z*-isomers of 3,4-didehydro-2-amino acids we made use of the known preferential *cis*-addition of hydrogen in heterogeneous catalytic hydrogenation of triple bonds. Addition of 3-(2-tetrahydropyranyloxy)-2-propynylmagnesium bromide to diethyl acetyliminomalonate afforded alkyne *XI* (Scheme 3). The tetrahydropyranyl group in *XI* was removed by hydrolysis and the obtained alcohol *XII* was converted in high yield into bromide *XIII* by treatment with triphenylphosphine dibromide. The compound *XIII* was then used for alkylations of potassium phthalimide and sodium salt of diethyl acetamidomalonate. The preparation of *Z*-isomers of 3,4-didehydroornithine and 3,4-didehydro-2,6-diaminopimelic acid is shown in Scheme 3.

An attempt to convert the alkyne *XII* into a triple bond-containing amino acid derivative resulted in quantitative formation of the oxazoline derivative *XVIII* (Scheme 4). The reaction took place already under very mild conditions and was studied by ^{1}H NMR spectroscopy. Spectrum of the reaction mixture (the reaction was performed in 10% D_2O/H_2O + 0·1% NH_4OH) exhibited temporary signals attributable to *E*/*Z*-isomers of the assumed intermediate *XVII* (Scheme 4). As



SCHEME 4

expected, the reaction in D_2O led to disappearance of olefinic proton signals. Also the splitting of the CH_2OH signal disappeared. The signals of the intermediate were observable already after 1 minute whereas the signals due to ethanol liberated by

the ester hydrolysis appeared only after 3 minutes. Attempted acid hydrolysis of derivative *XII* and/or *XV* (refluxing with 6M-HCl) gave a dark complex reaction mixture which was not further worked up.

Because of specific features of the enzymatic kinetic resolution of the described N-acetyl-3,4-didehydro-2-amino acids with acylase *I* (first of all the concurrent enzyme-catalyzed racemization of the liberated L-amino acid), it will be described in a separate article.

EXPERIMENTAL

Proton and ^{13}C NMR spectra were measured on a Jeol FX 60 spectrometer at 59·796 MHz and at 15·036 MHz, respectively, or on a Varian VXR-400 instrument at 400 MHz and 100 MHz, respectively. All spectra were obtained by the FT technique at 25°C, using tetramethylsilane as internal standard (or acetone in D_2O as solvent). The chemical shifts are given in the δ-scale. Melting points were determined on a Koffer block and are uncorrected. Column chromatography was performed on silica gel (Kieselgel 60, Merck, particle size 40–63 μm). Crystal water was not determined. The solvents were evaporated under diminished pressure at temperatures below 35°C. 1-Propenylmagnesium bromide was prepared by a described procedure from 1-bromopropene ($Z : E = 7 : 3$; 35·2 g) and magnesium (7·4 g) in anhydrous tetrahydrofuran (THF; 400 ml) in the atmosphere of an inert gas. Concentration of the reagent was determined titrimetrically. 3-(2-Tetrahydropyranyloxy)-2-propynylmagnesium bromide was prepared according to Henbest and coworkers[22] using anhydrous THF instead of ethyl ether.

Ethyl (*Z*)-2-Acetamido-2-ethoxycarbonyl-3-pentenoate (*Ia*)

Ethyldiisopropylamine (53 ml, 0·30 mol) was added during 30 min at −78°C to a stirred solution of diethyl 2-bromo-2-acetamidomalonate[17] (80·5 g, 0·27 mol) in anhydrous THF (1 l) under argon. The reaction mixture was warmed to 0°C during 1 h. After sedimentation of ethyldiisopropylamine hydrobromide, the reaction mixture was filtered in an inert atmosphere. The thus-obtained solution of acetyliminomalonate was cooled to −78°C and a solution of 1-propenylmagnesium bromide (1·05 equivalent) in THF was added under stirring in the course of 40 min. The reaction mixture was stirred at −78°C for 1 h, warmed to −15°C during 2 h and acidified to pH 4 with 0·5M-HCl. The aqueous phase was saturated with sodium chloride and separated. The organic solution was diluted with the same volume of diethyl ether and washed with saturated solution of sodium hydrogen carbonate. The solvent was evaporated and the obtained sirupy residue was dissolved in ethyl acetate and dried. The crude product was purified by column chromatography on silica gel (5·5 × 30 cm) in ethyl acetate–hexane (4 : 6); yield 39·6 g (57%) of a crude mixture of stereoisomers ($Z : E = 7 : 3$), m.p. 70–80°C. Crystallization from water and then from diethyl ether afforded 27·8 g (40%) of pure *Z*-isomer, m.p. 86–88°C. For $C_{12}H_{19}NO_5$ (257·3) calculated: 56·02% C, 7·44% H, 5·44% N; found: 55·80% C, 7·40% H, 5·35% N. ^1H NMR spectrum (60 MHz, $CDCl_3$): *Z* isomer: 1·26 t (6 H, $J = 7·3$, OCH_2CH_3); 1·64 dd, (3 H, $J = 7·3$, $J = 1·8$, H-5); 2·04 s (3 H, CH_3CO); 4·16 q (4 H, $J = 7·3$, CH_2CH_2O); 5·40 to 5·84 mt (1 H, H-4); 6·43 dq (1 H, $J = 10·3$, $J = 1·8$, H-3); 7·17 br s (NH); *E* isomer: 1·26 t (6 H, $J = 7·3$, OCH_2CH_3); 1·75 dd (3 H, $J = 7·3$, $J = 1·2$, H-5); 2·07 s (3 H, CH_3CO); 4·16 q (4 H, $J = 7·3$, OCH_2CH_3); 5·40–5·84 mt (1 H, H-4); 6·18 dq (1 H, $J = 16·5$, $J = 1·2$, H-3); 6·97 br s (NH). ^{13}C NMR spectrum (100 MHz, $CDCl_3$): *Z* isomer 13·32 q (C-5); 13·83 q (2 C, CH_3CH_2); 22·36 q (CH_3CO); 62·76 t (2 C, CH_3CH_2); 64·68 s (C-2); 125·65 d and 128·52 d

(C-3 and 4); 167·43 s (2 C, COO); 168·21 s (CONH). ^{13}C NMR spectrum (15 MHz, $CDCl_3$): mixture of *Z* and *E*: 13·5 q and 14·2 q (C-5); 17·7 q (CH_3CH_2); 22·5 q and 22·9 q (CH_3CO); 62·9 t (CH_2CH_3); 63·0 s and 64·6 s (C-2); 125·3 d and 125·8 d and 127·6 d and 128·6 d (C-3 and 4); 167·5 s and 168·4 s (COO and CO).

Diethyl 2,3-Bis(acetamido)-2,3-diethoxycarbonylsuccinate

The compound was isolated from further fractions in the above-described chromatography of *Ia*; yield 2·9 g (5%), m.p. 130–135°C (dichloromethane). For $C_{18}H_{28}N_2O_{10}$ (432·4) calculated: 50·00% C, 6·53% H, 6·49% N; found: 49·83% C, 6·55% H, 6·33% N. ^{1}H NMR spectrum (60 MHz, $CDCl_3$): 1·25 t (6 H, J = 7·3, OCH_2CH_3); 2·02 s (3 H, CH_3CO); 4·2 q (4 H, J = 7·3, OCH_2. .CH_3); 7·25 s (1 H, NH). ^{13}C NMR spectrum (15 MHz, $CDCl_3$): 13·8 q (4 C, CH_3CH_2); 23·3 q (2 C, CH_3CO); 62·9 t (4 C, CH_2CH_3); 68·6 s (2 C, C–NHCO); 165·2 s (4 C, 169·8 s (2 C, CH_3CO).

(*Z*)-3,4-Didehydro-D,L-norvaline (*IIa*)

A solution of *Ia* (1 g, 3·9 mmol) in 5M-HCl (5 ml) was refluxed for 2 h. After evaporation to dryness, the residue was dissolved in water (4 ml) and applied onto a column (3 × 1·5 cm) of Dowex 50 (H^+ form). The column was washed with water and the amino acid eluted with 3% NH_4OH. The eluate was concentrated and the product precipitated with ethanol; yield 0·327 g (73%), m.p. 214°C (decomp.). For $C_5H_9NO_2$ (115·1) calculated: 52·16% C, 7·88% H, 12·17% N; found: 52·07% C, 8·00% H, 12·20% N. ^{1}H NMR spectrum (400 MHz, D_2O): 1·765 dd (3 H, J(4·5) = 7·0, J(3·5) = 1·9, H-5); 4·589 dd (1 H, J(2·3) = 10·2, J(2·4) = 1·0, H-2); 5·436 ddd (1 H, J(3·4) = 10·7, J(3·2) = 10·2, J(3·5) = 1·9, H-3); 6·002 dqd (1 H, J = 10·7, J = 7·0, J = 1·0, H-4). ^{13}C NMR spectrum (100 MHz, D_2O): 13·07 q (C-5); 51·89 d (C-2); 122·17 d and 134·55 d (C-3 and 4); 174·23 s (COO).

(*Z*)-2-Acetamido-3-pentenoic Acid (*IIIa*)

To a stirred solution of *Ia* (6·2 g, 24·1 mmol) in methanol (50 ml) was added dropwise 1M-NaOH (60·3 ml) during 3 h, the mixture was stirred for further 4 h at room temperature, adjusted to pH 4·5 with acetic acid and concentrated. The sirupy residue was dissolved in ethyl acetate, filtered and applied onto a column of silica gel (4 × 8 cm). After washing the column with ethyl acetate (200 ml), the product was eluted with 30% acetic acid in chloroform. Crystallization from ethyl acetate–diethyl ether afforded 2·65 g (70%) of *IIIa*, m.p. 117–120°C. For $C_7H_{11}NO_3$ (157·2) calculated: 53·48% C, 7·05% H, 8·91% N; found: 53·13% C, 7·05% H, 8·90% N. ^{1}H NMR spectrum (60 MHz, $CDCl_3$ + CD_3OD): 1·82 d (3 H, J = 7·3, H-5); 2·02 s (3 H, CH_2CO); 5·14–6·18 mt (3 H, CH—CH=CH); 6·83 s (1 H, NH). ^{13}C NMR spectrum (15 MHz, $CDCl_3$ + CD_3OD): 13·5 q (C-5); 22·7 q (CH_3CO); 50·4 d (C-2); 124·5 d and 131·3 d (C-3 and 4); 170·9 s and 173·6 s (COO and CO).

Ethyl (*E*)-2-Acetamido-5-bromo-2-ethoxycarbonyl-3-pentenoate (*IVb*)

A mixture of *Ia* (2 g, 7·77 mmol), N-bromosuccinimide (1·494 g, 1·08 equiv.), dibenzoyl peroxide (about 20 mg) and anhydrous tetrachloromethane was placed into a quarz flask and irradiated in an argon atmosphere with a 400 W mercury lamp under vigorous stirring. The temperature was kept at 50–60°C and the reaction was complete after 10 min. The reaction mixture was filtered through a column of silica gel (2 × 1 cm) and the filtrate concentrated. The sirupy

residue was used in the next reaction without further purification. ^{1}H NMR spectrum (60 MHz, $CDCl_3$): 1·27 t (6 H, J = 7·3, CH_3CH_2); 2·09 s (3 H, CH_3CO); 3·99 d (2 H, J = 7·3, H-5); 4·26 q (4 H, J = 7·3, CH_2CH_3); 5·87 td (1 H, J = 7·3, J = 15·1, H-4); 6·46 d (1 H, J = 15·1, H-3); 7·27 s (1 H, NH). ^{13}C NMR spectrum (15 MHz, $CDCl_3$): 13·3 q (2 C, CH_3CH_2); 22·0 q (CH_3CO); 30·4 t (C-5); 62·4 t (2 C, CH_2CH_3); 65·6 s (C-2); 127·5 d and 128·5 d (C-3 and 4); 165·9 s (CO); 168·4 s (2 C, COO).

Ethyl (*E*)-2-Acetamido-5-phthalimido-2-ethoxycarbonyl-3-pentenoate (*Vb*)

Potassium phthalimide (1·73 g) was added to a solution of *IVb* (2·7 g) in anhydrous dimethylformamide (30 ml). The mixture was stirred at room temperature for 1 h and then at 55°C for another hour, cooled to room temperature, acidified with acetic acid and the solvent was evaporated. The product was purified by chromatography on silica gel (1·8 × 18 cm) in 20% ethyl acetate in toluene and subsequent crystallization from chloroform–hexane; yield 1·596 g (51% from *Ia*); m.p. 132–133°C. For $C_{20}H_{22}N_2O_7$ (402·4) calculated: 59·67% C, 5·51% H, 6·96% N; found: 59·37% C, 5·75% H, 6·89% N. ^{1}H NMR spectrum (60 MHz, $CDCl_3$): 1·25 t (6 H, J = 7·3, CH_3CH_2); 2·06 s (3 H, CH_3CO); 4·25 q (4 H, J = 7·3, CH_2CH_3); 4·37 d (2 H, J = 6·1, H-5); 5·74 td (1 H, J = 6·1, J = 15·3, H-4); 6·50 d (1 H, J = 15·3, H-3); 6·96 s (1 H, NH); 7·77 mt (4 H, Pht). ^{13}C NMR spectrum (15 MHz, $CDCl_3$): 13·9 q (2 C, CH_3CH_2); 22·9 q (CH_3CO) 39·0 t (C-5): 63·0 t (2 C, CH_2CH_3); 66·5 s (C-2); 123·3 d (2 C, Pht); 125·4 d and 129·0 d (C-3 and 4); 131·2 s (2 C, Pht); 134·3 d (2 C, Pht); 166·9 s (2 C) and 167·8 s and 168·7 s (2 C) correspond to CO and COO groups.

(*E*)-3,4-Didehydro-D,L-ornithine (*VIb*)

A solution of *Vb* (493 mg, 1·23 mmol) in 6M-HCl was refluxed for 3 h, cooled and the separated phthalic acid was filtered off. The filtrate was concentrated and the product purified by gradient chromatography in 0·2–3M-HCl on a 1 × 10 cm column of Dowex 50 (H^+ form). The product was dissolved in ethanol, pyridine was added dropwise (to turbidity) and the crystalline monohydrochloride *VIb* was collected; m.p. 220°C (decomp.); yield 96 mg (45%). For $C_5H_{10}N_2O_2$ .HCl.1/4 H_2O (171·1) calculated: 35·10% C, 6·76% H, 20·73% Cl, 16·37% N; found: 35·14% C, 6·76% H, 20·72% Cl, 16·06% N. ^{1}H NMR spectrum (400 MHz, D_2O): 3·74 d (2 H, J = 5·1, H-5); 4·38 d (1 H, J = 6·6, H-2); 5·98–6·14 mt (2 H, H-3 and 4). ^{13}C NMR spectrum (100 MHz, D_2O): 49·1 d (C-2); 64·72 t (C-5); 137·32 d and 137·62 d (C-3 and 4); 180·80 s (COO).

(*E*)-5-Amino-2-acetamido-3-pentenoic Acid (*VIIIb*)

A suspension of compound *Vb* (7·2 g, 17·9 mmol) in 0·36M-NaOH (200 ml, 4 equivalents) was stirred at 25°C for 12 h (after 2 h the reaction mixture became homogeneous). The solution was acidified with hydrochloric acid to pH 0·6 and allowed to stand at 25°C for 48 h. After freeze-drying, the residue was applied onto a column of Dowex 50 (H^+ form, 2·2 × 7·5 cm) and the phthalic acid was eluted with ethanol–water (7 : 3, 300 ml). The amino acid was eluted with 3% aqueous ammonia, the eluate concentrated and the residue crystallized from aqueous ethanol to give 1·392 g (44%) of *VIIIb*, m.p. 200–205°C (decomp.). For $C_7H_{12}N_2O_3.1/4\ H_2O$ (176·7) calculated: 47·58% C, 7·13% H, 15·85% N; found: 47·50% C, 7·10% H, 16·00% N. ^{1}H NMR spectrum (60 MHz, CD_3SOCD_3 + CF_3COOD): 1·93 s (3 H, CH_3CO); 3·51 mt (2 H, H-5); 4·96 mt (1 H, H-1); 5·73 dd (1 H, J = 15·6, J = 5·9, olefin); 6·00 dd (1 H, J = 15·6, J = 4·9, olefin); 8·48 d (1 H, J = 7·9, NH). ^{13}C NMR spectrum (15 MHz, CD_3SOCD_3 + CF_3COOD): 22·5 q (CH_3CO); 40·2 t (C-5); 53·7 d (C-2); 125·0 d and 131·1 d (C-3 and 4); 169·7 and 171·7 s (COO and CO).

Diethyl (*E*)-2,6-Bis(acetamido)-2,6-diethoxycarbonyl-3-heptenedioate (*IXb*)

A solution of *IVb* (2·7 g, 7·7 mmol) in anhydrous dimethylformamide (5 ml) was added at room temperature to a solution of sodium salt of diethyl acetamidomalonate (7·7 mmol) in anhydrous dimethylformamide (5 ml). The reaction mixture was gradually heated to 60°C in the course of 1 h, stirred for another hour, cooled to room temperature and acidified with acetic acid. The solvent was evaporated and the residue partitioned between water and ethyl acetate. The organic phase was concentrated and chromatographed on silica gel (2·5 × 30 cm) in 15% ethyl acetate in chloroform. Crystallization from diethyl ether afforded 1·468 g of the title product (40% from *Ia*), m.p. 95–97°C. For $C_{21}H_{32}N_2O_{10}$ (472·5) calculated: 53·36% C, 6·83% H, 5·93% N; found: 53·05% C, 6·94% H, 5·82% N. ^{1}H NMR spectrum (60 MHz, $CDCl_3$): 1·26 t (12 H, J = 7·3, 4 × CH_3CH_2); 2·06 s (6 H, 2 × CH_3CO); 3·05 d (2 H, J = 7·9, H-5); 4·24 q (8 H, J = 7·3, 4 × CH_2CH_3); 5·38 dd (1 H, J = 15·9, J = 7·9, H-4); 6·29 d (1 H, J = 15·9, H-3); 6·81 s (1 H, NH); 7·06 s (1 H, NH). ^{13}C NMR spectrum (15 MHz, $CDCl_3$): 13·5 q (2 C, CH_3CH_2); 13·6 q (2 C, CH_3CH_2); 22·4 q (2 C, CH_3CO); 34·8 t (C-5); 60·6 s (C-2 or 6); 62·2 t (2 C, CH_2CH_3); 62·5 t (2 C, CH_2CH_3); 65·9 s (C-2 or 6); 124·9 d and 129·8 d (C-3 and 4); 166·6 s and 167·0 s and 168·5 s and 169·5 s (COO and CO).

(*E*)-3,4-Didehydro-2,6-diaminopimelic Acid (*Xb*)

A solution of *IXb* (3·5 g, 7·40 mmol) in 6M-HCl was refluxed for 6 h. The mixture was evaporated to dryness, the residue dissolved in water and applied onto a column of Dowex 50 (H^+ form, 1 × 11 cm). The ion exchanger was washed with 50% aqueous methanol (100 ml) and then the amino acid *Xb* was eluted with 8% ammonium hydroxide. After evaporation, the residue was crystallized from water and ethanol, affording 1·07 g (75%) of the product, not melting up to 315°C (decomp.). For $C_7H_{12}N_2O_4.1/4\ H_2O$ (192·7) calculated: 43·61% C, 6·54% H, 14·54% N; found: 43·92% C, 6·52% H, 14·66% N. ^{1}H NMR spectrum (400 MHz, D_2O): 2·68–2·85 mt (2 H, H-5); 3·93 mt (1 H, H-6); 4·36 dmt (1 H, J(2, 3) = 7·6, J(2·4) = 1·2, H-2); 5·87 ddd (1 H, J(4·2) = 1·2, J = 4·8, J(3·4) = 15·0, H-4); 5·98 ddd (1 H, J = 1·4, J(2·3) = 7·6, J(3·4) = 15·0, H-3). ^{13}C NMR spectrum (100 MHz, D_2O): 34·10 t and 34·51 t (C-5); 54·80 d (CHCOOH); 57·17 d and 57·22 d (CHCOOH); 128·13 d and 128·37 d (—CH=); 132·28 d and 132·8 d (—CH=); 173·52 s and 173·54 s (COOH); 174·46 s and 174·57 s (COOH). According to the ^{13}C NMR spectrum, the ratio of diastereoisomers is about 1 : 1.

Ethyl 2-Acetamido-2-ethoxycarbonyl-5-(2-tetrahydropyranyloxy)-3-pentynoate (*XI*)

The title compound was prepared in the same manner as compound *Ia* except that 3-(2-tetrahydropyranyloxy)-2-propynylmagnesium bromide was used instead of 1-propenylmagnesium bromide. After warming to 0°C, the reaction mixture was decomposed with water and acidified with acetic acid. Further work-up was the same as for the compound *Ia* and the chromatography of *XI* was performed using gradient elution (5% to 10%) with ethyl acetate in chloroform. Yield 49·07 g (51%) of colourless noncrystalline oil. ^{1}H NMR spectrum (60 MHz, $CDCl_3$): 1·29 t (6 H, J = 7·3, CH_3CH_2); 0·98–1·63 mt (6 H, THP); 2·06 s (3 H, CH_3CO); 3·68 mt (2 H, THP); 4·31 q (4 H, J = 7·3, CH_2CH_3); 4·31 s (2 H, H-5); 5·06 mt (1 H, THP); 6·94 s (NH). ^{13}C NMR spectrum (15 MHz, $CDCl_3$): 13·9 q (2 C, CH_3CH_2); 19·1 t (THP); 23·1 q (CH_3CO); 25·5 t (THP); 30·3 t (THP); 54·5 t (C-5); 60·8 s (C-2); 62·7 t (major, THP); 63·2 t (minor, THP), 63·8 t (2 C, CH_2CH_3); 78·8 s and 81·0 s (C-3 and 4); 96·5 d (major, THP); 103·8 d (minor, THP); 164·5 s (CH_3CO); 168·5 s (2 C, COO).

Ethyl 2-Acetamido-2-ethoxycarbonyl-5-hydroxy-3-pentynoate (*XII*)

A suspension of compound *XI* (40 g) in 0·1M-HCl in aqueous methanol (1 : 10) was stirred for 3 h at 25°C. During this period of time the mixture gradually turned homogeneous. The solution was adjusted to pH 4 with pyridine and the solvents were evaporated. The residue was dissolved in ethyl acetate, washed with water and dried over sodium sulfate. Chromatography on silica gel (5·5 × 15 cm) in ethyl acetate–chloroform (2 : 8), followed by crystallization from ethyl acetate, afforded 20·9 g (35%) of the product *XII*, m.p. 88–90°C. For $C_{12}H_{17}NO_6$ (271·3) calculated: 53·13% C, 6·32% H, 5·16% N; found: 53·14% C, 6·40% H, 5·06% N. ^{1}H NMR spectrum (60 MHz, $CDCl_3$): 1·30 t (6 H, J = 7·3, CH_3CH_2); 2·07 s (3 H, CH_3CO); 4·30 s (2 H, H-5); 4·32 q (4 H, J = 7·3, CH_2CH_3); 7·13 s (1 H, NH). ^{13}C NMR spectrum (15 MHz, $CDCl_3$): 13·9 q (2 C, CH_3CH_2); 22·7 q (CH_3CO); 50·8 t (C-5); 58·9 s (C-2); 63·9 t (2 C, CH_2CH_3); 78·4 s and 83·8 s (C-3 and 4); 164·9 s (2 C, COO) and 169·5 s (CH_3CO).

2-Methyl-4-ethoxycarbonyl-5-(2-hydroxyethyl)-oxazoline (*XVIII*)

Triethylamine (0·1 ml) was added to a solution of compound *XII* (0·1 g) in ethanol (3 ml) and water (1 ml). After standing for 3 h, the volatile compounds were evaporated and the product was crystallized from ethyl ether–pentane, m.p. 58–61°C; yield 66 mg (90%). For $C_9H_{13}NO_4$ (199·2) calculated: 54·26% C, 6·58% H, 7·03% N; found: 54·52% C, 6·72% H, 6·88% N. ^{1}H NMR spectrum (400 MHz, $CDCl_3$): 1·393 t (3 H, J = 7·1, CH_3CH_2); 2·466 s (3 H, CH_3–C^2); 3·265 t (2 H, J = 6·2, CH_2–C^5); 3·995 t (2 H, CH_2OH); 4·376 q (2 H, J = 7·1, OCH_2CH_3). ^{13}C NMR spectrum (100 MHz, $CDCl_3$): 13·73 Q (J = 130·4, CH_3–C^2); 14·28 Qt (J = 127, J = 2·6, CH_3–CH_2); 29·64 Tt (J = 130·4, J = 2·0, CH_2–C^5); 60·58 Tt (J = 145·2, J = 5·2, OCH_2CH_2); 61·09 Tq (J = 148·1, J = 4·5, OCH_2CH_3); 128·44 t (J = 2·3, C^4); 156·97 t (J = 6·0, C^5); 159·97 q (J = 7·9, C^2); 162·62 t (J = 3·2, COO). Mass spectrum was measured on a Varian MAT 311 spectrometer (electron impact, 70 eV, direct inlet 190°C); m/z (rel. intensity, %): 199 (M^+, 1), 181 ($M^+ - H_2O$, 8), 170 (M^+ – CHO, 44), 123 ($M^+ - OC_2H_5 - OCH_2OH$, 100), 43 (C_2H_3O, 95).

Study of Mechanism of Cyclization of *XII* to *XVIII*

A solution of compound *XII* (8 mg) in 10% D_2O/H_2O (0·7 ml) was mixed with concentrated aqueous ammonia (3 mg). ^{1}H NMR spectrum of the intermediate (400 MHz): major: 1·18 t (6 H, J = 7·1, CH_2CH_3); 2·12 s (3 H, CH_3–C^2); 3·93 q (4 H, J = 7·1, CH_2CH_3); 4·28 d (2 H, J = 5·6, CH_2OH); 6·21 t (1 H, J = 5·6, CH=C^5); minor: 1·18 t (6 H, J = 7·1, CH_2CH_3); 2·03 s (3 H, CH_3–C^2); 3·93 q (4 H, J = 7·1, CH_2CH_3); 4·22 d (2 H, J = 7·3, CH_2OH); 6·16 t (1 H, J = 7·3, CH=C^5).

Ethyl 2-Acetamido-5-bromo-2-ethoxycarbonyl-3-pentynoate (*XIII*)

A solution of triphenylphosphine dibromide[23] (1 equiv.) in anhydrous dimethylformamide (80 ml) was added at room temperature to a solution of compound *XII* (12 g, 44·2 mmol) in anhydrous dimethylformamide (100 ml). The solvent was evaporated, the residue dissolved in ethyl acetate and filtered through a column of silica gel (4 × 4 cm). The filtrate was concentrated and chromatographed on silica gel (6 × 20 cm) in 30% ethyl acetate in hexane. Crystallization from ethyl acetate–diethyl ether afforded 13·67 g (93%) of the product, m.p. 73–74°C. For $C_{12}H_{16}NO_5Br$ (334·2) calculated: 43·12% C, 4·83% H, 23·91% Br, 4·21% N; found: 43·20% C, 4·92% H, 23·98% Br, 4·16% N. ^{1}H NMR spectrum (60 MHz, $CDCl_3$): 1·32 t (6 H, J = 7·3, CH_3CH_2); 2·08 s (3 H, CH_3CO); 3·96 s (2 H, H-5); 4·34 q (4 H, J = 7·3, CH_2CH_3); 6·97 s (1 H, NH).

^{13}C NMR spectrum (15 MHz, $CDCl_3$): 13·8 q + 13·8 t (3 C, 2 × $\mathbf{CH_3CH_2}$ + C-5); 22·7 q ($\mathbf{CH_3CO}$); 60·3 s (C-2); 63·9 t (2 C, $\mathbf{CH_2CH_3}$); 79·7 s and 80·0 s (C-3 and 4); 164·6 s and 168·9 s (COO and CO).

Ethyl 2-Acetamido-2-ethoxycarbonyl-5-phthalimido-3-pentynoate (*XIV*)

Potassium phthalimide (1·746 g, 1·0 equiv.) was added to a solution of compound *XIII* (3·2 g, 9·55 mmol) in anhydrous dimethylformamide (50 ml). The suspension was stirred vigorously at 45°C for 6 h, cooled to 25°C and acidified with acetic acid (0·8 ml). The solvents were evaporated and the residue was partitioned between water and chloroform and the organic phase was dried over magnesium sulfate. Crystallization from chloroform–diethyl ether and then from methanol afforded 2·55 g (67%) of the product *XIV*, m.p. 156–158°C. For $C_{20}H_{20}N_2O_7$ (400·4) calculated: 60·00% C, 5·03% H, 7·00% N; found: 59·68% C, 5·20% H, 7·04% N. ^{1}H NMR spectrum (60 MHz, $CDCl_3$): 1·27 t (6 H, J = 7·3, $\mathbf{CH_3CH_2}$); 2·05 s (3 H, CH_3CO); 4·29 q (4 H, J = 7·3, $\mathbf{CH_2CH_3}$); 4·52 s (2 H, H-5); 6·92 s (1 H, NH); 7·80 mt (4 H, Pht). ^{13}C NMR spectrum (15 MHz, $CDCl_3$): 13·8 q (2 C, $\mathbf{CH_3CH_2}$); 22·7 q ($\mathbf{CH_3CO}$); 27·6 t (C-5); 60·2 s (C-2); 63·7 t (2 C, $\mathbf{CH_2CH_3}$); 98·1 s (C-4); 123·5 d (2 C, Pht); 132·1 s (2 C, Pht); 134·2 d (2 C, Pht); 164·8 s and 166·98 s and 168·2 s and 168·8 s (COO and CO).

Ethyl (*Z*)-2-Acetamido-2-ethoxycarbonyl-5-phthalimido-3-pentenoate (*Va*)

Lindlar catalyst (1·036 g, Fluka) was added to a suspension of compound *XIV* (2·033 g, 5·08 mmol) in methanol (70 ml). The mixture was hydrogenated at atmospheric pressure for 1·5 h. During the hydrogenation the substrate dissolved. After filtration and evaporation of the solvent, the residue was crystallized from chloroform–diethyl ether to give 1·900 g (93%) of *Va*, m.p. 117–119°C. For $C_{20}H_{22}N_2O_7.1/3\ H_2O$ (408·4) calculated: 58·79% C, 5·60% H, 6·86% N; found: 58·73% C, 5·67% H, 6·76% N. ^{1}H NMR spectrum (60 MHz, $CDCl_3$): 1·29 t (6 H, J = 7·3, $\mathbf{CH_3CH_2}$); 2·14 s (3 H, CH_3CO); 4·32 q, (4 H, J = 7·3, $\mathbf{CH_2CH_3}$); 4·38 dd (2 H, J = 6·7, J = 1·8, H-5); 5·62 dt (1 H, J = 11·0, J = 6·7, H-4); 6·60 dt (1 H, J = 11·0, J = 1·8, H-3); 7·43 s (1 H, NH); 7·77 mt (4 H, AA'BB', Pht). ^{13}C NMR spectrum (15 MHz, $CDCl_3$): 13·9 q (2 C, $\mathbf{CH_3CH_2}$); 22·6 q ($\mathbf{CH_3CO}$); 35·2 t (C-5); 63·1 t (2 C, $\mathbf{CH_2CH_3}$); 64·7 s (C-2); 123·3 d (2 C, Pht); 128·1 d (2 C, C-3 and 4); 132·0 s (2 C, Pht); 134·1 d (2 C, Pht); 167·1 s (2 C) and 167·8 s and 169·1 s (2 C) (COO and CO).

(*Z*)-3,4-Didehydro-D,L-ornithine (*VIa*)

The *Z*-isomer *VIa* was prepared from the phthalimido derivative *Va* as described for the preparation of the *E*-isomer *VIb* from phthalimido derivative *Vb*; yield 44%, m.p. 192–193°C (decomp.). For $C_5H_{10}N_2O_2.HCl.1/4\ H_2O$ (171·1) calculated: 35·10% C, 6·76% H, 20·73% Cl, 16·37% N; found: 35·41% C, 6·66% H, 20·93% Cl, 16·40% N. ^{1}H NMR spectrum (400 MHz, D_2O): 3·75 ddd (1 H, J(5a, 5b) = 14·2, J(5a, 4) = 7·7, J(5a, 3) = 1·4, H-5a); 3·85 ddd (1 H, J(5b, 5a) = 14·2, J(5b, 4) = 7·1, J(5b, 3) = 1·1, H-5b); 4·64 dd (1 H, J(2, 3) = 9·8, J(2, 4) = 1·1, H-2); 5·86 mt (1 H, J(3, 2) = 9·8, J(3, 4) = 10·8, J(3, 5a) = 1·4, J(3, 5b) = 1·1, H-3); 5·99 mt (1 H, J(4, 2) = = 1·1, J(4, 3) = 10·8, J(4, 5a) = 7·7, J(4, 5b) = 7·1). ^{13}C NMR spectrum (100 MHz, D_2O): 37·00 t (C-5); 52·98 d (C-2); 128·46 d and 129·92 d (C-3 and 4); 172·60 s (C-1).

(*Z*)-5-Amino-2-acetamido-3-pentenoic Acid (*VIIIa*)

The *Z*-isomer *Va* was hydrolyzed to the amino acid *VIIIa* in the same manner as described for the *E*-isomer Vb; m.p. 175–180°C, yield 44%. For $C_7H_{12}N_2O_3.1/4\ H_2O$ (176·7) calculated:

47·58% C, 7·13% H, 15·86% N; found: 47·84% C, 7·17% H, 15·83% N. ^{1}H NMR spectrum (400 MHz, D_2O): 2·03 s (3 H, CH_3CO); 3·80 mt (2 H, H-5); 4·99 d (1 H, J = 8·3, H-2); 5·80 mt (2 H, H-3 and 4). ^{13}C NMR spectrum (100 MHz, D_2O): 22·36 q CH_3CO); 36·62 t (C-5); 53·65 d (C-2); 124·74 d and 134·05 d (C-3 and 4); 173·77 s and 176·30 s (CO and COO). IR spectrum (Nujol, UR-10 Carl Zeiss, Jena, G.D.R.): ($RCOO^-$) 1 585 cm^{-1}, (NH^+) 2 630 cm^{-1} and 2 790 cm^{-1}, (NH) 3 255 cm^{-1}.

Diethyl 2,6-Bis(acetamido)-2,6-diethoxycarbonyl-3-heptynedioate (*XV*)

The title compound was prepared in 48% yield from the bromo derivative *XIII* in the same manner as the alkene *IXb* from the bromo derivative *IVb*; m.p. 89—92°C. For $C_{21}H_{30}N_2O_{10}$ (470·5) calculated: 53·61% C, 6·43% H, 5·95% N; found: 53·54% C, 6·43% H, 5·87% N. ^{1}H NMR spectrum (60 MHz, $CDCl_3$): 1·26 t (6 H, J = 7·3, 2 × CH_3CH_2); 1·30 t (6 H, J = 7·3, 2 × CH_3CH_2); 2·07 s (6 H, CH_3CO); 3·26 s (2 H, H-5); 4·25 q (4 H, J = 7·3, 2 × CH_2CH_3); 4·30 q (4 H, J = 7·3, 2 × CH_2CH_3); 6·89 br s (1 H, NH); 7·10 br s (1 H, NH). ^{13}C NMR spectrum (15 MHz, $CDCl_3$): 13·9 q (4 C, CH_3CH_2); 22·8 q (2 C, CH_3CO); 24·5 t (C-5); 60·1 s (C-2); 63·7 t (2 C, CH_2CH_3); 65·4 s (C-6); 80·1 s and 89·0 s (C-3 and 4); 164·9 s (2 C) and 166·5 s (2 C) and 169·7 s (2 C) corresponds to groups CO and COO.

Diethyl (*Z*)-2,6-Bis(acetamido)-2,6-diethoxycarbonyl-3-heptenedioate (*IXa*)

This compound was prepared by hydrogenation (at atmospheric pressure) of alkyne *XV* in methanol (12 ml) over Lindlar catalyst (363 mg, Fluka). Under vigorous stirring, the reaction was over in 10 min. The catalyst was filtered off, the solvent evaporated and the product crystallized from diethyl ether; yield 556 mg (92%) of product *IXa*, m.p. 94—97°C. For $C_{21}H_{32}N_2O_{10}$ (472·5) calculated: 53·36% C, 6·83% H, 5·93% N; found: 53·14% C, 7·11% H, 5·95% N. ^{1}H NMR spectrum (400 MHz, $CDCl_3$): 1·245 t (12 H, J = 7·12, CH_3CH_2); 1·996 s (3 H, CH_3CO); 2·068 s (3 H, CH_3CO); 3·155 dd (2 H, J = 7·0 J = 2·2, H-5); 4·244 mt (8 H, CH_2CH_3); 5·327 dt (1 H, J = 11·7, J = 7·0, H-4); 6·493 dt (1 H, J = 11·7, J = 2·2, H-3); 6·745 s (1 H, NH); 7·157 s (1 H, NH). ^{13}C NMR spectrum (100 MHz, $CDCl_3$); 13·865 q (2 C, CH_3CH_2); 13·913 q (2 C, CH_3CH_2); 22·545 q (CH_3CO); 22·913 q (CH_3CO); 30·861 t (C-5); 62·693 t (2 C, CH_2CH_3); 62·911 t (2 C, CH_2CH_3); 64·971 s and 65·485 s (C-2 and 6); 126·118 d and 128·455 d (C-3 and 4); 166·924 s and 167·513 s and 168·909 and 169·107 s (CO and COO).

(*Z*)-3,4-Didehydro-2,6-diaminopimelic Acid (*Xa*)

Hydrolysis of the *Z*-isomer *IXa* to the amino acid *Xa* (and the isolation) was performed as described for the hydrolysis of the *E*-isomer *IXb* to amino acid *Xb*; yield 84%, m.p. 216°C (decomp.). For $C_7H_{12}N_2O_4.1/2\,H_2O$ (197·2) calculated: 42·61% C, 6·65% H, 14·21% N; found: 42·65% C, 6·92% H, 14·06% N. ^{1}H NMR spectrum (400 MHz, D_2O): 2·13—2·20 mt, 2·65 to 2·90 mt, 3·77 dd (J = 5·2, J = 8·8); 3·96 t (J = 4·9); 4·56 dd (J = 10·1, J = 1·1); 4·57 dd (J = 9·9, J = 0·9); 5·67 tmt (J = 10·1); 5·70 tmt (J = 9·7); 5·79 t (J = 7·9); 5·82 t (J = 8); 5·91 ddd (J = 9·8, J = 6·9, J = 1·1). ^{13}C NMR spectrum (100 MHz, D_2O): major: 29·21 t (C-5); 52·80 d and 54·52 d (C-2 and 6); 127·59 and 130·77 d (C-3 and 4); 173·39 and 174·50 s (COO); minor: 30·49 t (C-5); 52·75 d and 54·78 d (C-2 and 6); 126·6 d and 132·63 d (C-3 and 4); 173·48 s and 174·86 s (COO). The ^{13}C NMR spectrum exhibits two series of similarly distributed signals (in the ratio about 2 : 1) due to both the diastereoisomers. The ^{1}H NMR spectrum was complex and therefore was not studied in detail.

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