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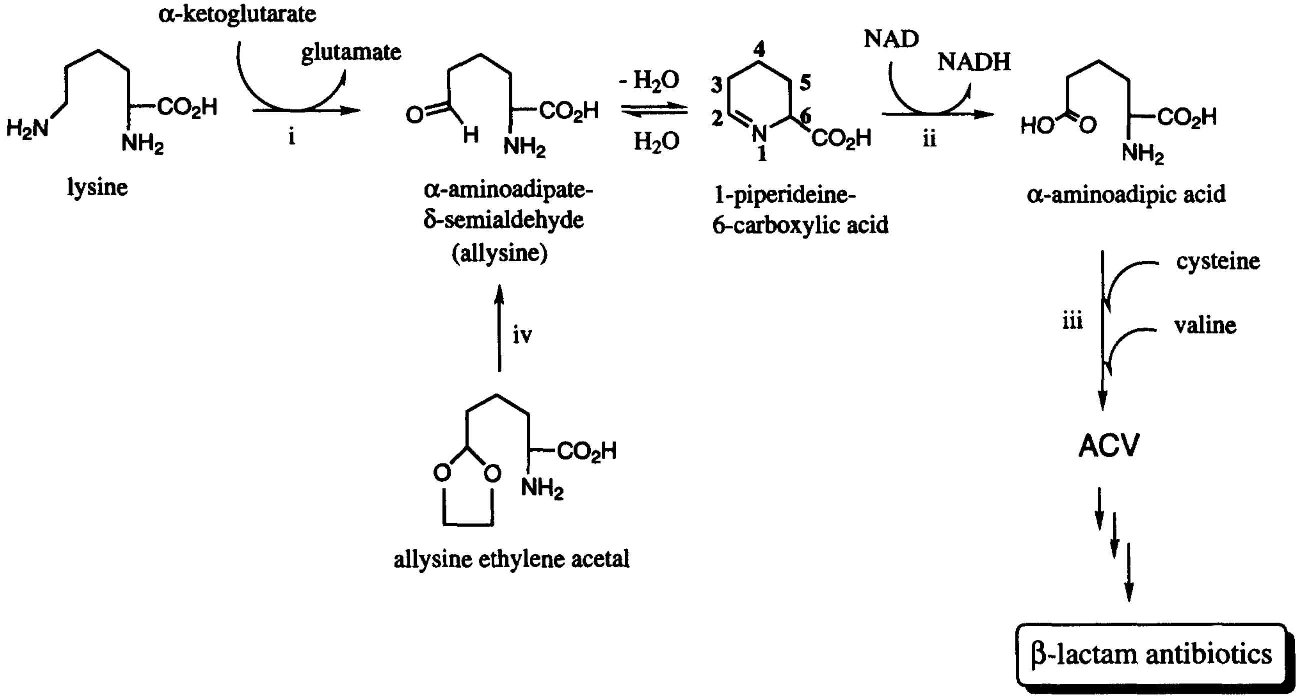
Chemical Synthesis of Allysine Ethylene Acetal and Conversion *in situ* Into 1-Piperideine-6-carboxylic Acid: Key Intermediate of the α-Aminoadipic Acid for β-Lactam Antibiotics Biosynthesis

Angel Rumbero,[a]* Juan Fco. Martín,[b]* M. Angeles Lumbreras,[b] Paloma Liras[b] and Cristina Esmahan[b]
[a]*Departamento de Química Orgánica, Universidad Autónoma de Madrid, Cantoblanco, 28049-Madrid, Spain*
[b]*Departamento de Ecología, Genética y Microbiología, Facultad de Biología, Universidad de León, 24071-León, Spain*

Abstract—Synthesis of α-amino acid allysine ethylene acetal from 3,4-dihydro-2*H*-pyran and its conversion *in situ* into 1-piperideine-6-carboxylic acid with an exceedingly simple work-up are described.

Introduction

β-Lactam antibiotics (penicillins, cephalosporins and cephamycins) are formed by condensation of L-α-aminoadipic acid, L-cysteine, and L-valine to form the tripeptide δ-(L-α-aminoadipyl)-L-cysteinyl-D-valine (ACV), which is further converted into cephalosporin or cephamycins.[1] α-Aminoadipic acid, which is a specific precursor of β-lactam antibiotics, is formed in actinomycetes by deamination of lysine. In this aminotransferase reaction, the ε-amino group of L-lysine is transferred to α-ketoglutarate to yield glutamate and α-aminoadipate-δ-semialdehyde (allysine) which is immediately converted into the intramolecularly dehydrated derivative (1-piperideine-6-carboxylic acid) (Scheme 1).[2] Enzymes which catalyze this reaction were found to be present in *Achromobacter liquidum*, *Flavobacterium flavescens*, *Flavobacterium lutescens*, *Streptomyces lactamdurans*, etc.[3,4]



Scheme 1. Reagents and conditions: (i) enzyme L-lysine-α-ketoglutarate-aminotransferase (LKAT); (ii) enzyme 1-piperideine-6-carboxylate dehydrogenase; (iii) enzyme ACV synthetase; (iv) Amberlyst-15, H_2O, rt, 1 h.

Allysine is also a very important crosslink precursor in the synthesis of proteins and, at the moment, it is synthesized enzymatically *in vivo* starting from lysine by the enzyme lysyl oxidase in the last step of the biosynthesis of collagen. In this communication, we report the first chemical synthesis of allysine ethylene acetal (**9**) (Scheme 2) and its conversion *in situ* into 1-piperideine-6-carboxylic acid.

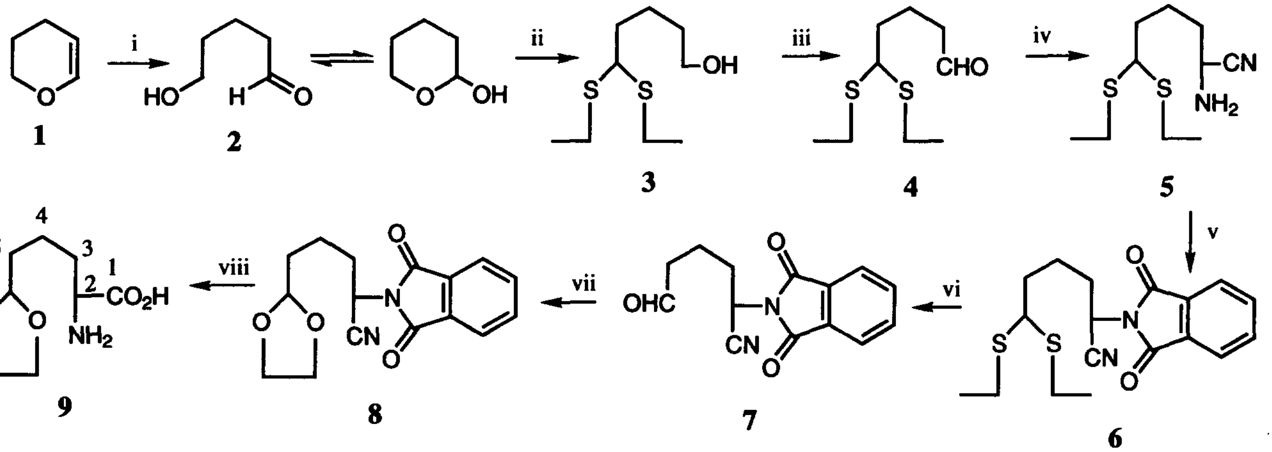
Results and Discussion

The first step of synthesis of compound **9** was the hydrolysis of 3,4-dihydro-2*H*-pyran (DHP) (**1**) with HNO_3 (0.2 N) at 80°C for 1 h, then trapping the aldehyde moiety with ethanethiol and HCl (8 M) from –70 °C to 25 °C for 3 h, to give compound **3** in 96% yield from **1**. The dithioethylacetal group is stable under conditions of deprotection of acid-labile groups and saponifications are possible without deblocking the aldehyde moiety. The oxidation of **3** was achieved with pyridinium chlorochromate (PCC) in CH_2Cl_2 at rt for 2 h to give aldehyde derivative **4** in 56% yield. Treatment of **4** with NaCN, NH_4Cl and NH_4OH at rt for 5 h gave compound **5** in 93% yield. Protection of the primary amine of compound **5** by using *N*-(ethoxycarbonyl)phthalimide (NECP) in triethylamine (TEA) and THF[5] gave the *N*-phthalimide **6** in 82% yield. Deprotection of the dithioacetal **6** with mercury (II) acetate in CH_3CN and water at rt for 30 min gave aldehyde **7** in 69% yield.

Conversion of the aldehyde into the acetal derivative **8**, was followed by simultaneous deprotection of the amino group and hydrolysis of the nitrile ($Ba(OH)_2 \cdot H_2O$, 24 h, reflux) to afford, after workup, allysine ethylene acetal in 50% yield. Its structure has been established from its spectral data and the assignments were accomplished by a combination of homo-COSY, DEPT experiments and 2D NMR heteronuclear spectroscopy. The purity of compound **9** was tested by HPLC.

HPLC: the derivatization of the amino acids with OPA (*o*-phthaldialdehyde) is frequently used for the determination of amino acids[6] in biological fluids and protein hydrolysates. The non-fluorescent reagent reacts in the presence of a reducing agent, such as mercaptoethanol, with a primary amino group forming derivatives with stronger fluorescence. For the derivatization we used the procedure described by Kabus *et al.*[7] For the separation of amino acid derivatives we used a continuous binary gradient which was run according to the Table 1 (see below). The flow rate was 1.0 mL min^{-1} and column temperature was maintained at a constant 32 °C. With this method the retention time of amino acid **9** is relatively constant (6.56–6.76 min).

Compound **9** is very stable and its hydrolysis to the corresponding carbonyl derivative has been successfully achieved by applying the macroreticular sulfonic acid based polystyrene cation exchange resin (Amberlyst-15).[8] Yield is excellent and work-up is exceedingly



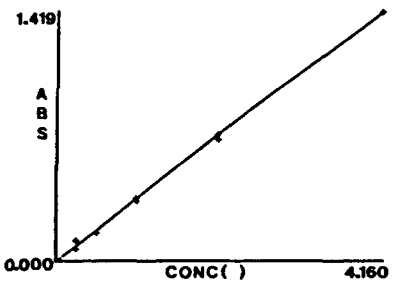
Scheme 2. Reagents and conditions: (i) HNO_3/H_2O, 80 °C; (ii) EtSH, –70 °C, 8 M HCl; (iii) PCC, CH_2Cl_2; (iv) NaCN, NH_4Cl, NH_4OH, rt, 5 h; (v) *N*-(ethoxycarbonyl)phthalimide, triethylamine, THF; (vi) $Hg(AcO)_2$, $CH_3CN–H_2O$; (vii) ethylene glycol, *p*-toluenesulfonic acid, C_6H_6, heat; (viii) $Ba(OH)_2 \cdot 8H_2O$, dioxane–H_2O, 24 h, reflux, $(NH_4)_2CO_3$, Lewatit MP600.

Table 1.

Time (min)	Methanol (%)	Na_2HPO_4, 50 mM, pH = 7.0, (%)
0	50	50
5	50	50
15	80	20
20	80	20
30	50	50

simple, only involving filtration of the resin state. Thus 1-piperideine-6-carboxylic acid can be prepared *in situ* very easily, although it has not been isolated because of its unstability. These types of compounds are characterized as derivatives, the corresponding five-member analog 1-pyrroline-5-carboxylic acid (intermediate in the biosynthesis of proline) is commercially available and characterized as a 2,4-dinitrophenylhydrazine hydrochloride double salt. However, the hydrolysis of **9** into 1-piperideine-6-carboxylic acid can be conveniently performed in a NMR tube. The reaction can be monitored by the disappearance of the signals of the acetal proton at δ 3.80 (*m*, 4H, H-7) and the appearance of a signal at δ 3.40 (*s*, 4H) assigned to ethylene glycol. The other signals were assigned to 1-piperideine-6-carboxylic acid: δ 1.41 (*m*, 2H, H-4), 1.70 (*m*, 2H, H-5), 1.97 (*m*, 2H, H-3), 3.82 (*dd*, 1H, *J* = 12.9 and 3.2 Hz, H-6) and 8.58 (*br s*, H-2, partly interchanged with D_2O).

Further evidence of the results obtained from NMR was provided by derivatizing with *o*-aminobenzaldehyde (*o*-AB). This compound and the analog 1-pyrroline-5-carboxylic acid were monitored by Soda *et al.*[9] and Yasuda *et al.*[10] by derivatizing with *o*-AB into an orange dihydroquinazolinium complex.[11] The *o*-AB adduct of both intermediates was distinguished spectrophotometrically, although such derivatives have not been isolated because of their unstability. We have monitored the formation of 1-piperideine-6-carboxylic acid by utilizing the assay of Kern *et al.*[12] for this compound. After the deblocking of the acetal **9**, the reaction mixture (200 μL) was combined with 10 μL of *o*-AB (0.4 M) up to a final volume of 1.2 mL. The orange color developed during the heating at 37 °C for 1 h was quantified by measuring the absorbance at 465 nm getting an extinction coefficient as well as that obtained by Fothergill and Guest[2] (Fig. 1).



STD	(mM)	ABS
1	0.000	0.000
2	0.260	0.075
3	0.260	0.121
4	0.520	0.169
5	0.520	0.163
6	1.040	0.351
7	1.040	0.341
8	2.080	0.681
9	2.080	0.712
10	4.160	1.419
11	4.160	1.417

K= 2.935 AO= -0.003

Figure 1.

Studies on biological activities corresponding to the L-lysine-6-aminotransferase (LAT) and 1-piperideine-6-carboxylate dehydrogenase enzymes in several microorganisms with the assistance of this compound are now in progress.

Experimental

5-Hydroxypentanal-1,1-dithioethyl acetal (3). Compound **2** [22.0 g (0.26 mol)], prepared following a reported procedure,[13] was added into 35.1 mL (0.475 mol) of ethanethiol. At -70 °C, 64.7 mL HCl (8 M) was added and the mixture stirred for 3 h reaching room temperature. The organic phase was separated and washed with 0.5 M NaOH. The resulting compound **3** was isolated as a colorless oil (43.0 g, 96%). ^{1}H NMR ($CDCl_3$): δ 1.26 (*t*, *J* = 7.3 Hz, 6H), 1.60 (*m*, 4H), 1.85 (*m*, 2H), 2.65 (*m*, 4H), 3.67 (*t*, *J* = 6.0 Hz, 2H), 3.80 (*t*, *J* = 6.8 Hz, 1H). ^{13}C NMR ($CDCl_3$): δ 14.5 (CH_3), 23.7 (CH_2), 24.1 (CH_2), 32.1 (CH_2), 35.8 (CH_2), 51.2 (CH), 62.4 (CH_2).

1,5-Pentanedial-1,1-dithioethyl acetal (4). Pyridinium chlorochromate (64.5 g, 0.3 mol) was suspended in 200 mL of dry CH_2Cl_2. At 0 °C, the alcohol **3** (43.0 g, 0.206 mol) in 25 mL of dry CH_2Cl_2 was then added in one portion to the magnetically stirred suspension. After 2 h, dry Et_2O (250 mL) was added and the supernatant liquid decanted from a black gum. The insoluble residue was washed with dry Et_2O (3 x 50 mL). The combined organic solution was filtered on a silica gel column and afterwards it was passed through a short pad of Florisil and the solvent was removed by distillation to give compound **4** as a colorless oil (23.2 g, 56%). ^{1}H NMR ($CDCl_3$): δ 1.28 (*t*, *J* = 6.8 Hz, 6H), 1.6–1.9 (*m*, 4H), 2.50 (*t*, *J* = 6.8 Hz, 2H), 2.64 (*m*, 4H), 3.80 (*t*, *J* = 6.1 Hz, 1H), 9.80 (*s*, 1H). ^{13}C NMR ($CDCl_3$): δ 14.4 (CH_3), 19.9 (CH_2), 22.8 (CH_2), 35.5 (CH_2), 43.1 (CH_2), 50.8 (CH), 201.8 (CO).

5-Amino-5-cyanopentanal-1,1-dithioethyl acetal (5). A solution of compound **4** in MeOH (30 mL) was dropwise added to a stirred mixture of NH_4Cl (8.80 g), NaCN (6.85 g), and conc aq. NH_3 (100 mL) at 15–20 °C. After being stirred for 5 h, the mixture was concentrated under reduced pressure at about 50 mL. The upper oily layer was separated, and the residual aqueous layer extracted with CH_2Cl_2. The combined oil and extracts were dried and the solvent was removed, under reduced pressure, the aminonitrile remained as an orange oil (29.5 g, 93%). ^{1}H NMR ($CDCl_3$): δ 1.25 (*t*, *J* = 7.9 Hz, 6H), 1.7–1.9 (*m*, 6H), 2.64 (*m*, 4H), 3.70 (*t*, *J* = 6.1, 1H), 3.80 (*t*, *J* = 6.4, 1H). ^{13}C NMR ($CDCl_3$): δ 14.3 (CH_3), 23.3 (CH_2), 24.0 (CH_2), 34.6 (CH_2), 35.2 (CH_2), 43.0 (CH), 50.7 (CH), 121.9 (CN).

*5-Cyano-5-*N*-phthaloylpentanal-1,1-dithioethyl acetal (6).* A solution of compound **5** (14.37 g, 61.9 mmol), *N*-carboethoxy phthalimide (13.58 g, 62 mmol), triethylamine (12.13 mL, 87.3 mmol) and THF dry (125 mL) was heated under reflux with stirring for 24 h. The reaction mixture was filtered at room temperature, and the filtrate concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 and washed with 10% HCl, H_2O, 5% $NaHCO_3$ and H_2O. CH_2Cl_2 was evaporated and the residue was chromatographied on silica gel using CH_2Cl_2 as eluent. Yield 18.36 g (82%). ^{1}H NMR ($CDCl_3$): δ 1.22 (*t*, *J* = 6.3 Hz, 6H), 1.72 (*m*, 2H), 1.83 (*m*, 2H), 2.20 (*m*, 2H), 2.62 (*m*, 4H), 3.75 (*t*, *J* = 6.5 Hz, 1H), 5.13 (*t*, *J* = 7.5 Hz, 1H), 7.81 (*dd*, *J* = 6.5 and 3.2 Hz, 2H), 7.92 (*dd*, *J* = 6.5 and 3.2 Hz, 2H). ^{13}C NMR ($CDCl_3$): δ 14.4 (CH_3), 23.5 (CH_2), 24.0 (CH_2), 30.8 (CH_2), 34.7 (CH_2), 39.4 (CH), 50.5 (CH), 115.8 (CN), 123.9 (CH), 131.1 (C), 134.7 (CH), 166.0 (CO).

*5-Cyano-5-*N*-phthaloyl-1-pentanal (7).* Dithioethyl acetal derivative (**6**) (17.17 g, 47.4 mmol) was dissolved in CH_3CN (480 mL). A solution of mercury(II) acetate (45.36 g, 142.2 mmol) in H_2O (137 mL) was added and the mixture stirred for 30 min. The reaction mixture was centrifugated and the supernatant evaporated under reduced pressure. The crude product was chromatographied on silica gel with hexane:AcOEt (1:1) as eluent. Yield 69%. ^{1}H NMR ($CDCl_3$): δ 1.72 (*m*, 2H), 2.18 (*m*, 2H), 2.55 (*t*, J = 7.5 Hz, 2H), 5.12 (*t*, J = 7.5 Hz, 1H), 7.80 (*dd*, J = 6.5 and 3.2 Hz, 2H), 7.89 (*dd*, J = 6.5 and 3.2 Hz, 2H), 9.72 (*s*, 1H). ^{13}C NMR ($CDCl_3$): δ 18.0 (CH_2), 30.6 (CH_2), 39.3 (CH), 42.2 (CH_2), 115.6 (CN), 123.9 (CH), 131.0 (C), 134.8 (CH), 166.0 (CO), 200.6 (CHO).

*2-(5-Cyano-5-*N*-phthaloyl)-1,3-dioxolane (8).* The aldehyde **7** (7.02 g, 27.4 mmol) was dissolved in benzene (100 mL), and ethylene glycol (1.59 mL, 28 mmol) and *p*-TsOH (50 mg, 0.29 mmol) were added. The mixture was refluxed for 2 h by using a Dean–Stark water separator. The cooled reaction mixture was washed with saturated $NaHCO_3$ solution and water. The organic phase was dried ($MgSO_4$) and evaporated under reduced pressure to give the corresponding acetal **8** (99%). ^{1}H NMR ($CDCl_3$): δ 1.50 (*m*, 2H), 1.62 (*m*, 2H), 2.18 (*m*, 2H), 3.80 (*m*, 4H), 4.75 (*t*, J = 4.8 Hz, 1H), 5.08 (*t*, J = 7.5 Hz, 1H), 7.72 (*dd*, J = 6.5 and 3.2 Hz, 2H), 7.83 (*dd*, J = 6.5 and 3.2 Hz, 2H). ^{13}C NMR ($CDCl_3$): δ 20.0 (CH_2), 31.2 (CH_2), 32.3 (CH_2), 39.5 (CH), 64.8 (CH_2), 103.6 (CH), 115.8 (CN), 123.9 (CH), 131.1 (C), 134.7 (CH), 166.0 (CO).

Allysine ethylene acetal (9). A solution of **8** (6.75 g, 22.5 mmol) in dioxane (50 mL) and barium hydroxide octahydrate (12.40 g, 39.4 mmol) in H_2O (100 mL) was heated under reflux for 24 h. The cooled reaction mixture was saturated with powdered $(NH_4)_2CO_3$ and the white powder ($BaCO_3$) filtered. The solvents were removed at reduced pressure and the residue was treated with H_2O and anion exchange resin Lewatit MP600 for separating the phthalic acid. After evaporation of H_2O at reduced pressure, the residue was treated with dry MeOH and a white powder corresponding to compound **9** was filtered. Yield 50%. Mp 216–217 °C; FAB$^+$ (MH$^+$: 190.1) (matrix *m*-NBA); ^{1}H NMR (D_2O): δ 1.31 (*m*, 2H, H-4), 1.55 (*td*, $J_{5,4}$ = 7.75 Hz, $J_{5,6}$ = 4.70 Hz, 2H, H-5), 1.72 (*m*, 2H, H-3), 3.59 (*t*, $J_{2,3}$ = 6.4 Hz, 1H, H-2), 3.80 (*m*, 4H, H-7), 4.79 (*t*, $J_{6,5}$ = 4.70 Hz, 1H, H-6). ^{13}C NMR (D_2O): δ 16.4 (C-4), 27.6 (C-3), 29.5 (C-5), 52.0 (C-2), 61.9 (C-7), 101.1 (C-6), 172.1 (CO).

*Hydrolysis of acetal **9** and assay method of 1-piperideine-6-carboxylic acid.* Amberlyst-15 (40 mg) was added to a solution of the acetal **9** (18.90 mg) in H_2O (1 mL) for 10 min. The resin was filtered and the filtrate was used to perform the assay of the formation of 1-piperideine-6-carboxylic acid.

The deblocking reaction mixture (200 μL) in the range of concentrations specified in the Figure 1, was mixed with 10 μL of *o*-AB (0.4 M) up to a final volume of 1.2 mL. The orange color developed during heating for 1 h at 37 °C, was quantitated by measuring the absorbance at 465 nm. The results are shown in Figure 1. The amount of 1-piperideine-6-carboxylic acid formed was calculated by using the extinction coefficient reported by Fothergill and Guest.[2]

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