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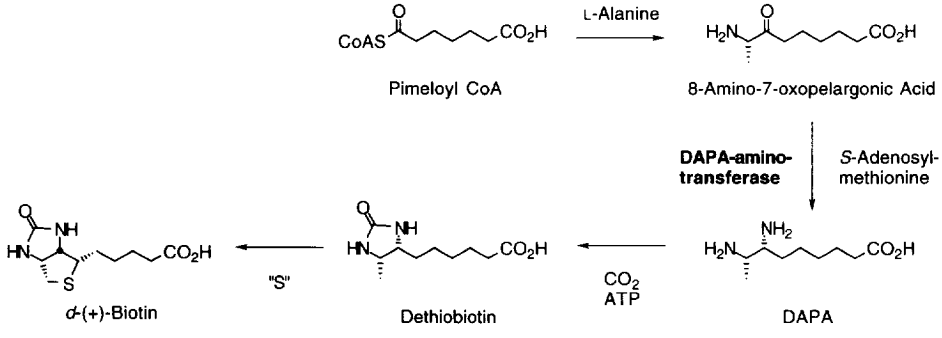
First Synthesis of Both Enantiomers of the Biotin Vitamer 8-Amino-7-oxopelargonic Acid

Denis Lucet[a], Thierry Le Gall[a], Charles Mioskowski[a]*, Olivier Ploux[b] and Andrée Marquet[b]

[a]CEA-Saclay, Service des Molécules Marquées, Département de Biologie Cellulaire et Moléculaire, F-91191 Gif-sur-Yvette, France
[b]Laboratoire de Chimie Organique Biologique, URA CNRS 493, Université Pierre et Marie Curie, Paris, France

Abstract : A short and efficient synthesis of both 8-amino-7-oxopelargonic acid enantiomers from D or L-alanine is presented. The key step of this first chemical synthesis is the non-racemizing Horner-Wadsworth-Emmons reaction of a β-ketophosphonate **3** and benzyl 4-formylbutanoate. The growth-promoting effect of the enantiomers was tested on *Saccharomyces cerevisiae*. Copyright © 1996 Elsevier Science Ltd

The vitamin biotin, which is an essential cofactor for carboxylase-catalyzed reactions, is synthesized by a multistep pathway in microorganisms[1] and plants[2] (**scheme 1**). Although biotin biosynthesis has been studied over a considerable period, the chemical synthesis of 8-amino-7-oxopelargonic acid enantiomers has not yet been described[3,4].

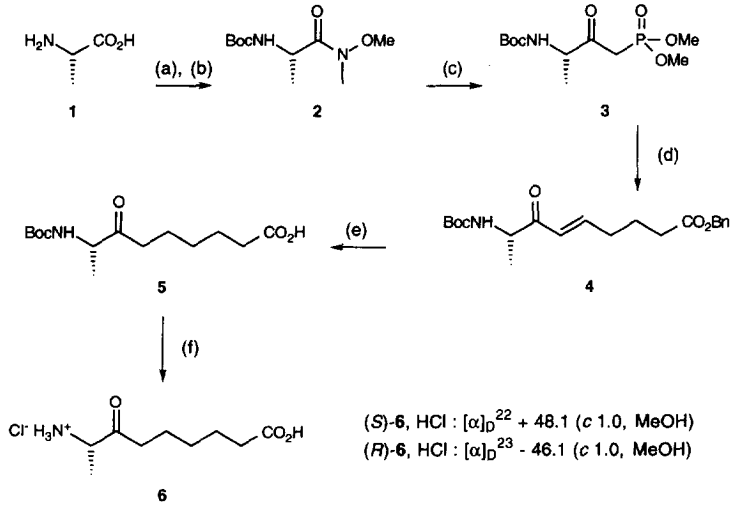


Scheme 1

In the course of studies on this pathway, and especially of the enzyme DAPA-aminotransferase[5], both enantiomers of 8-amino-7-oxopelargonic acid were needed.

Chemistry : We report herein the first chemical synthesis of both 8-amino-7-oxopelargonic acid enantiomers from L or D-alanine as the starting chiral template. The synthesis of (*S*)-8-amino-7-oxopelargonic acid is described in **scheme 2**. The known β–ketophosphonate **3**[6,7] was prepared using a different route, involving the addition of the lithium salt of dimethyl methylphosphonate on the Weinreb amide **2** derived from L-alanine. We noticed that **3** partially racemized during silica gel chromatography and that it has to be used as

crude material (NMR yield = 83% with dimethyl methylphosphonate as contaminating material) since it then displayed an enantiomeric excess greater than 96%[8]. The Horner-Wadsworth-Emmons (HWE) reaction of **3** with benzyl 4-formylbutanoate[9] was then studied. Conditions milder than the usual methods have been used to perform the HWE reaction of substrates which racemize easily or are base-sensitive[10,11,12]. Nevertheless, in our case the conventional method gave a satisfactory result : β-ketophosphonate **3** was first regioselectively deprotonated by 1 eq of NaH (THF, -15°C) and the aldehyde was then added. In this way, enantiomerically pure[8] enone **4**[13] was cleanly obtained. Interestingly, enone **4** did not racemize during silica gel chromatography, unlike L-serine-derived enones reported by Koskinen[11]. (*S*)-8-amino-7-oxopelargonic acid hydrochloride was obtained by a two-step procedure involving a quantitative one-pot hydrogenation-hydrogenolysis leading to **5**[14] and the cleavage of the Boc group using a AcOEt solution of hydrogen chloride. Recrystallization from a EtOH/Et_2O system afforded (*S*)-8-amino-7-oxopelargonic acid[15] as its HCl salt. It is noteworthy that the amine deprotection had to be conducted in strong acidic medium in order to avoid the self-condensation of the α-aminoketone leading to a pyrazine[16].



(*S*)-**6**, HCl : $[\alpha]_D^{22}$ + 48.1 (*c* 1.0, MeOH)
(*R*)-**6**, HCl : $[\alpha]_D^{23}$ - 46.1 (*c* 1.0, MeOH)

(a) NaOH / H_2O / *t*-BuOH ; Boc_2O ; rt ; 12 h ; 75% (b) *N*-Methylpiperidine ; ClC(O)OMe / CH_2Cl_2 ; -25°C ; 15 min ; HN(OMe)Me / CH_2Cl_2 ; -25°C->rt ; 3 h ; 93% (c) $LiCH_2P(O)(OMe)_2$ 2.0 eq / THF ; -78°C ; 10 min ; 83% ; 96% ee (d) 1) NaH 1.0 eq / THF ; -15°C ; 5 min 2) $CHO(CH_2)_3CO_2Bn$ / THF ; -10°C ; 2.5 h ; 86% ; 96% ee (e) H_2 50 bar / AcOEt ; Pd-C 10% ; 48 h ; 100% (f) HCl_{gas} / AcOEt ; 30 min ; EtOH/Et_2O recryst. ; 88 %

Scheme 2

The same procedure was used to synthesize the (*R*) isomer starting from D-alanine. The overall yield of the synthesis is 58% from the commercially available L or D-Boc-alanine. Both compounds were prepared on a 500-mg scale. The non-racemizing character of the last two steps leading to 8-amino-7-oxopelargonic acid hydrochloride and the opposite specific rotations of these compounds allow us to think that we thus obtained both 8-amino-7-oxopelargonic acid enantiomers enantiomerically pure although direct e.e. determination was not successful[17].

Biological studies : The growth-promoting effect of (*S*)-**6**, (*R*)-**6** and *rac*-**6**, on *Saccharomyces cerevisiae*, was tested using the diffusion agar plate[18, 19]. Plots of growth diameters versus concentrations on a

semilogarithmic scale were linear. The order of potencies is (*S*)-**6**>*rac*-**6**>(*R*)-**6** as shown in **Figure**. Assuming a potency of 1.00 for *rac*-**6** the potency of (*S*)-**6** is 1.55 and that of (*R*)-**6** is 0.77. Based on these data it is clear that *the biologically relevant enantiomer is* (*S*)-**6**, a result consistent with the known absolute configuration of (+)-biotin, and the reaction mechanism used by 8-amino-7-oxopelargonate synthase[20], the enzyme which forms 8-amino-7-oxopelargonic acid. It is not clear at this point why (*R*)-**6** can promote the growth of *S. cerevisiae*. A possibility is that compound **6** racemizes during incubation. Indeed, the fact that different values for the growth promoting activity of *rac*-**6** have been reported in the literature[4, 21, 22] (compared to (+)-biotine), shows that this bioassay can not give a good estimate of the enantiomeric purity of **6**. Therefore another assay, such as the *in vitro* transformation of **6** catalyzed by DAPA-aminotransferase, is needed for the complete assessment of the bioactivities of (*R*)- and (*S*)-**6**.

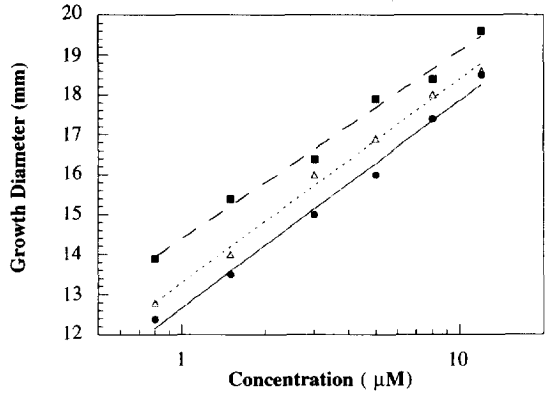


Figure. Growth response of *Saccharomyces cerevisiae* to (*S*)-6, (*R*)-6 and *rac*-6
Aliquots of known concentration of the different compounds were loaded on paper disks over the agar plate and the diameter of the growth circles were manually determined after 15 h incubation[18, 19]. Data were fitted to simple logarithmic function. Closed square (*S*)-**6** ; open triangle *rac*-**6** ; closed circle (*R*)-**6**.

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3. For a synthesis of 8-amino-7-oxopelargonic acid which gives no evidence of optically active product, see Suyama, T.; Kaneo, S. *Japanese Patent* **1963**, 19716 ; *Chem. Abstr.* **1964**, *60*, 4013g. Moreover, the last step of this synthesis involves the cleavage of a phthalimido protecting group using concentrated HCl at reflux and one of us (O.P.) showed[20] that *rac*-8-amino-7-oxopelargonic acid underwent a 3-deuterium exchange at the α-carbonyl position using 6M DCl at reflux, indicating that racemisation occured under these conditions.
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5. This enzyme catalyzes the conversion of 8-amino-7-oxopelargonic acid to 7,8-diaminopelargonic acid (DAPA).
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8. E.e. was determined by ^{1}H NMR spectroscopy of the compound in presence of Eu(hfc)$_3$. By this technique, a 2% precision was assumed.
9. Prepared in 52% yield from benzyl 5-bromopentanoate ($AgPF_6$, DMSO, 60h, then Et_3N).
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13. **4** : ^{1}H NMR (300 MHz, $CDCl_3$) δ(ppm) 7.34-7.25 (5H, m, Ph), 6.91 (1H, dt, *J* = 7.0, 15.7 Hz, C(O)CH=C<u>H</u>), 6.14 (1H, d, *J* = 15.7 Hz, C(O)C<u>H</u>=CH), 5.41 (1H, br d, *J* = 7.0 Hz, N<u>H</u>), 5.07 (2H, s, OC<u>H$_2$</u>Ph), 4.48 (1H, qd, *J* = 7.0, 7.0 Hz, NC<u>H</u>(CH$_3$)CO), 2.33 (2H, t, *J* = 7.4 Hz, C<u>H$_2$</u>CO$_2$Bn), 2.22 (2H, td, *J* = 7.0, 14.0 Hz, CH=CHC<u>H$_2$</u>), 1.78 (2H, tt, *J* = 7.0, 7.4 Hz, CH=CHCH$_2$C<u>H$_2$</u>), 1.39 (9H, s, *t*-Bu), 1.26 (3H, d, *J* = 7.0 Hz, NCH(C<u>H$_3$</u>)CO). ^{13}C NMR (75.4 MHz, $CDCl_3$) δ(ppm) 197.8 (COCH=CH), 172.4 (CO_2Bn), 154.9 (CO carbamate), 147.6 (COCH=<u>C</u>H), 135.7 (Ph quatern), 128.3 (Ph *meta*), 128.0 (Ph), 126.8 (C(O)<u>C</u>H=CH), 79.2 (*t*-Bu quatern), 66.0 (CO_2<u>C</u>H$_2$Ph), 53.1 (N<u>C</u>H(CH$_3$)CO), 33.1 (<u>C</u>H$_2$CO$_2$Bn), 31.5 (CH=CH<u>C</u>H$_2$), 28.1 (*t*-Bu), 22.9 (CH=CHCH$_2$<u>C</u>H$_2$), 18.2 (NCH(<u>C</u>H$_3$)CO). MS *m/z* (CI, NH_3) 376 $[M+H]^+$, 393 $[M+NH_4]^+$. m.p. = 38.7-40.0°C. (*R*)-enantiomer : $[\alpha]_D^{24}$ - 14.2 (*c* 2.4, MeOH).
14. **5** : ^{1}H NMR (300 MHz, $CDCl_3$) two rotamers *E*/*Z* 25:75 δ(ppm) 9.06 (1H, br s, CO_2H), 6.23 (1H, br s, NH *E*) 5.40 (1H, d, *J* = 6.9 Hz, NH *Z*) 4.17 (1H, qd, *J* = 6.9, 6.9 Hz, NC<u>H</u>(CH$_3$)CO *Z*), 3.93 (1H, m, NC<u>H</u>(CH$_3$)CO *E*), 2.45-2.36 (2H, m, COC<u>H$_2$</u>), 2.20 (2H, t, *J* = 7.4 Hz, C<u>H$_2$</u>CO$_2$), 1.55-1.43 (4H, m, COCH$_2$C<u>H$_2$</u>CH$_2$C<u>H$_2$</u>CH$_2$CO$_2$), 1.30 (9H, s, *t*-Bu), 1.30-1.18 (2H, m, COCH$_2$CH$_2$C<u>H$_2$</u>CH$_2$CH$_2$CO$_2$), 1.17 (3H, d, *J* = 6.9 Hz, NCH(C<u>H$_3$</u>)CO *Z*), 1.10 (3H, d, *J* = 7.0 Hz, NCH(C<u>H$_3$</u>)CO *E*). ^{13}C NMR (75.4 MHz, $CDCl_3$) two rotamers *E*/*Z* δ(ppm) 209.6 (CO ketone), 178.1 (CO_2H), 156.0 (CO carbamate *E*), 155.1 (CO carbamate *Z*), 80.8 (*t*-Bu quatern *E*), 79.4 (*t*-Bu quatern *Z*), 56.2 (N<u>C</u>H(CH$_3$)CO *E*), 54.7 (N<u>C</u>H(CH$_3$)CO *Z*), 38.4 (CO<u>C</u>H$_2$ *Z*), 37.5 (CO<u>C</u>H$_2$ *E*), 33.5 (<u>C</u>H$_2$CO$_2$), 28.2 (COCH$_2$CH$_2$C<u>H$_2$</u>CH$_2$CH$_2$CO$_2$), 28.0 (*t*-Bu), 24,1 and 22,7 (COCH$_2$<u>C</u>H$_2$CH$_2$<u>C</u>H$_2$CH$_2$CO$_2$), 17,2 (NCH(<u>C</u>H$_3$)CO *Z*), 16,8 (NCH(<u>C</u>H$_3$)CO *E*). MS *m/z* (CI, NH_3) 288 $[M+H]^+$, 305 $[M+NH_4]^+$, 592 $[M+2NH_4]^+$. (*S*)-enantiomer : $[\alpha]_D^{24}$ - 24.9 (*c* 2.9, MeOH). (*R*)-enantiomer : $[\alpha]_D^{24}$ + 22.5 (*c* 2.4, MeOH). Microanalysis C, 58.20, H, 8.70, N, 4.84, O, 28.26% $C_{14}H_{25}NO_5$ requires C, 58.52, H, 8.77, N, 4.87, O, 27.84%.
15. **6** : ^{1}H NMR (300 MHz, CD_4OD) δ(ppm) 4.10 (1H, q, *J* = 7.3 Hz, NC<u>H</u>(CH$_3$)CO), 2.70-2.48 (2H, m, COC<u>H$_2$</u>), 2.25 (2H, t, *J* = 7.3 Hz, C<u>H$_2$</u>CO$_2$), 1.64-1.51 (4H, m, COCH$_2$C<u>H$_2$</u>CH$_2$C<u>H$_2$</u>CH$_2$CO$_2$), 1.47 (3H, d, *J* = 7.3 Hz, NCH(<u>C</u>H$_3$)CO), 1.37-1.29 (2H, m, COCH$_2$CH$_2$C<u>H$_2$</u>CH$_2$CH$_2$CO$_2$). ^{13}C NMR (75.4 MHz, CD_4OD) δ(ppm) 207.3 (CO), 177.5 (CO_2H), 55.9 (N<u>C</u>H(CH$_3$)CO), 39.0 (CO<u>C</u>H$_2$), 34.7 (<u>C</u>H$_2$CO$_2$), 29.5 (COCH$_2$CH$_2$<u>C</u>H$_2$CH$_2$CH$_2$CO$_2$), 25.7 and 23.9 (COCH$_2$<u>C</u>H$_2$CH$_2$<u>C</u>H$_2$CH$_2$CO$_2$), 15.8 (NCH(<u>C</u>H$_3$)CO). MS *m/z* (CI, NH_3) 188 $[M-HCl+H]^+$, 337 $[Pyrazine+H]^+$, 375 $[2(M-HCl)+H]^+$. m.p. = 108.3-108.4 °C. (*S*)-enantiomer : $[\alpha]_D^{23}$ + 48.1 (*c* 1.0, MeOH). (*R*)-enantiomer : $[\alpha]_D^{22}$ - 46.1 (*c* 1.0, MeOH). Microanalysis C, 47.84, H, 7.67, N, 6.17% $C_9H_{18}ClNO_3$ requires C, 48.32, H, 8.11, N, 6.26%.
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17. ^{1}H NMR spectroscopy of **6** in CD_3CN in presence of Eu(hfc)$_3$ can not be used to determine the e.e. since no split signal was observed for a sample of the racemic compound ; HPLC analysis of (*R*)-1-(1-naphthyl)ethyl isocyanate-derived adducts of methyl esters of synthesized (+) or (-)-**6** led to inconsistent results with e.e. varying from 46 to 60%. Some racemisation may have occured during one of the steps required to carry out this analysis.
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