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TETRAHEDRON

A Stereocontrolled Synthesis of a New Class of 3,4,5,6-Tetrahydropyrimidine-based Chiral Amino Acids

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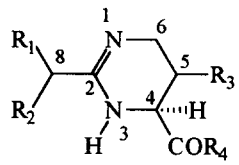
Abstract: *The stereocontrolled synthesis of seven 2-substituted-4-carboxy-3,4,5,6-tetrahydropyrimidines bearing either one chiral center at C-4 or two chiral centers at C-4 and C-8 was performed by condensation of (S)- or (R)-2,4-diaminobutyric acid (Daba) with iminoethers derived from glycine, (S)- and (R serine, (S)- and (R)-tyrosine. Under the conditions reported, epimerization was always completely prevented at the C-4 center, whereas at the C-8 center, it was completely avoided in the case of tyrosine derivatives and considerably diminished for the serine derivatives.*

INTRODUCTION

Among the 800 natural amino acids of (R) or (S) configuration reported in the literature, many are heterocyclic. Some of them such as proline or pipecolic acid include both groups of the amino acid function in the same saturated ring. Others, such as ectoine **1a** and hydroxyectoine **1b**, which have been found to have biological activities, possess a 3,4,5,6-tetrahydropyrimidine moiety, acting as agonists or antagonists for the receptors of peptidic molecules.[1]



1a $R_1 = R_2 = R_3 = H$, $R_4 = OH$
1b $R_1 = R_2 = H$, $R_3 = R_4 = OH$
1c R_1 = NH-Peptide, $R_2 = CH_2OH$, $R_3 = H$, R_4 = NH-Peptide
1d R_1 = NH-Peptide, $R_2 = CH_2CH_2CONH_2$, $R_3 = H$, R_4 = NH-Peptide
1e R_1 = NH-Peptide, $R_2 = CH_2C_6H_4OH$, $R_3 = H$, R_4 = NH-Peptide
1f R_1 = NH-Peptide, $R_2 = CH_2CH_2OH$, $R_3 = H$, R_4 = NH-Peptide
1g R_1 = NH-Peptide, R_2 = CHOHCOOH $R_3 = H$, R_4 = NH-Peptide
1h R_1 = ZNH, $R_2 = R_3 = H$, $R_4 = OH$

Figure 1

More complex 3,4,5,6-tetrahydropyrimidine-based structures have also been reported in all the chromophores of pyoverdins **2**, the chromopeptidic siderophores of the fluorescent *Pseudomonas*.[2] However for some pyoverdins, there is an additional 3,4,5,6 tetrahydropyrimidine ring on the peptide chain. This extra ring results from the condensation of 2,4-diaminobutyric acid with serine (Ser-CTHPMD) **1c**, glutamine (Gln-CTHPMD) **1d**, tyrosine (Tyr-CTHPMD) **1e**, homoserine (Hse-CTHPMD) **1f** and β-hydroxyaspartic acid **1g**. In these cases, this complex amino acid is located at the beginning (Tyr-CTHPMD), in the middle (Ser-CTHPMD & Gln-CTHPMD) or at the end (Hse-CTHPMD) of the peptidic chain of the siderophores.[2,3] In addition, the configuration of the components of this new amino acid has always been found to be (S) for 2,4-diaminobutyric acid (Daba) and (R) for serine,[4,5] glutamine,[6] tyrosine[7] and homoserine.[3]

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The role played by these additional tetrahydropyrimidine rings located on the peptide chain of the siderophores in the pyoverdin-mediated iron-transport has not yet been established and therefore their synthesis was undertaken in order to study their properties.

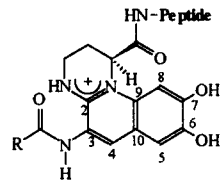

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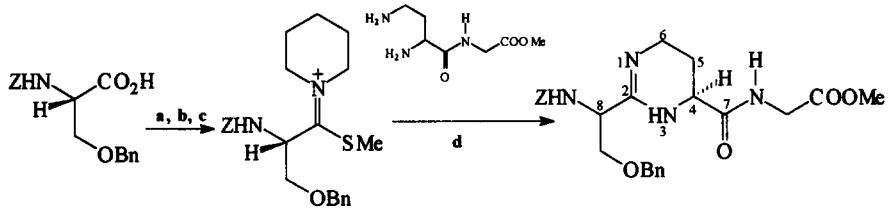
Figure 2: The chromophoric part of pyoverdins

Several synthetic procedures have been reported in the literature, starting from esters,[8-16] nitriles,[17] acids[18,19] or iminoethers.[20,21] The syntheses of 2-substituted-4-carboxy-3,4,5,6-tetrahydropyrimidines bearing a chiral center at C-4 (Figure 1) have been performed by condensation of Daba with various orthoesters or equivalents.[16,21,22,23] Attempts to introduce a second potential chiral center at position C-8 (Scheme 1) have been reported by Jones group who reacted thioiminoethers derived from serine or tyrosine both protected with a benzyloxycarbonyl group, with a derivative of Daba having the carboxyl group protected as its methoxycarbonylmethylamide derivative.[1] However the yields were low (11% & 42% respectively) and the products isolated as 1:1 mixtures of diastereoisomers, due to the total epimerization at the C-8 center.

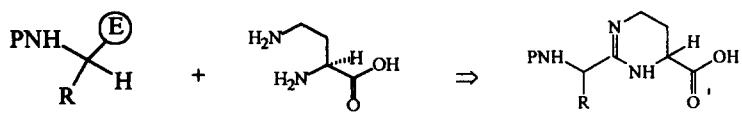


Scheme 1: a) DCC, pentafluorophenol, piperidine; b) Lawesson's reagent, toluene, 80°C; c) MeI, 40°C, d) EtOH, reflux

In view of these unsatisfactory results, we have studied a stereocontrolled synthesis of seven 2-substituted-4-carboxy-3,4,5,6-tetrahydropyrimidines performed by condensation of glycine, (S)- and (R)-serine, (S)-and (R)-tyrosine derivatives with (S)- or (R)-2,4-diaminobutyric acid, and report here the conditions where epimerization is avoided at the C-4 center and discuss those where it can be completely avoided (or otherwise considerably diminished) at the C-8 center.

RESULTS AND DISCUSSION

Our approach is based on the condensation of an amino acid derivative **3** bearing a reactive electrophilic group E such as an iminoether, in place of the initial α-carboxylic group, with the nucleophilic (S)- or (R)-2,4-diaminobutyric acid **4a** (or **4b**) (Scheme 2). The cyclization requires the protection of all the functional groups which are not involved in the reaction, except the carboxyl group of the Daba.

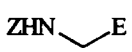


3 **4a (S) or 4b (R)** **1h, 1x,1y**

Scheme 2

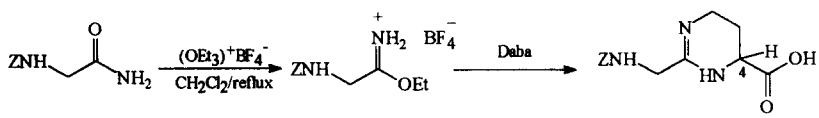
I) Synthesis of a tetrahydropyrimidine derived from glycine with one chiral center at C-4: (4S)- 2-(aminomethyl)-4-carboxy-3,4,5,6-tetrahydropyrimidine 1u

In order to protect the amine function of glycine, N-benzyloxycarbonylglycine **5** (Z-glycine) was reacted with N-hydroxysuccinimide (NHS) / dicyclohexylcarbodiimide (DCC) according to Anderson *et al.*[24] and the activated ester **6** treated with a solution of ammonia gas in dichloromethane at 0°C to furnish amide **7** in 74% yield.



5 E = COOH
6 E = COONSu
7 E = $CONH_2$

Weintraub *et al.*[25] have shown that Meerwein's reagent [$(OR_3)^+BF_4^-$] is far superior to dimethyl sulfate for the alkylation of amides. We have adapted this method for the preparation of the iminoether **8**, monitoring its formation by TLC and used the protocol of Brutsche & Hartke[26] in the treatment of amide **7**. The iminoether **8** was reacted straightaway with (S)- 2,4-diaminobutyric acid (S-Daba) **4a** in the presence of diisopropylethylamine (DIPEA) to give 2-(N-benzyloxyaminomethyl)-4-carboxy-3,4,5,6-tetrahydropyrimidine **1h**, though in only 30% yield. The iminoether **8** is probably unstable to air, since when carried out under argon and removing the solvent directly under reduced pressure, the overall yield rose to 66% (Scheme 3).



7 **8** **1h** (4S)

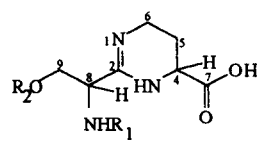
Scheme 3

Deprotection of the benzyloxycarbonyl group of **1h** by catalytic hydrogenation on Pd/C yielded (4S)- 2-(aminomethyl)-4-carboxy-3,4,5,6-tetrahydropyrimidine **1u** (90%).

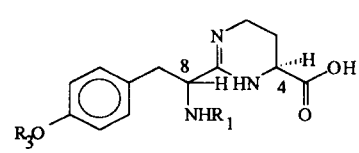
As no protection of the carboxylic group of 2,4-diaminobutyric acid was necessary, this prompted us to carry out similar syntheses of 2-substituted-4-carboxy-3,4,5,6-tetrahydropyrimidines.

II) Synthesis of 2-substituted-4-carboxy-3,4,5,6-tetrahydropyrimidines possessing two chiral centers respectively at C-4 & C-8: ($1x_1$-$1x_4$; $1y_1$-$1y_2$)

Similarly, reacting derivatives of serinamide and tyrosinamide with Meerwein's reagent and condensing the iminoethers obtained with either (S)- or (R)-Daba, gave us all the diastereoisomers of derivatives of 2-(1'-amino-2'-hydroxy)-ethyl- and 2-(1'-amino-2'-*p*-hydroxyphenyl)-ethyl-4-carboxy-3,4,5,6-tetrahydropyrimidines, namely: (8R,4S)-, (8S,4S)-, (8R,4R)- and (8S,4R)-2-(1'-benzyloxycarbonylamino-2'-hydroxy)-ethyl-4-carboxy-3,4,5,6-tetrahydropyrimidine $1x_1$-$1x_4$, as well as (8S,4S)- and (8R,4S)-2-(1'-benzyloxycarbonylamino-2'-p-methoxyphenyl)-ethyl-4-carboxy-3,4,5,6-tetrahydropyrimidine $1y_1$ & $1y_2$.



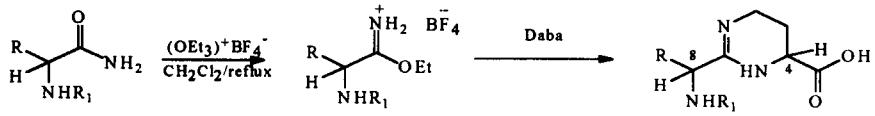
$1x_1$ -$1x_4$



$1y_1$ -$1y_2$

$1x_1$ (8R,4S) R_1 = Z, R_2 = Bn **$1x_3$** (8R,4R) R_1 = Z, R_2 = Bn **$1y_1$** (8S,4S) R_1 = Z, R_3 = CH_3
$1x_2$ (8S,4S) R_1 = Z, R_2 = Bn **$1x_4$** (8S,4R) R_1 = Z, R_2 = Bn **$1y_2$** (8R,4S) R_1 = Z, R_3 = CH_3

The two crucial steps were the conversion of the amide group into the iminoether group for serine or tyrosine, and the cyclization of these iminoethers with 2,4-diaminobutyric acid (Daba). The syntheses of the amides of serine and tyrosine required a previous protection of their amine and hydroxyl functions (Scheme 4).

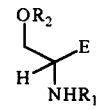


12 R = CH_2OR_2, R_1 = Z, R_2 = Bn
16 R=*p*-CH_2-C_6H_4-OR_3, R_1 =Z,R_3=CH_3
13 R = CH_2OR_2, R_1 = Z, R_2 = Bn
17 R=*p*-CH_2-C_6H_4-OR_3, R_1 =Z,R_3=CH_3
1x R = CH_2OR_2, R_1 = Z, R_2 = Bn
1y R=*p*-CH_2-C_6H_4-OR_3, R_1 =Z,R_3=CH_3

Scheme 4

A) 2-(1'-Benzyloxycarbonyl amino-2'-benzyloxy)-ethyl-4-carboxy-3,4,5,6-tetrahydro pyrimidines $1x_1$-$1x_4$

One of the major problems occurring in peptide synthesis is the epimerization at the Cα carbon atom of the amino acids. This epimerization is generally related to the nature of the protecting groups of the amine or acid functions and is strongly decreased when the protecting groups are of the urethane type.[27] Therefore we have performed several types of protections of the amine and the hydroxyl functions of serine either as an urethane (benzyloxycarbonyl) for the amine and a benzylic ether for the hydroxyl group. The starting point of this synthesis is the preparation of N-benzyloxycarbonyl-O-benzyl serinamide **11** (Scheme 4). This can be achieved starting from O-benzylserine **9**.

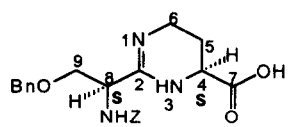


a S configuration
b R configuration

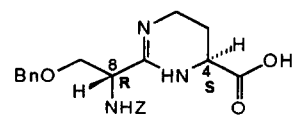
9 R_1 = H, R_2 = Bn, E = COOH
10 R_1 = Z, R_2 = Bn, E = COOH
11 R_1 = Z, R_2 = Bn, E = COONSu
12 R_1 = Z, R_2 = Bn, E = $CONH_2$

O-benzylserines **9a** and **9b** were treated with benzyloxycarbonyloxysuccinimide in the presence of triethylamine to give (S)- and (R)-N-benzyloxycarbonyl-O-benzylserines **10a** and **10b** with excellent yields 93% and 90% respectively. Acids **10a** and **10b** were further treated with N-hydroxysuccinimide to give the activated esters **11a** and **11b** which were then treated with ammonia gas in methylene chloride yielding amide **12a** and **12b** with an overall yield of 83% and 88% respectively. Iminoethers **13a** and **13b** were formed with excellent yields by reacting, in methylene chloride, triethyloxonium tetrafluoroborate with the amides **12a** and **12b** and used without further purification in the following step.

The synthesis of the (8R,4S)-3,4,5,6-tetrahydropyrimidine $\mathbf{1x_1}$ was performed by condensation of iminoether **13a** with (S)-2,4-diaminobutyric acid dihydrochloride **4a** in presence of DIPEA (2.5 equivalents with respect to 2,4-diaminobutyric acid dihydrochloride) in anhydrous methanol under reflux (Scheme 4). Starting from amide **13a**, a 1:1 mixture of diastereoisomers (8R,4S) $\mathbf{1x_1}$ and (8S,4S) $\mathbf{1x_2}$ occurring from a total epimerization at carbon C-8 was obtained with 80% yield (crude). This yield drops to 25% after purification, in agreement with the observation of Jones & Crockett[1], very likely due to the strong basic character of the amidine group of 3,4,5,6-tetrahydropyrimidine. In order to improve this yield, the crude reaction mixture was acidified to pH 3-4, then extracted with ethyl acetate: the yield was markedly improved to 69%.



$\mathbf{1x_1}$



$\mathbf{1x_2}$

The separation of diastereoisomers $\mathbf{1x_1}$ and $\mathbf{1x_2}$ being unsuccessful, we sought non-racemizing conditions for the synthesis. For this purpose several parameters were varied in the two successive reactions which give rise to the tetrahydropyrimidine ring: the formation of the iminoether and its condensation with 2,4-diaminobutyric acid. These parameters are the pH and the different ratios between triethyloxonium tetrafluoroborate, 2,4-diaminobutyric acid and the base (DIPEA) (Table 1).

Method	Amide **14a** Eq	$(OEt3)^+BF4^-$ Eq	(S)-Daba **4a** Eq	DIPEA Eq	pH	Yield %	$\mathbf{1x_1}/\mathbf{1x_2}$ %
A	1	1.1	1	2.5	8.0-8.2	69	50/50
B	1	1.1	1	2.0	7.4-7.6	62	50/50
C	1	1.5	1	2.5	6.8-7.0	54	56/44
D	1	1.5	0.8	1.5-2.0	6.0-6.4	39	87/13

Table 1: Ratio of the diastereoisomers $\mathbf{1x_1}$ and $\mathbf{1x_2}$ obtained under various conditions

The condensation reaction requires a basic pH for a satisfactory yield (69%, line A), however under these conditions there is a total epimerization. The acidity of the proton on the asymmetric center C-8 is definitely one of the determining factors of the epimerization. Therefore, performing the condensation reaction at slightly acidic pH (6.0-6.4) by varying the proportions of each reactant, we could set up reaction conditions where the epimerization was very low and obtain mostly $\mathbf{1x_1}$ (87% $\mathbf{1x_1}$ + 13% $\mathbf{1x_2}$) (Table 1, line D, 87/13): the decrease of the basicity of the amidine group in these conditions very likely prevents the epimerization of the asymmetric center C-8.[1,28]

In the same conditions, condensation of iminoether **13a** with (R)-2,4-diaminobutyric acid **4b** gave mostly $\mathbf{1x_3}$ (92% $\mathbf{1x_3}$ + 8% $\mathbf{1x_4}$) with 33% chemical yield. Similarly condensation of iminoether **13b** with (S)-2,4-diaminobutyric acid **4a** gave mostly $\mathbf{1x_2}$ (94% $\mathbf{1x_2}$ + 6% $\mathbf{1x_1}$) with 46% chemical yield). Finally the condensation of iminoethers **13b** with (R)-2,4-diaminobutyric acid **4b** by the same method (Scheme 5), gave mostly $\mathbf{1x_4}$ (89% $\mathbf{1x_4}$ + 11% $\mathbf{1x_3}$) respectively with 31% chemical yield.

Structures and configurations

The absolute configurations and hence the proportion of each diastereoisomer (Table 2) of the two asymmetric centers C-4 and C-8 were determined:

1. by GC-MS using a chiral stationary phase (Chirasil-D-Val) on the derivatized serine and Daba obtained by total acid hydrolysis of the mixtures containing each one predominant present diastereoisomer $\mathbf{1x_1}$- $\mathbf{1x_4}$;
2. by proton NMR at 500 MHz of the same samples.

Acid hydrolysis (4 days, 110°C, 6N HCl)[3] of the tetrahydropyrimidines $\mathbf{1x_1}$-$\mathbf{1x_4}$, and of the authentic amino acids as standards (R or S serine, R or S Daba) was followed by derivatization of the hydrolyzates as O-methyl, N-pentafluoropropionyl esters of the amino acids according to a method currently used in our laboratory.[29]

Compounds planned	Compounds obtained
$\mathbf{1x_1}$ + $\mathbf{1x_2}$	$\mathbf{1x_1}$ +$\mathbf{1x_2}$ (50/50)
$\mathbf{1x_1}$	$\mathbf{1x_1}$ +$\mathbf{1x_2}$ (87/13)
$\mathbf{1x_2}$	$\mathbf{1x_2}$ +$\mathbf{1x_1}$ (94/6)
$\mathbf{1x_3}$	$\mathbf{1x_3}$ +$\mathbf{1x_4}$ (92/8)
$\mathbf{1x_4}$	$\mathbf{1x_4}$ +$\mathbf{1x_3}$ (89/11)
$\mathbf{1x_1}$	$\mathbf{1x_1}$ +$\mathbf{1x_2}$ (50/50)

Table 2: Proportions of the various diastereoisomers of the tetrahydropyrimidines $\mathbf{1x_1}$-$\mathbf{1x_4}$ as measured from the % of epimerization of serine by GC-MS using a D-Chirasil-Val stationary phase after 4 days hydrolysis.

However, from GC-MS, it seems as if epimerization of Daba occurs in relatively high amounts. Actually it varies according to the condition of hydrolysis. Treatment of the amino acids with 6N hydrochloric acid for more than 24 hours at temperatures above 100° C is known to cause partial epimerization. This epimerization is a function of the nature of the amino acid, the concentration and the ionic strength of the salts in the medium, the presence of impurities and finally the particular position of the amino acid in the peptide or in the protein to which it belongs.[30-32]

Thus hydrolysis of the mixture of diastereoisomers $\mathbf{1x_1}$ + $\mathbf{1x_2}$ with 6N hydrochloric acid gives a 81/19 ratio of enantiomers (S) and (R) of Daba. If the deprotections of the benzylic and benzyloxycarbonyl groups of compound $\mathbf{1x_1}$ with trimethylsilyl iodide precede HCl 6N hydrolysis (4 days) the mixture of enantiomers (S) and (R) of Daba is in a 93/7 ratio. If the hydrolysis is continued for 8 days this ratio changes to 69/31. (4S)-2-(aminomethyl)-4-carboxy-3,4,5,6-tetrahydropyrimidine **1u** and authentic (S)-2,4-diaminobutyric acid alone give the same proportions of epimerization, showing the artefactual occurrence of the epimerization of the C-4 center in the tetrahydropyrimidines. On the contrary in the case of the serine enantiomers, the epimerization does not occur after hydrolysis in the

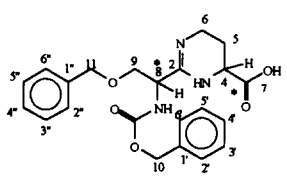
conditions we used for the tetrahydropyrimidines. Demange *et al.*[33] obtained the racemates of serine and Daba after acid hydrolysis (12 N HCl, 12 h, 180°C) of the 2-(1'-amino-2'-hydroxy)-ethyl-4-carboxy-3,4,5,6-tetrahydropyrimidine moiety of pyoverdin Pf CCM 2798, but the conditions which were used for hydrolysis were much more drastic. As the amidine group is very stable in this case, the quantitative hydrolytic cleavage of the C-N bonds in natural 3,4,5,6-tetrahydropyrimidine, can require drastic conditions.[34]

These results show clearly that epimerization of the asymmetric center C-4 (Daba moiety) does occur only in the course of the hydrolysis of the 3,4,5,6-tetrahydropyrimidines and not during the process of formation of the tetrahydropyrimidine ring. In this respect it is an artefact (besides no such epimerization for the C-4 center has ever been reported in the literature[1,21]). On the contrary epimerization of the asymmetric center C-8 is real and takes place during the synthesis of the 3,4,5,6-tetrahydropyrimidines. This is very likely related to factors such as pH, solvent, temperature, and probably to the very nature of the 3,4,5,6-tetrahydropyrimidine moiety.[1,28,35,36]

^{1}H NMR studies at 500 MHz confirmed these findings showing that the ratios of the major/minor diastereoisomers effectively present in compounds **1x$_1$-1x$_4$** are the same as those determined for serine by GC-MS after hydrolysis of the tetrahydropyrimidines. These have been identified by the presence of the signals of protons H-4 at 3.90 ppm - 3.96 ppm and of signals of protons H-9 at 3.75 ppm - 3.80 ppm (Table 3). H-8 is difficult to distinguish because of an overlap with the benzylic methylenic protons H-10. These latter occur as AB quartets differing in shape from one diastereoisomer to the other, in contrast to the H-11 benzyloxycarbonyl methylenic protons which occur also as AB quartets.

The structures of compounds **1x$_1$-1x$_4$** have been determined by 1D and 2D NMR (^{1}H-^{1}H, ^{1}H-^{13}C correlations at 500MHz). Using DEPT-135 and 2D heteronuclear ^{1}H-^{13}C correlations, the chemical shifts of the protons and carbon atoms of compounds **1x$_1$-1x$_4$** were assigned confirming the structures of the 2-(1'-benzyloxycarbonylamino-2'-benzyloxy)-ethyl-4-carboxy-3,4,5,6-tetrahydropyrimidines **1x$_1$-1x$_4$**.

The ^{13}C spectra of the four diastereoisomers are nearly identical and all show a resonance at 163 ppm assigned to the carbon C-2 atom of the tetrahydropyrimidine ring, with the same value as in related pyoverdins,[3,5-7] this signal being also present in the simplest 3,4,5,6-tetrahydropyrimidine **1u** and in the two diastereoisomeric tyrosine derivatives **1y$_1$** and **1y$_2$** (see below). The only significant difference shown in the chemical shifts of homologous carbon atoms was found in the ^{13}C spectrum of the 1:1 mixture of diastereoisomers **1x$_1$** and **1x$_2$** where carbon C-9 (70.04 & 70.16 ppm) and carbon C-11 (74.41 & 74.50 ppm) both occur as a set of two signals of equal intensity differing by ca 0.1 ppm.



Proton	1x$_1$ (8R,4S)	1x$_2$ (8S,4S)	1x$_3$ (8R,4R)	1x$_4$ (8S,4R)
H-4	3.92 (t, 5.1 Hz)	3.98 (t, 5.1 Hz)	3.98 (t, 5.1 Hz)	3.93 (t, 5.1 Hz)
H-9	3.76 (d, 4.8 Hz)	3.80 (d, 4.7 Hz)	3.80 (d, 4.7 Hz)	3.76 (d, 5.0 Hz)
H-11a	4.53 (d, 12 Hz)	4.55 (d, 11.5 Hz)	4.55 (d, 11.5 Hz)	4.53 (d, 12 Hz)
H-11b	4.60 (d, 12 Hz)	4.60 (d, 11.5 Hz)	4.59 (d, 11.5 Hz)	4.60 (d, 12 Hz)

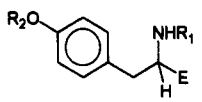
Table 3: Comparison of the chemical shifts and coupling constants of protons H-4, H-9 and H-11 of the four diastereoisomers of 2-(1'-benzyloxycarbonyl-amino-2'-benzyloxy)-ethyl-4-carboxy-3,4,5,6-tetrahydropyrimidine

B) Derivatives of 2-(1'-amino-2'-p-hydroxyphenyl)-ethyl-4-carboxy-3,4,5,6-tetrahydropyrimidines

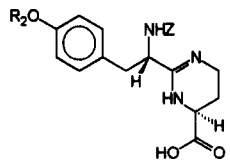
As mentioned above, (8S,4S)-2-(1'-amino-2'-p-hydroxyphenyl)-ethyl-4-carboxy-3,4,5,6-tetrahydropyrimidine was suggested to be a precursor of the chromophore of some pyoverdins, siderophores of the fluorescent *Pseudomonas*. In order to better describe the properties of this amino acid, the synthesis of (8S,4S)-2-(1'-benzyloxycarbonylamino-2'-p-methoxyphenyl)-ethyl-4-carboxy-3,4,5,6-tetrahydropyrimidine **1y$_1$** and (8R,4S)-2-(1'-benzyloxycarbonyl-amino-2'-p-methoxyphenyl)-ethyl-4-carboxy-3,4,5,6-tetrahydropyrimidine **1y$_2$**, was undertaken. This synthesis was performed in four steps identically to the synthesis of **1x$_1$-1x$_4$**, but using the iminoethers of the protected tyrosines instead of those of the protected serines.

The amine functions of (S)- and (R)-tyrosines **14a** and **14b** were first protected as N-benzyloxycarbonyl derivatives by reaction of N-benzyloxycarbonyloxysuccinimide with the (S)- and (R)-tyrosines. The free acid function was then esterified using NHS in the presence of DCC. The activated esters, treated with ammonia gas, gave respectively amides **15a** and **15b** with overall yields of 91% and 82%.

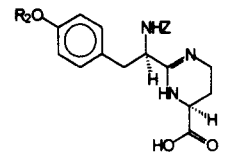
Since the phenolic hydroxyl group of tyrosine can be alkylated in the presence of an alkylating reagent such as triethyloxonium tetrafluoroborate in the course of the formation of the iminoethers,[37] and being able otherwise to give the phenoxide ion, a powerful nucleophile, it was essential to protect it. Among the various methods used, the protection as an ether, such as benzylic ether, is certainly the most widely used. Unfortunately this group can give rise to problems in structural studies concerning the determination by NMR of the absolute configuration of the asymmetric center C-8 of 2-(1'-amino-2'-*p*-hydroxyphenyl)-ethyl-4-carboxy-3,4,5,6-tetrahydropyrimidines, since there is overlap between the benzylic protons of the protecting group and the proton CHα of tyrosine in proton NMR. We therefore chose protection as a methyl ether.



a (S configuration) & b (R configuration)
14 R_1 = H, R_2 = H, E = COOH
15 R_1 = Z, R_2 = H, E = $CONH_2$
16 R_1 = Z, R_2 = CH_3,E = $CONH_2$
17 R_1 = Z, R_2 = CH_3, E = C(OEt)=NH



$1y_1$



$1y_2$

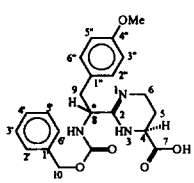
Methyl ethers **16a** and **16b**, were prepared from tyrosine derivatives **15a** and **15b** with 93% and 87 % yield respectively, and converted to the corresponding iminoethers **17a** and **17b**, analagously to procedures described above.

The condensation of iminoether **17a** with (S) Daba was performed identically to the condensation of iminoether **13a**, as described above, and yielded (8S,4S) 2-(1'-N-benzyloxycarbonylamino-2'-p-methoxyphenyl)-ethyl-4-carboxy-3,4,5,6-tetrahydropyrimidine **$1y_1$** (41%) as a single diastereoisomer (as determined by proton NMR and by GC-MS), in contrast to its serine homologue.

Similarly, starting from amide **17b**, the methoxy ether **$1y_2$** of (8R,4S)-2-(1'-benzyloxycarbonylamino-2'-p-hydroxyphenyl)-ethyl-4-carboxy-3,4,5,6-tetrahydropyrimidine was obtained with 45% yield, also as a single diastereisomer.

As for the serine derivatives, the ^{13}C spectra of the diastereisomers derived from tyrosine did not show large differences between each other, even when the spectra were determined at 125 MHz.

Proton NMR spectra at 200 MHz of compounds **$1y_1$** and **$1y_2$** (Table 4) showed clearly that the chemical shifts of protons H-4 and H-8 in each diastereoisomer **$1y_1$** and **$1y_2$** are different:



Proton	$1y_1$ (8S,4S)	$1y_2$ (8R,4S)
H-4	3.93 t (5.6 Hz)	4.00 t (5.7 Hz)
H-8	4.58 t (7.9 Hz)	4.65 dd (5.97 & 9.4 Hz)
H-9	3.05-3.09 m	2.93-3.21 m

Table 4: Comparison of the chemical shifts and coupling constants of the protons H-4, H-8 and H-9 of the two diastereoisomers of 2-(1'-benzyloxycarbonylamino-2'-*p*-methoxyphenyl)-ethyl-4-carboxy-3,4,5,6-tetrahydropyrimidine

- proton H-4 of compound **1y$_1$** occurs at 3.93 ppm as a triplet (J = 5.6 Hz) as it occurs, but at 4.00 ppm, for compound **1y$_2$** (J = 5.7 Hz);
- proton H-8 of compound **1y$_1$** occurs at 4.58 ppm as a triplet (J = 7.9 Hz) whereas for **1y$_2$** it occurs at 4.65 ppm as a double doublet (J = 5.7 Hz and J = 9.4 Hz).

These spectra show not only that both diastereoisomers (8S,4S) and (8R,4S) derived from tyrosine are easy to distinguish by proton NMR even at 200 MHz, but also that the synthesis of the derivatives of tyrosine occurs without any epimerization at carbon C-8 and that only one single diastereoisomer **1y$_1$** or **1y$_2$** is formed each time. Optical rotations of tetrahydropyrimidines **1y$_1$** and **1y$_2$** have been found to be respectively (+64°) and (-6,4°).

The reason for this stability is probably due not only to the weaker acidity of proton CHα of tyrosine with respect to the acidity of the corresponding proton of serine, but also to the lesser solubility of the 2-(1'-benzyloxycarbonylamino-2'-*p*-methoxyphenyl)-ethyl-4-carboxy-3,4,5,6-tetrahydro-pyrimidines **1y$_1$** and **1y$_2$** in the medium than their homologues derived from serine which can undergo a partial epimerization up to 13% under the same conditions.

In conclusion, this study has shown that 2-substituted-4-carboxy-3,4,5,6-tetrahydropyrimidines can be readily synthesized by condensation of unprotected 2,4-diaminobutyric acid with the protected iminoether tetrafluoroborates of amino acids. However, the carbon atom at the Cα position of the amidine ring (here carbon C-8) can easily be totally racemized. This epimerization can be considerably diminished or even avoided when the reaction is performed in slightly acidic conditions (pH 6.0-6.4).

EXPERIMENTAL

General indications

The melting points (mp) were measured in a capillary tube using a Büchi SMP-20 instrument and are not corrected. The proton NMR spectra were measured on Bruker AC-200 (200 MHz), AM-400 (400 MHz), ARX-500 (500 Mhz), AMX 500 (500 MHz) or with gradient instruments. GC-MS analyses were performed on a QMD 1000 Carlo Erba Instrument. The capillary column used was a Chirasil D-Val Chrompack of 25 m length. The temperature of the source was at 180°C. The temperature of the split-splitless injector was at 230°C. The mass spectra (MS) were determined using either an LKB 9000S or a Thomson THN 208 spectrometer. The FAB (Fast atom bombardment) spectra were performed using a ZAB-HF instrument (VG Analytical, Manchester, United Kingdom). The relative intensities of the signals are stated in parentheses after the mass m/z of the fragment considered. LSIMS-MS mass measurements were performed on an Autospec M instrument (Micromass, Altricham, UK) with a resolution of 10000 using PEG 400 as an internal satndard. The elemental analyses (microanalyses) were performed in Strasbourg by the Service de Microanalyse of the Institut Charles Sadron. The column chromatographies were performed using Merck 9385 silicagel (Darmstadt. Germany) 40 to 63 μm mesh. Thin layer chromatographies (TLC) were performed using silicagel analytical plates Merck 5715 (F_{254}) of 0.25 mm thickness. The detection on TLC plates was performed by UV light at 254 nm or 365 nm or using a spray and heating the plates. The sprays used were:

-a 0.3% solution of ninhydrin in a mixture of butanol/acetic acid (97:3, v/v).
-a 0.4% solution of 2.4-dinitrophenylhydrazine in 2M hydrochloric acid.
-a 10% solution of phosphomolybdic acid in ethanol.

The anhydrous solvents were obtained by distillation from an appropriate drying agent: phosphoric anhydride for methylene chloride and dimethylformamide; calcium hydride for toluene, ether and acetonitrile; sodium for methanol. The oxygen- or humidity sensitive reactions were performed under argon.

(R)-2,4-diamino butyric acid **4b** was prepared as described in reference 38.

A) Glycine derivatives

N-hydroxysuccinimide ester of N-benzyloxycarbonyl glycine **6** was prepared as described in reference 24. The yield was 83%.

mp 113-115 °C (methylene chloride/petroleum ether) (lit: 113-114°C).[24]

^{1}H NMR (200 MHz. CD_3OH): 2.82 (m, 4 H, CO-CH_2-CH_2CO); 4.25 (s, 2 H, -CH_2-N); 5.11 (s, 2 H, CH_2-benzyl); 7.27-7.37 (m, 5 H, H-aromatics).

N-Benzyloxycarbonylglycinamide 7

Compound **6** (2.04 g; 6.7 mmol) was dissolved in methylene chloride (20 mL). The solution was cooled down to 0°C then treated with ammonia gas during 15 minutes. The suspension was filtered and the residue crystallized in a mixture of methanol and ether (1.23 g; 5.9 mmol). The yield was 89%.

TLC: Rf = 0.38 (methylene chloride-methanol 85:15).
mp 134-135 °C (methanol/ether). (lit: 138-139°C).[39]
MS (EI): m/z: 208 (100), 191 (13), 108 (26). 91 (5).

^{1}H NMR (200 MHz. $CDCl_3$): 3.90 (d, J = 5.7 Hz, 2 H, CH_2-N); 5.14 (s, 2 H, CH_2-benzyl); 5.40 (broad s, 2 H, NH_2); 5.90 (broad s, 1 H, NH); 7.37-7.38 (m, 5 H, H-aromatics).

2-(N-benzyloxycarbonylaminomethyl)-4-carboxy-3,4,5,6-tetrahydropyrimidine 1h

Amide 7 (505 mg; 2.43 mmol) and triethyloxonium tetrafluoroborate (676 mg; 3.56 mmol) were dissolved in anhydrous methylene chloride (10 mL). The solution was refluxed for 30 minutes under argon. The solvent was eliminated under a stream of argon then under reduced pressure. To the oil obtained and maintained under argon were successively added (S)-2,4-diaminobutyric acid as a dihydrochloride (464 mg; 2.43 mmol), methanol (10 mL) and DIPEA (1.3 mL; 7.3 mmol). The mixture was refluxed for 5 hours under argon. The suspension was evaporated to dryness under reduced pressure and the mixture dissolved in water, acidified to pH 3-4 by addition of a 1N solution of hydrochloric acid. The aqueous phase was evaporated under reduced pressure, and the residue crystallized in water to yield colourless crystals (455 mg; 1.6 mmol. 66%).

TLC: Rf 0.52 (butanol-water-acetic acid 4:1:1, v/v).
mp 247-248 °C dec. (methanol/water). (lit: 251 °C).[23]
MS (CI): m/z $(M+H)^+$ = 292 (37), 246 (5), 184 (100), 108 (73).

^{1}H NMR (200 MHz; D_2O): 1.98-2.27 (m, 2 H, H-5); 3.33-3.58 (m, 2 H, H-6); 4.24 (s, 2 H, CH_2 glycine); 4.40 (t, J= 4.3 Hz, 1 H; H-4); 5.20 (s, 2 H, CH_2-benzyloxycarbonyl); 7.47 (s, 5 H, H-aromatics).

Calculated for $C_{14}H_{17}N_3O_4$	C% 57.61	H% 5.89	N% 14.39	O% 22.09
Found	C% 57.42	H% 5.84	N% 14.28	O% 22.14

(4S)-2-(aminomethyl)-4-carboxy-3,4,5,6-tetrahydropyrimidine 1u

5% palladium on charcoal (5 mg) was added to a solution of tetrahydropyrimidine **1h** (35 mg; 0.12 mmol) in a mixture of methanol (10 mL) and 1N aqueous hydrochloric acid (0.5 mL).The suspension was kept under argon then flushed with hydrogen, and finally stirred vigourously for 2.5 hours. Hydrogen was then replaced by argon and the suspension was filtered on a Celite column and the filtrate evaporated under reduced pressure to yield a white powder (16.9 mg; 0.11 mmol. 90%).

Rf: 0.2 (ethyl acetate /formic acid/water 4:1:1, v/v) (Detection with ninhydrin).
mp 203-206 °C (methanol/water).
MS (EI) m/z: 157 (27), 140 (5), 128 (5), 112 (100), 96 (69), 83 (63), 67 (25), 56 (41).
LSIMS-MS Mass Measurement: Found $(M+H)^+$ = 158.092694; $C_6H_{12}N_3O_2$ requires 158.092952

^{1}H NMR (200 MHz; CD_3OD):

	H-4	H-5	H-6	H-8
1u (this work)	3.98 (t; J=5.2 Hz)	2.13 (m)	3.42 (m)	3.71 (s)
From reference 1	4.04 (t)	2.19 (m)	3.47 (m)	3.67 (s)

^{13}C NMR (125 MHz, CD_3OD): 23.51 (C5); 38.66 (C6); 41.97 (C8) 54 53 (C4); 163.36 (HN-C=N); 175.21 (COOH).

B) Serine derivatives

(S)-N-benzyloxycarbonyl-O-benzyl serine 10a

(S)-O-benzylserine **9a** (1 g, 5.1 mmol, Fluka) was suspended in a mixture of dioxane (8 mL) and methanol (2 mL). To the suspension was added triethylamine (2.1 mL; 15.3 mmol) then, dropwise, a solution of (N-benzyloxycarbonyl) succinimide (1.396 g, 5.61 mmol) in 10 mL dioxane. The mixture was stirred for 1 hour after which the solution became clearer. The solvents were removed under reduced pressure and the residue was treated with 20 mL water acidified to pH 2-3 with 1N hydrochloric acid. The mixture was extracted with ethyl acetate (3 x 10 mL). The organic phase was washed with water (2 x 10 mL) then with a saturated solution of sodium chloride,

dried over magnesium sulphate, filtered and evaporated. The pale yellow oil obtained (1.75 g) was purified by chromatography on a column of silicagel (50 g) eluted successively with methylene chloride, then with a mixture of methylene chloride/methanol, 90:10, and yielded pure (S)-N-benzyloxycarbonyl-O-benzyl serine **10a** (1.56 g, 4.1 mmol; 93%) as colourless crystals.

TLC: Rf 0.71 (butanol-acetic acid-water 4:1:1, v/v) (Detection with ninhydrin).
mp 114-115°C (ethyl acetate-ether) (lit 118-119°C. ethyl acetate).[40]

^{1}H NMR (200MHz, $CDCl_3$): 3.67-3.98 (m, 2 H, CH_2-β serine); 4.52 (m, 1 H, CH-α serine); 5.12 (s, 2 H, CH_2-benzyl); 5.64 (s, 1 H, NH); 7.28-7.35 (m, 5 H, H-aromatics).

The same reaction performed in identical conditions on (R)-O-benzylserine **9b** gave (R)-N-benzyloxycarbonyl O-benzylserine **10b** with 90% yield (mp 94-96°C, chloroform).

(S)-N-benzyloxycarbonyl O-benzyl serinamide 12a
Compound **10a** (1.5 g, 4.6 mmol) and N-hydroxysuccinimide (0.52 g, 4.6 mmol) was dissolved in dioxane (10 mL), and the solution cooled down to 0°C. A solution of DCC (1.034 g, 5.0 mmol) in dioxane (4 mL) was then added dropwise in 15 minutes. The mixture was kept at 0°C for 45 more minutes and the solvent removed. The residue was dissolved in methylene chloride (25 mL), and treated at 0°C. with ammonia gas for 5 minutes, then warmed to room temperature, filtered and concentrated under reduced pressure. The residue was crystallized in a mixture of chloroform and diethyl ether to yield (S)-N-benzyloxycarbonyl O-benzyl serinamide **12a** as colourless needles (1.33 g, 4.05 mmol, 83%).

TLC: Rf: 0.5 (methylene chloride-methanol 9:1).
mp 141-142°C (chloroform/methanol).
MS (CI, NH_3) m/z: $(M+H)^+$= 329 (100), 312 (4), 285 (5), 221 (7.5), 195 (47.5), 107 (15.5).

^{1}H NMR (200 MHz; acetone-D6): 3.70-3.86 (m, 2 H, CH_2-β serine); 4.34-4.43 (m, 1 H, CH-α serine); 4.55 (s, 2 H, CH_2-benzyl); 5.1 (s, 2 H, CH_2-benzyloxycarbonyl); 6.45 (m, 2 H, NH_2); 7.07 (m, 1 H, NH); 7.28-7.37 (m, 10 H, H-aromatics).

Calculated for $C_{18}H_{20}N_2O_4$	C% 65.85	H% 6.09	N% 8.54	O% 19.51
Found	C% 66.05	H% 6.26	N% 8.66	O% 19.28

(R)-N-benzyloxycarbonyl O-benzyl serinamide 12b
This amide was prepared from compound **10b** using the same procedure used for compound **10a**. The yield was 88%.

mp 141-141.5°C.
LSIMS-MS Mass Measurement: Found $(M+H)^+$ = 329.149396; $C_{18}H_{21}N_2O_4$ requires 329.150132
^{1}H NMR of **16b** was identical to that of **16 a**.

Synthesis of a 1:1 mixture of diastereoisomers of 2-(1'-N-benzyloxycarbonylamino 2'-benzyloxy)-ethyl-4-carboxy-3,4,5,6-tetrahydropyrimidine $1x_1$ (8R,4S) & $1x_2$ (8S,4S)

Compound **12a** (156 mg, 0.48 mmol) and triethyloxonium tetrafluoroborate (100 mg, 0.52 mmol) were refluxed under argon for 2 hours in anhydrous methylene chloride (3 mL). After removal of the solvent under reduced pressure, (S)-2,4-diaminobutyric acid **4a** (92 mg, 0.48 mmol, Fluka) as its dihydrochloride was added under argon to the iminoether **13a** thus obtained. After addition of anhydrous methanol (5 mL), then DIPEA (210 µl, 1.2 mmol), the solution was refluxed for 2 hours, the solvent evaporated to dryness and the residue treated with water (25 mL) and acidified to pH 3-4 by addition of 1N HCl. The aqueous phase was extracted with ethyl acetate (3 x 10 mL), and the organic phase washed with a saturated solution of sodium chloride, dried over sodium sulphate, filtered and evaporated under reduced pressure. The residue was purified by chromatography. on a silicagel column (5 g) eluted first with methylene chloride, then with increasing amounts of methanol in the same solvent to yield a 1:1 mixture of diastereoisomers $1x_1$ & $1x_2$ (137 mg, 0.33 mmol, 69 %).

Rf: 0.36 (methylene chloride-methanol 8:2 v/v).
mp 182 -184 °C (mixture of diastereoisomers).
MS (CI, NH_3) m/z: $(M+H)^+$ = 412 (5), 304 (1), 260 (2), 196 (5), 152 (100), 126 (53), 108 (25), 91 (5).

^{1}H NMR (500 MHz; CD_3OD): 1.93-2.03 (m, 1 H, H-5a); 2.12-2.23 (m, 1H, H-5b); 3.34-3.39 (m, 2-H, H-6); 3.74 (d, J = 4.9 Hz, 1 H, H-9); 3.80 (d, J = 4.8 Hz; 1 H; H-9'); 3.89 (t, J = 5.6 Hz, 0.5 H, H-4); 3.96 (t, J = 6.2 Hz; 0.5

H; H-4'); 4.50- 4.67 (m, 3 H: H11 & H-8); 5.08 (d, J = 12.3 Hz, 1 H, H-10a); 5.14 (d, J = 12.3 Hz, 1 H, H-10b); 7.25-7.38 (m, 10 H; H-aromatics).

Protons H-4 et H-9 are each present as a set of two signals both in a ratio of 1:1.

^{13}C NMR (125 MHz CD3OD): 23.05 (C5); 39.37 (C6); 54.41 (C4); 54.81 (C8); 68.41 (C10); 70.04 & 70.16 (C9); 74.41 & 74.50 (C11); 129.16* (C2', C6', C2", C6"); 129.31 (C4', C4"); 129.60* (C3', C5', C3", C5"); 137.78** (C1'); 138.84** (C1"); 158.22 (O-CO-N); 163.07 (HN-C=N); 174.81 (COOH).

Calculated for $C_{22}H_{25}N_3O_5$	C% 64.22	H% 6.13	N% 10.21
Found	C% 63.82	H% 6.04	N% 10.05

$[\alpha]_D$: +37.6° for the 50:50 mixture of diastereoisomers (c 0.9; methanol/HCl 6N 2%).

(8R,4S)-2-(1'-N-benzyloxycarbonylamino 2'-benzyloxy)-ethyl-4-carboxy-3,4,5,6-tetrahydropyrimidine $\mathbf{1x_1}$
(S)-N-benzyloxycarbonyl-O-benzyl serinamide **12a** (250 mg, 0.76 mmol) and triethyloxonium tetrafluoroborate (217 mg, 1.14 mmol) were refluxed for 2 hours in anhydrous methylene chloride (5 mL) under argon. After removal of the solvent under reduced pressure, (S)-2,4-diaminobutyric acid **4a** as its dihydrochloride (116 mg, 0.61 mmol, Fluka) and methanol (10 mL) were added. The solution was then treated with DIPEA (260 µl, 1.53 mmol) and refluxed for 2 hours. After removal of the solvent under reduced pressure, the residue was treated with water (25 mL) and acidified to pH 3-4 using 1N HCl. The aqueous phase was extracted with ethyl acetate (3 x 15 mL). The organic phase was washed with a saturated solution of sodium chloride, dried over sodium sulphate and evaporated under reduced pressure. The residue was then purified by chromatography on a silica gel column (10 g) made up in methylene chloride, eluted with increasing amounts of methanol in the same solvent, to yield a solid product (123 mg, 0.30 mmol, 39%).

LSIMS-MS Mass Measurement: Found $(M+H)^+$ = 412.187595; $C_{22}H_{26}N_3O_5$ requires 412.187246

^{1}H MNR (500 MHz; CD_3OD): 1.94-2.05 (m, 1H, H-5a); 2.13 - 2.24 (m, 1H, H-5b); 3. 28 - 3.42 (m, 2 H, H-6); 3.76 (d, J = 4.8 Hz, 2 H, H-9); 3.92 (t, J = 5.1 Hz, 1 H, H-4); 4.55-4.65 (m, 1 H, H-8); 4.53 (d, J = 12 Hz, 1 H, H-11a); 4.60 (d, J = 12 Hz, 1 H, H-11b); 5.08 (d, J = 12 Hz, 1 H, H-10a); 5.14 (d, J = 12 Hz, 1 H, H-); 7.28 - 7.36 (m, 10 H, aromatics).

Protons H-4 and H-9 are each present as a set of two signals both in a ratio of 87/13.

Ratio of the diastereoisomers $\mathbf{1x_1/1x_2}$: 87/13 (determined from the ^{1}H NMR spectrum and from the GC-MS analyses).

^{13}C NMR (125 MHz CD3OD): 22.96 (C5); 39.34 (C6); 54.45 (C4); 54.69 (C8); 68.43 (C10); 70.04 (C9); 74.41 (C11); 129.15* (C2', C6', C2", C6"); 129.31 (C4', C4"); 129.60* (C3', C5', C3", C5"); 137.75** (C1'); 138.80** (C1"); 158.28 (O-CO-N); 163.36 (HN-C=N); 175.21 (COOH).

$[\alpha]_D$: +33.8° (c 0.9; methanol/HCl 6N 2%).

(8S,4S)-2-(1'-N-benzyloxycarbonylamino-2'-benzyloxy)-ethyl-4-carboxy-3,4,5,6-tetrahydropyrimidine $\mathbf{1x_2}$
The same reaction performed under identical conditions on (R)-N-benzyloxycarbonyl-O-benzyl serinamide **12b** and (S)-2,4-diaminobutyric acid **4a** yielded mainly (8S,4S) $\mathbf{1x_2}$ (46%).

LSIMS-MS Mass Measurement: Found $(M+H)^+$ = 412.187620; $C_{22}H_{26}N_3O_5$ requires 412.187246

^{1}H NMR (500 MHz; CD_3OD): 1.94-2.05 (m, 1 H, H-5a); 2.13 - 2.24 (m, 1H, H-5b); 3.29 - 3.42 (m, 2 H, H-6); 3.80 (d, J = 4.7 Hz, 2 H, H-9); 3.98 (t, J = 5.1 Hz, 1 H, H-4); 4.53-4.65 (m, 1 H, H-8); 4.55 (d, J = 11.56 Hz, 1 H, H-11a); 4.60 (d, J = 11.56 Hz, 1 H, H-11b); 5.075 (d, J = 12.2 Hz, 1 H, H-10a); 5.15 (d, J = 12.2 Hz, 1 H, H-10b); 7.28 - 7.36 (m, 10 H, aromatics).

Protons H-4 and H-9 are each present as a set of two signals both in a ratio of 94/6.

Ratio of the diastereoisomers $\mathbf{1x_2/1x_1}$: 94/6 (determined from the ^{1}H NMR spectrum and from the GC-MS analyses).

^{13}C NMR (100 MHz CD_3OD): 23.05 (C5); 39.37 (C6); 54.39 (C4); 54.81 (C8); 68.46 (C10); 70.16 (C9); 74.50 (C11); 129.15* (C2', C6', C2", C6"); 129.31 (C4', C4"); 129.60* (C3', C5', C3", C5"); 137.78** (C1'); 138.84** (C1"); 158.22 (O-CO-N); 163.07 (HN-C=N); 174.81 (COOH).
$[\alpha]_D$ = -41.6° (c 0.9; methanol/HCl 6N 2%).

(8R,4R)-2-(1'-N-benzyloxycarbonylamino-2'-benzyloxy)-ethyl-4-carboxy-3,4,5,6-tetrahydropyrimidine $1x_3$
The same reaction performed under identical conditions on (S)-N-benzyloxycarbonyl-O-benzyl serinamide **12a** and (R)-2,4-diaminobutyric acid **4b** yielded mainly (8R,4R) $1x_3$ (33%).

LSIMS-MS Mass Measurement: Found $(M+H)^+$ = 412.187536; $C_{22}H_{26}N_3O_5$ requires 412.187246

^{1}H NMR (500 MHz; CD_3OD): 1.94 -2.05 (m, 1 H, H-5a); 2.13 - 2.24 (m, 1 H, H-5b); 3.28 - 3.43 (m, 2 H, H-6); 3.80 (d, J = 4.7 Hz, 2 H, H-9); 3.98 (t, J = 5.1 Hz, 1 H, H-4); 4.53-4.65 (m, 1 H, H-8); 4.55 (d, J = 11.56 Hz, 1 H, H-11a); 4.59 (d, J = 11.56 Hz, 1 H, H-11b); 5.06 (d, J = 13 Hz, 1 H, H10a); 5.14 (d, J = 13 Hz, 1 H,); 7.28 - 7.36 (m, 10 H, aromatics).

Protons H-4 and H-9 are each present as a set of two signals both in a ratio of 92/8.
Ratio of the diastereoisomers $1x_3/1x_4$: 92/8 (determined from the ^{1}H NMR spectrum and from the GC-MS analyses).

^{13}C NMR (100 MHz CD3OD): 22.80 (C5); 39.25 (C6); 54.45 (C-4); 54.51 (C-8); 68.44 (C10); 70.18 (C9); 74.40 (C11); 128.98* (C2', C6', C2", C6"); 129.28 (C4', C4"); 129.54* (C3', C5', C3", C5"); 137.65** (C1'); 138.78** (C1"); 158.36 (O-CO-N); 163.14 (HN-C=N); 175.80 (COOH).
$[\alpha]_D$ = -43.9° (c 0.9; methanol/HCl 6N 2%).

(8S,4R)-2-(1'-N-benzyloxycarbonylamino-2'-benzyloxy)-ethyl-4-carboxy-3,4,5,6-tetrahydropyrimidine $1x_4$
The same reaction performed in identical condition on (R)-N-benzyloxycarbonyl-O-benzyl serinamide **12b** and (R)-2,4-diaminobutyric acid **4b** yielded mainly (8S,4R) $1x_4$ (31%).

LSIMS-MS Mass Measurement: Found $(M+H)^+$ = 412.187900; $C_{22}H_{26}N_3O_5$ requires 412.187246

^{1}H NMR (500 MHz; CD_3OD): 1.94- 2.05 (m; 1 H; H-5a); 2.13 - 2.24 (m, 1 H, H-5b); 3.29 - 3.43 (m; 2 H; H-6); 3.76 (d; J = 4.8 Hz, 2 H, H-9); 3.93 (t, J = 5.1 Hz, 1 H, H-4); 4.52-4.65 (m, 1 H, H-8); 4.53 (d, J = 12 Hz, 1 H, H-11a); 4.60 (d, J = 12 Hz, 1 H, H11b); 5.07 (d, J = 13 Hz, 1 H, H10a); 5.13 (d, J = 13 Hz, 1 H, H10b); 7.28 - 7.36 (m, 10 H, aromatics).

Protons H-4 et H-9 are each present as a set of two signals both in a ratio of 89/11.
Ratio of the diastereoisomers $1x_4/1x_3$: 89/11 (determined from the ^{1}H MNR spectrum and from the GC-MS analyses).

^{13}C NMR (125 MHz CD3OD): 22.78 (C5); 39.19 (C6); 54.31 (C4); 54.42 (C8); 68.41 (C10); 70.05 (C9); 74.35 (C11); 129.10* (C2', C6', C2", C6"); 129.29 (C4', C4"); 129.57* (C3', C5', C3", C5"); 137.69** (C1'); 138.77** (C1"); 158.20 (O-CO-N); 163.23 (HN-C=N); 174.63 (COOH).

$[\alpha]_D$ = -30.8°C (c 0.9; methanol-HCl 6N 2%).

C) Tyrosine derivatives

(S)- and (R)-N-benzyloxycarbonyl tyrosinamides
Using the same sequence of reactions by which we prepared the serinamides, we made the tyrosinamide derivatives **15a** and **15b** starting from (S)- and (R)-tyrosine **14a** and **14b** (Jansen chemicals). The yields of **15a** and **15b** were 91% and 82% respectively

TLC: Rf 0.45 (methylene chloride/methanol 9:1, v/v).

FAB-MS: $(M+H)^+$ = 315.2 m.u.

^{1}H NMR (200 MHz; CD_3OD): 2.69-3.30 (m, 2 H, CH_2-β); 4.03 (dd, 1 H, J = 5.4 and 9.1 Hz, CHα); 5.00 (d, J = 12;5 Hz, H10a); 5.02 (d, J = 12;5 Hz, H10b);); 6.68 (d, J = 8.3 Hz, 2 H, H-3" and H-5" Tyr); 7.04 (d, 2 H, J = 8.4 Hz, H-2" and H-6" Tyr) 7.28 (m, 5 H, H-2', H-3', H-4', H-5' & H-6').

Calculated for $C_{17}H_{18}N_2O_4$	C% 64.97	H% 5.73	N% 8.92	O% 20.38
Found	C% 64.84	H% 5.73	N% 8.88	O% 20.37

Protection of the hydroxyl group of N-benzyloxycarbonyl tyrosinamides
(S)- or (R)-N-benzyloxycarbonyl tyrosinamide **15a** (or **15b**) (1.0 g, 3.18 mmol) and potassium carbonate (878 mg, 6.36 mmol) were suspended in anhydrous dimethylformamide (6mL). Methyl iodide was added (0.59 mL, 9.6 mmol) and the mixture stirred for 5 hours. After filtration and addition of water (15 mL), the filtrate was acidified to

pH 2-3. The precipitate was filtered, washed with diethyl ether and dried under reduced pressure to give a colourless solid with 93% yield for **16a** (and 87% for **16b**).

TLC: Rf 0.58 (methylene chloride-methanol 9:1, v/v).
mp 138-139°C (**16a**) and 169-170°C (**16b**).

LSIMS-MS Mass Measurement: Found $(M+H)^+$ = 329.150029; $C_{18}H_{21}N_2O_4$ requires 329.150132

^{1}H NMR (200 MHz, CD_3OD): 2.72-3.12 (m, 2 H, CH_2-β); 3.75 (s, 3 H, OCH_3); 4.31 (dd, *J* = 5.3 and 9.3 Hz, 1 H, CHα); 5.00 (d, *J* = 12;5 Hz, H10a); 5.11 (d, *J* = 12;5 Hz, H10b); 6.68 (d, *J* = 8.3 Hz, 2 H, H-3" & H-5" tyrosine); 7.04 (d, 2 H, *J* = 8.4 Hz, H-2" & H-6" Tyr) 7.28 (m, 5 H, H-2', H-3', H-4', H-5' & H-6').

(8S,4S)- and (8R,4S)-2-(1'-N-benzyloxycarbonylamino-2'-p-methoxyphenyl)-ethyl-4-carboxy-3,4,5,6-tetrahydropyrimidines ($1y_1$) & ($1y_2$)

(S)- or (R)-N-benzyloxycarbonyl methoxytyrosinamide **16a** (or **16b**) (443 mg, 1.35 mmol) and triethyloxonium tetrafluoroborate (384 mg, 2 mmol) were dissolved in methylene chloride (5 mL). The mixture was refluxed for 2 hours under argon. After removal of the solvent under reduced pressure, (S)-2,4-diaminobutyric acid **4a** (257 mg, 1.34 mmol) and DIPEA (0.58 mL, 3.38 mmol) were added under argon. The mixture was refluxed for another 4 hours then acidified to pH 2-3 and evaporated under reduced pressure. The residue was purified by chromatography on a column of silicagel (10 g) eluted with increasing amounts of methanol in methylene chloride to yield (8S,4S)-2-(1'-N-benzyloxycarbonylamino-2'-*p*-methoxyphenyl)-ethyl-4-carboxy-3,4,5,6-tetrahydro-pyrimidine $1y_1$ (226 mg, 0.55 mmol, 41 %) (or (8R,4S)-2-(1'-N-benzyloxycarbonylamino-2'-p-methoxyphenyl)-ethyl-4-carboxy-3,4,5,6-tetrahydropyrimidine $1y_2$ (45 %)).

TLC: Rf 0.32 (methylene chloride-methanol 9:1, v/v).

(8S,4S)-2-(1'-N-benzyloxycarbonylamino-2'-p-methoxyphenyl)-ethyl-4-carboxy-3,4,5,6-tetrahydro-pyrimidine ($1y_1$)

LSIMS-MS Mass Measurement: Found $(M+H)^+$ = 412.187765; $C_{22}H_{26}N_3O_5$ requires 412.187246

^{1}H NMR (200 MHz, CD_3OD): 1.90 -2.20 (m, 2 H, H-5); 2.92 - 3.15 (m, 2 H, H-9); 3.20 - 3.35 (m, 2 H, H-6); 3.74 (s, 3 H, OMe); 3.93 (t, *J* = 5.6 Hz, 1 H, H-4); 4.58 (t, *J*= 7.9 Hz, 1 H, H-8); 4.99 (d, *J* = 12;5 Hz,); 5.09 (d, *J* = 12;5 Hz, H10b); 6.83 (d, *J* = 8.7 Hz, 2 H, H-3" and H-5" tyrosine); 7.22 (d, *J* = 8.7 Hz, 2 H, H-2" & H-6" tyrosine) 7.25 - 7.32 (m, 5 H, H-2', H-3', H-4', H-5' & H-6').

^{13}C NMR (125 MHz CD_3OD): 22.63 (C5); 38.46 (C9); 38.82 (C6); 54.14 (C4); 55.72 (OMe); 56.51 (C8); 68.19 (C10); 115.18 (C3", C5" Tyr); 128.34 (C1" Tyr); 128.81* (C2', C6'); 129.25 (C4'); 129.55* (C3', C5'); 131.59 (C2", C6" Tyr); 137.67 (C1'); 158.07 (C4" Tyr); 160.58 (O-CO-N); 164.65 (HN-C=N); 175.93 (COOH).

$[\alpha]_D$ = +64° (c 0.9 methanol/HCl 2%).

Calculated for $C_{22}H_{25}N_3O_5$, 1.5 HCl, $1H_2O$	C% 54.57	H% 5.93	N% 8.69
Found	C% 54.52	H% 5.75	N% 8.66

(8R,4S)-2-(1'-N-benzyloxycarbonylamino-2'-p-methoxyphenyl)-ethyl-4-carboxy-3,4,5,6-tetrahydro-pyrimidine ($1y_2$)

LSIMS-MS Mass Measurement: Found $(M+H)^+$ = 412.187345; $C_{22}H_{26}N_3O_5$ requires 412.187246

^{1}H NMR (200 MHz, CD_3OD): 1.88-2.20 (m, 2 H, H-5); 2.90-3.24 (m, 2 H, H-9); 3.27 - 3.38 (m, 2 H, H-6); 3.75 (s, 3 H, OMe); 4.00 (t, *J* = 5.67 Hz, 1 H, H-4); 4.65 (dd, *J*= 5.97 Hz & 9.4 Hz, 1 H, H-8); 4.95 (d, *J* = 12;5 Hz, H10a CH_2-benzyloxycarbonyl); 5.08 (d, *J* = 12;5 Hz, H10b CH_2-benzyloxycarbonyl); 6.84 (d, *J* = 8.6 Hz, 2 H, H-3" and H-5" tyrosine); 7.21 (d, *J* = 8.6 Hz, 2 H, H-2" & H-6" tyrosine); 7.24 - 7.33 (m, 5 H H-2', H-3', H-4', H-5' & H-6').

^{13}C NMR (125 MHz CD3OD): 22.68 (C5); 38.46 (C9); 39.00 (C6); 54.18 (C4); 55.73 (OMe); 56.08 (C8); 68.11 (C10); 115.17 (C3", C5" Tyr); 128.57 (C1" Tyr); 128.81* (C2', C6'); 129.15 (C4'); 129.53* (C3', C5'); 131.47 (C2", C6" Tyr); 137.67 (C1'); 158.04 (C4" Tyr); 160.49 (O-CO-N); 164.63 (HN-C=N); 175.54 (COOH).

$[\alpha]_D$ = -6.4° (c 0.9 methanol/HCl 2%).

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