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(54) **Titre : CELLULES RECOMBINANTES FABRIQUANT DES ACIDES ω -AMINOCARBOXYLIQUES, DES ESTERS D'ACIDES ω -AMINOCARBOXYLIQUES OU LEURS LACTAMES**

(54) **Title: RECOMBINANT CELLS PRODUCING ω -AMINOCARBOXYLIC ACIDS, ω -AMINOCARBOXYLIC ACID ESTERS OR THEIR LACTAMS**

(57) **Abrégé/Abstract:**

The present invention relates to a cell, which has been genetically modified relative to its wild type, so that in comparison with its wild type it is able to produce more ω -aminocarboxylic acids, more ω -aminocarboxylic acid esters or more lactams derived from ω -aminocarboxylic acids, starting from carboxylic acids or carboxylic acid esters. Furthermore, the present invention relates to a method for the production of a genetically modified cell, the cells obtainable by this method, a method for the production of ω -aminocarboxylic acids, of ω -aminocarboxylic acid esters or of lactams derived from ω -aminocarboxylic acids, the ω -aminocarboxylic acids, ω -aminocarboxylic acid esters or lactams derived from ω -aminocarboxylic acids obtainable by this method, a method for the production of polyamides based on ω -aminocarboxylic acids or based on lactams and the polyamides obtainable by this method.



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ABSTRACT

The present invention relates to a cell, which has been genetically modified relative to its wild type, so that in comparison with its wild type it is able to produce more ω -aminocarboxylic acids, more ω -aminocarboxylic acid esters or more lactams derived from ω -aminocarboxylic acids, starting from carboxylic acids or carboxylic acid esters. Furthermore, the present invention relates to a method for the production of a genetically modified cell, the cells obtainable by this method, a method for the production of ω -aminocarboxylic acids, of ω -aminocarboxylic acid esters or of lactams derived from ω -aminocarboxylic acids, the ω -aminocarboxylic acids, ω -aminocarboxylic acid esters or lactams derived from ω -aminocarboxylic acids obtainable by this method, a method for the production of polyamides based on ω -aminocarboxylic acids or based on lactams and the polyamides obtainable by this method.

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RECOMBINANT CELLS PRODUCING ω -AMINOCARBOXYLIC ACIDS, ω -AMINOCARBOXYLIC ACID ESTERS OR THEIR LACTAMS

The present invention relates to cells that are genetically modified relative to their wild type, a method for the production of a genetically modified cell, the cells obtainable by this method, a method for the production of ω -aminocarboxylic acids, ω -aminocarboxylic acid esters or of lactams derived from ω -aminocarboxylic acids, the ω -aminocarboxylic acids, ω -aminocarboxylic acid esters or lactams derived from ω -aminocarboxylic acids obtainable by this method, a method for the production of polyamides based on ω -aminocarboxylic acids or on lactams and the polyamides obtainable by this method.

Polyamides are polymers whose repeating units (monomers) possess the amide group as a characteristic feature. The designation "polyamides" is usually used to designate synthetic, commercially usable thermoplastics and therefore demarcates this class of substances from the chemically related proteins. Nearly all the important polyamides are derived from primary amines, i.e. the functional group $-\text{CO}-\text{NH}-$ occurs in their repeat units. Polyamides of secondary amines ($-\text{CO}-\text{NR}-$, R = organic residue) also exist. Aminocarboxylic acids, lactams and/or diamines and dicarboxylic acids in particular find application as monomers for the polyamides.

The production of polyamides on the basis of lactams is particularly important. Thus, "polyamide 6", a product that is widely used in industry, is obtained by ring opening polymerization of ϵ -caprolactam, whereas "polyamide 12", which is also industrially important, is obtained by ring opening polymerization of laurinelactam. Copolymers of lactams, such as copolymers of ϵ -caprolactam and laurinelactam ("polyamide 6/12") are also of considerable commercial importance.

The production of ϵ -caprolactam is usually carried out by reacting cyclohexanone with the hydrogensulphate or the hydrochloride of hydroxylamine with formation of cyclohexanone oxime. This is converted by a Beckmann rearrangement into ϵ -caprolactam, often with the use of concentrated sulphuric acid as catalyst. Cyclohexanone is usually produced by catalytic

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oxidation of cyclohexane with oxygen of the air, cyclohexane being obtained in its turn by hydrogenation of benzene.

The production of laurinlactam is particularly expensive. On an industrial scale this first involves the trimerization of butadiene, with formation of cyclododecatriene. The cyclododecatriene is then hydrogenated with formation of cyclododecane and the cyclododecane obtained is oxidized with formation of cyclododecanone. The cyclododecanone thus obtained is then reacted with hydroxylamine to cyclododecane oxime, which is then converted in a Beckmann rearrangement to laurinlactam.

The disadvantage of these methods known from the prior art for the production of lactams by Beckmann rearrangement of oximes is, among other things, that large amounts of salts, for example sodium sulphate, are formed as by-product, which requires disposal. Therefore other methods for the production of lactams are also described in the prior art, which do not have these disadvantages. Thus, EP-A-0 748 797 describes a method for the production of lactams from dinitriles, in which the dinitrile is hydrogenated to aminonitrile and the aminonitrile is converted by cyclizing hydrolysis to the lactam. Molecular sieves, such as acid zeolites, silicates and non-zeolitic molecular sieves, metal phosphates and metal oxides or mixed metal oxides have been disclosed as catalyst for cyclizing hydrolysis. However, this method has, among other drawbacks, the disadvantage that the selectivity of the conversion of the aminonitrile by cyclizing hydrolysis is rather low and therefore large amounts of by-products are formed. Furthermore, in the methods for the production of lactams described from this prior art, hydrocarbons such as benzene or butadiene are used, which are obtained by cracking gasoline or petroleum and therefore are not derived from renewable raw materials. The production of polyamides, which are based on lactams produced in this way, is therefore to be regarded as disadvantageous from the environmental standpoint.

The present invention was based on the aim of overcoming the disadvantages arising from the prior art.

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In particular the present invention was based on the aim of providing a method by which lactams, in particular laurinlactam, can be formed in the fewest possible steps and with formation of the minimum possible amount of by-products.

Another aim of the present invention was to provide a method by which lactams, in particular
5 laurinlactam, can be produced from renewable raw materials.

A contribution to achievement of the aforementioned aims is provided by a cell, which has been genetically modified relative to its wild type so that, in comparison with its wild type, it is able to produce more ω -aminocarboxylic acids, ω -aminocarboxylic acid esters or more lactams derived from ω -aminocarboxylic acids, starting from carboxylic acids or carboxylic acid esters. Such a
10 cell can be used in order to produce ω -aminocarboxylic acids, ω -aminocarboxylic acid esters or lactams derived from ω -aminocarboxylic acids by fermentation from carboxylic acids or carboxylic acid esters, for example from lauric acid or lauric acid esters.

In an embodiment, the present invention relates to a cell, which has been genetically modified relative to its wild type so that, in comparison with its wild type, it is able to produce more
15 ω -aminocarboxylic acids, more ω -aminocarboxylic acid esters or more lactams derived from ω -aminocarboxylic acids, starting from carboxylic acids or carboxylic acid esters, wherein the cell has increased activity, in comparison with its wild type, of the enzymes E_I and E_{III} or of the enzymes E_I , E_{II} and E_{III} : i) an enzyme E_I , which catalyses the conversion of carboxylic acids or carboxylic acid esters to the corresponding ω -hydroxycarboxylic acids or ω -hydroxycarboxylic acid esters,
20 selected from the group consisting of the alkane mono-oxygenases: *alkBGT*-encoded alkane monooxygenase from *Pseudomonas putida* GPo1 and cytochrome P450 monooxygenases from *Candida tropicalis*; ii) an enzyme E_{II} , which catalyses the conversion of ω -hydroxycarboxylic acids or ω -hydroxycarboxylic acid esters to the corresponding ω -oxocarboxylic acids or ω -oxocarboxylic acid esters, selected from the group consisting of the alcohol dehydrogenases encoded by the *alkJ*
25 gene; iii) an enzyme E_{III} , which catalyses the conversion of ω -oxocarboxylic acids or ω -oxocarboxylic acid esters to the corresponding ω -aminocarboxylic acids or ω -aminocarboxylic acid esters, selected from the group of the ω -transaminases.

In another embodiment, the present invention relates to a method for producing ω -aminocarboxylic acids, of ω -aminocarboxylic acid esters or of lactams derived from
30 ω -aminocarboxylic acids, containing the process steps: I) contacting the cell as described herein

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with a culture medium containing a carboxylic acid or a carboxylic acid ester or with a culture medium contiguous with an organic phase containing a carboxylic acid or a carboxylic acid ester in conditions that make it possible for the cell to form ω -aminocarboxylic acids, ω -aminocarboxylic acid esters or lactams derived from ω -aminocarboxylic acids, starting from
5 carboxylic acid or from carboxylic acid esters; and II) optionally isolation of the resultant ω -aminocarboxylic acids, the resultant ω -aminocarboxylic acid esters or the lactams derived from ω -aminocarboxylic acids.

In another embodiment, the present invention relates to a method for producing polyamides based on ω -aminocarboxylic acids, comprising the process steps: (α 1) production of ω -aminocarboxylic
10 acids by the method as described herein; and (α 2) polymerization of the ω -aminocarboxylic acid, obtaining a polyamide.

In another embodiment, the present invention relates to a method for producing polyamides based on lactams, comprising the process steps: (β 1) production of lactams by the method as described herein; and (β 2) ring opening polymerization or polycondensation of the lactam, obtaining a
15 polyamide.

The formulation "*that in comparison with its wild type it is able to produce more ω -aminocarboxylic acids, ω -aminocarboxylic acid esters or more lactams derived from ω -aminocarboxylic acids, starting from carboxylic acids or carboxylic acid esters*" also applies to the case when the wild type of the genetically modified cell is not able to form any
20 ω -aminocarboxylic acids, ω -aminocarboxylic acid esters or any lactams derived from ω -aminocarboxylic acids, or at least no detectable amounts of these compounds and it is only after the genetic modification that detectable amounts of these components can be formed.

A "*wild type*" of a cell preferably denotes a cell whose genome is in a state such as arose naturally by evolution. The term is used both for the whole cell and for individual genes. The term
25 "*wild type*" therefore in particular does not include such cells or such genes whose gene sequences have been altered at least partially by man by recombinant methods.

It is preferable according to the invention for the genetically modified cell to have been genetically modified so that in a defined time interval, preferably within 2 hours, still more preferably within 8 hours and most preferably within 24 hours, it forms at least twice, especially

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preferably at least 10 times, even more preferably at least 100 times, and yet more preferably at least 1000 times and most preferably at least 10000 times more ω -aminocarboxylic acids, ω -aminocarboxylic acid esters or lactams derived from ω -aminocarboxylic acids than the wild-type cell. The increase in product formation can be determined for example by cultivating the cell according to the invention and the wild-type cell each separately under the same conditions (same cell density, same nutrient medium, same culture conditions) for a specified time interval in a suitable nutrient medium and then determining the amount of target product (ω -aminocarboxylic acids, ω -aminocarboxylic acid esters or lactams derived from ω -aminocarboxylic acids) in the nutrient medium.

The cells according to the invention can be prokaryotes or eukaryotes. They can be mammalian cells (such as human cells), plant cells or microorganisms such as yeasts, fungi or bacteria, microorganisms being especially preferred and bacteria and yeasts being most preferred.

Suitable bacteria, yeasts or fungi are in particular those bacteria, yeasts or fungi that have been deposited in the German Collection of Microorganisms and Cell Cultures (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, abbreviated to DSMZ), Brunswick, Germany, as strains of bacteria, yeasts or fungi. Suitable bacteria according to the invention belong to the genera listed at

<http://www.dsmz.de/species/bacteria.htm>

suitable yeasts according to the invention belong to the genera listed at

<http://www.dsmz.de/species/yeasts.htm>

and suitable fungi according to the invention are those listed at

<http://www.dsmz.de/species/fungi.htm>

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Cells that are especially preferred according to the invention are derived from cells of the genera *Corynebacterium*, *Brevibacterium*, *Bacillus*, *Lactobacillus*, *Lactococcus*, *Candida*, *Pichia*, *Kluveromyces*, *Saccharomyces*, *Escherichia*, *Zymomonas*, *Yarrowia*, *Methylobacterium*, *Ralstonia*, *Pseudomonas*, *Burkholderia* and *Clostridium*, with *Escherichia coli*, *Corynebacterium glutamicum* and *Pseudomonas putida* being especially preferred and *Escherichia coli* being most preferred.

According to a preferred embodiment of the cell according to the invention the latter displays, in comparison with its wild type, increased activity of at least one of the following enzymes:

- i) an enzyme E_I, which catalyses the conversion of carboxylic acids or carboxylic acid esters to the corresponding ω -hydroxycarboxylic acids or ω -hydroxycarboxylic acid esters;
- ii) an enzyme E_{II}, which catalyses the conversion of ω -hydroxycarboxylic acids or ω -hydroxycarboxylic acid esters to the corresponding ω -oxocarboxylic acids or ω -oxocarboxylic acid esters;
- iii) an enzyme E_{III}, which catalyses the conversion of ω -oxocarboxylic acids or ω -oxocarboxylic acid esters to the corresponding ω -aminocarboxylic acids or ω -aminocarboxylic acid esters.

The term "*increased activity of an enzyme*", as used above in connection with the enzyme E_I and hereinafter in connection with the enzymes E_{II} etc., is preferably to be understood as increased intracellular activity.

The following account regarding the increase in enzyme activity in cells applies both to the increase in activity of the enzyme E_I and to all the enzymes stated subsequently, whose activity can possibly be increased.

Basically, an increase in enzymatic activity can be achieved by increasing the copy number of the gene sequence or gene sequences that code for the enzyme, using a strong promoter or employing a gene or allele that codes for a corresponding enzyme with increased activity and optionally by combining these measures. Genetically modified cells according to the invention

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are for example produced by transformation, transduction, conjugation or a combination of these methods with a vector that contains the desired gene, an allele of this gene or parts thereof and a vector that makes expression of the gene possible. Heterologous expression is in particular achieved by integration of the gene or of the alleles in the chromosome of the cell or an extrachromosomally replicating vector.

A review of possible ways of increasing the enzyme activity in cells for the example of pyruvate carboxylase is given in DE-A-100 31 999, and whose disclosures with respect to the possibilities for increasing the enzyme activity in cells forms part of the disclosure of the present invention.

The expression of the aforementioned and all subsequently mentioned enzymes or genes can be detected by means of 1- and 2-dimensional protein gel separation and subsequent optical identification of the protein concentration in the gel using appropriate evaluation software. If the increase in enzyme activity is based exclusively on an increase in expression of the corresponding gene, the increase in enzyme activity can be quantified in a simple way by comparing the 1- or 2-dimensional protein separations between wild type and genetically modified cell. A usual method for the preparation of protein gels in the case of coryneform bacteria and for identification of the proteins is the procedure described by Hermann et al. (*Electrophoresis*, 22: 1712-23 (2001)). The protein concentration can also be analysed by Western blot hybridization with an antibody that is specific for the protein that is to be detected (Sambrook et al., *Molecular Cloning: a laboratory manual*, 2nd Ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. USA, 1989) followed by optical evaluation with appropriate software for determination of concentration (Lohaus and Meyer (1989) *Biospektrum*, 5: 32-39; Lottspeich (1999), *Angewandte Chemie* 111: 2630-2647). The activity of DNA-binding proteins can be measured by DNA-Band-Shift-Assays (also called gel retardation) (Wilson et al. (2001) *Journal of Bacteriology*, 183: 2151-2155). The action of DNA-binding proteins on the expression of other genes can be detected by various well-described methods of reporter gene assay (Sambrook et al., *Molecular Cloning: a laboratory manual*, 2nd Ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. USA, 1989). Intracellular enzymatic activities can be determined by various methods that have been described (Donahue et al. (2000) *Journal of*

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Bacteriology 182 (19): 5624-5627; Ray et al. (2000) *Journal of Bacteriology* 182 (8): 2277-2284; Freedberg et al. (1973) *Journal of Bacteriology* 115 (3): 816-823). If in the subsequent account no concrete methods are stated for determination of the activity of a particular enzyme, the increase in enzyme activity as well as the decrease in enzyme activity are preferably determined by the methods described in Hermann et al., *Electrophoresis*, 22: 1712-23 (2001), Lohaus et al., *Biospektrum* 5 32-39 (1998), Lottspeich, *Angewandte Chemie* 111: 2630-2647 (1999) and Wilson et al., *Journal of Bacteriology* 183: 2151-2155 (2001).

If the increase in enzyme activity is brought about by mutation of the endogenous gene, such mutations can either be produced undirected according to classical methods, such as by UV-irradiation or by mutation-causing chemicals, or purposefully by genetic engineering methods such as deletion(s), insertion(s) and/or nucleotide exchange(s). Genetically modified cells are obtained as a result of these mutations. Especially preferred mutants of enzymes are in particular also enzymes for which feedback inhibition is no longer present or at least is reduced in comparison with the wild-type enzyme.

If the increase in enzyme activity is brought about through an increase in expression of an enzyme, then for example we increase the copy number of the corresponding genes or mutate the promoter and regulating region or the ribosome binding site, which is located upstream of the structural gene. Expression cassettes that are inserted upstream of the structural gene work in this way. By means of inducible promoters it is additionally possible to increase the expression at any time. Moreover, the enzyme gene can also be assigned, as regulatory sequences, so-called "*enhancers*", which as a result of improved interaction between RNA-polymerase and DNA also bring about increased gene expression. Expression is also improved by measures for extending the life of the m-RNA. Furthermore, by preventing the degradation of the enzyme protein, enzyme activity is also intensified. The genes or gene constructs are then either contained in plasmids with varying copy number or are integrated in the chromosome and amplified. Alternatively, overexpression of the relevant genes can in addition be achieved by altering the composition of the medium and the culture conditions. A person skilled in the art will find instructions for this in, *inter alia*, Martin et al. (*Bio/technology* 5, 137-146 (1987)), Guerrero et al. (*Gene* 138, 35-41 (1994)), Tsuchiya and Morinaga (*Bio/technology* 6, 428-430 (1988)),

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Eikmanns et al. (*Gene* 102, 93-98 (1991)), in EP-A-0 472 869, in US 4,601,893, in Schwarzer and Pühler (*Bio/technology* 9, 84-87 (1991), in Reinscheid et al. (*Applied and Environmental Microbiology* 60, 126-132 (1994)), in LaBarre et al. (*Journal of Bacteriology* 175, 1001-1007 (1993)), in WO-A-96/15246, in Malumbres et al. (*Gene* 134, 15-24 (1993), in JP-A-10-229891, in Jensen and Hammer (*Biotechnology and Bioengineering* 58, 191-195 (1998)) and in known textbooks of genetics and molecular biology. The measures described above also lead, like mutations, to genetically modified cells.

Episomal plasmids, for example, are used for increasing the expression of the genes in question. Suitable plasmids are in particular those that are replicated in coryneform bacteria. Numerous known plasmid vectors, for example pZ1 (Menkel et al., *Applied and Environmental Microbiology* 64: 549-554 (1989)), pEKEx1 (Eikmanns et al., *Gene* 107: 69-74 (1991)) or pHS2-1 (Sonnen et al., *Gene* 107: 69-74 (1991)) are based on the cryptic plasmids pHM1519, pBL1 or pGA1. Other plasmid vectors, for example those based on pCG4 (US 4,489,160) or pNG2 (Serwold-Davis et al., *FEMS Microbiology Letters* 66: 119-124 (1990)) or pAG1 (US 5,158,891), can be used in the same way.

Furthermore, plasmid vectors are also suitable, by means of which we can apply the method of gene amplification by integration into the chromosome, as was described for example by Reinscheid et al. (*Applied and Environmental Microbiology* 60: 126-132 (1994)) for the duplication or amplification of the *hom-thrB* operon. In this method the complete gene is cloned into a plasmid vector, which can be replicated in a host (typically *Escherichia coli*), but not in *Corynebacterium glutamicum*. Vectors that may be considered are for example pSUP301 (Simon et al., *Bio/Technology* 1: 784-791 (1983)), pK18mob or pK19mob (Schäfer et al., *Gene* 145: 69-73 (1994)), pGEM-T (Promega Corporation, Madison, Wisconsin, USA), pCR2.1-TOPO (Shuman, *Journal of Biological Chemistry* 269: 32678-84 (1994)), pCR[®]Blunt (Invitrogen, Groningen, The Netherlands), pEM1 (Schrumpf et al., *Journal of Bacteriology* 173: 4510-4516)) or pBGS8 (Spratt et al., *Gene* 41: 337-342 (1986)). The plasmid vector that contains the gene to be amplified is then transferred by conjugation or transformation into the desired strain of *Corynebacterium glutamicum*. The method of conjugation is described for example in Schäfer et al., *Applied and Environmental Microbiology* 60: 756-759 (1994). Methods for transformation

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are described for example in Thierbach et al., *Applied Microbiology and Biotechnology* 29: 356-362 (1988), Dunican and Shivnan, *Bio/Technology* 7: 1067-1070 (1989) and Tauch et al., *FEMS Microbiology Letters* 123: 343-347 (1994). After homologous recombination by a "cross-over" event, the resultant strain contains at least two copies of the relevant gene.

The formulation used in the above and hereinafter "*increased activity of an enzyme E_x relative to its wild type*" preferably always means activity of the respective enzyme E_x that is increased by a factor of at least 2, especially preferably of at least 10, even more preferably of at least 100, yet more preferably of at least 1000 and most preferably of at least 10000. Furthermore, the cell according to the invention, which has "*increased activity of an enzyme E_x relative to its wild type*", in particular also comprises a cell whose wild type has no or at least no detectable activity of this enzyme E_x and only displayed a detectable activity of this enzyme E_x after the enzyme activity was increased, for example through overexpression. In this connection the term "*overexpression*" or the formulation "*increase in expression*" used hereinafter also includes the case when a starting cell, for example a wild-type cell, has no or at least no detectable expression and it is only by recombinant methods that a detectable expression of the enzyme E_x is induced.

Furthermore, according to the invention it is preferable for the cell to have increased activity of enzymes E_I and E_{II} , enzymes E_I and E_{III} , enzymes E_{II} and E_{III} or even increased activity of all the enzymes E_I , E_{II} and E_{III} .

Furthermore, in connection with the aforementioned preferred embodiment of the cell according to the invention it is preferable for

- enzyme E_I to be an alkane monooxygenase or a xylene monooxygenase, or, preferably and
- enzyme E_{II} to be an alkane monooxygenase, an alcohol dehydrogenase or an alcohol oxidase, or, preferably and
- enzyme E_{III} to be a ω -transaminase.

A preferred enzyme E_I , in particular a preferred alkane monooxygenase is the alkane monooxygenase encoded by the *alkBGT* gene from *Pseudomonas putida* GPO1. The isolation of

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the *alkBGT* gene sequence is described for example by van Beilen et al. in "*Functional Analysis of Alkane Hydroxylases from Gram-Negative and Gram-Positive Bacteria*", Journal of Bacteriology, Vol. 184 (6), pages 1733-1742 (2002). Furthermore, cytochrome P450 monooxygenases, in particular cytochrome P450 monooxygenases from *Candida*, for example from *Candida tropicalis*, or from plants, for example from the chick-pea (*Cicer arietinum L.*), can also be used as alkane monooxygenases. The gene sequences of suitable cytochrome P450 monooxygenases from *Candida tropicalis* are for example disclosed in WO-A-00/20566, whereas the gene sequences of suitable cytochrome P450 monooxygenases from the chick-pea are given for example by Barz et al. in "*Cloning and characterization of eight cytochrome P450 cDNAs from chickpea (Cicer arietinum L.) cell suspension cultures*", Plant Science, Vol. 155, pages 101-108 (2000). Other homologues of the *alkB* gene are also given by van Beilen et al. in "*Oil & Gas Science and Technology*", Vol. 58 (4), pages 427-440 (2003). A suitable gene for a xylene monooxygenase is for example the *xylM* or the *xylA* gene, and a plasmid containing these two genes has the GENBANK Accession No. M37480.

A preferred enzyme E_{II}, in particular a preferred alcohol dehydrogenase is for example the alcohol dehydrogenase encoded by the *alkJ* gene (EC 1.1.99-2), in particular the alcohol dehydrogenase encoded by the *alkJ* gene from *Pseudomonas putida* GPo1. The gene sequences the alcohol dehydrogenase encoded by the *alkJ* gene from *Pseudomonas putida* GPo1, *Alcanivorax borkumensis*, *Bordetella parapertussis*, *Bordetella bronchiseptica* or from *Roseobacter denitrificans* can be found for example in the KEGG gene databank.

Suitable ω -transaminases are for example the ω -transaminases that are characterized in US-A-2007/0092957 by the sequence numbers 248, 250, 252 and 254.

A preferred enzyme E_{III}, in particular a preferred ω -transaminase is in particular the ω -transaminase from *Chromobacterium violaceum* DSM30191 (Kaulmann et al., 2007; "*Substrate spectrum of ω -transaminase from Chromobacterium violaceum DSM30191 and its potential for biocatalysis*", *Enzyme and Microbial Technology*, Vol. 41, pages 628-637), which is encoded by the gene sequence according to SEQ ID No. 01.

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It can be advantageous to use, as enzyme E_{III}, ω -transaminases that can be isolated from plants.

The ω -transaminases from plants selected from the group comprising *Arabidopsis thaliana*, *Avena sativa*, *Beta vulgaris*, *Glycine max*, *Hordeum vulgare*, *Lotus japonicus*, *Solanum lycopersicum*, *Manihot esculenta*, *Oryza sativa*, *Triticum aestivum*, *Zea mays*, *Spinacia oleracea*, *Arum maculatum*, *Mercurialis perennis* and *Urtica dioica*, are preferred here, and *Arabidopsis thaliana* is especially preferred. Enzymes that are encoded by nucleic acids that have 90%, preferably 95%, especially preferably 99 and quite especially preferably 100% identity to the sequence according to SEQ ID No. 39, are suitable in particular as ω -transaminases. The "nucleotide identity" relative to SEQ ID No. 39 is determined using known methods. In general, special computer programs with algorithms are used, taking into account special requirements. Preferred methods for determination of identity first produce the greatest agreement between the sequences to be compared. Computer programs for determination of identity comprise, but are not restricted to, the GCG software package, including

- GAP (Deveroy, J. et al., Nucleic Acid Research 12 (1984), page 387, Genetics Computer Group University of Wisconsin, Madison (WI), and
- BLASTP, BLASTN and FASTA (Altschul, S. et al., Journal of Molecular Biology 215 (1990), pages 403-410. The BLAST program can be obtained from the National Center for Biotechnology Information (NCBI) and from other sources (BLAST Manual, Altschul S. et al., NCBI NLM NIH Bethesda ND 22894; Altschul S. et al., as above).

The well-known Smith-Waterman algorithm can also be used for determining nucleotide identity.

Preferred parameters for nucleotide comparison comprise the following:

- Algorithm Needleman and Wunsch, Journal of Molecular Biology 48 (1970), pages 443-453

- Comparison matrix

Matches = + 10

Mismatches = 0

Gap penalty = 50

Gap length penalty = 3

The GAP program is also suitable for use with the parameters given above. The aforementioned parameters are the default parameters in the nucleotide sequence comparison.

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Moreover, enzymes from the subgroup of the β -Ala:pyruvate transaminases are suitable. These include e.g. transaminases from *Pseudomonas putida* W619 (gi: 119860707, gi: 119855857, gi: 119856278), from *Pseudomonas putida* KT2440 (gi: 24984391), from *Pseudomonas aeruginosa* PA01 (gi 15595330, gi: 15600506, gi 15595418, gi 9951072); *Streptomyces coelicolor* A3(2) (gi: 3319731), *Streptomyces avermitilis* MA 4680 (gi: 29831094, gi: 29829154) and *Chromobacterium violaceum* ATCC 12472 (gi 34102747). The amino acid sequences of the aforementioned transaminases are presented in the sequences according to SEQ ID No. 19 to SEQ ID No. 30.

For the case when the cells according to the invention are to be used for the production of ω -aminocarboxylic acids, ω -aminocarboxylic acid esters or lactams based on ω -aminocarboxylic acids starting from carboxylic acid esters, it is moreover advantageous if the cell according to the invention has, in addition to increased activity of at least one of the enzymes E_I , E_{II} and E_{III} , preferably in addition to increased activity of the enzymes E_I and E_{III} or E_I , E_{II} and E_{III} , also increased activity of an enzyme E_{IV} , which catalyses the conversion of ω -aminocarboxylic acid esters to the corresponding ω -aminocarboxylic acids, said enzyme E_{IV} preferably being an esterase, which preferably is secreted by the cell. Secretion of the esterase by the cell has the advantage that the ester bond is only cleaved outside of the cell. This ensures that, owing to the better membrane permeability of the ω -aminocarboxylic acid ester compared with ω -aminocarboxylic acid, sufficient target product leaves the cell and can be transferred to the nutrient medium surrounding the cell.

Preferred esterases according to the invention are in particular lipase, and as an example of a suitable lipase we may mention the lipase LipA from *Pseudomonas fluorescens* HU380 (ACC Code Q76D26, Kojima and Shimizu, "Purification and Characterization of the Lipase from *Pseudomonas fluorescens* HU380", *Journal of Bioscience and Bioengineering*, Volume 96 (3), pages 219-226 (2003)). In order to ensure that the esterases are secreted, they can be provided, in a manner known by a person skilled in the art, with corresponding signal sequences, which establish secretion. If for example the aforementioned lipase LipA from *Pseudomonas fluorescens* HU380 is overexpressed in *E. coli*, it can be provided advantageously with signal

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sequences from EstA, an esterase that occurs naturally on the cell surface of *Pseudomonas aeruginosa* (Becker et al., "A generic system for the *Escherichia coli* cell-surface display of lipolytic enzymes", *FEBS Letters*, Vol. 579, pages 1177-1182 (2005)). Other suitable enzymes are lipases from *C. antarctica*, *M. miehei* and *P. cepacia* (Vaysse et al., "Enzyme and Microbial Technology", Vol. 31, pages 648-655 (2002)).

Alternatively the secreted ω -aminocarboxylic acid ester can also be cleaved conventionally, to obtain the ω -aminocarboxylic acid, for example by saponification, i.e. hydrolysis of the ω -aminocarboxylic acid ester by the aqueous solution of a hydroxide, e.g. by sodium hydroxide.

Furthermore, it may prove advantageous according to the invention if the cell according to the invention, in addition to increased activity of at least one of the enzymes E_I , E_{II} and E_{III} , preferably in addition to increased activity of the enzymes E_I and E_{III} or E_I , E_{II} and E_{III} and optionally also in addition to increased activity of the aforementioned enzyme E_{IV} , also has increased activity of an enzyme E_V , which catalyses the conversion of ω -aminocarboxylic acids to the corresponding lactams, and it can also be advantageous here if this enzyme E_V is secreted by the cell. In this way it can be possible for the ω -aminocarboxylic acids formed directly by the cell or the ω -aminocarboxylic acid that is only formed after extracellular cleavage of ω -aminocarboxylic acid esters to be converted to the corresponding lactam, thus optionally facilitating purification of the target product.

According to another, special embodiment of the cell according to the invention, it has, in addition to increased activity of one or more of the enzymes E_I , E_{II} or E_{III} and optionally increased activity of the enzyme E_{IV} and/or E_V , also increased activity of an enzyme E_{VI} , which catalyses the conversion of an α -ketocarboxylic acid to an amino acid, said enzyme E_{VI} preferably being an amino acid dehydrogenase. Such a modification of the cell would have the advantage that in the case when amino acids are used as donor for the NH_2 group, which is consumed during the transaminase (E_{III})–mediated reaction of an ω -oxocarboxylic acid or an ω -oxocarboxylic acid ester to the corresponding ω -aminocarboxylic acid, to the corresponding ω -aminocarboxylic acid ester or to the corresponding ω -aminocarboxylic acid ester, can be correspondingly regenerated. Preferred, as amino acid dehydrogenase, is the alanine dehydrogenase from *B. subtilis* (EC No. 1.4.1.1; Gene ID: 936557), which is encoded by the

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gene sequence according to SEQ ID No. 02. Other suitable amino acid dehydrogenases are serine dehydrogenases, aspartate dehydrogenases, phenylalanine dehydrogenases and glutamate dehydrogenases.

A contribution to achievement of the aims stated at the beginning is also provided by a method for the production of a genetically modified cell, comprising the process step of increasing the activity of at least one of the following enzymes:

- i) an enzyme E_I, which catalyses the conversion of carboxylic acids or carboxylic acid esters to the corresponding ω -hydroxycarboxylic acids or ω -hydroxycarboxylic acid esters,
- ii) an enzyme E_{II}, which catalyses the conversion of ω -hydroxycarboxylic acids or ω -hydroxycarboxylic acid esters to the corresponding ω -oxocarboxylic acids or ω -oxocarboxylic acid esters, or
- iii) an enzyme E_{III}, which catalyses the conversion of ω -oxocarboxylic acids or ω -oxocarboxylic acid esters to the corresponding ω -aminocarboxylic acids or ω -aminocarboxylic acid esters,

in a cell, with the enzyme activities preferably being increased by the methods described at the beginning.

According to a special embodiment of the method described above, in this method, in addition to the increase in activity of the enzymes E_I, E_{II} and/or E_{III}, the activity of an enzyme E_{IV}, which catalyses the conversion of ω -aminocarboxylic acid esters to the corresponding ω -aminocarboxylic acids, and/or of an enzyme E_V, which catalyses the conversion of ω -aminocarboxylic acids to the corresponding lactams, is also increased by increasing the expression of these enzymes, with the enzymes E_{IV} and/or E_V preferably being secreted by the cell.

A contribution to achievement of the aims stated at the beginning is also provided by the genetically modified cells that are obtainable by the method described above.

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Another contribution to achievement of the cells stated at the beginning is provided by a method for the production of ω -aminocarboxylic acids, of ω -aminocarboxylic acid esters or of lactams derived from ω -aminocarboxylic acids, containing the process steps:

- I) contacting a cell according to the invention with a culture medium containing a carboxylic acid or a carboxylic acid ester or with a culture medium contiguous with an organic phase containing a carboxylic acid or a carboxylic acid ester in conditions that enable the cell to form ω -aminocarboxylic acids, ω -aminocarboxylic acid esters or lactams derived from ω -aminocarboxylic acids, from the carboxylic acid or from the carboxylic acid esters;
- II) optionally isolation of the resultant ω -aminocarboxylic acids, ω -aminocarboxylic acid esters or lactams derived from ω -aminocarboxylic acids.

In step I) of the method according to the invention the cells are first brought into contact with a culture medium containing a carboxylic acid or a carboxylic acid ester or with a culture medium contiguous with an organic phase containing a carboxylic acid or a carboxylic acid ester, and this contacting takes place under conditions that make it possible for the cell to form ω -aminocarboxylic acids, ω -aminocarboxylic acid esters or lactams derived from ω -aminocarboxylic acids, from the carboxylic acid or from the carboxylic acid esters.

The genetically modified cells according to the invention can be brought into contact with the nutrient medium, and therefore cultivated continuously or discontinuously in a *batch process* or in a *fed-batch process* or in a *repeated-fed-batch process*, for the purpose of producing ω -aminocarboxylic acids or lactams derived from ω -aminocarboxylic acids. A semi-continuous process is also conceivable, as described in GB-A-1009370. Known culture methods are described in Chmiel's textbook ("*Bioprozesstechnik 1. Einführung in die Bioverfahrenstechnik*" [Bioprocess Techniques 1. Introduction to Bioprocess Engineering] (Gustav Fischer Verlag, Stuttgart, 1991)) or in the textbook by Storhas ("*Bioreaktoren und periphere einrichtungen*" [Bioreactors and Peripheral Equipment], Vieweg Verlag, Brunswick/Wiesbaden, 1994).

The culture medium to be used must be suitable for the requirements of the particular strains. Descriptions of culture media for various microorganisms are given in "*Manual of Methods for*

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General Bacteriology" of the American Society for Bacteriology (Washington D.C., USA, 1981).

Apart from the carboxylic acids or carboxylic acid esters, the carbon source used can be carbohydrates, e.g. glucose, sucrose, lactose, fructose, maltose, molasses, starch and cellulose, oils and fats, e.g. soya oil, sunflower oil, peanut oil and coconut oil, fatty acids e.g. palmitic acid, stearic acid and linoleic acid, alcohols e.g. glycerol and methanol, hydrocarbons such as methane, amino acids such as L-glutamate or L-valine or organic acids e.g. acetic acid. These substances can be used separately or as a mixture. The use of carbohydrates, especially monosaccharides, oligosaccharides or polysaccharides, is especially preferred, as described in US 6,013,494 and US 6,136,576, and of C₅-sugars or glycerol.

Organic nitrogen-containing compounds such as peptones, yeast extract, meat extract, malt extract, corn-steep liquor, soybean flour and urea or inorganic compounds such as ammonium sulphate, ammonium chloride, ammonium phosphate, ammonium carbonate and ammonium nitrate can be used as the nitrogen source. The nitrogen sources can be used separately or as a mixture.

Phosphoric acid, potassium dihydrogen phosphate or dipotassium hydrogen phosphate or the corresponding sodium-containing salts can be used as the source of phosphorus. The culture medium must in addition contain salts of metals, for example magnesium sulphate or iron sulphate, which are required for growth. Finally, essential growth substances such as amino acids and vitamins are used in addition to the substances mentioned above. Furthermore, suitable precursors can be added to the culture medium. The stated substances can be added to the culture in the form of a single preparation, or they can be supplied in a suitable manner during cultivation.

Basic compounds such as sodium hydroxide, potassium hydroxide, ammonia or ammonia water or acid compounds such as phosphoric acid or sulphuric acid are used in a suitable manner for controlling the pH of the culture. Antifoaming agents, e.g. fatty acid polyglycol esters, are used for controlling foaming. To maintain plasmid stability, suitable selectively acting substances, e.g.

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antibiotics, can be added to the medium. To maintain aerobic conditions, oxygen or oxygen-containing gas mixtures, e.g. air, are fed into the culture. The temperature of the culture is normally in the range from 20°C to 45°C and preferably 25°C to 40°C.

According to an especially preferred embodiment of the method according to the invention for the production of ω -aminocarboxylic acids, of ω -aminocarboxylic acid esters or of lactams derived from ω -aminocarboxylic acids, in which a recombinant cell, derived from an *E. coli* cell, is used as the cell according to the invention, a mineral salt medium supplemented with ampicillin, chloramphenicol and kanamycin according to Riesenberget al., "*High cell density fermentation of recombinant Escherichia coli expressing human interferon alpha 1*", *Appl Microbiol and Biotechnol*, Vol. 34 (1), pages 77-82 (1990)) is used as the nutrient medium.

The contacting of the cells according to the invention with the culture medium in step I) preferably takes place in conditions that enable the cell to form ω -aminocarboxylic acids, ω -aminocarboxylic acid esters or lactams derived from ω -aminocarboxylic acids starting from carboxylic acid or from carboxylic acid esters. As carboxylic acids or carboxylic acid esters, consideration may be given in particular to carboxylic acids with number of carbons in the range from 6 to 20, especially preferably from 6 to 15, in particular from 6 to 12, lauric acid being especially preferred as carboxylic acid. As carboxylic acid esters, consideration may be given in particular to the methyl or ethyl esters of the aforementioned carboxylic acids, with the methyl ester of lauric acid being especially preferred as carboxylic acid ester.

In the production of the ω -aminocarboxylic acids, ω -aminocarboxylic acid esters or lactams derived from ω -aminocarboxylic acids, various procedures are conceivable in step I).

According to one embodiment of the method according to the invention, the cells are first cultivated, for the purpose of biomass production, in a nutrient medium that does not contain carboxylic acids or carboxylic acid esters, and in particular does not contain the aforementioned, preferred carboxylic acids or carboxylic acid esters. It is only after a certain biomass has been obtained that the carboxylic acids or the carboxylic acid esters are added to the nutrient medium or the cells are brought into contact with a new nutrient medium containing the carboxylic acids

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or carboxylic acid esters. In this connection it is in particular preferable for the content of carboxylic acids or carboxylic acid esters during the formation of ω -aminocarboxylic acids, of ω -aminocarboxylic acid esters or of lactams derived from ω -aminocarboxylic acids to be in the range from 1 to 200 g/l, especially preferably in the range from 20 to 200 g/l.

According to another embodiment of the method according to the invention, it is carried out in a two phase system, containing

- A) an aqueous phase, and
- B) an organic phase,

with the formation of the ω -aminocarboxylic acids, ω -aminocarboxylic acid esters or lactams derived from ω -aminocarboxylic acids by the cells in step I) taking place in the aqueous phase and with the resultant ω -aminocarboxylic acids, the resultant ω -aminocarboxylic acid esters or the resultant lactams derived from ω -aminocarboxylic acids accumulating in the organic phase. In this way it is possible for the resultant ω -aminocarboxylic acids, the resultant ω -aminocarboxylic acid esters or the resultant lactams derived from ω -aminocarboxylic acids to be extracted *in situ*.

Also in this embodiment of the method according to the invention, it may prove advantageous for the cells first to be cultivated, for the purpose of biomass production, in a nutrient medium that does not contain carboxylic acids or carboxylic acid esters, and in particular does not contain the aforementioned, preferred carboxylic acids or carboxylic acid esters. It is only after a certain biomass has been obtained that the cell suspension as aqueous phase A) is brought into contact with the organic phase B), where in particular the organic phase B) contains the carboxylic acid or the carboxylic acid esters preferably in an amount in the range from 1 to 200 g/l, especially preferably in the range from 20 to 200 g/l. However, if substrates that are not toxic to the cells used, such as methyl laurate, are employed as carboxylic acids or carboxylic acid esters, then the content of these carboxylic acids or carboxylic acid esters in the organic phase can also be significantly higher. In such a case it may also be possible to use the pure carboxylic acid or the pure carboxylic acid esters, for example pure methyl laurate, as organic phase.

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As organic phase, it is possible to use alkanes of medium chain length, preferably those with a logP value of more than 4 (little foam formation), or physically similar aromatics or aromatic esters, though preferably, as mentioned above, lauric acid esters, especially preferably methyl laurate, BEHP (bis(2-ethylhexyl)phthalate) or long-chain fatty acid esters (biodiesel).

Furthermore it is preferable according to the invention if, at least during the phase of formation of ω -aminocarboxylic acids, of ω -aminocarboxylic acid esters or of lactams derived from ω -aminocarboxylic acids, the culture medium used in step I) contains amino group donors, such as ammonia or ammonium ions or even amino acids, though in particular alanine or aspartate, which function as amine donors in the transaminase-catalysed conversion of the ω -oxocarboxylic acids or the ω -oxocarboxylic acid esters to the corresponding ω -aminocarboxylic acids or ω -aminocarboxylic acid esters.

In step II) of the method according to the invention, the resultant ω -aminocarboxylic acids, the resultant ω -aminocarboxylic acid esters or the lactams derived from the ω -aminocarboxylic acids are optionally isolated, and it is preferable for this isolation to take place in an at least two-stage purification process, comprising

- a) an extraction step, in which the ω -aminocarboxylic acids, the ω -aminocarboxylic acid esters or the lactams derived from ω -aminocarboxylic acids are extracted from the culture medium, and
- b) a fine purification step, in which the extract obtained in step a) is purified further by a distillation process or selective crystallization, obtaining an ω -aminocarboxylic acid phase, an ω -aminocarboxylic acid ester phase or a lactam phase with a purity of at least 99.8%.

The extraction in step a) can in particular be designed as so-called "*in situ*" extraction, in which steps I) and II) of the method according to the invention for the production of ω -aminocarboxylic acids, of ω -aminocarboxylic acid esters or of lactams derived from ω -aminocarboxylic acids are carried out simultaneously. This "*in situ*" extraction has already been described above.

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The fine purification in step II) can for example take place by distillation or crystallization.

In a special embodiment of the method according to the invention for the production of ω -aminocarboxylic acids, of ω -aminocarboxylic acid esters or of lactams derived from ω -aminocarboxylic acids, the ω -aminocarboxylic acid esters formed in step I) are reacted in another process step by conventional chemical methods to ω -aminocarboxylic acids; a preferred, conventional chemical method is saponification, in which the ω -aminocarboxylic acid ester is reacted with an aqueous solution of a base, preferably a hydroxide, preferably sodium hydroxide, to the ω -aminocarboxylic acid.

Preferably this method is used for the production of ω -aminolauric acid from lauric acid esters, preferably methyl laurate;

A contribution to achievement of the aims stated at the beginning is also provided by ω -aminocarboxylic acids, ω -aminocarboxylic acid esters or by lactams derived from ω -aminocarboxylic acids, which are obtainable by the method described above, the lactam preferably being laurinlactam, which is obtained if lauric acid or lauric acid esters are used as carboxylic acid or as carboxylic acid esters in step I) of the method according to the invention for the production of lactams derived from ω -aminocarboxylic acids, wherein the ω -aminocarboxylic acid is preferably ω -aminolauric acid and the ω -aminocarboxylic acid ester is preferably ω -aminomethyl laurate.

A contribution to achievement of the aims stated at the beginning is also provided by a method for the production of polyamides based on ω -aminocarboxylic acids, comprising the process steps:

- (α 1) production of ω -aminocarboxylic acids by one of the methods described above for the production of ω -aminocarboxylic acids, in particular by the method described above for the production of ω -aminolauric acid from lauric acid or lauric acid esters;
- (α 2) polymerization of the ω -aminocarboxylic acid, obtaining a polyamide.

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In step ($\alpha 2$) of the method according to the invention for the production of polyamides based on ω -aminocarboxylic acids, the ω -aminocarboxylic acids obtained in step ($\alpha 1$), in particular the ω -aminolauric acids obtained in step ($\alpha 1$), are converted in a polymerization to a polyamide, and optionally mixtures of various ω -aminocarboxylic acids can also be used, for which at least one of the ω -aminocarboxylic acids, but optionally all ω -aminocarboxylic acids were produced by the method according to the invention for the production of ω -aminocarboxylic acids.

The production of the polyamides from the ω -aminocarboxylic acids can be carried out by well-known methods, as described for example in L. Notarbartolo, Ind. Plast. Mod. 10(1958)2, p. 44, JP 01-074224, JP 01-051433, JP63286428, JP58080324 or JP60179425.

A contribution to achievement of the aims stated at the beginning is also provided by a method for the production of polyamides based on lactams, comprising the process steps:

- ($\beta 1$) production of lactams by the method described above for the production of lactams derived from ω -aminocarboxylic acids, in particular by the method described above for the production of laurinlactam from lauric acid or lauric acid esters;
- ($\beta 2$) ring opening polymerization or polycondensation of the laurinlactam, obtaining a polyamide.

In step ($\beta 2$) of the method according to the invention for the production of polyamides based on lactams, the lactams obtained in step ($\beta 1$), in particular the laurinlactam obtained in step ($\beta 1$), are converted in a ring opening polymerization or by polycondensation to a polyamide, and optionally it is also possible to use mixtures of various lactams, for example mixtures of laurinlactam and ϵ -caprolactam, for which at least one of the lactams, but optionally all lactams were produced by the method according to the invention for the production of lactams derived from ω -aminocarboxylic acids.

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The production of the polyamides from the lactams can be carried out by well-known methods, as described for example in DE-A-14 95 198, DE-A-25 58 480, EP-A-0 129 196 or also in "*Polymerization Processes*", Interscience, New York, 1977, pages 424-467, especially pages 444-446.

A contribution to achievement of the aims stated at the beginning is also provided by polyamides, which are obtainable by the methods described above. It is especially preferable for these polyamides to be based, up to at least 10 wt.%, especially preferably up to at least 25 wt.%, still more preferably up to at least 50 wt.% and most preferably up to at least 75 wt.%, on lauric acid, lauric acid ester or laurinlactam, obtained by the method according to the invention for the production of lauric acid, of lauric acid ester or of laurinlactam from lauric acid or lauric acid esters.

The invention will now be explained with the aid of non-limiting diagrams and examples.

Fig. 1 shows, schematically, a recombinant plasmid for chromosomal integration of the *alk* genes in *Escherichia coli* (tnp: transposase gene; bla: ampicillin resistance gene; oriT RP4: mobilization region; I and O mark the "*inverted repeats*" of the mini-transposon Tn5).

Fig. 2 shows, schematically, a recombinant plasmid for expression of transaminase and of alanine dehydrogenase under the control of an arabinose inducible promoter (bla: ampicillin resistance gene; CV2025: gene for ω -transaminase from *Chromobacterium violaceum*; ald: gene for alanine dehydrogenase from *B. subtilis*; araC: transcription regulator).

Fig. 3 shows, schematically, a recombinant plasmid for the expression of LipA in *E. coli* and presentation of the enzyme on the cell surface (colE1, ColE1: replication origin; *estA**, *estA*: gene with amino acid exchange alanine for serine (codon #38), cat: chloramphenicol resistance gene; phoA: gene segment that encodes the leader sequence of alkaline phosphatase).

Fig. 4 shows determination of the activity of *C. violaceum*-transaminase from the enzyme assay. The activity was determined in duplicate (active1, active2) with a photometer. A batch without

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the co-substrate L-alanine (w.Cos), a batch with heat-inactivated enzyme (inactive) and a batch from *E. coli* expression culture with empty vector (empty vector), purified similarly to the omega-TA, were used as negative controls.

Fig. 5 shows chromatograms of the substrate 12-aminomethyl laurate at the start of reference measurement (top) and after 2 h incubation with the purified transaminase (bottom).

Fig. 6 shows chromatograms of the substrate 12-aminomethyl laurate after 24 h (top) of the enzyme assay and after spiking the substrate (control, bottom).

Fig. 7 shows 6 chromatograms of the substrate 12-aminomethyl laurate after heat inactivation of the enzyme (top) and after 0h (bottom).

Fig. 8 shows the starting plasmid pGEc47, which was used as template for the amplification of alkBGTS.

Fig. 9 shows the primers used and the resultant PCR products alkBFG and alkT.

Fig.10 shows the recombinant vector pBT10, which was used for the synthesis of hydroxymethyl laurate and oxomethyl laurate.

Fig.11 shows a GC chromatogram of the 12-oxo-methyl laurate standard.

Fig.12 shows a GC chromatogram of the 12-hydroxymethyl laurate standard.

Fig.13 shows a chromatogram of the organic phase from a resting cell biotransformation in the bioreactor of methyl laurate, at time 0 min.

Fig.14 shows a chromatogram of the organic phase from a resting cell biotransformation in the bioreactor of methyl laurate, at time 150 min.

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Fig.15 shows the expression vector pGJ3130 with AT3G22200.

Fig. 16 shows detection of enzyme activity by coupled enzyme assay (inactive, heat-inactivated protein; w.Cos., without addition of alanine; akt, the purified, active enzyme).

Fig. 17 shows detection of the heterologously expressed protein AT3G22200 by HPLC. Top: product after 20 min incubation at 10.8 min; bottom: reference substance at 10.8 min.

Fig.18 shows the plasmid map of the expression vector pET-21a(+) with the transaminase gene ppta5 (pPPTA5).

Fig.19 shows the plasmid map of the expression vectors pET-21a(+) with the transaminase gene psta (pPSTA).

Fig. 20 shows the amination of 5 mM 12-oxomethyl laurate with the transaminases PPTA5. For the evaluation, the peak areas of 12-oxo- and 12-aminomethyl laurate from the chromatograms obtained from neutral and acid extraction were added together and the percentage of educt or product was calculated.

Fig.21 shows the amination of 5 mM 12-oxomethyl laurate with the transaminases PSTA. For the evaluation, the peak areas of 12-oxo- and 12-aminomethyl laurate from the chromatograms obtained from neutral and acid extraction were added together and the percentage of educt or product was calculated.

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EXAMPLES

A. Conversion of lauric acid or methyl laurate to laurinlactam

For the conversion of lauric acid or of methyl laurate to laurinlactam, *E. coli* is supplemented with the necessary enzymes monooxygenase, alcohol dehydrogenase, ω -transaminase, alanine dehydrogenase and a lipase. The enzymes are overexpressed in *E. coli*; the expression levels of the individual enzymes are dependent on the kinetics of the individual reactions and require optimum adjustment to one another. The expression level is adjusted by adding the appropriate amount of inductor. The expression of monooxygenase and of alcohol dehydrogenase is induced with n-octane; transaminase and alanine dehydrogenase are induced with arabinose and the lipase with IPTG.

A1. Cloning of the individual enzymes

Hydroxylation and aldehyde formation

The alkane hydroxylase system *alkBGT* from *Pseudomonas putida* GPo1 is used for the hydroxylation of lauric acid or of methyl laurate. The second step to the aldehyde is catalysed by the alcohol dehydrogenase *alkJ*.

The genes *alkBGJT* necessary for these reactions are integrated in *E. coli* by insertion into the mini-transposon Tn5 chromosomally into the genome of *E. coli* (de Lorenzo et al., *J Bacteriol.*, Vol. 172 (11), pages 6568-6572 and 6557-6567 (1990); Panke et al., *Appl and Environm. Microbiol.*, pages 5619-5623 (1999)). The genes are to be expressed under the control of the *alkB* promoter and of the positive regulator *alkS*. Transfer of the Tn5-*alkBGFJST* construct to the *E. coli* target organism is effected with the aid of the mobilizable plasmid pUT-Km (Panke et al., 1999, see above).

The *alkST* locus with the expression-relevant regulator *alkS* is organized outside of the *alkBFGHJKL* operon and arranged in the opposite direction in the genome of

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Pseudomonas putida. The arrangement of the genes is preserved during cloning into the transposon-bearing plasmid. The fragments *alkST* and *alkBFGJ* are integrated into the plasmid one after another.

The genes to be cloned *alkB* (SEQ ID No. 03), *alkG* (SEQ ID No. 04) and *alkJ* (SEQ ID No. 05) are indeed organized in *P. putida* together in the operon *alkBFGHJKL*, but are separated by the gene *alkH*, which encodes an aldehyde dehydrogenase. The desired intermediate, the aldehyde of lauric acid, would be broken down again by this enzyme and the latter must therefore be excluded from the cloning of the *alkBGJ* genes.

To simplify the cloning of *alkB* and *alkG*, the gene *alkF* located between them is amplified and cloned together with *alkB* and *alkG*. AlkF is of no significance for the reaction that is to be catalysed. The genes *alkBFG* and *alkJ* are amplified in two separate PCR steps and fused together by SOE-PCR (Horton et al., *Gene*, Vol. 77, pages 61-68 (1989)). The OCT plasmid from *Pseudomonas putida* GPo1 serves as target DNA.

A2. Cloning strategy:

PCR amplification of the fragments *alkST* = 4077 bp (SEQ ID No. 06 (*alkS*) and SEQ ID No. 07 (*alkT*)) with *NotI* cleavage site upstream of *alkT*:

Primers

alkT-forward-*NotI* (SEQ ID No. 08)

5' ACGTAGCGGCCGCCTAATCAGGTAATTTTATAC

alkS-reverse (SEQ ID No. 09)

5' GAGCGAGCTATCTGGT

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The PCR fragment is cloned into the transposon-bearing vector pUT-Km. For this, the vector is cut within *Tn5* with *NotI* and ligated with the *alkST* fragment by blunt end ligation. The recombinant plasmid is designated pUT-Km-*alkST*.

A3. Synthesis of *alkBFGJ* constructs by the SOE-PCR technique:

Synthesis of fragment 1: *alkBFG* + promoter upstream *alkB* and *NotI* cleavage site at the 5'-end (product size: 1409 bp):

Primers

alkBFG-forward-*NotI* (SEQ ID No. 10)

5' TCGTAGCGGCCCGCCCAGCAGACGACGGAGCAA

alkBFG-reverse-SOE (SEQ ID No. 11)

5' ATTTTATCTTCTCGAGGCTTTTCCTCGTAGAGCACAT

Synthesis of fragment 3, *alkJ* with complementary end to *alkG* at the 5'-end and *NotI* cleavage site at the 3'-end (product size: 1723 bp):

Primers

alkJ-forward-SOE (SEQ ID No. 12)

5' TGCTCTAACGAGGAAAAGCCTCGAGAAGATAAAATGTA

alkJ-reverse-*NotI* (SEQ ID No. 13)

5' ATTGACGCGGCCGCTTACATGCAGACAGCTATCA

The two separate fragments are fused together by SOE-PCR. (3 separate PCR reactions required). The recombinant plasmid pUT-Km-*alkST* and the *alkBFGJ* construct are cut with *NotI* and ligated. The new recombinant plasmid pUT-Km-*alkBGJST* (see Fig. 1) is

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transformed in *E. coli* HB101 and transferred to *E. coli* JM101 by conjugative plasmid transfer.

A4. Amination and amino donor regeneration

For amination to the ω -aminolauric acid ester and regeneration of the amino donor, the Tn5:*alkBGJST*-bearing *E. coli* strain JM101 is additionally transformed with the recombinant plasmid pBAD-CV2025-*ald*. This plasmid is based on the pBAD30 vector (Guzman et al., "Tight Regulation, Modulation, and High-Level Expression by Vectors Containing the Arabinose P_{BAD} Promoter", *J. Bacteriol.*, Vol. 177 (14), pages 4121-4130 (1995)). pBAD-CV2025-*ald* carries the gene for the transaminase CV2025 from *Chromobacterium violaceum* DSM30191 (SEQ ID No. 01; Kaulmann et al., *Enzyme and Microbial Technology* Vol. 41, pages 628-637 (2007) and the gene *ald*, which codes for an alanine dehydrogenase from *Bacillus subtilis subsp. Subtilis* (SEQ ID No. 02; NP_391071). The genes are under the control of an arabinose inducible promoter.

A5. Cloning strategy

PCR amplification of the transaminase gene with chromosomal DNA from *Chromobacterium violaceum* DSM30191 (product size: 1415 bp):

Primers

CV2025-forward-*SacII* (SEQ ID No. 14)

5' CGAGGAGCTCAGGAGGATCCAAGCATGCAGAAGCAACGTACG

CV2025-reverse-*KpnI* (SEQ ID No. 15)

5' GTCATGTACCCCTAAGCCAGCCCGCGCGCCT

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For the cloning into the pBAD30 vector, the *forward*-primer contains a ribosome binding site in addition to the *Xba*I cleavage site. Ligation into the pBAD30 vector takes place via the cleavage sites *Sac*I and *Kpn*I. The recombinant vector is designated pBAD-CV2025.

PCR amplification of the alanine dehydrogenase gene *ald* with chromosomal DNA from *B. subtilis subsp. Subtilis* (NP_391071) (product size: 1171 bp).

Primers

*Ala*DH-forward-*Xba*I (SEQ ID No. 16)

5' ACCTATCTAGAAGGAGGACGCATATGATCATAGGGGTTTCCT

*Ala*DH-reverse-*Pst*I (SEQ ID No. 17)

5' AACCTCTGCAGTTAAGCACCCGCCAC

For the cloning into the recombinant vector pBAD-CV2025, the *forward*-primer contains a ribosome binding site in addition to the *Xba*I cleavage site. The cloning into the vector takes place via the cleavage sites *Xba*I and *Pst*I. The resultant plasmid is designated pBAD-CV2025-*ald* (see Fig. 2).

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A6. Alternative cloning of the omega-transaminase gene from *Chromobacterium violaceum*DSM 30191 for codon-optimized expression in *E. coli*

The gene coding for omega-transaminase was synthesized by the company Geneart, optimized for *E. coli* codon usage (SEQ ID No. 42) and cloned into the vector pGA15 (Geneart). During synthesis of the gene, the cleavage sites *SacI* and *KpnI* were incorporated in flanking positions and, after digestion with *SacI* and *KpnI*, were cloned into the vector pGA15, linearized beforehand with *SacI* and *KpnI*. The resultant vector was digested with the restriction endonucleases *SacI* and *KpnI*, the fragment (transaminase) was purified and was ligated into the expression vector paCYCDuet-1 (Novagen). The correct plasmids were verified by restriction analysis. The resultant vector is called paCYCDuet-1::omega transaminase.

A7. Purification of heterologously expressed protein by means of 6xHis-Tag

After transformation of the vector (paCYCDuet-1::omega transaminase) in *E. coli* XL1blue, the transformed strain was cultivated in double YT-medium (dYT) with the antibiotic ampicillin (100 µg/ml) at 28°C up to a density of OD_{600 nm}=0.3-0.4. Expression takes place under the control of the *P_{lac}* promoter and is induced with IPTG (final concentration 1 mM). Lysis of the bacterial expression culture: 50 ml of culture was centrifuged at 2360 x g, and then resuspended in 5 ml Na-phosphate buffer (pH 8) with 5 mM EDTA, 300 mM NaCl and 1 mg/ml lysozyme, and incubated for 1h at RT. The lysate was centrifuged at 2360 x g for 10 min and the supernatant was purified on a Protino Ni-TED 2000 packed column (following the instructions of the manufacturer; Macherey-Nagel, Düren). The protein concentration was determined according to Bradford

A8. Detection of enzyme activity by coupled assay

The activity was determined in a coupled assay, in which the pyruvate formed as by-product of the transaminase reaction is reacted further in a second step, and NADH is oxidized to NAD⁺. The decrease in NADH concentration (principle: measurement of the decrease in extinction) is measured in the photometer at 340 nm and provides a measure of the activity.

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Preparation	
50 mM	Na-phosphate pH 7.5
50 mM	L-alanine
100 μ M	Pyridoxal phosphate
250 μ g	12-oxomethyl dodecanoate
1.25 mM	NADH
10 U	Lactate dehydrogenase
10 μ g	heterologously expressed protein
Make up to 1 ml with doubly distilled water	

The assay was started by adding 5 μ l 12-ODME (50 mg/ml). Measurement is performed continuously every minute at 340 nm at RT up to max. 20 minutes.

Inactivated protein and a preparation without co-substrate were used for control.

Fig. 4 shows the variation in extinction, determined photometrically.

A9. Detection of the heterologously expressed protein by HPLC

Preparation	
50 mM	Na-phosphate pH 7.5
50 mM	L-alanine
100 μ M	Pyridoxal phosphate
250 μ g	12-oxomethyl dodecanoate
50 μ g	heterologously expressed protein
Make up to 500 μ l with doubly distilled water	

After incubation for 4h at RT, the reaction was stopped with 1 Vol. MeOH.

For HPLC analysis, the preparation was derivatized with o-phthalic aldehyde (oPA) and 250 μ l thereof was analysed. 50 mM NaAC pH 4:acetonitrile 4:1 (v:v) was used as solvent A.

Solvent B was acetonitrile with 5% 50 mM NaAC pH 4. The gradient was from 30% B to 60% B in 4 min, from 60% B to 100% B in 2 min. The flow rate was 1.2 ml/min.

Separation took place in an Agilent Zorbax RP18 column (Agilent Technologies, USA), the column temperature was 40°C.

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Figs. 5 and 6 show the standard and the decrease of the 12-oxomethyl laurate. Heat-inactivated enzyme was used as negative control (Fig. 7).

A6. Ester cleavage

The lipase LipA (Q76D26) from *Pseudomonas fluorescens* (Kojima & Shimizu, *J. of Bioscience and Bioengin.*, Vol. 96 (3), pages 219-226 (2003)) is used for the cleavage of ω -aminomethyl laurate to ω -aminolauric acid. The gene is amplified with the primers LipA-SfiI-up and LipA-SfiI-down with chromosomal DNA from *Pseudomonas fluorescens* and cloned via the *Sfi*I cleavage sites into the vector pEST100. The recombinant plasmid is designated pEST-*lipA* (see Fig. 3).

The cloning fuses *lipA* (SEQ ID No. 18) to the signal sequence of alkaline phosphatase *phoA* and the autotransporter domain of EstA, an esterase from *P. aeruginosa*, so that the lipase is transferred across the cytoplasmic membrane and is displayed on the cell surface of *E. coli*. For the procedure see Becker et al., "A generic system for the *Escherichia coli* cell-surface display of lipolytic enzymes", *FEBS Letters* Vol. 579, pages 1177-1182 (2005). Expression takes place under the control of the P_{lac} promoter and is induced with IPTG (final concentration 1 mM) (product size: 1894 bp).

Primers

Primer *lipA*-Sfi-up (SEQ ID No. 19)

5' AACAAAAGGGCCGCAATGGCCATGGGTGTGTATGACTAC

Primer *lipA*-Sfi-down (SEQ ID No. 20)

5' TACAGGGGCCACCACGGCCTCAGGCGATCACAATTCC

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B Synthesis of 12-hydroxymethyl laurate and 12-oxomethyl laurate starting from methyl laurate with the AlkBGT alkane hydroxylase system from *Pseudomonas putida* GPo1

B1. Construction of the *alkBGT* Expression vectors

The construct pBT10 (Fig. 10, SEQ ID No. 31), which contains the three components alkane hydroxylase (AlkB), rubredoxin (AlkG) and rubredoxin reductase (AlkT) from *Pseudomonas putida* that are necessary for the oxidation to the aldehyde, was produced starting from the pCOM systems (Smits et al., 2001 Plasmid 64:16-24). For expression of the three genes, the *alkBFG* gene sequence was put under the control of the *alkB*-promoter and the *alkT* gene under the control of the *alkS*-promoter.

B2. Cloning strategy:

To simplify the cloning of *alkB* and *alkG*, the gene *alkF* located between them was amplified and cloned together with *alkB* and *alkG*. *AlkF* is of no significance for the reaction that is to be catalysed.

PCR amplification of the fragment *alkBFG* = 2524 bp (cf. SEQ ID No. 03 (*alkB*) and SEQ ID No. 04 (*alkG*)) with *NdeI* cleavage site upstream of *alkB* and *SalI* cleavage site downstream of *alkG*:

Primer: *alkBFG* forward (SEQ ID No. 32)

AAGGGAATTCCATATGCTTGAGAAACACAGAGTTC

Primer: *alkBFG* reverse (SEQ ID No. 33)

AAAATTCGCGTCGACAAGCGCTGAATGGGTATCGG

PCR amplification of the fragment *alkT* (2958 bp) (cf. SEQ ID No. 07 (*alkT*))

Primer *alkT* forward (SEQ ID No. 34)

TGAGACAGTGGCTGTTAGAG

Primer *alkT* reverse (SEQ ID No. 35)

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TAATAACCGCTCGAGAACGCTTACCGCCAACACAG

The fragments alkBFG and alkT were amplified by PCR. The plasmid pGEc47 (Fig. 12) (Eggink et al. (1987) J. Biol. Chem. 262, 17712-17718) was used as template.

The clonings were carried out by means of the subcloning vector pSMART® HCKan (Lucigen Corporation). This additional step was necessary because direct cloning had not been successful. For this, the commercially available vector pSMART® HCKan (Lucigen), which was already linearized and provided with blunt ends, was ligated with the respective blunt-end PCR product (Fig. 9).

Next, the alkBFG fragment with the restriction enzymes NdeI and Sall and the alkT fragment with the restriction enzymes PacI and XhoI were cut out of the subcloning vectors. The fragments were separated in agarose gel (1%), cut out of the gel and isolated using a gel extraction kit.

The fragments were ligated one after another into the vector pCOM10 (Smits, T. H. M., Seeger, M. A., Witholt, B. & van Beilen, J. B. (2001) Plasmid 46, 16–24). In the first step alkBFG was inserted in pCOM10 via the cleavage sites NdeI and Sall, and in a second step alkT was then cloned via the cleavage sites PacI and XhoI.

The recombinant plasmid was first transformed in *E. coli* DH5 α . Plasmid-bearing clones were selected on kanamycin-containing LB medium. The isolated plasmid was checked by restriction analysis and sequencing. It is designated pBT10 (Fig. 10).

B3. Biotransformation in the bioreactor of methyl laurate to hydroxymethyl laurate and 12-oxomethyl laurate

For the biotransformation, the plasmid pBT10 was transformed by heat shock at 42°C for 2 min in the chemically competent strain *E. coli* W3110. For the synthesis of hydroxymethyl laurate and 12-oxomethyl laurate, *E. coli* W3110-pBT10 was cultivated overnight at 30°C and 180 rpm in M9 medium and harvested. The biomass was taken up in M9 medium with 0.5% glucose up to OD₄₅₀ = 0.2. After a growth time of 4 h, expression was induced with dicyclopropyl ketone and it was incubated for a further 4 hours. The cells were centrifuged,

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the cell pellet was resuspended in KPi-buffer (50 mM, pH 7.4) and put in a bioreactor. A biomass concentration of about 1.8 gCDW/L was established. Stirring vigorously (1500 min⁻¹), the substrate methyl laurate in the ratio 1:3 was added to the cell suspension (100 ml cell suspension, 50 ml methyl laurate). The temperature was kept constant at 30°C. Formation of the products hydroxymethyl laurate and 12-oxomethyl laurate was detected by GC analysis of the reaction mixture. For this, a sample was taken after 0 min as negative control (Fig. 13) and after 150 min (Fig. 14) from the organic phase of the reaction mixture, and was analysed by GC (Thermo Trace GC Ultra). The column was a Varian Inc. FactorFour™ VF-5m, length: 30 m, film thickness: 0.25 µm, inside diameter: 0.25 mm.

Analysis conditions:

Furnace temperature	80 – 280°C
Ramp	15°C/min
Split ratio	15
Injection volume	1 µl
Carrier flow	1.5 ml/min
PTV injector	80 – 280°C at 15°C/s
Detector base temperature:	320°C

The detection of 12-oxomethyl laurate (Fig. 11) and 12-hydroxymethyl laurate (Fig. 12) was demonstrated by injection of the pure substances.

C Conversion of 12-oxomethyl laurate to 12-aminomethyl laurate

C 1: Isolation and expression of an aminotransferase from *Arabidopsis thaliana*

A known aminotransferase from *Arabidopsis thaliana* was analysed. Surprisingly, 4-aminobutyrate transaminase (at3g22200, SEQ ID No. 38) displayed an activity of about 14 U/mg heterologously expressed protein versus 12-oxomethyl dodecanoate. The product 12-aminomethyl dodecanoate was confirmed by HPLC.

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Unless stated otherwise, all methods were carried out in accordance with the protocols in Sambrook, J., Fritsch, E. F., & Maniatis, T (1989). Molecular Cloning, 2nd Ed. New York: Cold Spring Harbor Laboratory Press.

Isolation of 4-aminobutyrate transaminase from *A.thaliana* (at3g22200)

The RNA was isolated with the RNeasy Mini Kit from a whole, flowering plant of the species *A. thaliana* following the instructions of the manufacturer: QIAGEN GmbH, Hilden.

Then cDNA synthesis was carried out with the RT Skript Kit (USB Europe GmbH, Staufien) following the manufacturer's instructions. RNA quality/quantity determination was performed by Nanodrop following the instructions of the manufacturer (Thermo Fisher Scientific Inc. Waltham USA).

PCR from *A.thaliana* cDNA for insertion of cleavage sites

Using the following primers, the DNA coding for the 4-aminobutyrate transaminase with SEQ ID No. 39 was cloned in the NaeI, BamHI digested vector

Forward-primer inserts protease cleavage site and NaeI, SEQ ID No. 36

GCCGGCGAGAACCTGTACTTTCAGATGGCAAGTAAGTATGCCACTTG

Reverse-primer inserts BamHI, SEQ ID No. 37

GGATCCTCACTTCTTGCTGAGCCTTG

PCR was carried out according to the following protocol:

Preparation	Programme
2 µl cDNA	95°C 3 min
5 µl 10x Pfu buffer MgSO ₄	94°C 45 sec
5 µl dNTPs 2 mM	58°C 1 min 30 cycles } 72°C 4 min 72°C 10 min
2 µl primer forward 10 µM	
2 µl primer reverse 10 µM	
0.5 µl Pfu	
36.5 µl H ₂ O	

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The resultant PCR product was purified with the NucleoSpin® Extract II Kit (Macherey-Nagel, Germany, following the manufacturer's instructions).

Expression of the heterologous protein

Using the PCR product described above, the vector pGJ3130 (Fig. 15, SEQ ID No. 43) was produced by standard methods of molecular biology and transformed in *E. coli* XL1blue. The transformed *E. coli* strain was cultivated in double YT-medium (dYT) with the antibiotic ampicillin (100 µg/ml) and addition of 0.5 mM IPTG at 28°C up to a density of OD600 nm=0.3-0.4.

Purification of heterologously expressed protein by means of 6xHis-Tag

Lysis of the bacterial expression culture: 50 ml culture was centrifuged at 2360 x g, and then resuspended in 5 ml Na-phosphate buffer (pH 8) with 5 mM EDTA, 300 mM NaCl and 1 mg/ml lysozyme, and incubated for 1h at RT. The lysate was centrifuged at 2360 x g for 10 min and the supernatant was purified on a Protino Ni-TED 2000 packed column (following the instructions of the manufacturer; Macherey-Nagel, Düren). The protein concentration was determined according to Bradford.

Detection of enzyme activity by means of a coupled assay

The activity was determined in a coupled assay, in which the pyruvate that formed as by-product of the transaminase reaction is reacted further in a second step, and NADH is oxidized to NAD⁺. The decrease in NADH concentration (principle: measurement of the decrease in extinction) is measured in the photometer at 340 nm and provides a measure of the activity.

Preparation	
50 mM	Na-phosphate pH 7.5
50 mM	L-alanine
100 µM	Pyridoxal phosphate
250 µg	12-oxomethyl dodecanoate
1.25 mM	NADH

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10 U	Lactate dehydrogenase
10 µg	heterologously expressed protein
Make up to 1 ml with doubly distilled water	

The assay was started by adding 5 µl 12-ODME (50 mg/ml). Measurement is performed continuously every minute at 340 nm at RT for up to max. 20 minutes.

Inactivated protein and a preparation without co-substrate were used as the control.

Fig. 16 shows the variation in extinction, determined photometrically.

Detection of the heterologously expressed protein by HPLC

Preparation	
50 mM	Na-phosphate pH 7.5
50 mM	L-alanine
100 µM	Pyridoxal phosphate
250 µg	12-oxomethyl dodecanoate
50 µg	heterologously expressed protein
Make up to 500 µl with doubly distilled water	

After incubation for 4h at RT, the reaction was stopped with 1 Vol. MeOH

For the HPLC analysis, the preparation was derivatized with o-phthalic aldehyde (oPA) and 250 µl was analysed. 50 mM NaAC pH 4:acetonitrile 4:1 (v:v) was used as solvent A. Solvent B was acetonitrile with 5% 50 mM NaAC pH 4. The gradient was from 30% B to 60% B in 4 min, from 60% B to 100% B in 2 min. The flow rate was 1.2 ml/min.

Separation took place in an Agilent Zorbax RP18 column (Agilent Technologies, USA), the column temperature was 40°C. Fig. 17 (top) shows the formation of 12-aminomethyl laurate. The reference sample is shown at the bottom in Fig. 17.

D Amination of 12-oxomethyl laurate with PPTA5 and PSTA from *Pseudomonas*

D1. Cloning of PPTA5 and PSTA

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The strains *E. coli* BL21(DE3)/PPTA5 and *E. coli* BL21(DE3)/PSTA were used for the amination of 12-oxomethyl laurate. These strains were constructed as follows. The expression vector pET-21a(+) (Novagen) was selected for the cloning of both transaminase genes. For the PPTA5 gene, SEQ ID No. 40, primers were constructed, which were intended to add the restriction cleavage sites NdeI and XhoI to the ends of the gene; primer PPTA5_NdeI: GGAATTCCATATGAGCGTCAACAACCCGCAAACCCG (SEQ ID No. 44) and

Primer PPTA5_XhoI: CCGCTCGAGTTATCGAATCGCCTCAAGGGTCAGGTCC (SEQ ID No. 45). For the psta gene, SEQ ID No. 41, primers with NdeI and BamHI at the ends; primer PSTA_NdeI:

GGAATTCCATATGAGCGATTCGCAAACCCTGCACTGGC (SEQ ID No. 46) and

Primer PSTA_BamHI: CGCGGATCCTCAGCCCAGCACATCCTTGGCTGTCTG (SEQ ID No. 47)

These primers were used in PCRs. The purified PCR products and the vector pET-21a(+) were then submitted to restriction with the restriction enzymes NdeI and XhoI or NdeI and BamHI. The cut vector was dephosphorylated with alkaline phosphatase from shrimp. The vector cut with NdeI and XhoI and the PPTA5 gene, and the vector cut with NdeI and BamHI and the psta gene, were, after ligation with T4 DNA ligase, transformed with the competent expression strain *E. coli* XL1-Blue. After some clones had been grown, the plasmids were isolated and then underwent restriction and gel electrophoretic analysis. The transaminase sequences of the clones obtained (pPPTA5 or pPSTA) were confirmed by sequence analysis. Fig. 18 shows the plasmid maps of the expression vectors.

D2. Expression of PPTA5 and PSTA

For expression, the vectors pPPTA5 and pPSTA were transformed in competent *E. coli* BL21(DE3) cells. One individual colony of each was inoculated in 5 ml LB-Amp medium (ampicillin concentration 100µg/ml) and shaken overnight at 37°C. Then 1% was inoculated in 200 ml LB-Amp medium, shaken at 37°C and after reaching an OD₆₀₀ of 0.5, gene expression was induced with 0.5 mM IPTG. After shaking for 20 hours at 30°C, the cells were harvested and stored at -20°C.

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For digestion of the strains *E. coli* BL21(DE3)/pPPTA5 and *E. coli* BL21(DE3)/pPSTA, 0.4 g of cells from each were processed with 100 mM Tris-HCl buffer pH 7.2 to 25% cell suspensions, which were treated twice for 90 sec with ultrasound (Bandelin Sonoplus HD2070; probe MS73; 50% intensity). After centrifugation, the supernatants were removed. The raw extracts obtained were used in conversions of 12-oxomethyl laurate. The 400 µl preparations contained 5 mM 12-oxomethyl laurate, dissolved in N,N-dimethylformamide, 500 mM DL-alanine, 1 mM pyridoxal-5'-phosphate and 80 µl raw extract in 10 mM Kpi-buffer pH 7.0. It was shaken at 25°C. After specified times, 20 µl samples were taken from each, one portion was made alkaline with 1 µl 1% NaOH solution and was shaken out with 100 µl ethyl acetate. The organic phases were investigated by gas chromatography (gas chromatograph from Perkin Elmer, Clarus 500 with flame ionization detector). For this, an Optima 5-column (0.25 µm, 30 m, 0.25 mm, Macherey-Nagel) was used with programme:

80°C	
25°C/min	180°C
5°C/min	215°C
20°C/min	280°C

The retention times of 12-oxo- and 12-aminomethyl laurate are 7.2 and 7.7 min, respectively.

The results of the reactions are presented in Figs. 20 and 21. For the evaluation, the peak areas of 12-oxo- and 12-aminomethyl laurate from the chromatograms obtained for neutral and acid extraction were added together and the percentage of educt or product was calculated.

SEQUENCE LISTING IN ELECTRONIC FORM

In accordance with Section 111(1) of the Patent Rules, this description contains a sequence listing in electronic form in ASCII text format (file: 23443-1015 Seq 25-JUN-10 v1.txt).

A copy of the sequence listing in electronic form is available from the Canadian Intellectual Property Office.

The sequences in the sequence listing in electronic form are reproduced in the following table.

SEQUENCE TABLE

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Gly Arg Met Gly Ala Met Thr Gly Ala Glu Ala Phe Gly Val Thr Pro
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Asp Leu Met Cys Ile Ala Lys Gln Val Thr Asn Gly Ala Ile Pro Met
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Arg Asn Tyr Gly Leu Ala Gly Ala Ile Gln Ile Ala Ala Arg Asp Gly
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Asp Ala Ile Val Arg Pro Tyr Glu Val Ala Met Lys Leu Trp Lys Ala
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Gly Phe Tyr Val Arg Phe Gly Gly Asp Thr Leu Gln Phe Gly Pro Thr
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65                                70                75                80
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His Pro Ala Val Val Glu Leu Ser Glu Met Leu Phe Ser Leu Leu Pro
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Pro Gln Lys Lys Ile Met Ile Gly Arg Trp Asn Gly Tyr His Gly Ser
145                               150                155                160
Thr Leu Gly Ser Thr Ala Leu Gly Gly Met Lys Phe Met His Glu Met
    165                               170                175
Gly Gly Met Leu Pro Asp Phe Ala His Ile Asp Glu Pro Tyr Trp Tyr
    180                               185                190
Ala Asn Gly Gly Glu Leu Ser Pro Ala Glu Phe Gly Arg Arg Ala Ala
195                               200                205

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Leu Gln Leu Glu Glu Lys Ile Leu Glu Leu Gly Ala Glu Asn Val Ala
 210          215          220
Ala Phe Val Ala Glu Pro Phe Gln Gly Ala Gly Gly Met Ile Phe Pro
225          230          235          240
Pro Gln Ser Tyr Trp Pro Glu Ile Gln Arg Ile Cys Arg Gln Tyr Asp
          245          250          255
Val Leu Leu Cys Ala Asp Glu Val Ile Gly Gly Phe Gly Arg Thr Gly
          260          265          270
Glu Trp Phe Ala His Glu His Phe Gly Phe Gln Pro Asp Thr Leu Ser
          275          280          285
Ile Ala Lys Gly Leu Thr Ser Gly Tyr Ile Pro Met Gly Gly Leu Val
          290          295          300
Leu Gly Lys Arg Ile Ala Glu Val Leu Val Glu Gln Gly Gly Val Phe
305          310          315          320
Ala His Gly Leu Thr Tyr Ser Gly His Pro Val Ala Ala Ala Val Ala
          325          330          335
Ile Ala Asn Leu Lys Ala Leu Arg Asp Glu Gly Val Val Thr Arg Val
          340          345          350
Arg Glu Glu Thr Gly Pro Tyr Leu Gln Arg Cys Leu Arg Glu Val Phe
          355          360          365
Gly Asp His Pro Leu Val Gly Glu Val Gln Gly Ala Gly Phe Val Ala
          370          375          380
Ala Leu Gln Phe Ala Glu Asp Lys Val Thr Arg Lys Arg Phe Ala Asn
385          390          395          400
Glu Asn Asp Leu Ala Trp Arg Cys Arg Thr Ile Gly Phe Glu Glu Gly
          405          410          415
Val Ile Ile Arg Ser Thr Leu Gly Arg Met Ile Met Ala Pro Ala Leu
          420          425          430
Val Ala Gly Arg Ala Glu Ile Asp Glu Leu Ile Asp Lys Thr Arg Ile
          435          440          445
Ala Val Asp Arg Thr Ala Arg Glu Ile Gly Val Leu
          450          455          460

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<210> 21
<211> 448
<212> PRT
<213> Pseudomonas aeruginosa PA01

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<400> 21
Met Asn Gln Pro Leu Asn Val Ala Pro Pro Val Ser Ser Glu Leu Asn
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Leu Arg Ala His Trp Met Pro Phe Ser Ala Asn Arg Asn Phe Gln Lys
          20          25          30
Asp Pro Arg Ile Ile Val Ala Ala Glu Gly Ser Trp Leu Thr Asp Asp
          35          40          45
Lys Gly Arg Lys Val Tyr Asp Ser Leu Ser Gly Leu Trp Thr Cys Gly
          50          55          60
Ala Gly His Ser Arg Lys Glu Ile Gln Glu Ala Val Ala Arg Gln Leu
65          70          75          80
Gly Thr Leu Asp Tyr Ser Pro Gly Phe Gln Tyr Gly His Pro Leu Ser
          85          90          95
Phe Gln Leu Ala Glu Lys Ile Ala Gly Leu Leu Pro Gly Glu Leu Asn
          100          105          110
His Val Phe Phe Thr Gly Ser Gly Ser Glu Cys Ala Asp Thr Ser Ile
          115          120          125
Lys Met Ala Arg Ala Tyr Trp Arg Leu Lys Gly Gln Pro Gln Lys Thr
          130          135          140
Lys Leu Ile Gly Arg Ala Arg Gly Tyr His Gly Val Asn Val Ala Gly
145          150          155          160
Thr Ser Leu Gly Gly Ile Gly Gly Asn Arg Lys Met Phe Gly Gln Leu
          165          170          175
Met Asp Val Asp His Leu Pro His Thr Leu Gln Pro Gly Met Ala Phe
          180          185          190

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Thr	Arg	Gly	Met	Ala	Gln	Thr	Gly	Gly	Val	Glu	Leu	Ala	Asn	Glu	Leu
		195					200					205			
Leu	Lys	Leu	Ile	Glu	Leu	His	Asp	Ala	Ser	Asn	Ile	Ala	Ala	Val	Ile
	210					215					220				
Val	Glu	Pro	Met	Ser	Gly	Ser	Ala	Gly	Val	Leu	Val	Pro	Pro	Val	Gly
225					230					235					240
Tyr	Leu	Gln	Arg	Leu	Arg	Glu	Ile	Cys	Asp	Gln	His	Asn	Ile	Leu	Leu
			245						250					255	
Ile	Phe	Asp	Glu	Val	Ile	Thr	Ala	Phe	Gly	Arg	Leu	Gly	Thr	Tyr	Ser
		260					265					270			
Gly	Ala	Glu	Tyr	Phe	Gly	Val	Thr	Pro	Asp	Leu	Met	Asn	Val	Ala	Lys
		275					280					285			
Gln	Val	Thr	Asn	Gly	Ala	Val	Pro	Met	Gly	Ala	Val	Ile	Ala	Ser	Ser
	290					295					300				
Glu	Ile	Tyr	Asp	Thr	Phe	Met	Asn	Gln	Ala	Leu	Pro	Glu	His	Ala	Val
305					310					315					320
Glu	Phe	Ser	His	Gly	Tyr	Thr	Tyr	Ser	Ala	His	Pro	Val	Ala	Cys	Ala
			325						330					335	
Ala	Gly	Leu	Ala	Ala	Leu	Asp	Ile	Leu	Ala	Arg	Asp	Asn	Leu	Val	Gln
			340					345					350		
Gln	Ser	Ala	Glu	Leu	Ala	Pro	His	Phe	Glu	Lys	Gly	Leu	His	Gly	Leu
		355					360					365			
Gln	Gly	Ala	Lys	Asn	Val	Ile	Asp	Ile	Arg	Asn	Cys	Gly	Leu	Ala	Gly
	370					375					380				
Ala	Ile	Gln	Ile	Ala	Pro	Arg	Asp	Gly	Asp	Pro	Thr	Val	Arg	Pro	Phe
385					390					395					400
Glu	Ala	Gly	Met	Lys	Leu	Trp	Gln	Gln	Gly	Phe	Tyr	Val	Arg	Phe	Gly
			405						410					415	
Gly	Asp	Thr	Leu	Gln	Phe	Gly	Pro	Thr	Phe	Asn	Ala	Arg	Pro	Glu	Glu
			420					425				430			
Leu	Asp	Arg	Leu	Phe	Asp	Ala	Val	Gly	Glu	Ala	Leu	Asn	Gly	Ile	Ala
		435					440					445			

<210> 22
 <211> 444
 <212> PRT
 <213> Pseudomonas aeruginosa PA01

<400> 22

Met	Thr	Met	Asn	Asp	Glu	Pro	Gln	Ser	Ser	Ser	Leu	Asp	Asn	Phe	Trp
1			5					10						15	
Met	Pro	Phe	Thr	Ala	Asn	Arg	Gln	Phe	Lys	Ala	Arg	Pro	Arg	Leu	Leu
		20					25					30			
Glu	Ser	Ala	Glu	Gly	Ile	His	Tyr	Ile	Ala	Gln	Gly	Gly	Arg	Arg	Ile
		35				40					45				
Leu	Asp	Gly	Thr	Ala	Gly	Leu	Trp	Cys	Cys	Asn	Ala	Gly	His	Gly	Arg
50					55					60					
Arg	Glu	Ile	Ser	Glu	Ala	Val	Ala	Arg	Gln	Ile	Ala	Thr	Leu	Asp	Tyr
65				70						75					80
Ala	Pro	Pro	Phe	Gln	Met	Gly	His	Pro	Leu	Pro	Phe	Glu	Leu	Ala	Ala
			85					90						95	
Arg	Leu	Thr	Glu	Ile	Ala	Pro	Pro	Ser	Leu	Asn	Lys	Val	Phe	Phe	Thr
		100					105					110			
Asn	Ser	Gly	Ser	Glu	Ser	Ala	Asp	Thr	Ala	Leu	Lys	Ile	Ala	Leu	Ala
		115				120						125			
Tyr	Gln	Arg	Ala	Ile	Gly	Gln	Gly	Thr	Arg	Thr	Arg	Leu	Ile	Gly	Arg
	130					135					140				
Glu	Leu	Gly	Tyr	His	Gly	Val	Gly	Phe	Gly	Gly	Leu	Ser	Val	Gly	Gly
145					150					155					160
Met	Val	Asn	Asn	Arg	Lys	Ala	Phe	Ser	Ala	Asn	Leu	Leu	Pro	Gly	Val
			165					170						175	
Asp	His	Leu	Pro	His	Thr	Leu	Asp	Val	Ala	Arg	Asn	Ala	Phe	Thr	Val
			180					185					190		

Gly Leu Pro Glu His Gly Val Glu Lys Ala Glu Glu Leu Glu Arg Leu
 195 200 205
 Val Thr Leu His Gly Ala Glu Asn Ile Ala Ala Val Ile Val Glu Pro
 210 215 220
 Met Ser Gly Ser Ala Gly Val Val Leu Pro Pro Lys Gly Tyr Leu Gln
 225 230 235 240
 Arg Leu Arg Glu Ile Thr Arg Lys His Gly Ile Leu Leu Ile Phe Asp
 245 250 255
 Glu Val Ile Thr Gly Phe Gly Arg Val Gly Glu Ala Phe Ala Ala Gln
 260 265 270
 Arg Trp Gly Val Val Pro Asp Leu Leu Thr Cys Ala Lys Gly Leu Thr
 275 280 285
 Asn Gly Ser Ile Pro Met Gly Ala Val Phe Val Asp Glu Lys Ile His
 290 295 300
 Ala Ala Phe Met Gln Gly Pro Gln Gly Ala Ile Glu Phe Phe His Gly
 305 310 315 320
 Tyr Thr Tyr Ser Gly His Pro Val Ala Cys Ala Ala Ala Leu Ala Thr
 325 330 335
 Leu Asp Ile Tyr Arg Arg Asp Asp Leu Phe Gln Arg Ala Val Glu Leu
 340 345 350
 Glu Gly Tyr Trp Gln Asp Ala Leu Phe Ser Leu Arg Asp Leu Pro Asn
 355 360 365
 Val Val Asp Ile Arg Ala Val Gly Leu Val Gly Gly Val Gln Leu Ala
 370 375 380
 Pro His Ala Asp Gly Pro Gly Lys Arg Gly Tyr Asp Val Phe Glu Arg
 385 390 395 400
 Cys Phe Trp Glu His Asp Leu Met Val Arg Val Thr Gly Asp Ile Ile
 405 410 415
 Ala Met Ser Pro Pro Leu Ile Ile Asp Lys Pro His Ile Asp Gln Ile
 420 425 430
 Val Glu Arg Leu Ala Gln Ala Ile Arg Ala Ser Val
 435 440

<210> 23

<211> 468

<212> PRT

<213> Pseudomonas aeruginosa PA01

<400> 23

Met Asn Ala Arg Leu His Ala Thr Ser Pro Leu Gly Asp Ala Asp Leu
 1 5 10 15
 Val Arg Ala Asp Gln Ala His Tyr Met His Gly Tyr His Val Phe Asp
 20 25 30
 Asp His Arg Val Asn Gly Ser Leu Asn Ile Ala Ala Gly Asp Gly Ala
 35 40 45
 Tyr Ile Tyr Asp Thr Ala Gly Asn Arg Tyr Leu Asp Ala Val Gly Gly
 50 55 60
 Met Trp Cys Thr Asn Ile Gly Leu Gly Arg Glu Glu Met Ala Arg Thr
 65 70 75 80
 Val Ala Glu Gln Thr Arg Leu Leu Ala Tyr Ser Asn Pro Phe Cys Asp
 85 90 95
 Met Ala Asn Pro Arg Ala Ile Glu Leu Cys Arg Lys Leu Ala Glu Leu
 100 105 110
 Ala Pro Gly Asp Leu Asp His Val Phe Leu Thr Thr Gly Gly Ser Thr
 115 120 125
 Ala Val Asp Thr Ala Ile Arg Leu Met His Tyr Tyr Gln Asn Cys Arg
 130 135 140
 Gly Lys Arg Ala Lys Lys His Val Ile Thr Arg Ile Asn Ala Tyr His
 145 150 155 160
 Gly Ser Thr Phe Leu Gly Met Ser Leu Gly Gly Lys Ser Ala Asp Arg
 165 170 175
 Pro Ala Glu Phe Asp Phe Leu Asp Glu Arg Ile His His Leu Ala Cys
 180 185 190

Pro Tyr Tyr Tyr Arg Ala Pro Glu Gly Leu Gly Glu Ala Glu Phe Leu
 195 200 205
 Asp Gly Leu Val Asp Glu Phe Glu Arg Lys Ile Leu Glu Leu Gly Ala
 210 215 220
 Asp Arg Val Gly Ala Phe Ile Ser Glu Pro Val Phe Gly Ser Gly Gly
 225 230 235 240
 Val Ile Val Pro Pro Ala Gly Tyr His Arg Arg Met Trp Glu Leu Cys
 245 250 255
 Gln Arg Tyr Asp Val Leu Tyr Ile Ser Asp Glu Val Val Thr Ser Phe
 260 265 270
 Gly Arg Leu Gly His Phe Phe Ala Ser Gln Ala Val Phe Gly Val Gln
 275 280 285
 Pro Asp Ile Ile Leu Thr Ala Lys Gly Leu Thr Ser Gly Tyr Gln Pro
 290 295 300
 Leu Gly Ala Cys Ile Phe Ser Arg Arg Ile Trp Glu Val Ile Ala Glu
 305 310 315 320
 Pro Asp Lys Gly Arg Cys Phe Ser His Gly Phe Thr Tyr Ser Gly His
 325 330 335
 Pro Val Ala Cys Ala Ala Ala Leu Lys Asn Ile Glu Ile Ile Glu Arg
 340 345 350
 Glu Gly Leu Leu Ala His Ala Asp Glu Val Gly Arg Tyr Phe Glu Glu
 355 360 365
 Arg Leu Gln Ser Leu Arg Asp Leu Pro Ile Val Gly Asp Val Arg Gly
 370 375 380
 Met Arg Phe Met Ala Cys Val Glu Phe Val Ala Asp Lys Ala Ser Lys
 385 390 395 400
 Ala Leu Phe Pro Glu Ser Leu Asn Ile Gly Glu Trp Val His Leu Arg
 405 410 415
 Ala Gln Lys Arg Gly Leu Leu Val Arg Pro Ile Val His Leu Asn Val
 420 425 430
 Met Ser Pro Pro Leu Ile Leu Thr Arg Glu Gln Val Asp Thr Val Val
 435 440 445
 Arg Val Leu Arg Glu Ser Ile Glu Glu Thr Val Glu Asp Leu Val Arg
 450 455 460
 Ala Gly His Arg
 465

<210> 24
 <211> 460
 <212> PRT
 <213> Pseudomonas putida W619

<400> 24
 Met Asn Ala Pro Phe Gln Pro Gln Arg Asp Thr Arg Asp Tyr Gln Ala
 1 5 10 15
 Ser Asp Ala Ala His His Ile His Ala Phe Leu Asp Gln Lys Ala Leu
 20 25 30
 Asn Ala Glu Gly Pro Arg Val Ile Thr Arg Gly Glu Arg Leu Tyr Leu
 35 40 45
 Trp Asp Asn Asp Gly Arg Arg Tyr Leu Asp Gly Met Ser Gly Leu Trp
 50 55 60
 Cys Thr Gln Leu Gly Tyr Gly Arg Lys Asp Leu Thr Ala Ala Ala Ala
 65 70 75 80
 Ala Gln Met Asp Gln Leu Ala Tyr Tyr Asn Met Phe Phe His Thr Thr
 85 90 95
 His Pro Ala Val Ile Glu Leu Ser Glu Leu Leu Phe Ser Leu Leu Pro
 100 105 110
 Gly His Tyr Ser His Ala Ile Tyr Thr Asn Ser Gly Ser Glu Ala Asn
 115 120 125
 Glu Val Leu Ile Arg Thr Val Arg Arg Tyr Trp Gln Val Val Gly Gln
 130 135 140
 Pro Asn Lys Lys Val Met Ile Gly Arg Trp Asn Gly Tyr His Gly Ser
 145 150 155 160

Thr	Leu	Ala	Ala	Thr	Ala	Leu	Gly	Gly	Met	Lys	Phe	Met	His	Glu	Met	
				165					170					175		
Gly	Gly	Leu	Ile	Pro	Asp	Val	Ala	His	Ile	Asp	Glu	Pro	Tyr	Trp	Tyr	
			180					185					190			
Ala	Glu	Gly	Gly	Glu	Leu	Thr	Pro	Ala	Glu	Phe	Gly	Arg	Arg	Cys	Ala	
		195					200					205				
Leu	Gln	Leu	Glu	Glu	Lys	Ile	Leu	Glu	Leu	Gly	Ala	Glu	Asn	Val	Ala	
	210					215					220					
Gly	Phe	Val	Ala	Glu	Pro	Phe	Gln	Gly	Ala	Gly	Gly	Met	Ile	Phe	Pro	
225					230					235					240	
Pro	Glu	Ser	Tyr	Trp	Pro	Glu	Ile	Gln	Arg	Ile	Cys	Arg	Gln	Tyr	Asp	
				245					250					255		
Val	Leu	Leu	Cys	Ala	Asp	Glu	Val	Ile	Gly	Gly	Phe	Gly	Arg	Thr	Gly	
			260					265					270			
Glu	Trp	Phe	Ala	His	Glu	Tyr	Phe	Gly	Phe	Glu	Pro	Asp	Thr	Leu	Ser	
		275					280					285				
Ile	Ala	Lys	Gly	Leu	Thr	Ser	Gly	Tyr	Val	Pro	Met	Gly	Gly	Leu	Val	
	290					295					300					
Leu	Ser	Lys	Arg	Ile	Ala	Glu	Ala	Leu	Val	Glu	Arg	Gly	Gly	Val	Phe	
305					310					315					320	
Ala	His	Gly	Leu	Thr	Tyr	Ser	Gly	His	Pro	Val	Ala	Ala	Ala	Val	Ala	
				325					330					335		
Ile	Ala	Asn	Leu	Lys	Ala	Leu	Arg	Asp	Glu	Gly	Ile	Val	Arg	Gln	Val	
			340					345					350			
Lys	Asp	Asp	Thr	Gly	Pro	Tyr	Leu	Gln	Arg	Ile	Leu	Arg	Glu	Val	Phe	
		355					360					365				
Ala	Asn	His	Pro	Leu	Ile	Gly	Gln	Val	Gln	Gly	Ala	Gly	Leu	Val	Ala	
	370					375					380					
Ala	Leu	Gln	Phe	Ala	Glu	Asp	Lys	Gly	Ser	Arg	Lys	Arg	Tyr	Ala	Asn	
385					390					395					400	
Glu	Asn	Asp	Leu	Ala	Trp	Gln	Cys	Arg	Thr	Tyr	Gly	Phe	Glu	Glu	Gly	
			405						410					415		
Val	Ile	Ile	Arg	Ser	Thr	Leu	Gly	Arg	Met	Ile	Met	Ala	Pro	Ala	Leu	
			420					425					430			
Val	Ala	Thr	His	Gly	Glu	Leu	Asp	Glu	Leu	Val	Asp	Lys	Thr	Arg	Ile	
		435					440					445				
Ala	Val	Asp	Arg	Thr	Ala	Arg	Gly	Leu	Gly	Ile	Leu					
	450					455					460					

<210> 25
 <211> 459
 <212> PRT
 <213> Pseudomonas putida KT2440

<400> 25
 Met Ser Thr His Ser Ser Thr Val Gln Asn Asp Leu Ala Ala Leu Ile
 1 5 10 15
 His Pro Asn Thr Asn Leu Ala Gln His Arg Glu Val Gly Pro Leu Val
 20 25 30
 Ile Ala Arg Gly Asp Gly Val Arg Val Phe Asp Glu Gln Gly Asn Ala
 35 40 45
 Tyr Ile Glu Ala Met Ser Gly Leu Trp Ser Ala Ala Leu Gly Phe Ser
 50 55 60
 Glu Gln Arg Leu Val Asp Ala Ala Val Glu Gln Phe Lys Gln Leu Pro
 65 70 75 80
 Tyr Tyr His Ser Phe Ser His Lys Thr Asn Ala Pro Ala Ala Leu
 85 90 95
 Ala Ala Lys Leu Ala Ala Leu Ala Pro Gly Asp Leu Asn His Val Phe
 100 105 110
 Phe Thr Asn Ser Gly Ser Glu Ala Asn Asp Ser Val Val Lys Met Val
 115 120 125
 Trp Tyr Val Asn Asn Ala Leu Gly Arg Pro Ala Lys Lys Lys Phe Ile
 130 135 140

Ser	Arg	Gln	Gln	Ala	Tyr	His	Gly	Ala	Thr	Val	Ala	Ala	Ala	Ser	Leu
145					150					155					160
Thr	Gly	Ile	Pro	Ser	Met	His	Arg	Asp	Phe	Asp	Leu	Pro	Ala	Ile	Pro
				165					170					175	
Val	His	His	Leu	Thr	Cys	Pro	Asn	Phe	Tyr	Arg	Phe	Ala	Arg	Pro	Gly
			180					185					190		
Glu	Ser	Gln	Glu	Ala	Phe	Thr	Val	Arg	Leu	Ala	Asn	Glu	Leu	Glu	Arg
		195					200				205				
Tyr	Ile	Leu	Ala	Glu	Gly	Pro	Glu	Thr	Ile	Ala	Ala	Phe	Ile	Gly	Glu
	210					215					220				
Pro	Val	Ile	Ala	Ala	Gly	Gly	Val	Ile	Pro	Pro	Pro	Thr	Gly	Tyr	Trp
225					230					235					240
Ala	Ala	Ile	Gln	Ala	Val	Cys	Lys	Arg	Tyr	Asp	Ile	Leu	Val	Val	Ile
			245						250					255	
Asp	Glu	Ile	Ile	Thr	Gly	Phe	Gly	Arg	Leu	Gly	Thr	Met	Phe	Gly	Ser
			260					265					270		
Gln	Leu	Tyr	Gly	Ile	Gln	Pro	Asp	Ile	Met	Val	Leu	Ser	Lys	Gln	Leu
		275					280					285			
Thr	Ser	Ser	Tyr	Gln	Pro	Leu	Ala	Ala	Val	Val	Val	Ser	Asp	Ala	Met
	290					295					300				
Asn	Asp	Val	Leu	Val	Ser	Gln	Ser	Gln	Arg	Leu	Gly	Ala	Phe	Ala	His
305					310					315					320
Gly	Leu	Thr	Cys	Thr	Gly	His	Pro	Val	Ala	Thr	Ala	Val	Ala	Leu	Glu
			325						330					335	
Asn	Ile	Arg	Ile	Ile	Glu	Glu	Arg	Asp	Leu	Val	Gly	His	Val	Gln	His
			340					345					350		
Leu	Ala	Pro	Val	Phe	Gln	Arg	His	Leu	Arg	Ala	Phe	Glu	Asp	His	Pro
		355					360					365			
Leu	Val	Gly	Asn	Val	Arg	Gly	Val	Gly	Leu	Met	Gly	Gly	Ile	Glu	Leu
	370					375					380				
Val	Ala	Asp	Lys	Ala	Thr	Arg	Gln	Pro	Phe	Ala	Gln	Pro	Gly	Thr	Leu
385					390					395					400
Gly	Gly	Tyr	Val	Phe	Lys	Gln	Ala	His	Lys	His	Gly	Leu	Ile	Ile	Arg
				405					410					415	
Ala	Ile	Tyr	Asp	Thr	Ile	Ala	Phe	Cys	Pro	Pro	Leu	Ile	Thr	Thr	Gln
			420					425					430		
Asp	Asp	Ile	Glu	Ala	Ile	Phe	Ser	Ala	Phe	Glu	Arg	Thr	Leu	Ala	Asp
		435					440					445			
Ala	Thr	Asp	Trp	Ala	Arg	Ser	Gln	His	Leu	Leu					
	450					455									

<210> 26
 <211> 490
 <212> PRT
 <213> Pseudomonas putida W619

<400> 26

Met	Arg	Ile	Ser	Ser	Val	Ser	Ser	Ala	Pro	Gly	Ser	Val	Ser	Ser	Cys
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Cys	Leu	Leu	Cys	Asp	Gln	Pro	Leu	Arg	Pro	Pro	Ala	Gly	Gly	Leu	Trp
		20					25					30			
Thr	Leu	Glu	Lys	His	Met	Ser	Val	Lys	Asn	Pro	Gln	Thr	Arg	Asp	Trp
	35						40					45			
Gln	Thr	Leu	Ser	Gly	Glu	His	His	Leu	Ala	Pro	Phe	Ser	Asp	Tyr	Lys
	50					55					60				
Gln	Leu	Lys	Glu	Lys	Gly	Pro	Arg	Ile	Ile	Thr	Lys	Ala	Gln	Gly	Val
65					70					75					80
His	Leu	Trp	Asp	Ser	Glu	Gly	His	Lys	Ile	Leu	Asp	Gly	Met	Ala	Gly
			85					90					95		
Leu	Trp	Cys	Val	Ala	Val	Gly	Tyr	Gly	Arg	Glu	Glu	Leu	Val	Gln	Ala
		100						105					110		
Ala	Glu	Lys	Gln	Met	Arg	Glu	Leu	Pro	Tyr	Tyr	Asn	Leu	Phe	Phe	Gln
		115					120					125			

Thr	Ala	His	Pro	Pro	Ala	Leu	Glu	Leu	Ala	Lys	Ala	Ile	Thr	Asp	Val
	130					135					140				
Ala	Pro	Glu	Gly	Met	Thr	His	Val	Phe	Phe	Thr	Gly	Ser	Gly	Ser	Glu
145					150					155					160
Gly	Asn	Asp	Thr	Val	Leu	Arg	Met	Val	Arg	His	Tyr	Trp	Ala	Leu	Lys
				165					170					175	
Gly	Lys	Pro	Gln	Lys	Gln	Thr	Ile	Ile	Gly	Arg	Ile	Asn	Gly	Tyr	His
			180					185					190		
Gly	Ser	Thr	Val	Ala	Gly	Ala	Ser	Leu	Gly	Gly	Met	Ser	Gly	Met	His
	195						200					205			
Glu	Gln	Gly	Gly	Leu	Pro	Ile	Pro	Gly	Ile	Val	His	Ile	Pro	Gln	Pro
	210					215					220				
Tyr	Trp	Phe	Gly	Glu	Gly	Gly	Asp	Met	Ser	Pro	Asp	Asp	Phe	Gly	Val
225					230					235					240
Trp	Ala	Ala	Glu	Gln	Leu	Glu	Lys	Lys	Ile	Leu	Glu	Val	Gly	Glu	Asp
				245					250					255	
Asn	Val	Ala	Ala	Phe	Ile	Ala	Glu	Pro	Ile	Gln	Gly	Ala	Gly	Gly	Val
			260				265						270		
Ile	Ile	Pro	Pro	Glu	Thr	Tyr	Trp	Pro	Lys	Val	Lys	Glu	Ile	Leu	Ala
	275						280					285			
Lys	Tyr	Asp	Ile	Leu	Phe	Val	Ala	Asp	Glu	Val	Ile	Cys	Gly	Phe	Gly
	290				295						300				
Arg	Thr	Gly	Glu	Trp	Phe	Gly	Ser	Asp	Tyr	Tyr	Asp	Leu	Lys	Pro	Asp
305					310					315					320
Leu	Met	Thr	Ile	Ala	Lys	Gly	Leu	Thr	Ser	Gly	Tyr	Ile	Pro	Met	Gly
				325					330					335	
Gly	Val	Ile	Val	Arg	Asp	Lys	Val	Ala	Lys	Val	Leu	Ser	Glu	Gly	Gly
			340					345					350		
Asp	Phe	Asn	His	Gly	Phe	Thr	Tyr	Ser	Gly	His	Pro	Val	Ala	Ala	Ala
	355						360					365			
Val	Gly	Leu	Glu	Asn	Leu	Arg	Ile	Leu	Arg	Glu	Glu	Lys	Ile	Val	Glu
	370					375					380				
Lys	Val	Arg	Thr	Glu	Val	Ala	Pro	Tyr	Leu	Gln	Lys	Arg	Leu	Arg	Glu
385					390					395					400
Leu	Gln	Asp	His	Pro	Leu	Val	Gly	Glu	Val	Arg	Gly	Leu	Gly	Leu	Leu
				405					410					415	
Gly	Ala	Ile	Glu	Leu	Val	Lys	Asp	Lys	Ala	Ser	Arg	Ser	Arg	Tyr	Glu
			420					425					430		
Gly	Lys	Gly	Val	Gly	Met	Val	Cys	Arg	Asn	Phe	Cys	Phe	Asp	Asn	Gly
	435						440					445			
Leu	Ile	Met	Arg	Ala	Val	Gly	Asp	Thr	Met	Ile	Ile	Ala	Pro	Pro	Leu
	450					455					460				
Val	Ile	Ser	His	Ala	Glu	Val	Asp	Glu	Leu	Val	Glu	Lys	Ala	Arg	Lys
465					470					475					480
Cys	Leu	Asp	Leu	Thr	Leu	Glu	Ala	Ile	Arg						
			485						490						

<210> 27

<211> 457

<212> PRT

<213> Streptomyces coelicolor A3(2)

<400> 27

Met	Ser	Thr	Asp	Ser	Pro	Lys	Asp	Leu	Ser	Arg	Thr	Ala	Tyr	Asp	His
1				5					10					15	
Leu	Trp	Met	His	Phe	Thr	Arg	Met	Ser	Ser	Tyr	Glu	Asn	Ala	Pro	Val
			20					25					30		
Pro	Thr	Ile	Val	Arg	Gly	Glu	Gly	Thr	His	Ile	Tyr	Asp	Asp	Lys	Gly
			35				40					45			
Arg	Arg	Tyr	Leu	Asp	Gly	Leu	Ala	Gly	Leu	Phe	Val	Val	Gln	Ala	Gly
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His	Gly	Arg	Gln	Glu	Leu	Ala	Glu	Thr	Ala	Ser	Lys	Gln	Ala	Gln	Glu
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Leu Ala Phe Phe Pro Val Trp Ser Tyr Ala His Pro Lys Ala Val Glu
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Leu Ala Glu Arg Leu Ala Asn Glu Ala Pro Gly Asp Leu Asn Lys Val
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Phe Phe Thr Thr Gly Gly Gly Glu Ala Val Glu Thr Ala Trp Lys Leu
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Ala Lys Gln Tyr Phe Lys Leu Thr Gly Lys Pro Thr Lys Tyr Lys Val
      130        135        140
Ile Ser Arg Ala Val Ala Tyr His Gly Thr Pro Gln Gly Ala Leu Ser
145      150      155      160
Ile Thr Gly Leu Pro Ala Leu Lys Ala Pro Phe Glu Pro Leu Val Pro
      165        170        175
Gly Ala His Lys Val Pro Asn Thr Asn Ile Tyr Arg Ala Pro Ile His
      180        185        190
Gly Asp Asp Pro Glu Ala Tyr Gly Arg Trp Ala Ala Asp Gln Ile Glu
      195        200        205
Gln Gln Ile Leu Phe Glu Gly Pro Glu Thr Val Ala Ala Val Phe Leu
      210        215        220
Glu Pro Val Gln Asn Ala Gly Gly Cys Phe Pro Pro Pro Pro Gly Tyr
225      230      235      240
Phe Gln Arg Val Arg Glu Ile Cys Asp Gln Tyr Asp Val Leu Leu Val
      245        250        255
Ser Asp Glu Val Ile Cys Ala Phe Gly Arg Leu Gly Thr Thr Phe Ala
      260        265        270
Cys Asp Lys Phe Gly Tyr Val Pro Asp Met Ile Thr Cys Ala Lys Gly
      275        280        285
Met Thr Ser Gly Tyr Ser Pro Ile Gly Ala Cys Val Ile Ser Asp Arg
      290        295        300
Leu Ala Glu Pro Phe Tyr Lys Gly Asp Asn Thr Phe Leu His Gly Tyr
305      310      315      320
Thr Phe Gly Gly His Pro Val Ser Ala Ala Val Gly Ile Ala Asn Leu
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Asp Leu Phe Glu Arg Glu Gly Leu Asn Gln His Val Leu Asp Asn Glu
      340        345        350
Gly Ala Phe Arg Ala Thr Leu Glu Lys Leu His Asp Leu Pro Ile Val
      355        360        365
Gly Asp Val Arg Gly Asn Gly Phe Phe Tyr Gly Ile Glu Leu Val Lys
      370        375        380
Asp Lys Ala Thr Lys Glu Ser Phe Asp Glu Glu Glu Thr Glu Arg Val
385      390      395      400
Leu Tyr Gly Phe Leu Ser Lys Lys Leu Phe Glu Asn Gly Leu Tyr Cys
      405        410        415
Arg Ala Asp Asp Arg Gly Asp Pro Val Ile Gln Leu Ala Pro Pro Leu
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Ile Ser Asn Gln Glu Thr Phe Asp Glu Ile Glu Gln Ile Leu Arg Ala
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Thr Leu Thr Glu Ala Trp Thr Lys Leu
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<210> 28
<211> 459
<212> PRT
<213> Streptomyces avermitilis MA 4680

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Pro Val Pro Thr Ile Val Arg Gly Glu Gly Thr Tyr Ile Tyr Asp Asp
      35      40      45
Lys Gly Lys Arg Tyr Leu Asp Gly Leu Ser Gly Leu Phe Val Val Gln
50      55      60

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Gln	Glu	Leu	Ala	Phe	Phe	Pro	Val	Trp	Ser	Tyr	Ala	His	Pro	Lys	Ala
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Val	Glu	Leu	Ala	Glu	Arg	Leu	Ala	Asn	Tyr	Ala	Pro	Gly	Asp	Leu	Asn
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Lys	Val	Phe	Phe	Thr	Thr	Gly	Gly	Gly	Glu	Ala	Val	Glu	Thr	Ala	Trp
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Lys	Leu	Ala	Lys	Gln	Tyr	Phe	Lys	Leu	Gln	Gly	Lys	Pro	Thr	Lys	Tyr
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Lys	Val	Ile	Ser	Arg	Ala	Val	Ala	Tyr	His	Gly	Thr	Pro	Gln	Gly	Ala
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Leu	Ser	Ile	Thr	Gly	Leu	Pro	Ala	Leu	Lys	Ala	Pro	Phe	Glu	Pro	Leu
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Val	Pro	Gly	Ala	His	Lys	Val	Pro	Asn	Thr	Asn	Ile	Tyr	Arg	Ala	Pro
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Leu	Phe	Gly	Asp	Asp	Pro	Glu	Ala	Phe	Gly	Arg	Trp	Ala	Ala	Asp	Gln
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Ile	Glu	Gln	Gln	Ile	Leu	Phe	Glu	Gly	Pro	Glu	Thr	Val	Ala	Ala	Val
	210					215					220				
Phe	Leu	Glu	Pro	Val	Gln	Asn	Ala	Gly	Gly	Cys	Phe	Pro	Pro	Pro	Pro
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Gly	Tyr	Phe	Gln	Arg	Val	Arg	Glu	Ile	Cys	Asp	Gln	Tyr	Asp	Val	Leu
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Phe	Ala	Cys	Asp	Lys	Phe	Gly	Tyr	Val	Pro	Asp	Met	Ile	Thr	Cys	Ala
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Lys	Gly	Met	Thr	Ser	Gly	Tyr	Ser	Pro	Ile	Gly	Ala	Cys	Ile	Val	Ser
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Asp	Arg	Ile	Ala	Glu	Pro	Phe	Tyr	Lys	Gly	Asp	Asn	Thr	Phe	Leu	His
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Gly	Tyr	Thr	Phe	Gly	Gly	His	Pro	Val	Ser	Ala	Ala	Val	Gly	Val	Ala
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Asn	Leu	Asp	Leu	Phe	Glu	Arg	Glu	Gly	Leu	Asn	Gln	His	Val	Leu	Asp
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Asn	Glu	Ser	Ala	Phe	Leu	Thr	Thr	Leu	Gln	Lys	Leu	His	Asp	Leu	Pro
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Val	Lys	Asp	Lys	Ala	Thr	Lys	Glu	Thr	Phe	Thr	Asp	Glu	Glu	Ser	Glu
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Arg	Val	Leu	Tyr	Gly	Phe	Val	Ser	Lys	Lys	Leu	Phe	Glu	Tyr	Gly	Leu
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Tyr	Cys	Arg	Ala	Asp	Asp	Arg	Gly	Asp	Pro	Val	Ile	Gln	Leu	Ser	Pro
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Pro	Leu	Ile	Ser	Asn	Gln	Ser	Thr	Phe	Asp	Glu	Ile	Glu	Ser	Ile	Ile
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Arg	Gln	Val	Leu	Thr	Glu	Ala	Trp	Thr	Lys	Leu					
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<210> 29
 <211> 450
 <212> PRT
 <213> Streptomyces avermitilis MA 4680

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 35 40 45

Gly Arg Arg Tyr Leu Asp Phe Thr Ser Gly Leu Val Phe Thr Asn Ile
 50 55 60
 Gly Tyr Gln His Pro Lys Val Val Ala Ala Ile Gln Glu Gln Ala Ala
 65 70 75 80
 Ser Leu Thr Thr Phe Ala Pro Ala Phe Ala Val Glu Ala Arg Ser Glu
 85 90 95
 Ala Ala Arg Leu Ile Ala Glu Arg Thr Pro Gly Asp Leu Asp Lys Ile
 100 105 110
 Phe Phe Thr Asn Gly Gly Ala Asp Ala Ile Glu His Ala Val Arg Met
 115 120 125
 Ala Arg Ile His Thr Gly Arg Pro Lys Val Leu Ser Ala Tyr Arg Ser
 130 135 140
 Tyr His Gly Gly Thr Gln Gln Ala Val Asn Ile Thr Gly Asp Pro Arg
 145 150 155 160
 Arg Trp Ala Ser Asp Ser Ala Ser Ala Gly Val Val His Phe Trp Ala
 165 170 175
 Pro Tyr Leu Tyr Arg Ser Arg Phe Tyr Ala Glu Thr Glu Gln Gln Glu
 180 185 190
 Cys Glu Arg Ala Leu Glu His Leu Glu Thr Thr Ile Ala Phe Glu Gly
 195 200 205
 Pro Gly Thr Ile Ala Ala Ile Val Leu Glu Thr Val Pro Gly Thr Ala
 210 215 220
 Gly Ile Met Val Pro Pro Pro Gly Tyr Leu Ala Gly Val Arg Glu Leu
 225 230 235 240
 Cys Asp Lys Tyr Gly Ile Val Phe Val Leu Asp Glu Val Met Ala Gly
 245 250 255
 Phe Gly Arg Thr Gly Glu Trp Phe Ala Ala Asp Leu Phe Asp Val Thr
 260 265 270
 Pro Asp Leu Met Thr Phe Ala Lys Gly Val Asn Ser Gly Tyr Val Pro
 275 280 285
 Leu Gly Gly Val Ala Ile Ser Gly Lys Ile Ala Glu Thr Phe Gly Lys
 290 295 300
 Arg Ala Tyr Pro Gly Gly Leu Thr Tyr Ser Gly His Pro Leu Ala Cys
 305 310 315 320
 Ala Ala Ala Val Ala Thr Ile Asn Val Met Ala Glu Glu Gly Val Val
 325 330 335
 Glu Asn Ala Ala Asn Leu Gly Ala Arg Val Ile Glu Pro Gly Leu Arg
 340 345 350
 Glu Leu Ala Glu Arg His Pro Ser Val Gly Glu Val Arg Gly Val Gly
 355 360 365
 Met Phe Trp Ala Leu Glu Leu Val Lys Asp Arg Glu Thr Arg Glu Pro
 370 375 380
 Leu Val Pro Tyr Asn Ala Ala Gly Glu Ala Asn Ala Pro Met Ala Ala
 385 390 395 400
 Phe Gly Ala Ala Ala Lys Ala Asn Gly Leu Trp Pro Phe Ile Asn Met
 405 410 415
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 Lys Glu Gly Leu Ala Ala Leu Asp Ala Ala Leu Ser Val Ala Asp Glu
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 Tyr Thr
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<210> 30

<211> 446

<212> PRT

<213> Chromobacterium violaceum ATCC 12472

<400> 30

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 20 25 30

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Pro Arg Leu Ile Val Ser Gly Glu Gly Asn Tyr Leu Thr Asp Ala Asp
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Gly Arg Arg Ile Phe Asp Ser Leu Ser Gly Leu Trp Cys Cys Gly Ala
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Gly His Ser Arg Lys Glu Ile Ala Glu Ala Ala Tyr Arg Gln Leu Ser
65      70      75      80
Thr Leu Asp Tyr Ser Pro Gly Phe Gln Phe Gly His Pro Leu Ser Phe
      85      90      95
Arg Leu Ala Glu Arg Val Ala Ala Met Ala Pro Gly Ala Leu Asn His
      100      105      110
Val Phe Phe Thr Asn Ser Gly Ser Glu Cys Ala Asp Thr Ala Val Lys
      115      120      125
Met Ala Arg Ala Tyr Trp Arg Leu Lys Gly Gln Ala Ser Lys Thr Lys
      130      135      140
Leu Ile Gly Arg Ala Arg Gly Tyr His Gly Val Asn Ile Ala Gly Thr
145      150      155      160
Ser Leu Gly Gly Met Asn Gly Asn Arg Lys Leu Phe Gly Pro Leu Met
      165      170      175
Asp Ala Asp His Leu Pro His Thr Leu Leu Pro Ala Asn Ala Phe Ser
      180      185      190
Arg Gly Leu Pro Glu Gln Gly Ala Glu Leu Ala Asp Asp Leu Leu Arg
      195      200      205
Leu Ile Glu Leu His Asp Ala Ser Asn Ile Ala Ala Val Ile Val Glu
      210      215      220
Pro Met Ala Gly Ser Ala Gly Val Ile Val Pro Pro Gln Gly Tyr Leu
225      230      235      240
Gln Arg Leu Arg Glu Ile Cys Thr Gln His Gly Ile Leu Leu Ile Phe
      245      250      255
Asp Glu Val Ile Thr Gly Phe Gly Arg Thr Gly Ser Leu Phe Gly Ala
      260      265      270
Asp His Phe Gly Val Thr Pro Asp Ile Met Asn Leu Ala Lys Gln Leu
      275      280      285
Thr Asn Gly Ala Val Pro Met Gly Ala Val Val Ala Ser Ser Glu Ile
      290      295      300
Tyr Asp Ala Phe Met Ala Gln Ala Thr Pro Glu Tyr Ala Val Glu Phe
305      310      315      320
Ala His Gly Tyr Thr Tyr Ser Ala His Pro Val Ala Cys Ala Ala Ala
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Leu Ala Ala Leu Asp Val Leu Glu Gln Glu Asn Leu Val Ala Arg Ala
      340      345      350
Ala Glu Leu Ala Pro His Phe Glu Arg Gly Ile His Gly Leu Lys Gly
      355      360      365
Leu Pro His Val Ile Asp Ile Arg Asn Cys Gly Leu Ala Gly Ala Val
      370      375      380
Gln Ile Ala Pro Ser Gly Gly Asp Ala Ile Val Arg Pro Tyr Glu Ala
385      390      395      400
Ala Met Ala Leu Trp Arg Lys Gly Phe Tyr Val Arg Tyr Gly Gly Asp
      405      410      415
Ala Leu Gln Phe Gly Pro Pro Phe Thr Ala Thr Pro Gln Glu Leu Asp
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Ser Leu Phe Asp Ala Val Gly Glu Thr Leu Ala Lys Leu Ala
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<210> 31
<211> 11539
<212> DNA
<213> Artificial Sequence

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<220>
<223> Vector

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35

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<220>
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20

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<220>
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<400> 35
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35

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<220>
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47

<210> 37
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<220>

<223> Primer

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28

<210> 38

<211> 446

<212> PRT

<213> Arabidopsis thaliana

<400> 38

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Arg	Arg	Ile	Phe	Thr	Thr	Glu	Ala	Ala	Pro	Glu	Lys	Gly	Asn	Glu	Pro	35	40	45	
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His	Ser	Phe	Trp	Asn	Arg	Thr	Thr	Lys	Pro	Ser	Leu	Asp	Leu	Ala	Lys	65	70	75	80
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Leu	His	Thr	Asp	Cys	Pro	His	Tyr	Trp	Arg	Phe	His	Leu	Pro	Gly	Glu	165	170	175	
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Glu	Val	Ile	Cys	Ala	Phe	Gly	Arg	Leu	Gly	Thr	Met	Phe	Gly	Cys	Asp	245	250	255	
Lys	Tyr	Asn	Ile	Lys	Pro	Asp	Leu	Val	Thr	Leu	Ala	Lys	Ala	Leu	Ser	260	265	270	
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Leu	Lys	Ile	Tyr	Lys	Glu	Arg	Asn	Ile	Pro	Glu	Tyr	Val	Ala	Lys	Val	325	330	335	
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<211> 1359

<212> DNA

<213> Pseudomonas sp.

<400> 41

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<211> 1380

<212> DNA

<213> Artificial Sequence

<220>

<223> codon optimized gene for expression in E coli

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<211> 5143

<212> DNA

<213> Artificial Sequence

<220>

<223> Vector

<400> 43

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23443-1015

CLAIMS:

1. Cell, which has been genetically modified relative to its wild type so that, in comparison with its wild type, it is able to produce more ω -aminocarboxylic acids, more ω -aminocarboxylic acid esters or more lactams derived from ω -aminocarboxylic acids,
5 starting from carboxylic acids or carboxylic acid esters, wherein the cell has increased activity, in comparison with its wild type, of the enzymes E_I and E_{III} or of the enzymes E_I, E_{II} and E_{III}:
 - i) an enzyme E_I, which catalyses the conversion of carboxylic acids or carboxylic acid esters to the corresponding ω -hydroxycarboxylic acids or
10 ω -hydroxycarboxylic acid esters, selected from the group consisting of the alkane mono-oxygenases:

alkBGT-encoded alkane monooxygenase from *Pseudomonas putida* GPo1 and cytochrome P450 monooxygenases from *Candida tropicalis*;
 - ii) an enzyme E_{II}, which catalyses the conversion of ω -hydroxycarboxylic acids
15 or ω -hydroxycarboxylic acid esters to the corresponding ω -oxocarboxylic acids or ω -oxocarboxylic acid esters, selected from the group consisting of the alcohol dehydrogenases encoded by the *alkJ* gene;
 - iii) an enzyme E_{III}, which catalyses the conversion of ω -oxocarboxylic acids or ω -oxocarboxylic acid esters to the corresponding ω -aminocarboxylic acids or
20 ω -aminocarboxylic acid esters, selected from the group of the ω -transaminases.
2. Cell according to Claim 1, wherein the enzyme E_I is the *alkBGT*-encoded alkane monooxygenase from *Pseudomonas putida* GPo1.
3. Cell according to Claim 1 or 2, wherein the enzyme E_{II} is encoded by the *alkJ* gene from *Pseudomonas putida* GPo1.
- 25 4. Cell according to any one of Claims 1 to 3, wherein the enzyme E_{III} is the ω -transaminase CV2025 from *Chromobacterium violaceum* DSM30191.

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5. Cell according to any one of Claims 1 to 4, wherein the expression of an enzyme E_{IV}, which catalyses the conversion of ω -aminocarboxylic acid esters to the corresponding ω -aminocarboxylic acids, is increased in the cell in comparison with its wild type.
- 5 6. Cell according to Claim 5, wherein the enzyme E_{IV} is the lipase LipA (Q76D26) from *Pseudomonas fluorescens*, which is secreted by the cell.
7. Cell according to any one of Claims 1 to 6, wherein the expression of an enzyme E_V, which catalyses the conversion of ω -aminocarboxylic acids to the corresponding lactams, is increased in the cell in comparison with its wild type.
- 10 8. Cell according to Claim 7, wherein the enzyme E_V is secreted by the cell.
9. Cell according to any one of Claims 1 to 8, wherein the cell is a genetically modified *Escherichia coli* cell, a genetically modified *Corynebacterium glutamicum* cell or a genetically modified *Pseudomonas putida* cell.
10. Method for producing ω -aminocarboxylic acids, of ω -aminocarboxylic acid
 15 esters or of lactams derived from ω -aminocarboxylic acids, containing the process steps:
- I) contacting the cell according to any one of Claims 1 to 9 with a culture medium containing a carboxylic acid or a carboxylic acid ester or with a culture medium contiguous with an organic phase containing a carboxylic acid or a carboxylic acid ester in conditions that make it possible for the cell to form ω -aminocarboxylic acids,
 20 ω -aminocarboxylic acid esters or lactams derived from ω -aminocarboxylic acids, starting from carboxylic acid or from carboxylic acid esters; and
- II) optionally isolation of the resultant ω -aminocarboxylic acids, the resultant ω -aminocarboxylic acid esters or the lactams derived from ω -aminocarboxylic acids.
11. Method according to Claim 10, wherein the ω -aminocarboxylic acid esters
 25 formed in step I) are converted in another process step by conventional chemical methods to ω -aminocarboxylic acids.

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12. Method according to Claim 10 or 11, wherein the cell is a genetically modified *Escherichia coli* cell, a genetically modified *Corynebacterium glutamicum* cell or a genetically modified *Pseudomonas putida* cell.

13. Method according to any one of Claims 10 to 12, wherein the culture medium
5 used in step I) contains amino acids, which function as amine donor in the transaminase-catalysed conversion of the ω -oxocarboxylic acids or the ω -oxocarboxylic acid esters to the corresponding ω -aminocarboxylic acids or ω -aminocarboxylic acid esters.

14. Method according to any one of Claims 10 to 13, wherein the method is carried out in a two-phase system, containing

10 A) an aqueous phase, and

B) an organic phase,

where the formation of the ω -aminocarboxylic acids, the ω -aminocarboxylic acid esters or the lactams derived from ω -aminocarboxylic acids by the cells in step I) takes place in the aqueous phase and the resultant ω -aminocarboxylic acids, the resultant
15 ω -aminocarboxylic acid esters or the resultant lactams derived from ω -aminocarboxylic acids accumulate in the organic phase.

15. Method according to any one of Claims 10 to 14, wherein the isolation of the resultant ω -aminocarboxylic acids, the resultant ω -aminocarboxylic acid esters or the lactams derived from ω -aminocarboxylic acids takes place by an at least two-stage purification
20 process, comprising

a) an extraction step, in which the ω -aminocarboxylic acids, the ω -aminocarboxylic acid esters or the lactams derived from ω -aminocarboxylic acids are extracted from the culture medium, and

b) a fine purification step, in which the extract obtained in step a) is purified
25 further by distillation methods or additional extraction processes, obtaining an

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ω -aminocarboxylic acid phase, an ω -aminocarboxylic acid ester phase or a lactam phase with a purity of at least 99.8%.

16. Method according to Claim 15, wherein the extraction in step a) is a reactive extraction.

5 17. Method according to any one of Claims 10 to 16, wherein the carboxylic acid is lauric acid or the carboxylic acid ester is methyl laurate and in that the lauric acid or the methyl laurate is converted in step II) to laurinolactam.

18. Method for producing polyamides based on ω -aminocarboxylic acids, comprising the process steps:

10 (α1) production of ω -aminocarboxylic acids by the method according to any one of Claims 10 to 17; and

(α2) polymerization of the ω -aminocarboxylic acid, obtaining a polyamide.

19. Method for producing polyamides based on lactams, comprising the process steps:

15 (β1) production of lactams by the method according to any one of Claims 10 to 17; and

(β2) ring opening polymerization or polycondensation of the lactam, obtaining a polyamide.

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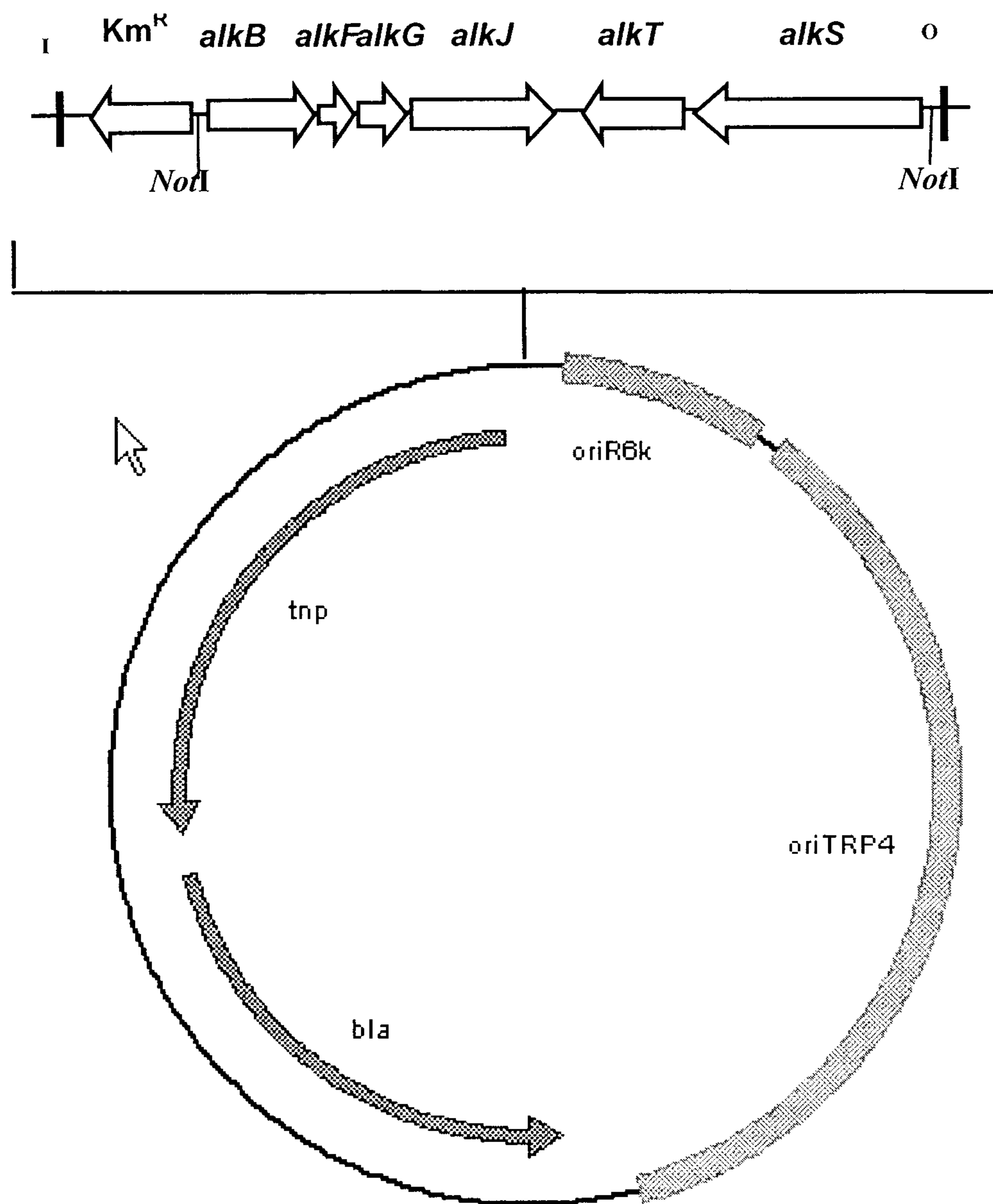


Fig. 1

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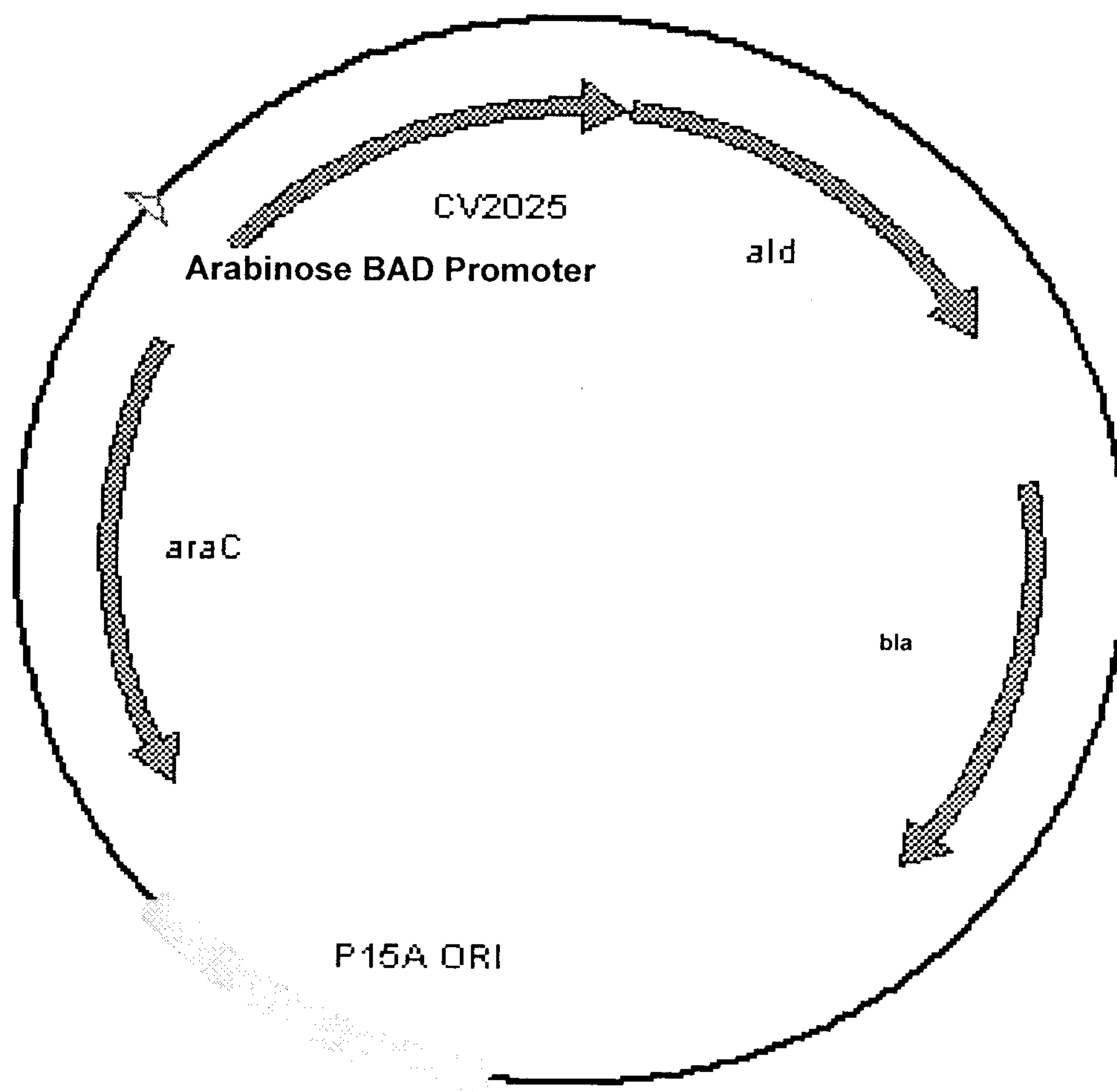


FIG. 2

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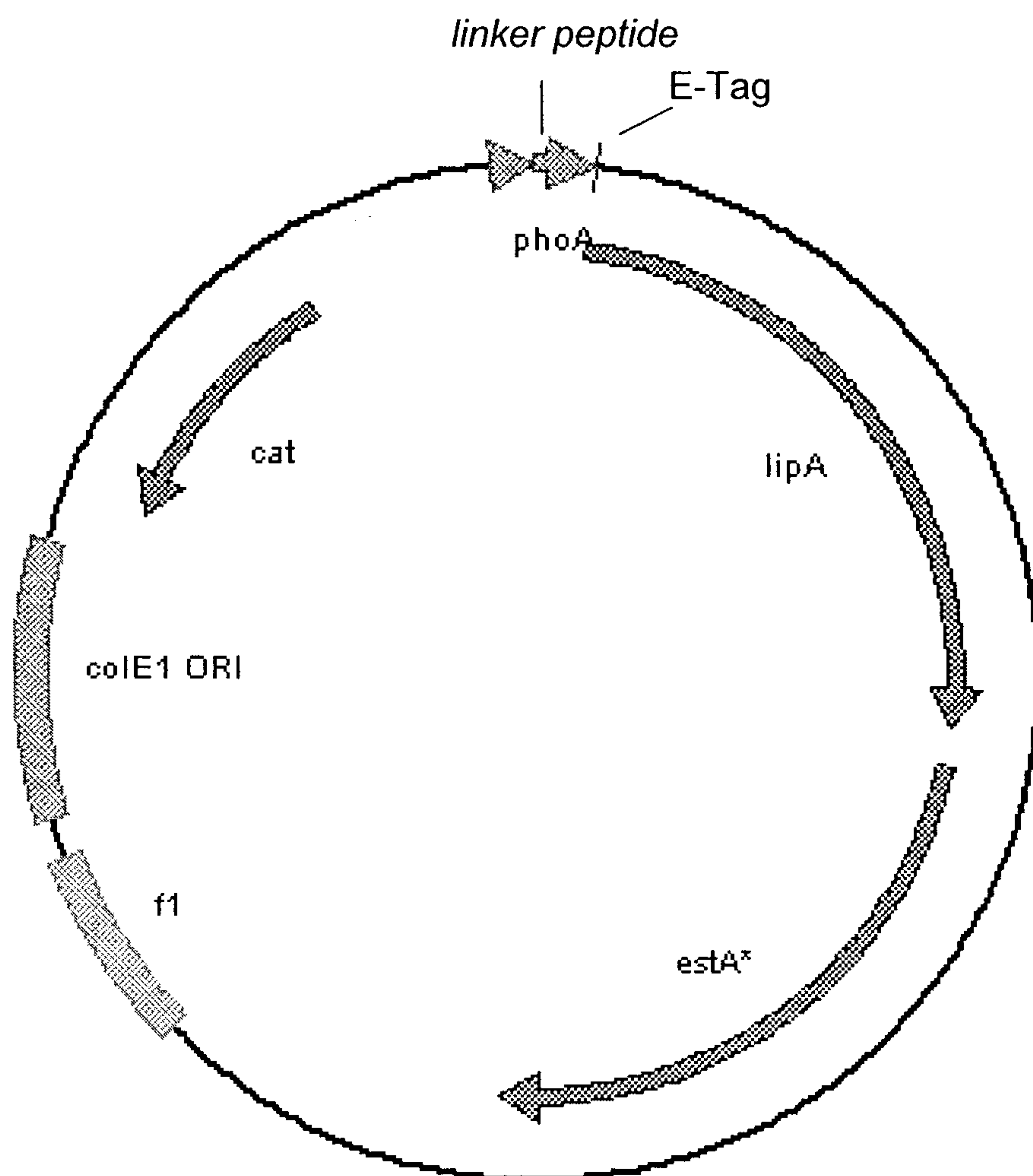


FIG. 3

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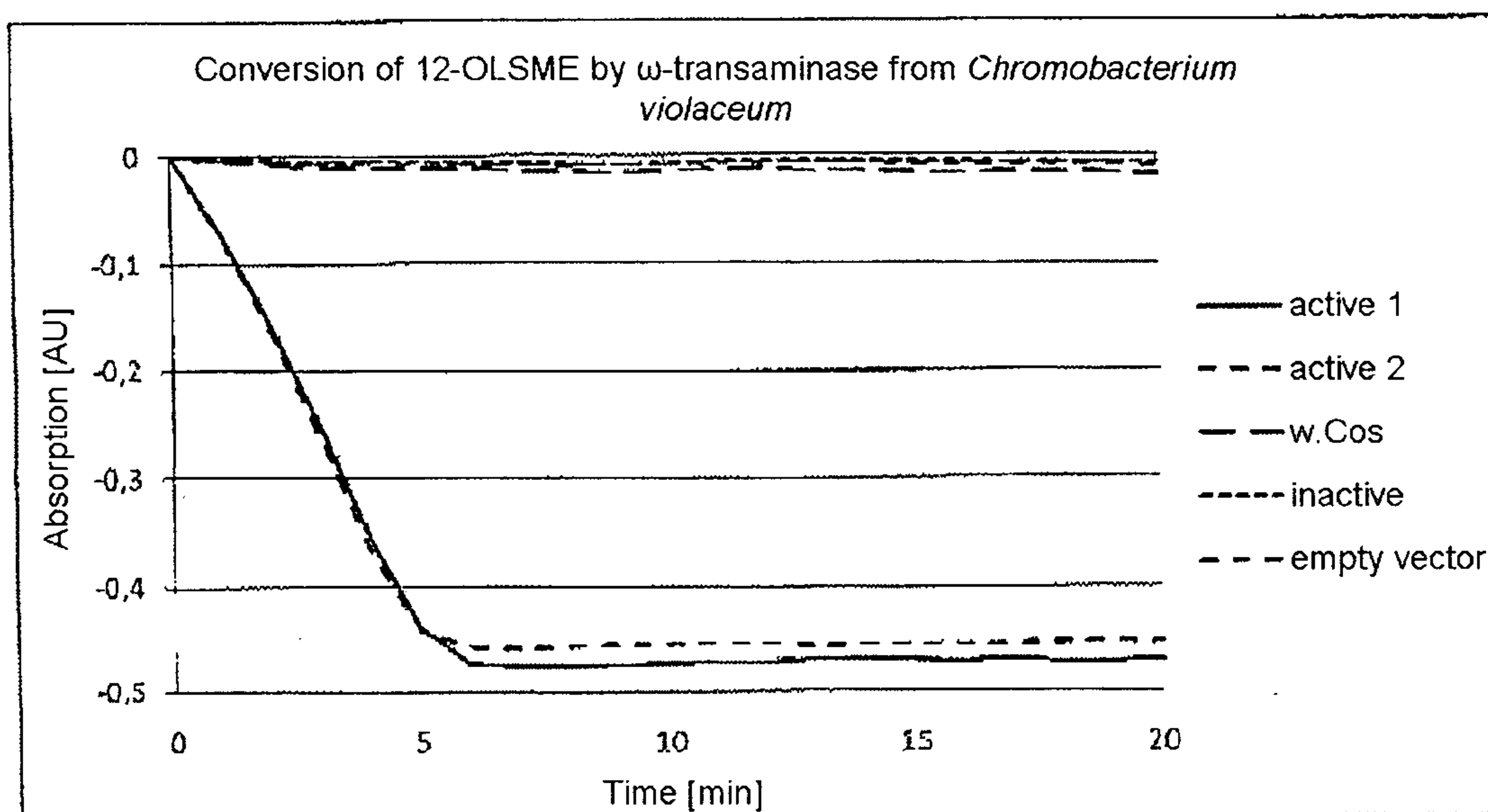
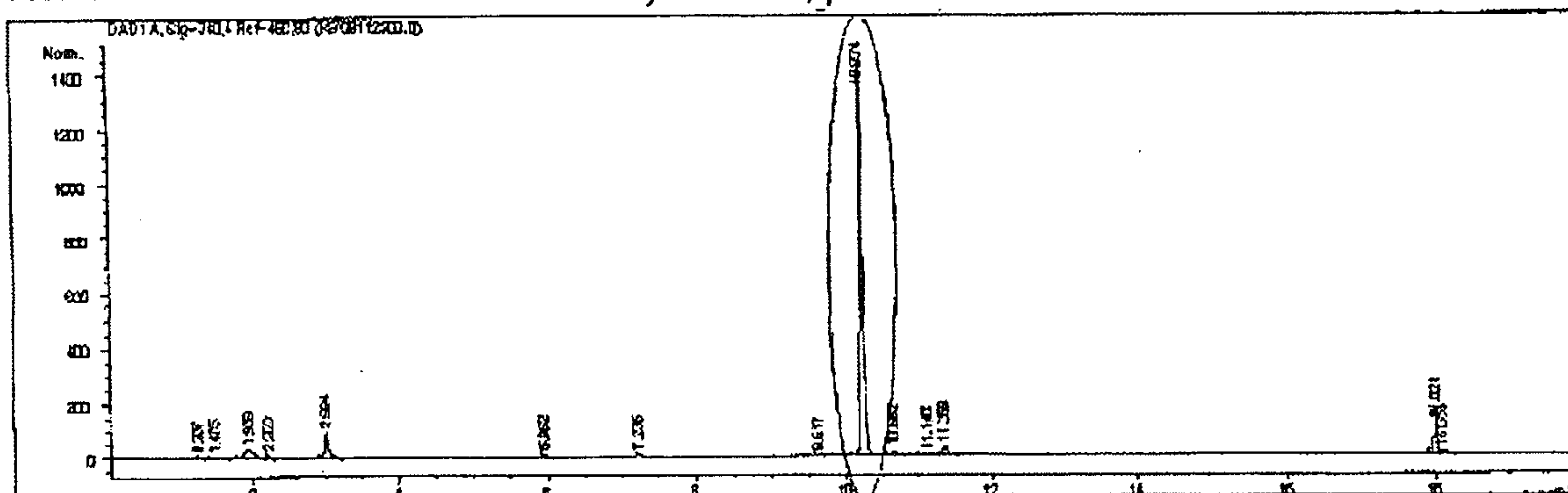


FIG. 4

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Reference substance 12-aminomethyl laurate, peak at 10.2 min



Conversion after 2h, peak at 10.2 min

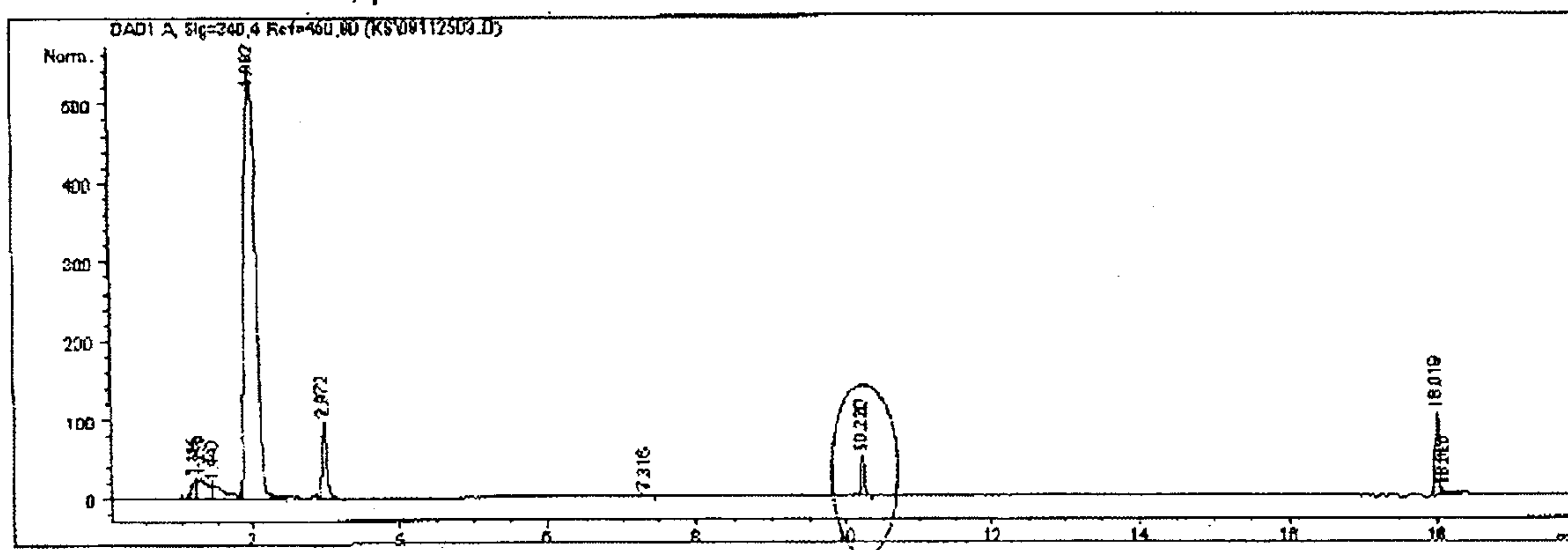
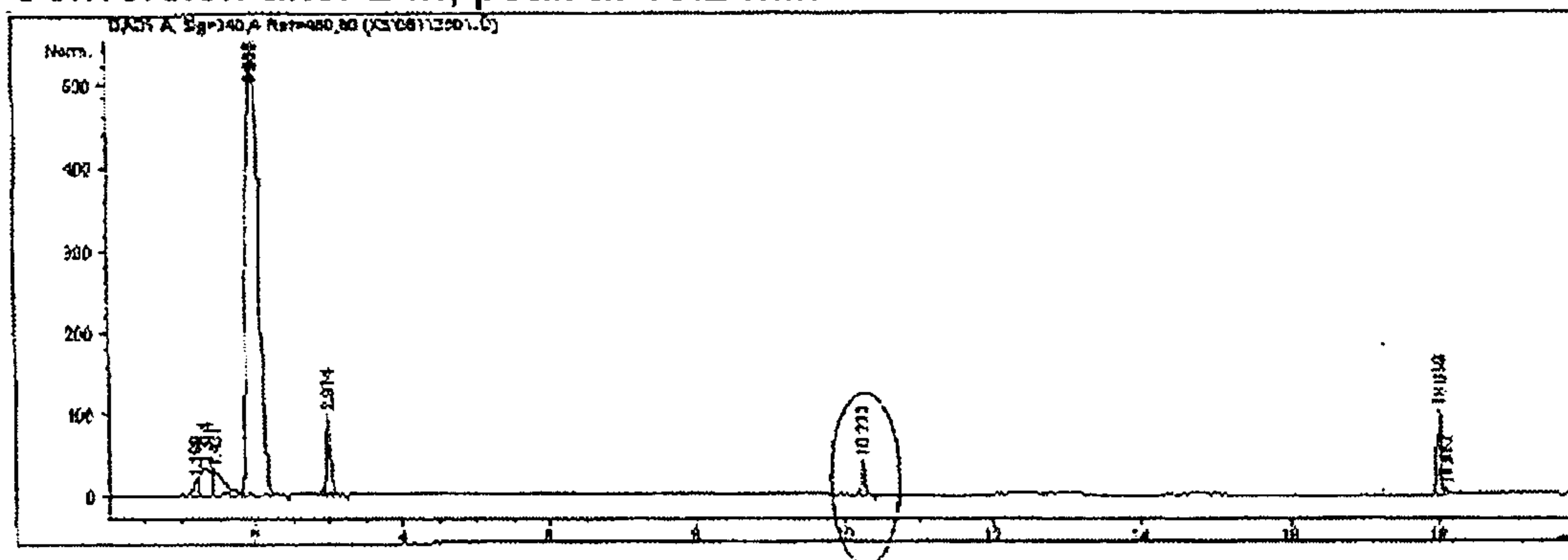


FIG. 5

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Conversion after 24h, peak at 10.2 min



Conversion after 24h, spiked with reference substance, peak at 10.2 min

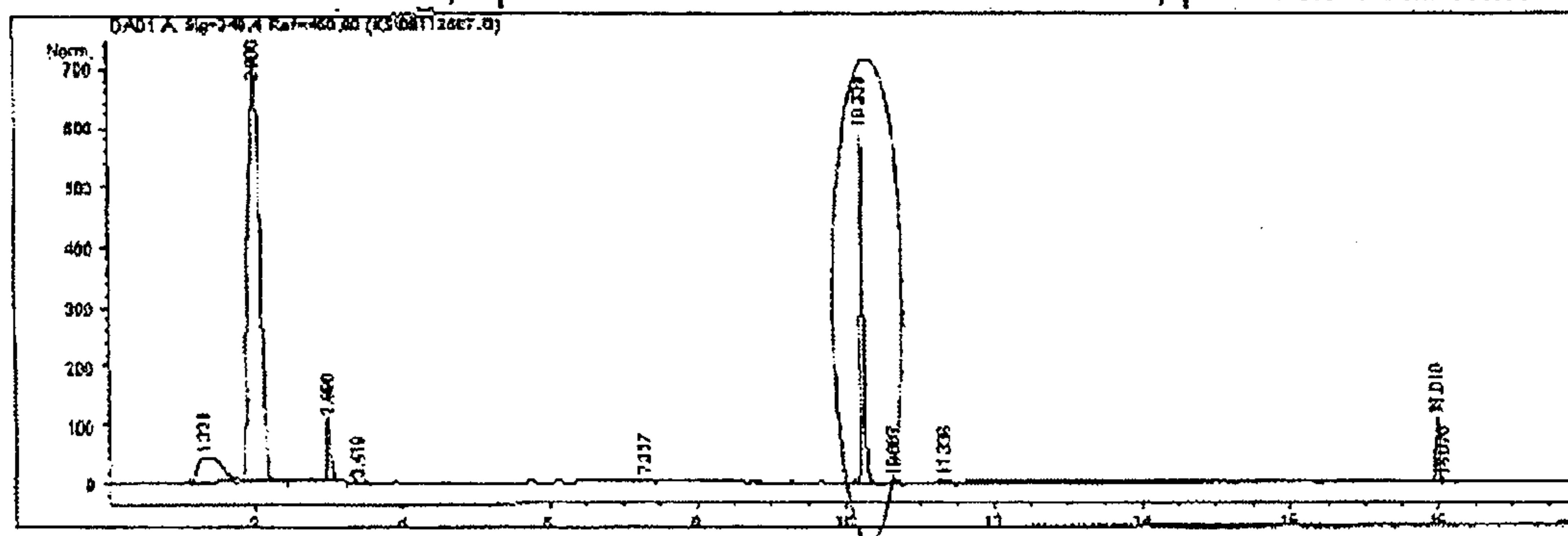
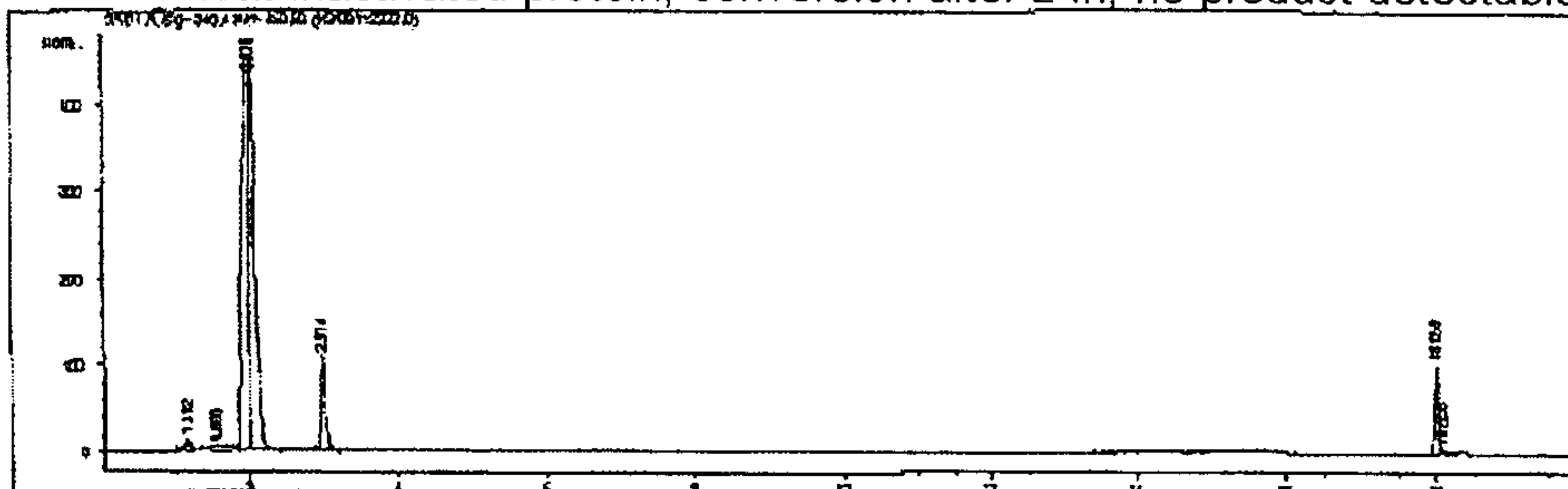


FIG. 6

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Control: heat-inactivated protein, conversion after 24h, no product detectable



Control: conversion after 0h, no product detectable

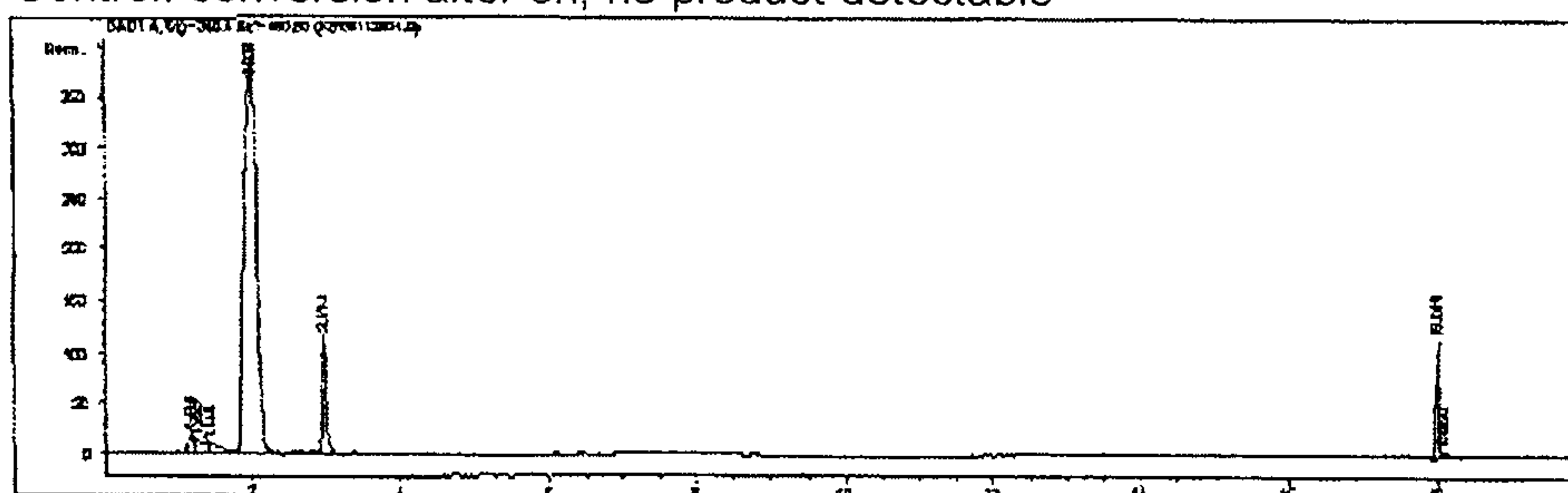


FIG. 7

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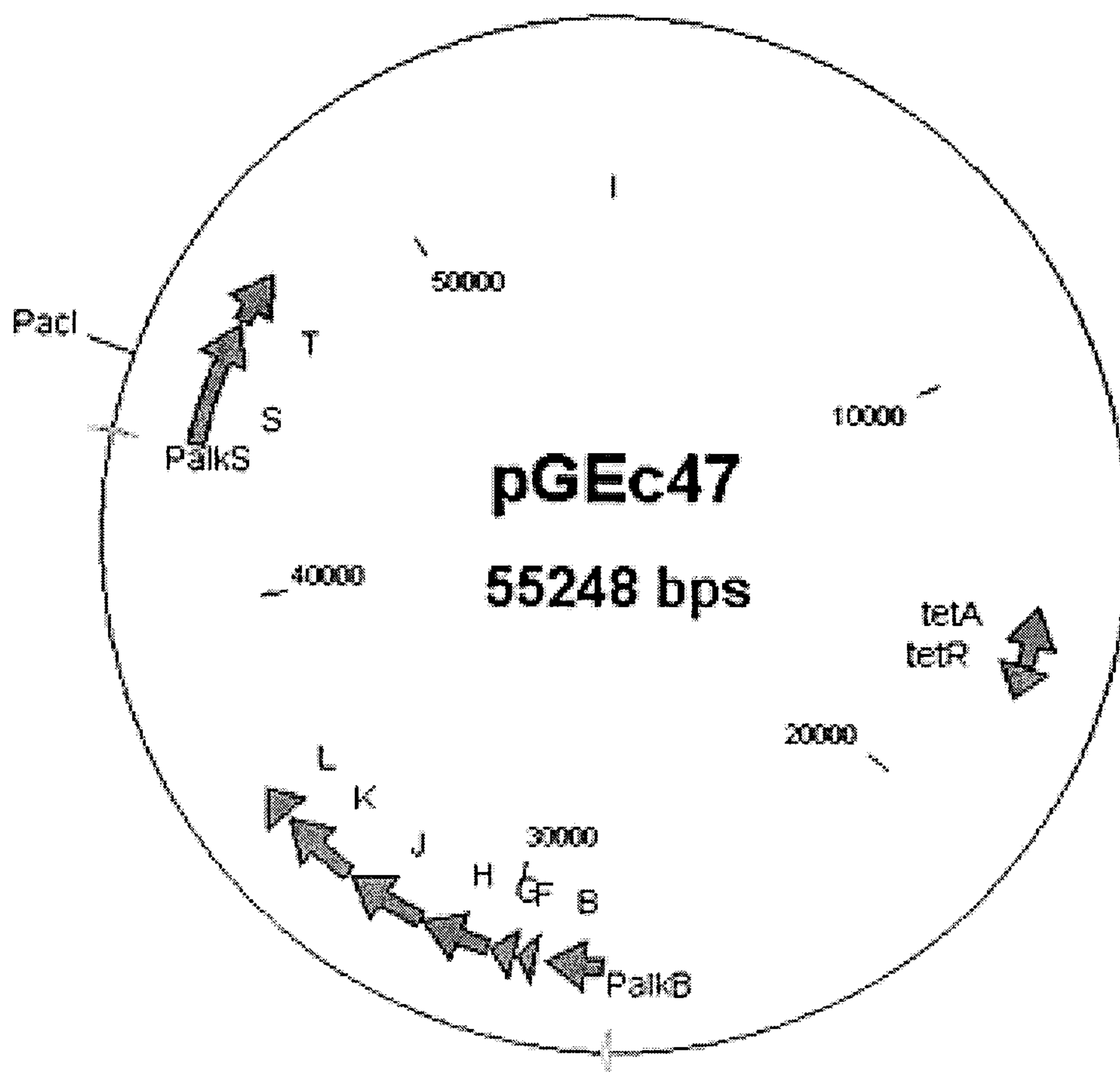


FIG. 8

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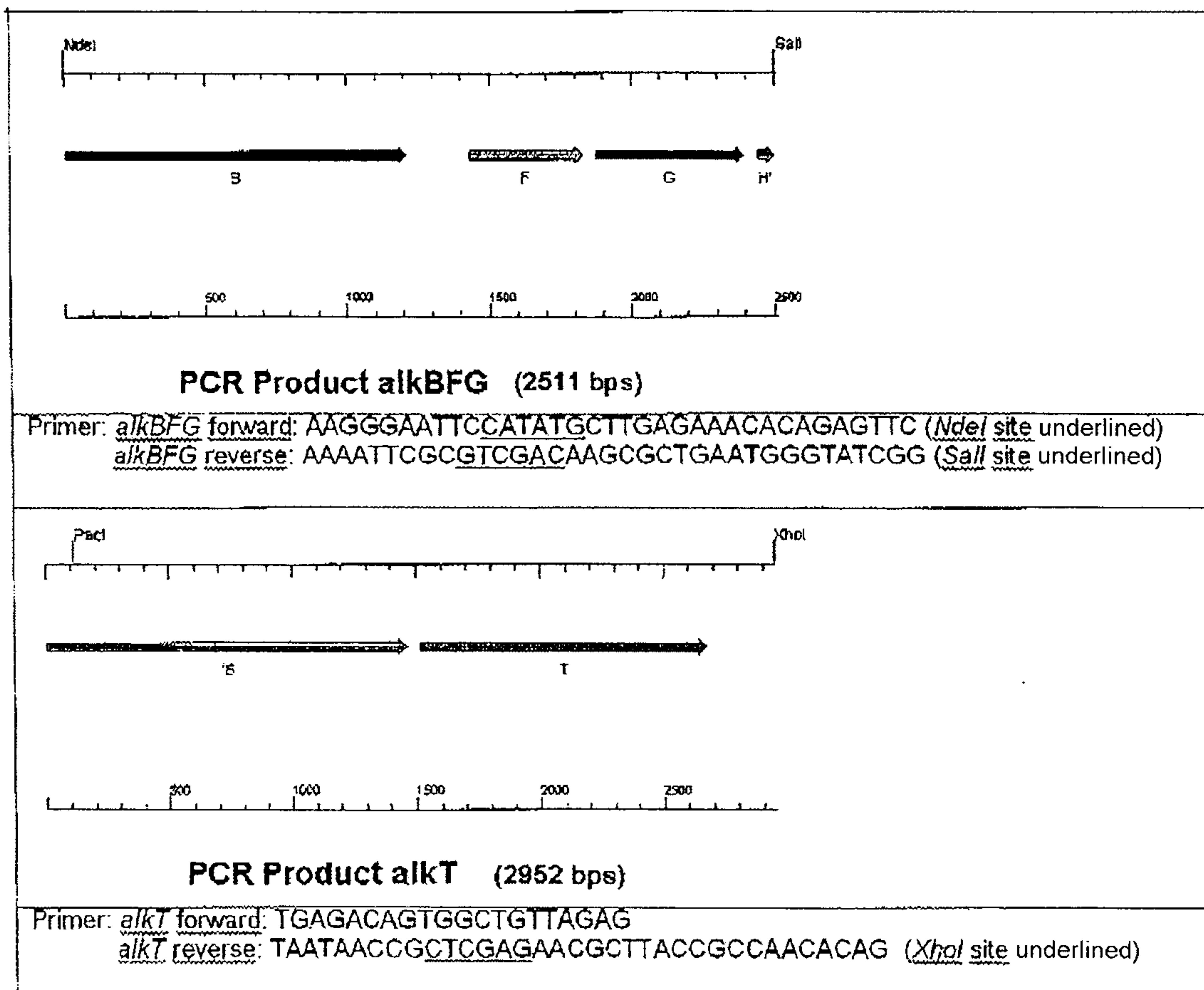


FIG. 9

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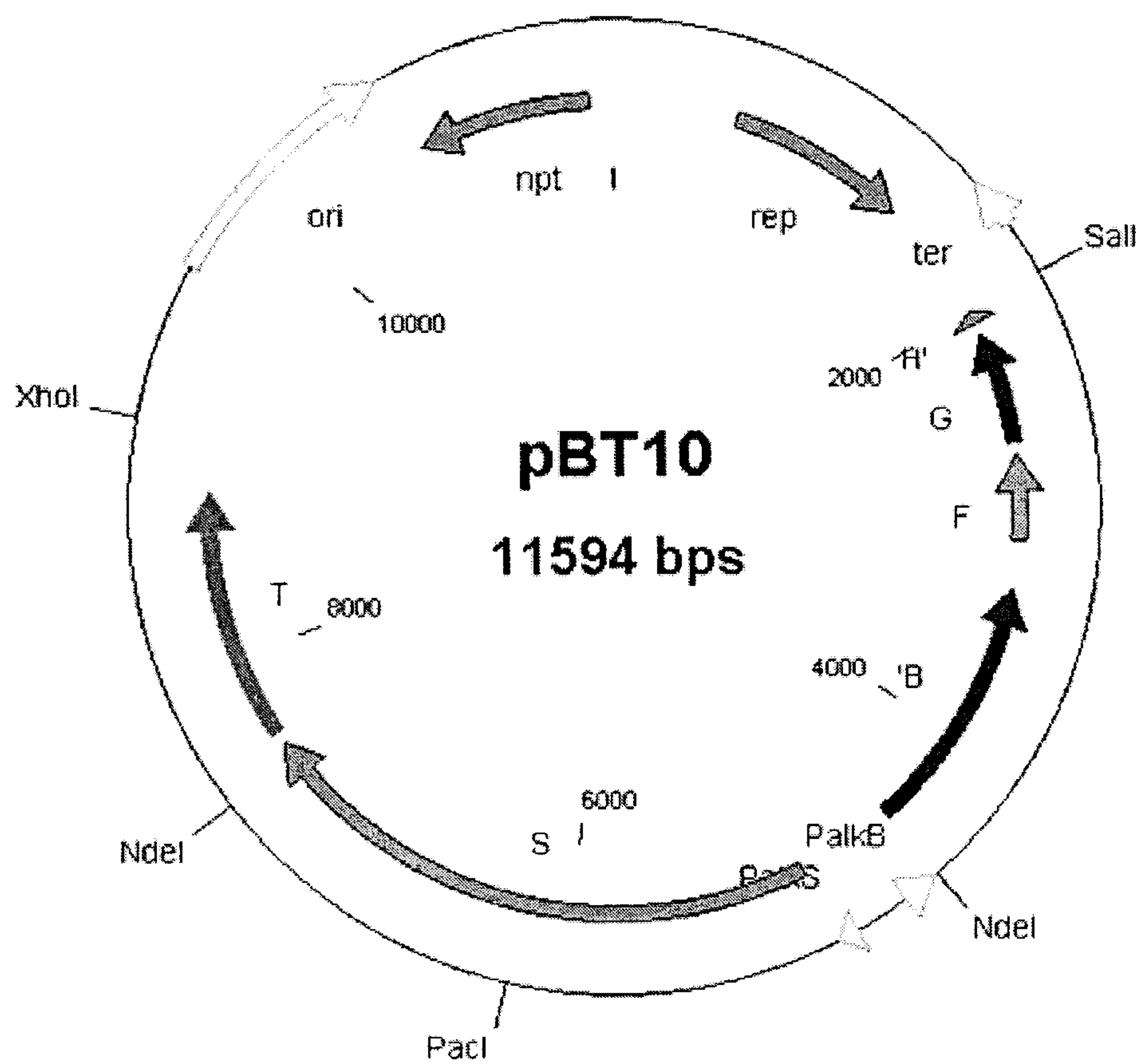


FIG. 10

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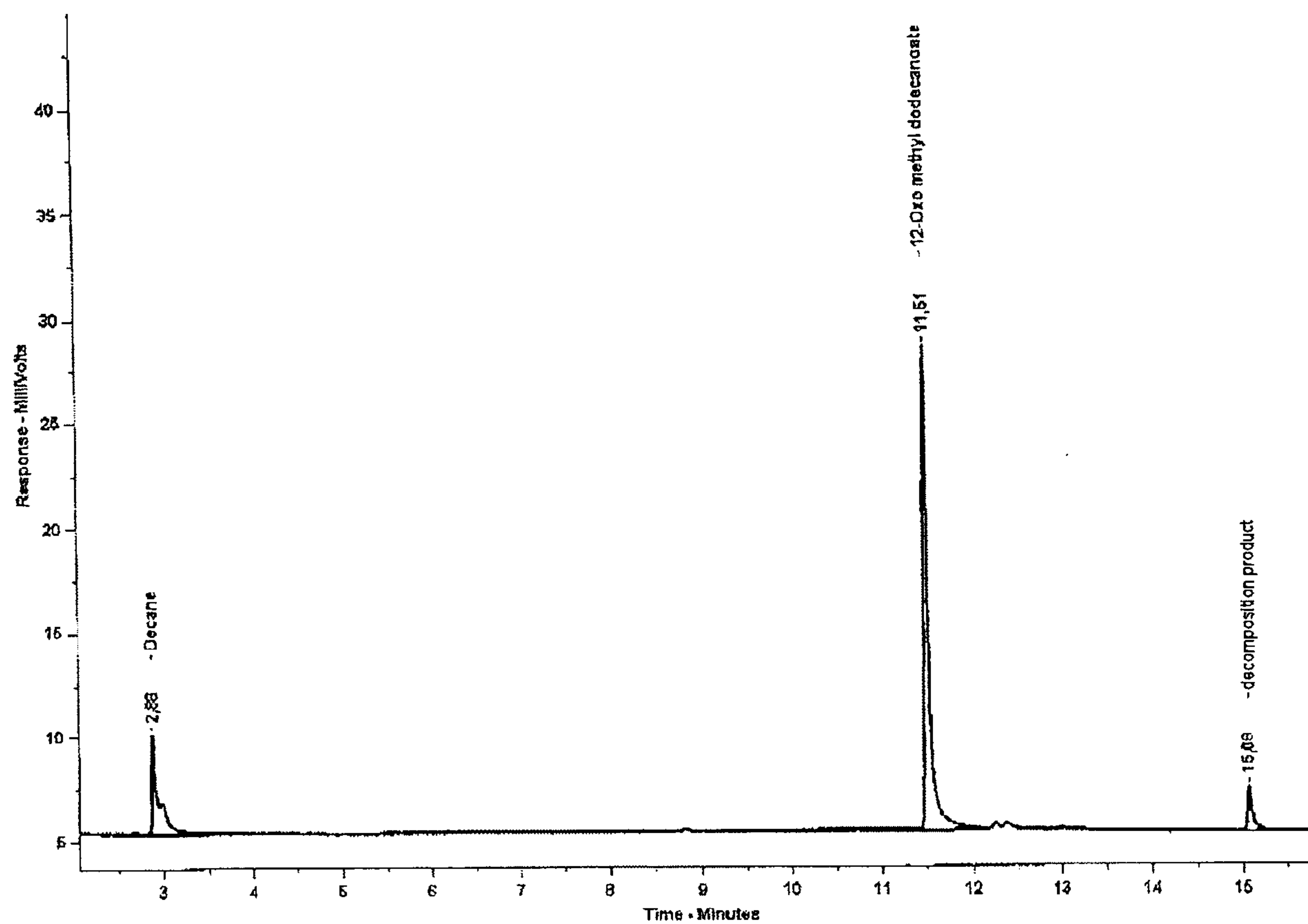


FIG. 11

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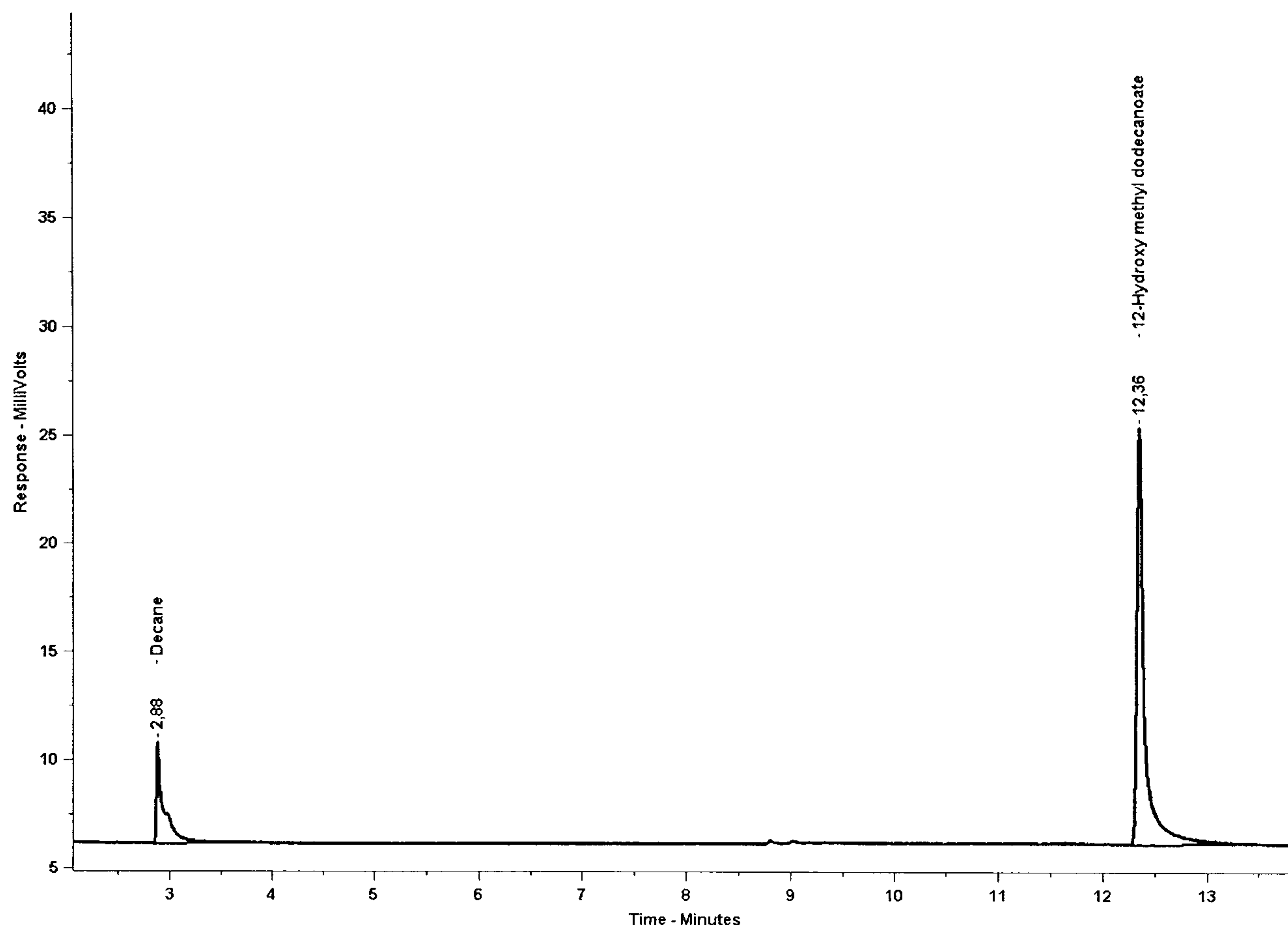
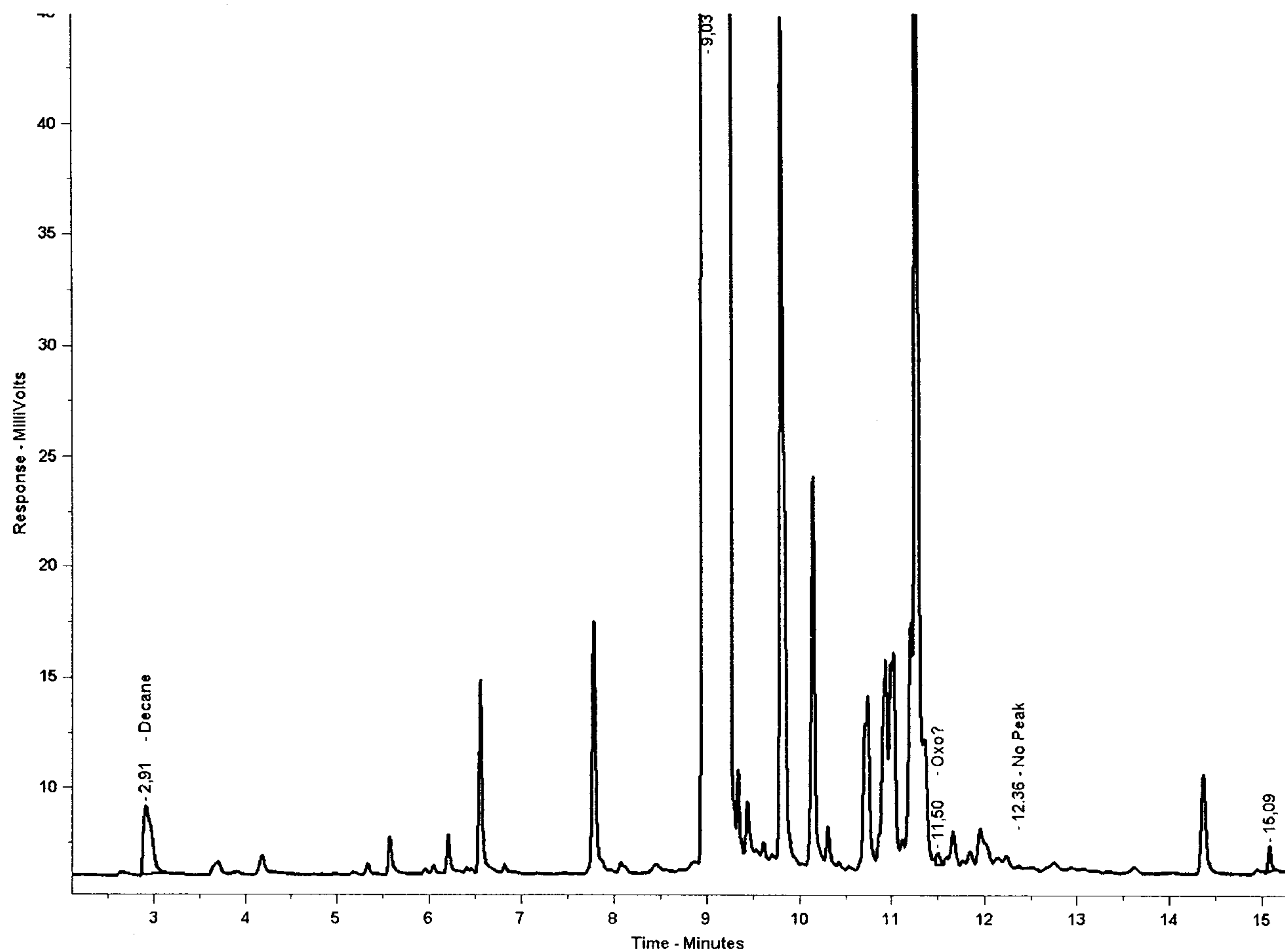


FIG. 12

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FIGUR13

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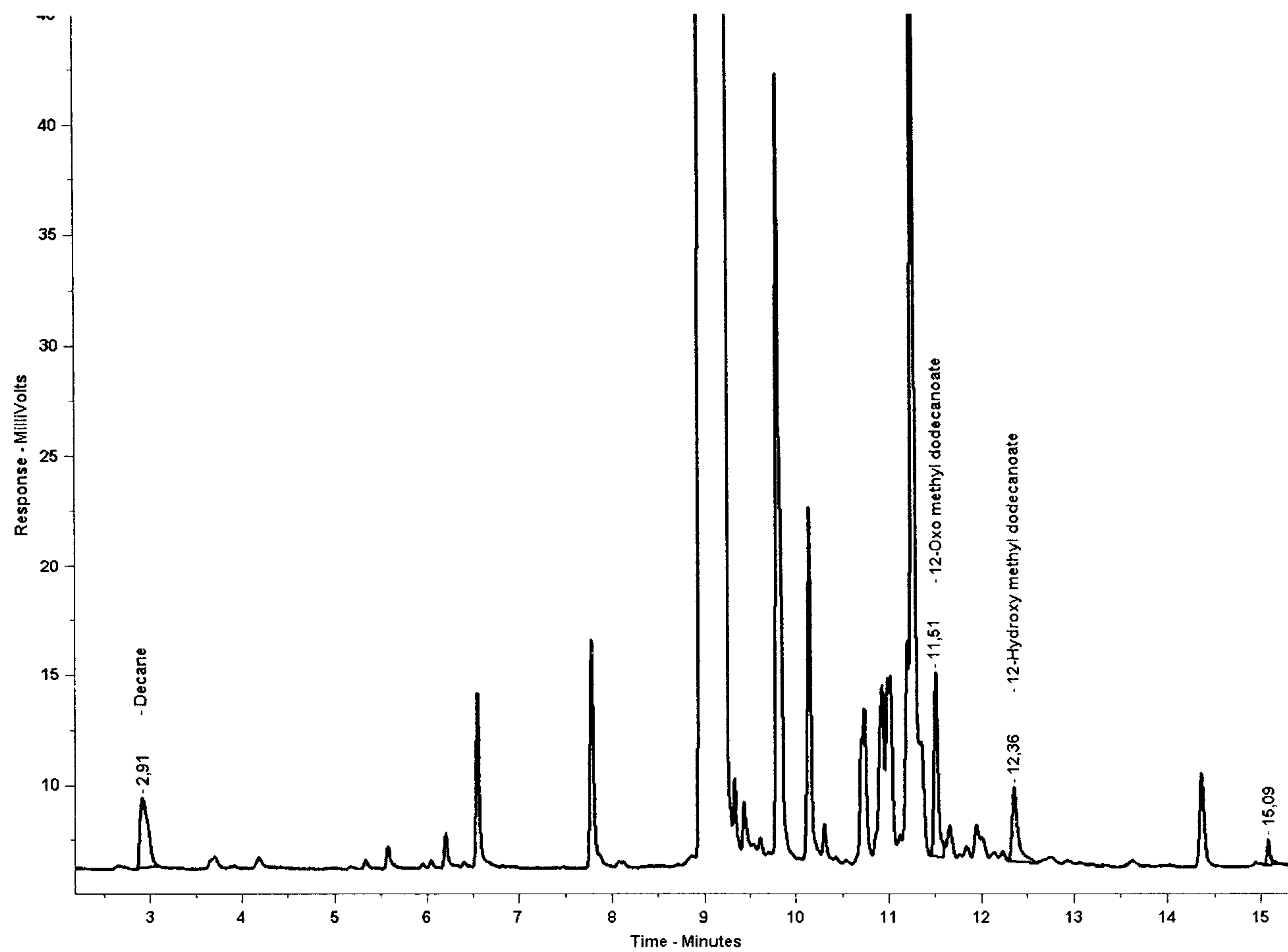


FIG. 14

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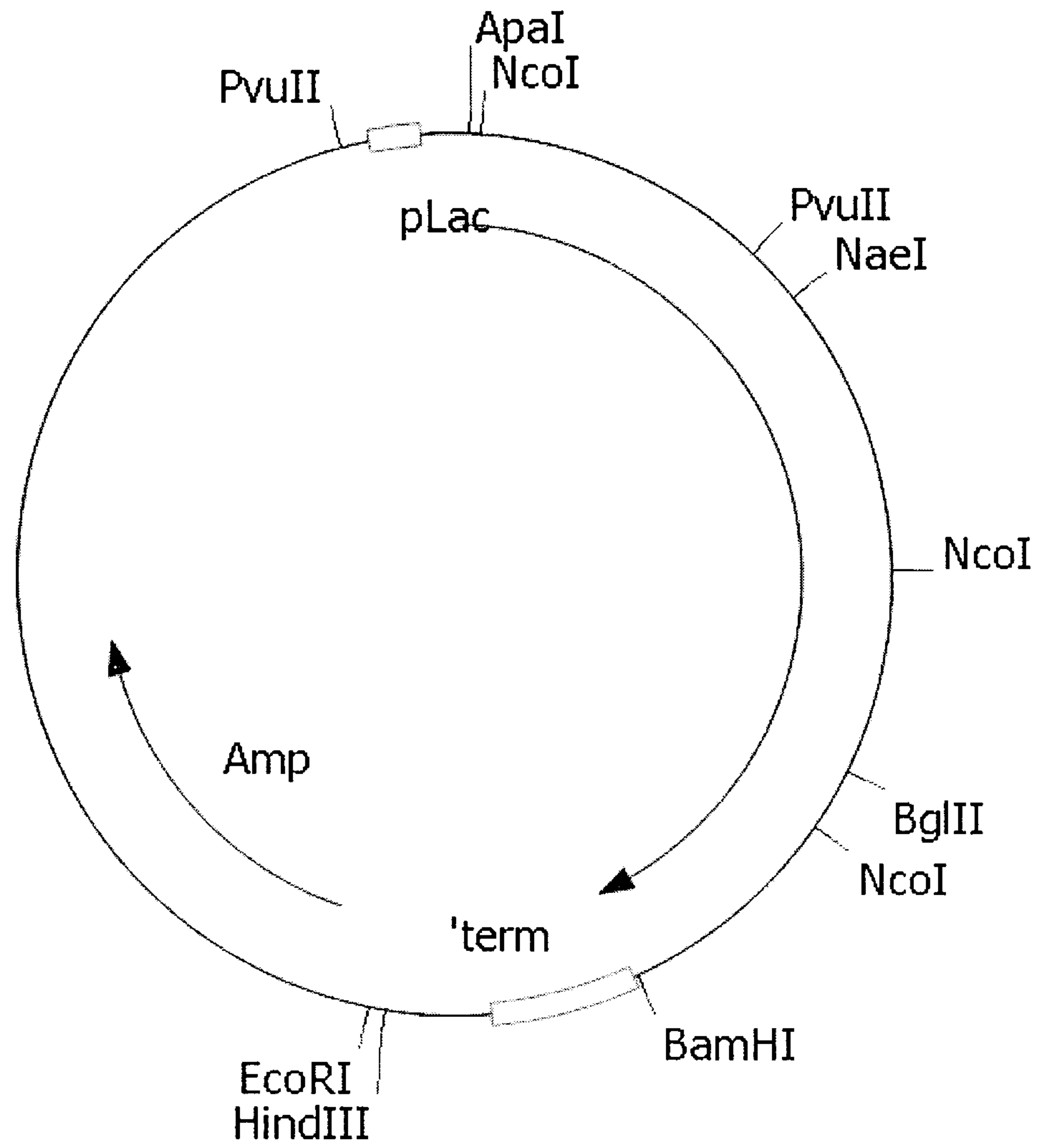


FIG. 15

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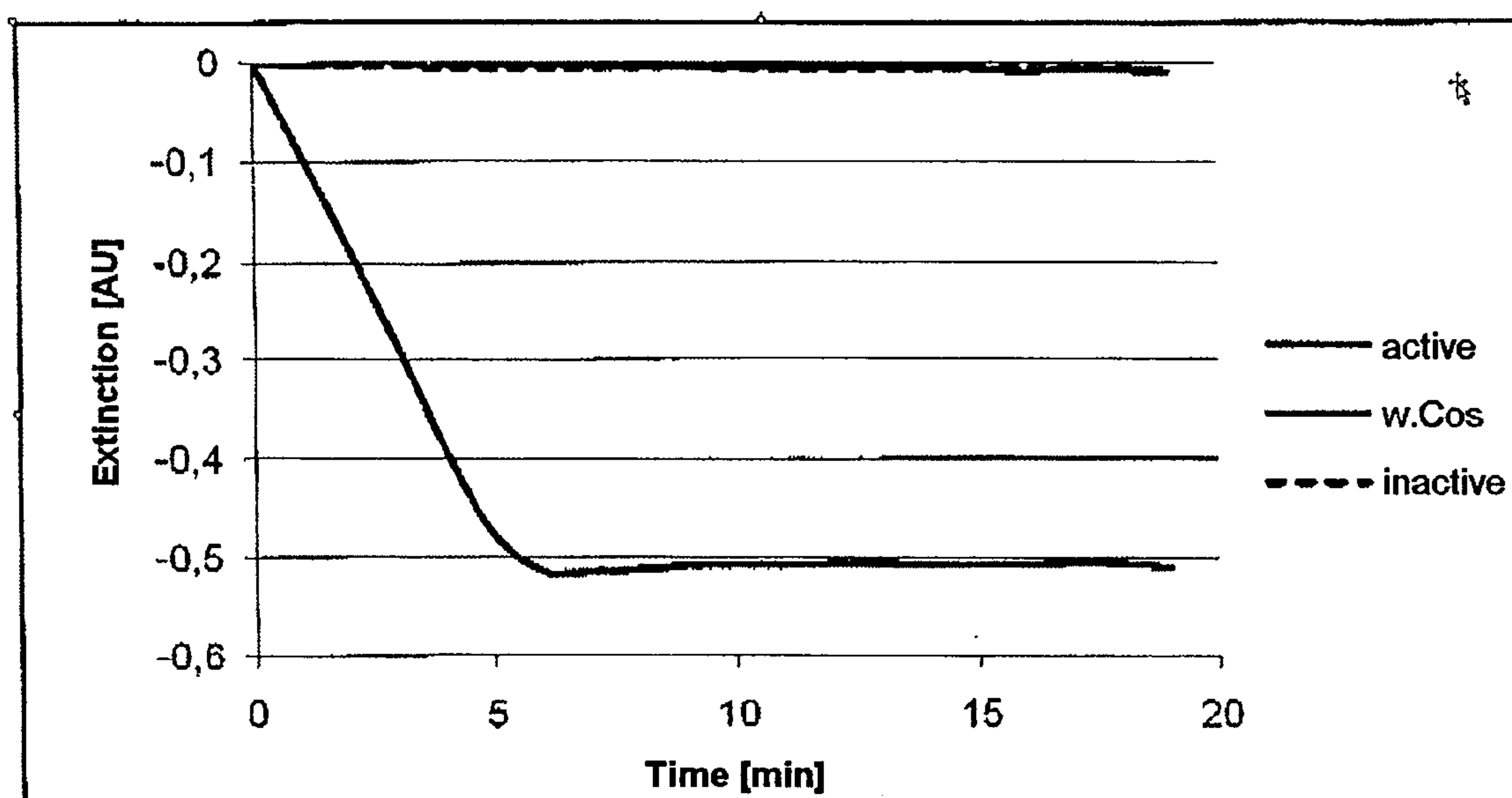


FIG. 16

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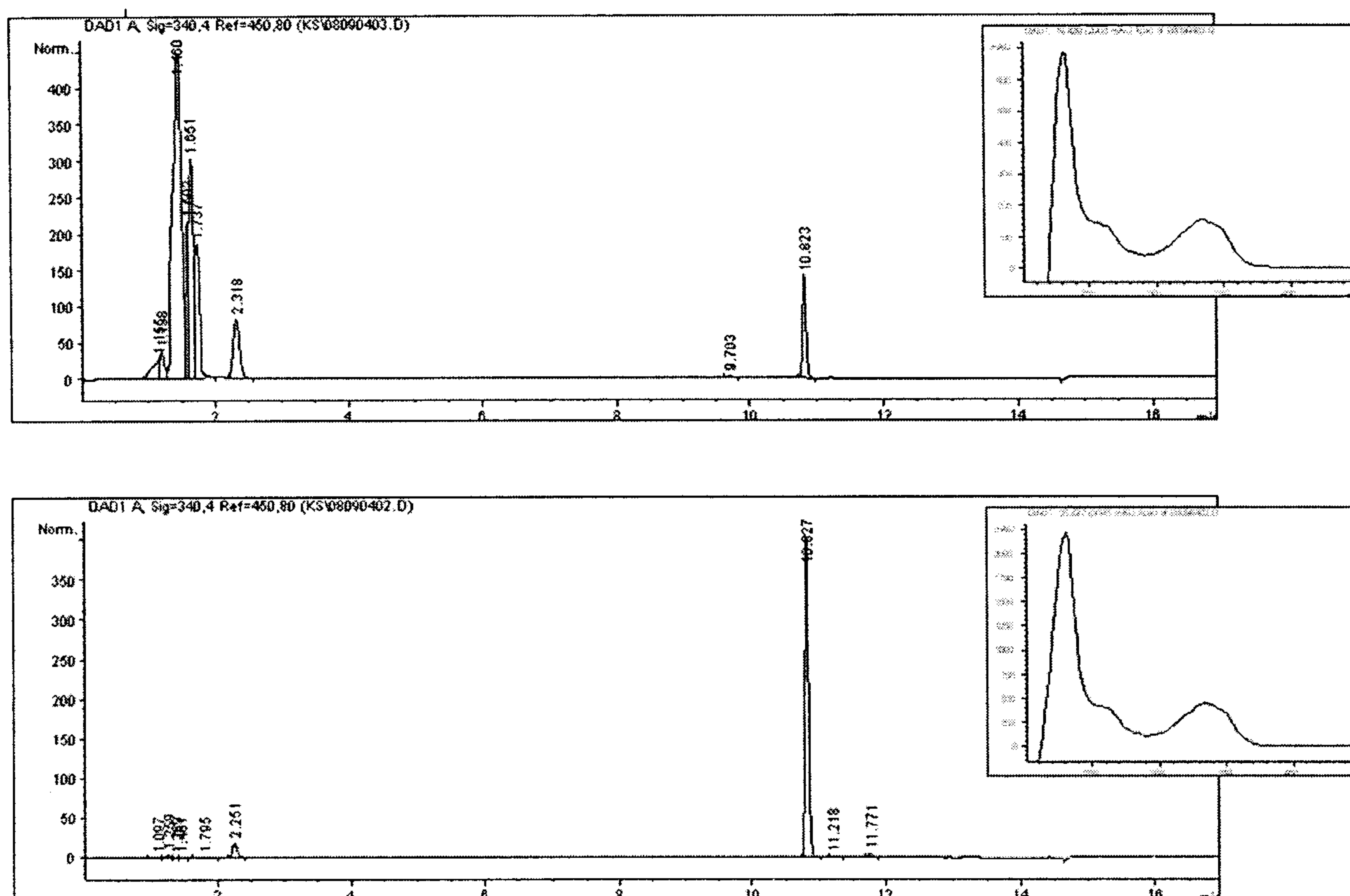


FIG. 17

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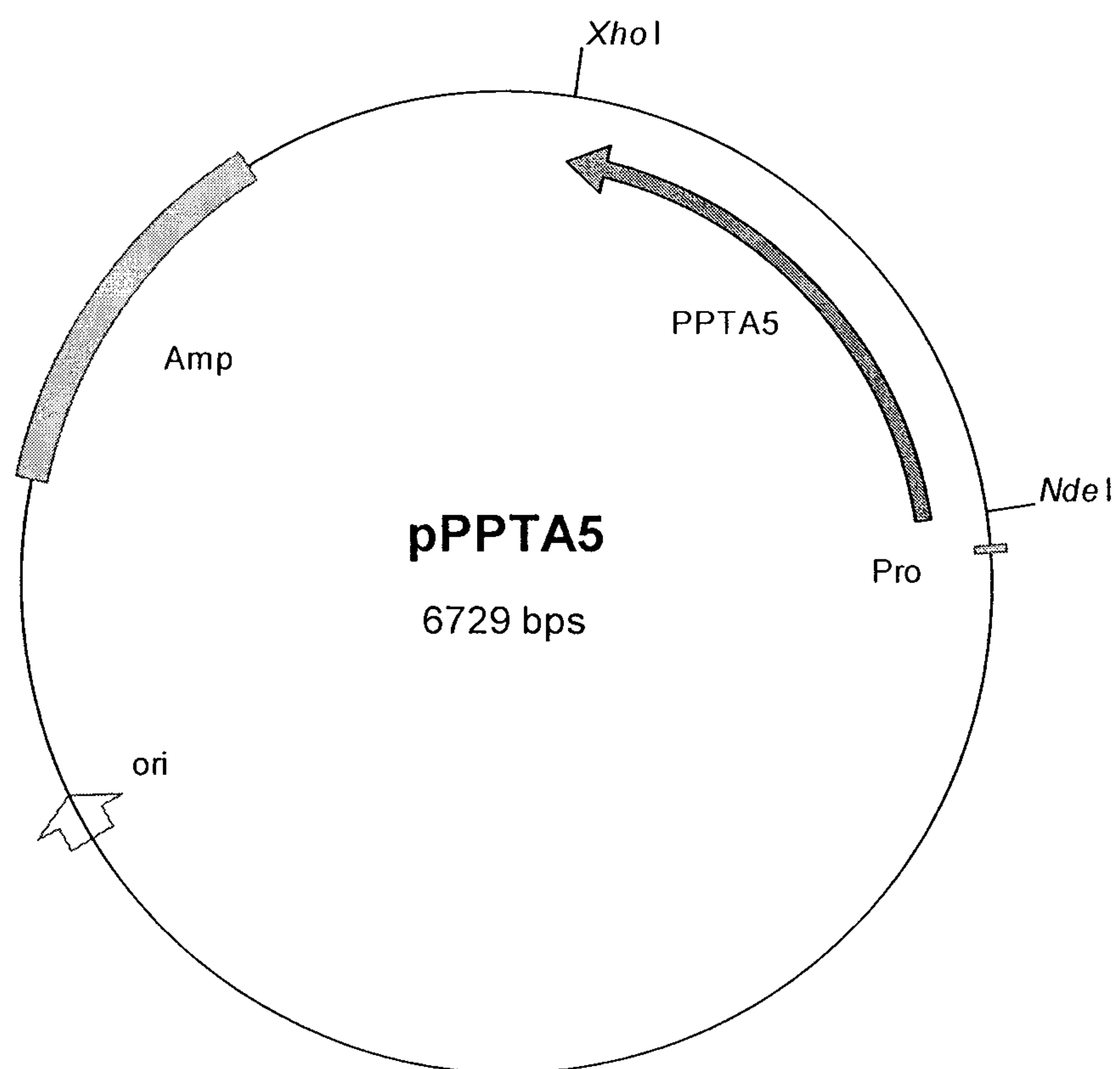


FIG. 18

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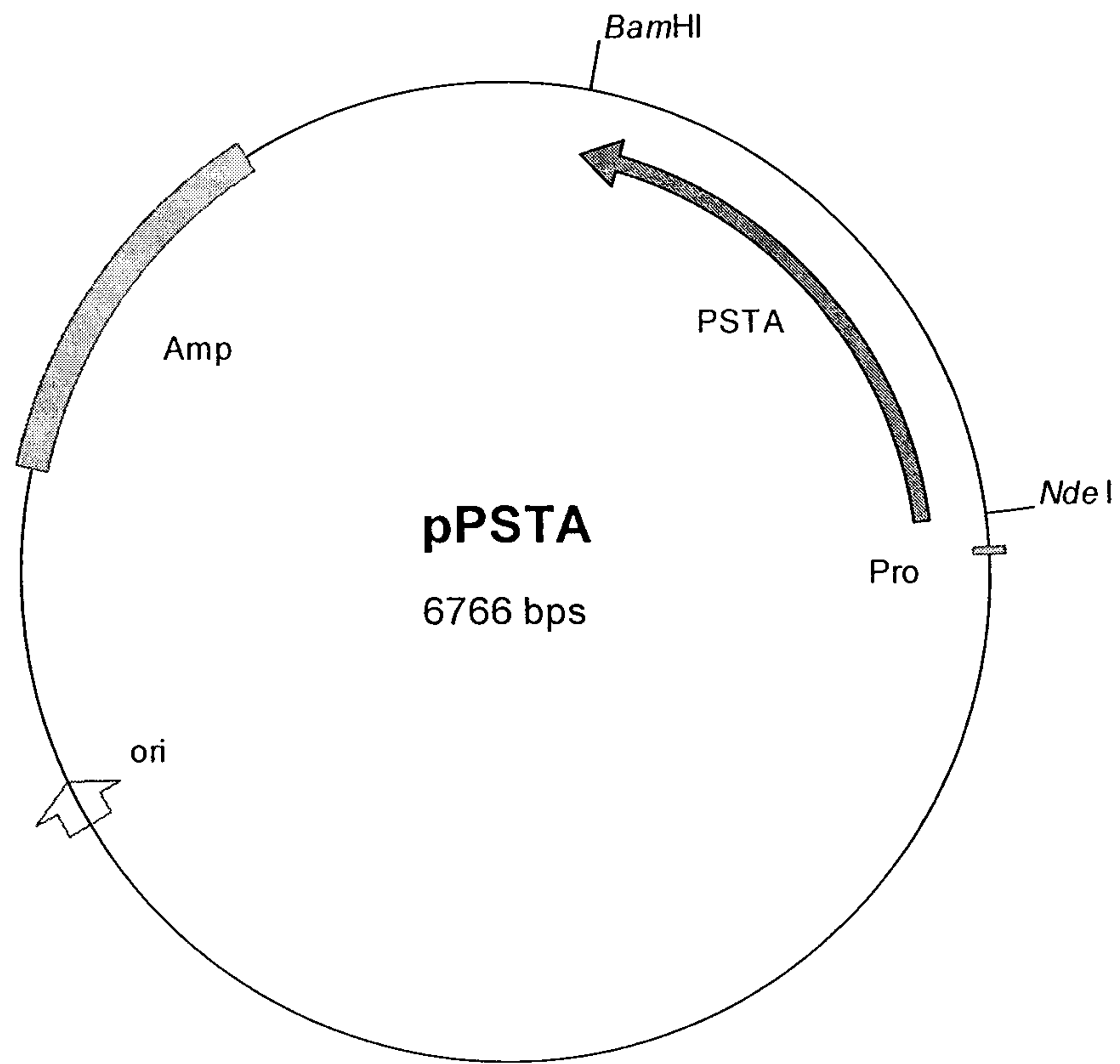


FIG. 19

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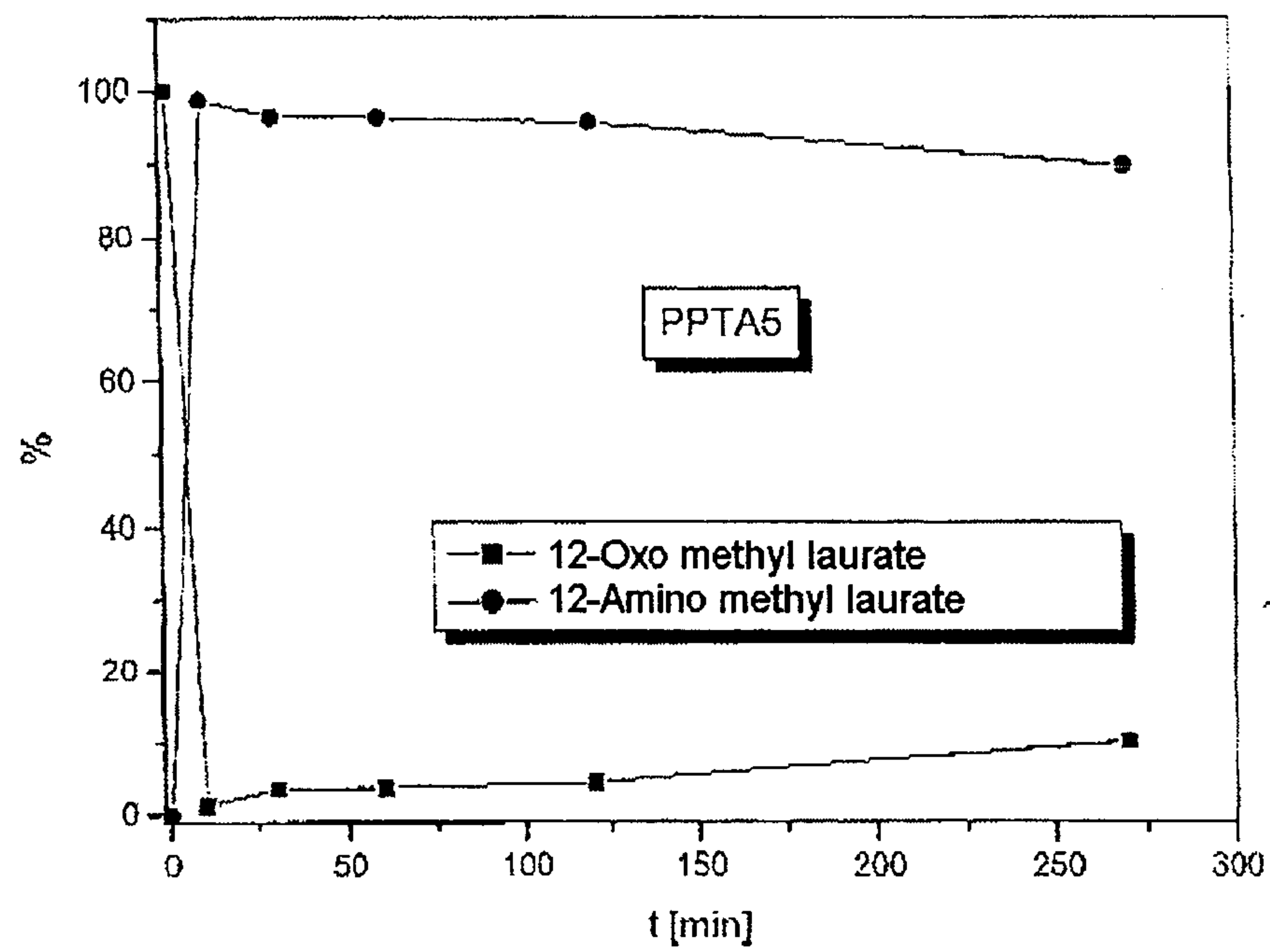


FIG. 20

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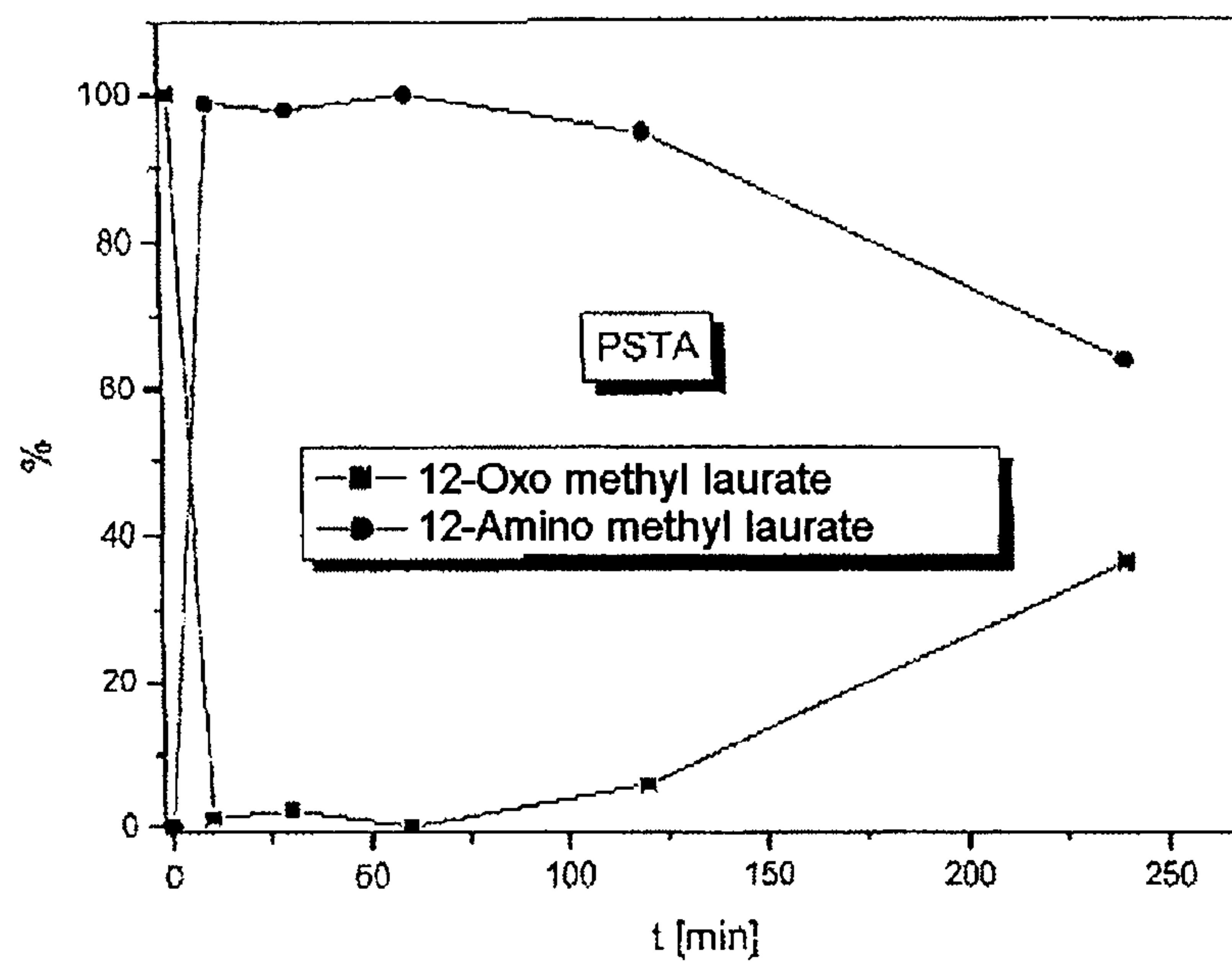


FIG. 21