

Producing Amines And Diamines From A Carboxylic Acid Or Dicarboxylic Acid Or A Monoester Thereof

5 The invention relates to a whole-cell catalyst which expresses a recombinant α -dioxxygenase and/or the combination of a recombinant fatty acid reductase and of a phosphopantetheinyl transferase which phosphopantetheinylates the fatty acid reductase, and which additionally expresses a transaminase, wherein the phosphopantetheinyl transferase and/or transaminase is preferably recombinant; and also to a process for converting a carboxylic acid or dicarboxylic
10 acid or a monoester thereof to an amine or diamine, comprising the steps of contacting the carboxylic acid or dicarboxylic acid or the monoester thereof with a phosphopantetheinylated fatty acid reductase or an α -dioxxygenase and contacting the product with a transaminase.

Polyamides are a class of polymers which are characterized by repeating amide groups. In
15 contrast to the chemically related proteins, the term "polyamide" usually relates to synthetic, commercially available, thermoplastic polymers. Polyamides are derived from primary amines or from secondary amines which are conventionally obtained via cracked hydrocarbons. However, it is also possible to use derivatives, more precisely aminocarboxylic acids, lactams and diamines, for producing the polymer. Also of interest as starting materials are short-chain,
20 gaseous alkanes which can be obtained proceeding from renewable raw materials using biotechnological processes.

The conventional chemical-technical production of such amines and diamines is dependent on the supply of fossil raw materials, inefficient, and in the process large amounts of undesired by-
25 products are produced, in some steps of the synthesis up to 80%. An example of such a process is the production of lauro lactam. Conventionally, this is carried out via a multi-stage process which not only delivers a low yield, but also simultaneously requires the provision of a complex infrastructure.

30 In view of the said disadvantages, processes have been developed in order to obtain amines and diamines using biocatalysts starting from renewable raw materials. Suitable renewable raw materials are in particular sources of fatty acids which can be obtained in the form of rapeseed oil, globe thistle oil, palm kernel oil, coconut oil, sunflower kernel oil and similar natural products from a large number of biological sources, in particular from plants.

PCT/EP 2008/067447 describes a biotechnological system for producing chemically related products, more precisely ω -aminocarboxylic acids, using a cell which has a series of suitable enzymatic activities and is able to convert carboxylic acids to corresponding ω -aminocarboxylic acid. The process comprises a cascade of enzymatically catalysed reactions, more particularly
5 the oxidation of a fatty acid at the terminal carbon atom to the aldehyde and the subsequent amination using a transaminase and an amino acid as amine donor, which can be regenerated via an amino acid dehydrogenase.

A known disadvantage of the AlkBGT oxidase system from *Pseudomonas putida* GPO1 used
10 therein, however, is that it is not able to achieve selective oxidation of aliphatic alkanes to primary alcohols. Rather, a multitude of oxidation products arise; in particular, the fraction of more highly oxidized products, such as the corresponding aldehyde, ketone or the corresponding carboxylic acid, increases with increasing reaction time (C. Grant, J. M. Woodley and F. Baganz (2011), *Enzyme and Microbial Technology* 48, 480-486), which correspondingly
15 reduces the yield of desired amine.

The problem of the relatively unselective oxidation is exacerbated by the fact that the corresponding oxidation products are structurally very similar. This means that it is very difficult to separate them off from the desired oxidation products efficiently and without a significant loss
20 in yield.

A further disadvantage of this process is that overoxidized by-products, for example the dicarboxylic acid of the fatty acid used as reactant, the recycling of hydrophobic solvents and hydrophobic liquid cation exchangers which can be used as per PCT/EP2011/071491 for
25 removing the product from the aqueous reaction mixture, are damaging to efficiency in resource utilization.

In this connection, it should be emphasized that the complexity of biotechnological systems having a cascade of reactions such as the one described in PCT/EP 2008/067447, wherein
30 each reaction is catalysed by a particular enzyme, makes it difficult to optimize the reaction conditions. For instance, in the case of the fundamentally reactive ω -amino fatty acids as product, there is the possibility that, from a certain critical concentration inside the cell, they react with essential constituents of the organism and thus have a toxic effect. If this is the case, the growth and synthesis capacity of the organism is impaired through to cell death, without the

developer being able to immediately recognize the toxicity or to even assign it to a particular reactant, intermediate product or product. Which organism tolerates which concentration of a chemically reactive substance is likewise not predictable.

- 5 Also with regard to a product yield that is to be improved and a by-product development that is to be reduced, a person skilled in the art cannot routinely identify limiting and critical factors in a system such as the one described in PCT/EP2008/067447. If the yield of product is too low, this can be due to the fact that one of the enzymes is present in an excessively low concentration, without it being known which of the possible enzymes it is, i.e. the reactant is not converted
10 within the intended period or before degradation by competing enzymes owing to insufficient synthesis capacity. Alternatively, it is by all means possible that an enzyme is indeed detectable in the cell in the form of a polypeptide, but, specifically in this cell, does not have the folding essential to the activity or lacks a hitherto unknown cofactor that is, however, essential to the activity. Similarly, as already mentioned, the metabolic product may be toxic to the cell or be
15 degraded. Lastly, interfering interactions with endogenous enzymes, i.e. enzymes naturally present in a cell used as whole-cell catalyst, should be expected.

There is therefore a need for processes for producing alkyl monoamines and diamines from fatty acids, in which the enzymatically catalysed reactions proceed more selectively and the
20 formation of undesired by-products is minimized.

Against this background, it is an object of the invention to provide a biotechnological process for producing alkyl monoamines and diamines from fatty acids that is as efficient as possible with regard to yield, carbon and/or nitrogen balance and/or purity.

- 25 It is also an object of the invention to provide a biotechnological process for producing alkyl monoamines and diamines from fatty acids that is as efficient as possible with regard to yield, carbon and/or nitrogen balance, reusability of agents used and/or purity of the product. In this connection, an efficient carbon and/or nitrogen balance is preferably understood as meaning
30 that a very high proportion of the carbon and/or nitrogen fed to a cell for converting a carboxylic acid or a carboxylic ester in the form of suitable substrates is recovered in the desired final product, instead of, for example, being converted to products other than the one desired.

It is also an object of the invention to improve the ability of a multi-phase reaction mixture from the production of alkyl monoamines and diamines from fatty acids to be worked up, especially with regard to reusability relating to work-up of used hydrophobic solvent and liquid cation exchanger, and also with regard to phase formation and separation in a biphasic system comprising an aqueous phase in which the conversion of the carboxylic acid or the carboxylic ester proceeds, and an organic phase containing organic solvents and/or liquid cation exchangers.

These and further objects are achieved by the subject matter of the present application and in particular also by the subject matter of the accompanying independent claims, the dependent claims specifying embodiments of the invention.

In a first aspect, the object underlying the invention is achieved by a whole-cell catalyst which expresses a recombinant α -dioxygenase or the combination of a recombinant fatty acid reductase and of a phosphopantetheinyl transferase which phosphopantetheinylates the fatty acid reductase, and which additionally expresses a transaminase, wherein the phosphopantetheinyl transferase and/or transaminase is preferably recombinant.

In a first embodiment of the first aspect, the object is achieved by a whole-cell catalyst which additionally expresses an amino acid dehydrogenase which is preferably recombinant.

In a second embodiment, which is also an embodiment of the first embodiment, the object is achieved by a whole-cell catalyst which additionally expresses an alkane hydroxylase which is preferably recombinant.

In a third embodiment, which is also an embodiment of the first to second embodiment, the object is achieved by a whole-cell catalyst which additionally expresses a polypeptide of the AlkL family, which polypeptide is preferably recombinant.

In a fourth embodiment, which is also an embodiment of the second embodiment, the object is achieved by a whole-cell catalyst which additionally expresses an alcohol dehydrogenase which is preferably recombinant.

In a fifth embodiment, which is also an embodiment of the first to fourth embodiment, the object is achieved by a whole-cell catalyst, wherein the activity of at least one enzyme involved in the β -oxidation is reduced with respect to the wild type of the whole-cell catalyst.

5 In a sixth embodiment, which is also an embodiment of the first to fifth embodiment, the object is achieved by a whole-cell catalyst, wherein the activity of BioH or a variant thereof is reduced or elevated with respect to the wild type of the whole-cell catalyst.

10 In a seventh embodiment, which is also an embodiment of the first to sixth embodiment, the object is achieved by a whole-cell catalyst, wherein the activity of FadL or a variant thereof is elevated with respect to the wild type of the whole-cell catalyst.

In a second aspect, the object underlying the invention is achieved by a process for converting
15 a carboxylic acid or dicarboxylic acid or a monoester thereof to an amine or diamine, comprising the steps of

a) providing a carboxylic acid or dicarboxylic acid or a monoester thereof, in the case of a dicarboxylic acid, preferably by contacting a carboxylic acid with an alkane
20 hydroxylase and/or alcohol dehydrogenase,

b) contacting the carboxylic acid or dicarboxylic acid or the monoester thereof with a phosphopantetheinylated fatty acid reductase or an α -dioxxygenase to form an aldehyde product, and
25

c) contacting the product from step a) with a transaminase.

In a second aspect, the object underlying the invention is achieved by a process, wherein an amino acid dehydrogenase is present in step c).

30 In a first embodiment of the second aspect, the object is achieved by a process, wherein at least one enzyme from the group comprising phosphopantetheinylated fatty acid reductase, α -dioxxygenase, transaminase, amino acid dehydrogenase and alkane hydroxylase, preferably all

the enzymes used from said group, are provided in the form of a whole-cell catalyst the first aspect of the present invention.

In a second embodiment, which is also an embodiment of the first embodiment, the object is achieved by a process, wherein the carboxylic acid or dicarboxylic acid or the monoester thereof is a compound of the formula (I)



10 where R^1 is selected from the group comprising -H and $COOR^3$,

where R^2 and R^3 are each independently selected from the group comprising H, methyl, ethyl and propyl,

15 with the proviso that at least one of the radicals R^2 and R^3 is H,

where A is an unbranched, branched, linear, cyclic, substituted or unsubstituted hydrocarbon group having at least four carbon atoms.

20 In a third embodiment, which is also an embodiment of the first to second embodiment, the object is achieved by a process, wherein A has the formula $-(CH_2)_n-$, where n is at least 4, preferably at least 10.

In a third aspect, the object underlying the invention is achieved by using the whole-cell catalyst according to the first aspect or the process according to the second aspect for aminating a fatty acid, ω -hydroxy fatty acid, ω -oxo fatty acid or a monoester thereof.

In a fourth aspect, the object underlying the invention is achieved by a reaction mixture comprising the whole-cell catalyst according to the first aspect in aqueous solution and also a fatty acid, ω -hydroxy fatty acid, ω -oxo fatty acid or a monoester thereof of the formula (I)



where R^1 is selected from the group comprising -H, -CHO, -OH and $COOR^3$,

where R^2 and R^3 are each independently selected from the group comprising H, methyl, ethyl and propyl,

5 with the proviso that at least one of the radicals R^2 and R^3 is H,

where A is an unbranched, branched, linear, cyclic, substituted or unsubstituted hydrocarbon group having at least four carbon atoms, preferably the formula $-(CH_2)_n-$, where n is at least 4, particularly preferably at least 10.

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The present invention is based on the finding by the inventor that a functionally recombinant fatty acid reductase or α -dioxygenase in a whole-cell catalyst which is used to produce amines and diamines from fatty acids and has a corresponding enzyme environment surprisingly
15 increases the yield of amines and diamines.

Furthermore, the present invention is based on the finding by the inventor that a functionally recombinant fatty acid reductase or α -dioxygenase in a whole-cell catalyst which is used to produce amines and diamines from fatty acids and has a corresponding enzyme environment
20 surprisingly reduces the concentration of interfering by-products, more particularly overoxidized fatty acids in the form of dicarboxylic acids and esters thereof, in the product which arises.

Furthermore, the present invention is based on the finding by the inventor that a functionally recombinant fatty acid reductase or α -dioxygenase in a whole-cell catalyst which is used to
25 produce amines and diamines fatty acids and has a corresponding enzyme environment improves the purity and reusability of liquid cation exchangers such as oleic acid, which are used to remove the amine and diamine from a fermentation solution comprising the whole-cell catalyst.

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The present invention provides an improved process for converting a carboxylic acid or dicarboxylic acid or a monoester thereof to an amine or diamine, which process is notable for the fact that, besides the enzymes catalysing the transfer of the fatty acid via its various oxidation stages to the amine, a fatty acid reductase or α -dioxygenase is also present,

preferably when a whole-cell catalyst is used to carry out the process. In a preferred embodiment, the term "fatty acid reductase", as used herein, is understood to mean an enzyme which catalyses the conversion of a ω -carboxylic acid, also referred to as dicarboxylic acid or ω -carboxy fatty acid, to the corresponding ω -oxo fatty acid with consumption of ATP and NAD(P)H. In the prior art, for example in WO/2010/135624, fatty acid reductases for producing ω -hydroxy fatty acids are described, but not as part of a system for producing ω -amino fatty acids. In an even more preferred embodiment, the fatty acid reductase is selected from the group of fatty acid reductases comprising the amino acid sequences YP_887275.1, ZP_11001941.1, ZP_06852401.1, NP_959974.1, YP_001070587.1, ZP_05217435.1, YP_882653.1, YP_639435.1, ZP_10800193.1, YP_006452763.1, YP_006730440.1, ZP_11196216.1, YP_005349252.1, ZP_05224908.1, YP_005338837.1, YP_006307000.1, YP_005343991.1, ZP_11001942.1, ZP_09979565.1, YP_005003162.1, YP_953393.1, YP_001850422.1, ZP_11011489.1, ZP_12689264.1, YP_905678.1, ZP_09976919.1, YP_004746059.1, NP_217106.1, YP_004525443.1, NP_337166.1, ZP_09685823.1, YP_978699.1, ZP_06437984.1, ZP_06514086.1, NP_856267.1, CAA19077.1, NP_301424.1, ZP_06522140.1, ZP_06518098.1, ZP_11008938.1, ZP_07432374.2, AAR91681.1, YP_006808747.1, YP_001851230.1, ZP_15327751.1, ZP_15455857.1, ZP_12874284.1, ZP_15332534.1, ZP_15512956.1, ZP_14244106.1, ZP_15470899.1, ZP_11439367.1, YP_001703694.1, ZP_15446742.1, YP_006808978.1, ZP_07964926.1, YP_006521379.1, ZP_10796908.1, ZP_15512957.1, ZP_12874283.1, YP_005350955.1, ZP_14243341.1, YP_001705436.1, ZP_15329649.1, YP_006522325.1, YP_006732197.1, YP_003658971.1, ZP_05227804.1, YP_001703695.1, YP_006308707.1, ZP_15342047.1, YP_006521380.1, ZP_15327752.1, YP_005340557.1, ZP_11439578.1, ZP_15392943.1, ZP_15514789.1, ZP_12996178.1, ZP_09412214.1, ZP_06849686.1, YP_889972.1, YP_006570321.1, ZP_15375693.1, YP_006308219.1, YP_006521600.1, YP_005340029.1, YP_005350457.1, ZP_11439836.1, ZP_12994664.1, ZP_14240588.1, ZP_14236860.1, ZP_09410830.1, YP_006731697.1, YP_005264225.1, YP_001704097.1, ZP_15328186.1, ZP_09402885.1, ZP_12690463.1, AFO59871.1, ZP_07966879.1, YP_118225.1, YP_001828302.1, YP_006566873.1, YP_003660169.1, ZP_15337407.1, ZP_08240521.1, ZP_10456477.1, YP_001537947.1, YP_004016539.1, ZP_07664024.1, ZP_14244107.1, ZP_09794557.1, ZP_09274211.1, ZP_05224899.1, ZP_15484175.1, AAA17105.1, ZP_11437924.1, ZP_15446621.1, YP_003646340.1, ZP_15382134.1, ZP_14237669.1, ZP_09165547.1, YP_004019203.1, ZP_14240225.1, YP_001220863.1, CBA74242.1, ZP_12994240.1, EIE27140.1, ZP_15354547.1, ZP_15432557.1, ZP_15500132.1, ZP_15478632.1,

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 10 EIE21044.1, ZP_15375054.1, NP_334518.1, 4DQV_A, ZP_06435375.1, YP_003030020.1,
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 ZP_06452908.1, ZP_06519578.1, YP_004721827.1, CAJ77696.1, ZP_09680854.1,
 ZP_09686453.1, YP_884815.1, YP_884815.1,
 15 CAB55600.1, ZP_09081423.1, YP_006521568.1, ZP_11440626.1, ZP_15513309.1,
 ZP_09410778.1, ZP_15374248.1, ZP_15405954.1, YP_001704047.1, ZP_14236911.1,
 ZP_12873916.1, ZP_14242094.1, ZP_12994610.1, ZP_07664023.1, ZP_15446620.1,
 ZP_15484174.1, ZP_14240245.1, YP_005358845.1 and XP_002669159.1,
 more particularly YP_006731697.1, ZP_09839660.1, YP_001704097.1, YP_889972.1,
 20 ZP_05045132.1, ZP_09794557.1, ZP_08240521.1, NP_959974.1, ZP_10456477.1,
 YP_118225.1, NP_217106, YP_905678.1, YP_887275.1, ZP_11001941.1, YP_953393.1 and
 YP_005349252.1 variants thereof.

Fatty acid reductases are a group of enzymes which require for their activity a
 25 phosphopantetheinylation, i.e. the covalent attachment of a phosphopantetheinyl cofactor to the
 enzyme. Accordingly, the fatty acid reductase used according to the invention is
 phosphopantetheinylated, and a whole-cell catalyst expressing the fatty acid reductase
 expresses, either as part of its environment of endogenously expressed enzymes or in
 recombinant form, a phosphopantetheinyl transferase which phosphopantetheinylates the fatty
 30 acid reductase. In a preferred embodiment, the term "phosphopantetheinyl transferase", as
 used herein, is understood to mean an enzyme which transfers a phosphopantetheinyl moiety
 from a phosphopantetheinyl-CoA to an enzyme, preferably to the fatty acid reductase. In a
 particularly preferred embodiment, the phosphopantetheinyl transferase is selected from the
 group of phosphopantetheinyl transferases comprising the amino acid sequences ABI83656.1,

YP_006811024.1, YP_120266.1, YP_005265173.1, YP_004006671.1, ZP_08152482.1,
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 ZP_09788717.1, ZP_09799863.1, ZP_10961877.1, YP_003273299.1, GAB86168.1,
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 10 NP_302077.1, YP_005003342.1, YP_005349465.1, ZP_10800435.1, ZP_06564430.1,
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 5 ZP_14659796.1, ZP_14383679.1, YP_005058606.1, YP_001221073.1, ZP_08231568.1,
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 ZP_08516197.1, ZP_10160483.1, ZP_01987188.1, ZP_01755304.1, ZP_08825027.1,
 15 ZP_05077116.1, YP_001444606.1, ZP_03392800.1, ZP_01057781.1, AFB69889.1,
 ZP_08815097.1 and AAO17175.1 and variants thereof. In a particularly preferred embodiment,
 the phosphopantetheinyl transferase is the one having the database code ABI83656.1 or a
 variant thereof.

20 As an alternative or in addition to the combination of fatty acid reductase and
 phosphopantetheinyl transferase, the whole-cell catalyst can also comprise an α -dioxxygenase.
 In a preferred embodiment, the term " α -dioxxygenase", as used herein, is understood to mean
 an enzyme which catalyses the conversion of a carboxylic acid and/or dicarboxylic acid or a
 monoester thereof with consumption of a molecule of oxygen and with elimination of a carbon
 25 dioxide molecule to a carboxylic acid and/or dicarboxylic acid or a monoester thereof which
 bears an aldehyde group at the terminal ω -carbon atom and is shortened by one carbon atom at
 the terminal ω -carbon atom with respect to the carboxylic acid and/or dicarboxylic acid or a
 monoester thereof used as reactant. In a particularly preferred embodiment, the α -dioxxygenase
 is selected from the group of α -dioxxygenases comprising the amino acid sequences
 30 NP_001066718.1, EAY82977.1, BAH79993.1, ABG22011.1, BAJ90503.1, AFD04418.1,
 AFD04417.1, BAJ87736.1, AFW75180.1, ABG22012.1, XP_002311389.1, CAH05011.1,
 XP_002279884.1, CBI34957.3, AAG59584.1, NP_001234414.1, NP_001234410.1,
 XP_003553942.1, XP_002275161.1, XP_003553937.1, CBI34960.3, CAA07589.1,

XP_003543402.1, XP_002517402.1, XP_002882184.1, NP_186791.1, AAK85133.1, CAN77070.1, XP_002529555.1, CAH64542.1, NP_001234061.1, XP_002281357.1, ADM21465.1, XP_002318527.1, NP_177509.1, CAN74266.1, XP_002888940.1, NP_001185393.1, XP_003631072.1, BAJ33800.1, XP_002517377.1, XP_003530944.1, 5 BAJ34623.1, ABG22013.1, ABP02610.1, XP_001773135.1, XP_002960339.1, ABK95279.1, ABD73303.1, ABD73304.1, YP_001805721.1, ZP_08971815.1, ZP_08430366.1, YP_823013.1, ZP_05026427.1, ZP_11003953.1, YP_007064484.1, YP_007113008.1, YP_633369.1, ZP_18906570.1, ZP_09251410.1, ZP_10050808.1, ZP_01306662.1, YP_001516886.1, ZP_05042862.1, AAC49625.1, ZP_09648375.1, ZP_09792714.1, ZP_09788527.1, 10 XP_001728273.1, AAC83355.1, YP_890542.1, ZP_11000891.1, XP_002605323.1, EGO58341.1, YP_006249145.1, YP_001507004.1, YP_001704637.1, ZP_12876141.1, ZP_11150830.1, ZP_14236257.1, ZP_09411385.1, ZP_14243118.1, EKD16664.1, ZP_15416799.1, ZP_15338016.1, ZP_10080295.1, ZP_11438929.1, ZP_12995210.1, ZP_10946648.1, YP_003409541.1, XP_001637870.1, YP_005451221.1, XP_001212758.1, 15 ZP_07290489.1, ZP_05781329.1, ZP_19187748.1, ZP_06574534.1, XP_002605322.1, NP_822950.1, YP_006366425.1, EJP63377.1, EKD21217.1, XP_001795927.1, XP_003042615.1, ZP_06566152.1, EGU88116.1, EFY94417.1, XP_388327.1, EKJ68934.1, ZP_07290463.1, CCC10458.1, YP_001107201.1, XP_003348248.1, T49753, CAD31840.1, XP_001229975.1, CBN77040.1, YP_004813753.1, XP_002513273.1, XP_001627136.1, 20 AFG52858.1, AFG52857.1, AEW08450.1, NP_841291.1, YP_004512343.1, ACG75701.1 and ZP_03500906.1 and variants thereof. In a particularly preferred embodiment, the α -dioxxygenase is the one having the database code NP_001066718.1 or a variant thereof.

Besides the α -dioxxygenase or the combination of fatty acid reductase and the 25 phosphopantetheinyl transferase, the whole-cell catalyst according to the invention needs to have a transaminase which aminates terminal aldehyde groups. In a preferred embodiment, the term "transaminase", as used herein, is understood to mean an enzyme which catalyses the transfer of α -amino groups from a donor molecule, preferably an amino acid, to an acceptor molecule, preferably an α -ketocarboxylic acid. In a particularly preferred embodiment, the 30 transaminase is selected from the group of transaminases comprising the amino acid sequences 3HMU_A, AAD41041.1, AAK15486.1, ABE03917.1, ADR60699.1, ADR61066.1, ADR62525.1, AEL07495.1, CAZ86955.1, EFW82310.1, EFW87681.1, EGC99983.1, EGD03176.1, EGE58369.1, EGH06681.1, EGH08331.1, EGH24301.1,

EGH32343.1, EGH46412.1, EGH55033.1, EGH62152.1, EGH67339.1, EGH70821.1,
EGH71404.1, EGH78772.1, EGH85312.1, EGH97105.1, EGP57596.1, NP_102850.1,
NP_106560.1, NP_248912.1, NP_248990.1, NP_354026.2, NP_421926.1,
NP_637699.1, NP_642792.1, NP_744329.1, NP_744732.1, NP_747283.1,
5 NP_795039.1, NP_901695.1, XP_002943905.1, YP_001021095.1, YP_001059677.1,
YP_001061726.1, YP_001066961.1, YP_001074671.1, YP_001120907.1,
YP_001140117.1, YP_001170616.1, YP_001185848.1, YP_001188121.1,
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YP_001412573.1, YP_001417624.1, YP_001526058.1, YP_001579295.1,
10 YP_001581170.1, YP_001668026.1, YP_001669478.1, YP_001671460.1,
YP_001685569.1, YP_001747156.1, YP_001749732.1, YP_001765463.1,
YP_001766294.1, YP_001790770.1, YP_001808775.1, YP_001809596.1,
YP_001859758.1, YP_001888405.1, YP_001903233.1, YP_001977571.1,
YP_002229759.1, YP_002231363.1, YP_002280472.1, YP_002297678.1,
15 YP_002543874.1, YP_002549011.1, YP_002796201.1, YP_002801960.1,
YP_002875335.1, YP_002897523.1, YP_002912290.1, YP_002974935.1,
YP_003060891.1, YP_003264235.1, YP_003552364.1, YP_003578319.1,
YP_003591946.1, YP_003607814.1, YP_003641922.1, YP_003674025.1,
YP_003692877.1, YP_003755112.1, YP_003896973.1, YP_003907026.1,
20 YP_003912421.1, YP_004086766.1, YP_004142571.1, YP_004147141.1,
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YP_004472442.1, YP_004590892.1, YP_004612414.1, YP_004676537.1,
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25 YP_108931.1, YP_110490.1, YP_168667.1, YP_237931.1, YP_260624.1,
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30 YP_622254.1, YP_625753.1, YP_680590.1, YP_751687.1, YP_767071.1,
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YP_983084.1, YP_995622.1, ZP_00948889.1, ZP_00954344.1, ZP_00959736.1,
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 5 ZP_01763880.1, ZP_01769626.1, ZP_01865961.1, ZP_01881393.1, ZP_01901558.1,
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 ZP_03456092.1, ZP_03517291.1, ZP_03529055.1, ZP_03571515.1, ZP_03572809.1,
 10 ZP_03587785.1, ZP_03588560.1, ZP_03697266.1, ZP_03697962.1, ZP_04521092.1,
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 ZP_04964181.1, ZP_05053721.1, ZP_05063588.1, ZP_05073059.1, ZP_05077806.1,
 ZP_05082750.1, ZP_05091128.1, ZP_05095488.1, ZP_05101701.1, ZP_05116783.1,
 15 ZP_05121836.1, ZP_05127756.1, ZP_05637806.1, ZP_05742087.1, ZP_05783548.1,
 ZP_05786246.1, ZP_05843149.1, ZP_05945960.1, ZP_06459045.1, ZP_06487195.1,
 ZP_06492453.1, ZP_06493162.1, ZP_06703644.1, ZP_06731146.1, ZP_06839371.1,
 ZP_07007312.1, ZP_07266194.1, ZP_07374050.1, ZP_07662787.1, ZP_07778196.1,
 ZP_07797983.1, ZP_08099459.1, ZP_08138203.1, ZP_08141719.1, ZP_08142973.1,
 20 ZP_08177102.1, ZP_08185821.1, ZP_08186468.1, ZP_08208888.1, ZP_08266590.1,
 ZP_08402041.1, ZP_08406891.1, ZP_08522175.1, ZP_08527488.1, ZP_08631252.1,
 ZP_08636687 and variants thereof.

The fatty acid reductase used according to the invention and preferably also other enzymes
 25 used according to the invention are recombinant enzymes. In a preferred embodiment, the term
 "recombinant", as used herein, is understood to mean that the nucleic acid molecule encoding
 the corresponding enzyme does not occur in the natural cell and/or it has been produced using
 gene technology methods. In a preferred embodiment, the term recombinant protein is used if
 the corresponding polypeptide is encoded by a recombinant nucleic acid. In a preferred
 30 embodiment, a recombinant cell, as used herein, is understood to mean a cell which has at
 least one recombinant nucleic acid or recombinant polypeptide. Methods suitable for producing
 recombinant molecules or cells are known to the person skilled in the art, for example those

described in Sambrook/Fritsch/Maniatis (1989): Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, 2nd edition. Recombinant enzymes are preferably overexpressed, for example by using pET or pGEX vector systems, which are known to the person skilled in the art.

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With regard to the choice of organism, the whole-cell catalyst usable according to the invention is not subject to any restrictions, provided it is culturable, stable and amenable to possible modifications introducible by gene technology methods, for example methods for attenuating enzyme activities, an example being knock-outs. For instance, the cell can equally be a

10 prokaryotic cell or eukaryotic cell. In the case of a eukaryotic cell, particular preference is given to unicellular eukaryotes, especially yeasts such as *Saccharomyces cerevisiae*, *Candida tropicalis*, *Candida albicans* and *Pichia pastoris*. In the case of prokaryotic cells, the cell can, for example, be a bacterium selected from the group comprising *Magnetococcus*, *Mariprofundus*, *Acetobacter*, *Acetobacterium*, *Acidiphilium*, *Afipia*, *Ahrensia*, *Asticcacaulis*, *Aurantimonas*,

15 *Azorhizobium*, *Azospirillum*, *Bacillus*, *Bartonella*, *tribocorum*, *Beijerinckia*, *Bradyrhizobium*, *Brevundimonas*, *subvibrioides*, *Brucella*, *Caulobacter*, *Chelativorans*, *Citricella*, *Citromicrobium*, *Clostridium*, *Corynebacterium*, *Dinoroseobacter*, *Erythrobacter*, *Fulvimarina*, *Gluconacetobacter*, *Granulibacter*, *Hirschia*, *Hoeflea*, *Hyphomicrobium*, *Hyphomonas*, *Ketogulonicigenium*, *Labrenzia*, *Loktanella*, *Magnetospirillum*, *Maricaulis*, *Maritimibacter*,

20 *Mesorhizobium*, *Methylobacterium*, *Methylocystis*, *Methylosinus*, *Nitrobacter*, *Novosphingobium*, *Oceanibulbus*, *Oceanicaulis*, *Oceanicola*, *Ochrobactrum*, *Octadecabacter*, *Oligotropha*, *Paracoccus*, *Parvibaculum*, *Parvularcula*, *Pelagibaca*, *Phaeobacter*, *Phenylobacterium*, *Polymorphum*, *Pseudovibrio*, *Rhodobacter*, *Rhodomicrobium*, *Rhodopseudomonas*, *Rhodospirillum*, *Roseibium*, *Roseobacter*, *Roseomonas*, *Roseovarius*, *Ruegeria*, *Sagittula*,

25 *Silicibacter*, *Sphingobium*, *Sphingomonas*, *Sphingopyxis*, *Starkeya*, *Sulfitobacter*, *Thalassiobium*, *Xanthobacter*, *Zymomonas*, *Agrobacterium*, *Rhizobium*, *Sinorhizobium*, *Anaplasma*, *Ehrlichia*, *Neorickettsia*, *Orientia*, *Rickettsia*, *Wolbachia*, *Bordetella*, *Burkholderia*, *Cupriavidus*, *Taiwanensis*, *Lautropia*, *Limnobacter*, *Polynucleobacter*, *Ralstonia*, *Chromobacterium*, *Eikenella*, *corrodens*, *Basfia*, *Kingella*, *Laribacter*, *Lutiella*, *Neisseria*,

30 *Simonsiella*, *Achromobacter*, *Acidovorax*, *Alicyclophilus*, *Aromatoleum*, *Azoarcus*, *Comamonas*, *Dechloromonas*, *Delftia*, *Gallionella*, *Herbaspirillum*, *Herminiimonas*, *Hylemonella*, *Janthinobacterium*, *Leptothrix*, *Methylibium*, *Methylobacillus*, *Methylophilales*, *Methyloversatilis*, *Methylovorus*, *Nitrosomonas*, *Nitrosospira*, *Oxalobacter*, *Parasutterella*, *Polaromonas*, *Polaromonas*, *Pusillimonas*, *Rhodoferax*, *Rubrivivax*, *Sideroxydans*, *Sutterella*, *wadsworthensis*,

Taylorella, *Thauera*, *Thiobacillus*, *Thiomonas*, *Variovorax*, *Verminephrobacter*,
Anaeromyxobacter, *Bdellovibrio*, *bacteriovorus*, *Bilophila*, *Desulfarculus*, *Desulfatibacillum*,
Desulfobacca, *Desulfobacterium*, *Desulfobulbus*, *Desulfococcus*, *Desulfohalobium*,
Desulfitobacterium, *Desulfomicrobium*, *Desulfonatronospira*, *Desulfotalea*, *Desulfovibrio*,
5 *Desulfuromonas*, *Geobacter*, *Haliangium*, *Hipaea*, *Lawsonia*, *Myxococcus*, *Pelobacter*,
Plesiocystis, *Sorangium*, *Stigmatella*, *Syntrophobacter*, *Syntrophus*, *Arcobacter*, *Caminibacter*,
Campylobacter, *Helicobacter*, *Nitratifractor*, *Nitratiruptor*, *Sulfuricurvum*, *Sulfurimonas*,
Sulfurospirillum, *Sulfurovum*, *Wolinella*, *Buchnera*, *Blochmannia*, *Hamiltonella*, *Regiella*, *Riesia*,
Citrobacter, *Cronobacter*, *Dickeya*, *Edwardsiella*, *Enterobacter*, *Erwinia*, *Escherichia*, *Klebsiella*,
10 *Pantoea*, *Pectobacterium*, *Proteus*, *Providencia*, *Rahnella*, *Salmonella*, *Serratia*, *Shigella*,
Sodalis, *Wigglesworthia*, *Glossina*, *Xenorhabdus*, *Yersinia*, *Acidithiobacillus*, *Acinetobacter*,
Aeromonas, *Alcanivorax*, *Alkalilimnicola*, *Allochromatium*, *Alteromonadales*, *Alteromonas*,
Baumannia, *Beggiatoa*, *Bermanella*, *Carsonella*, *Ruthia*, *Vesicomysocius*, *Cardiobacterium*,
Chromohalobacter, *Colwellia*, *Congregibacter*, *Coxiella*, *Dichelobacter*, *Endoriftia*,
15 *Enhydrobacter*, *Ferrimonas*, *Francisella*, *Glaciecola*, *Hahella*, *Halomonas*, *Halorhodospira*,
Halothiobacillus, *Idiomarina*, *Kangiella*, *Legionella*, *Marinobacter*, *Marinomonas*, *Methylobacter*,
Methylococcus, *Methylomicrobium*, *Methylophaga*, *Moraxella*, *Moritella*, *Neptuniibacter*,
Nitrococcus, *Pseudoalteromonas*, *Psychrobacter*, *Psychromonas*, *Reinekea*, *Rickettsiella*,
Saccharophagus, *Shewanella*, *Succinatimonas*, *Teredinibacter*, *Thioalkalimicrobium*,
20 *Thioalkalivibrio*, *Thiomicrospira*, *Tolumonas*, *Vibrionales*, *Actinobacillus*, *Aggregatibacter*,
Gallibacterium, *Haemophilus*, *Histophilus*, *Mannheimia*, *Pasteurella*, *Azotobacter*, *Cellvibrio*,
Pseudomonas, *Aliivibrio*, *Grimontia*, *Photobacterium*, *Photobacterium*, *Vibrio*,
Pseudoxanthomonas, *Stenotrophomonas*, *Xanthomonas*, *Xylella*, *Borrelia*, *Brachyspira*,
Leptospira, *Spirochaeta*, *Treponema*, *Hodgkinia*, *Puniceispirillum*, *Liberibacter*, *Pelagibacter*,
25 *Odyssella*, *Accumulibacter*, more particularly *B. subtilis*, *B. megaterium*, *C. glutamicum*, *E. coli*,
Pseudomonas sp., *Pseudomonas fluorescens*, *Pseudomonas putida*, *Pseudomonas stutzeri*,
Acinetobacter sp., *Burkholderia* sp., *Burkholderia thailandensis*, cyanobacteria, *Klebsiella* sp.,
Klebsiella oxytoca, *Salmonella* sp., *Rhizobium* sp. and *Rhizobium meliloti*. In a particularly
preferred embodiment, the cell is an enterobacterium, most preferably *Escherichia coli*.

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It is advantageous when the whole-cell catalyst according to the invention comprises not only
the fatty acid reductase, phosphopantetheinyl transferase and the transaminase, but also an
alanine dehydrogenase in order to regenerate from inorganic nitrogen-containing molecules the
alanine consumed by the transaminase during the amination of the terminal aldehyde group. In

a preferred embodiment, the term "alanine dehydrogenase", as used herein, is understood to mean an enzyme which catalyses the conversion of L-alanine with consumption of water and NAD⁺ to pyruvate, ammonia and NADH and the reverse reaction. In a particularly preferred embodiment, the alanine dehydrogenase is selected from the group of alanine dehydrogenases comprising the amino acid sequence of the alanine dehydrogenase from *Bacillus subtilis* (database code L20916), *Rhizobium leguminosarum* (database code CP001622), *Vibrio proteolyticus* (database code AF070716), *Mycobacterium tuberculosis* (database code X63069), *Enterobacter aerogenes* (database code AB013821), EGR93259.1, YP_003654745.1, YP_003651439.1, YP_003637111.1, YP_003631815.1, YP_001327051.1, YP_001262560.1, YP_886996.1, YP_882850.1, YP_704410.1, YP_703508.1, ZP_08624689.1, YP_001230376.1, P17557.1, P17556.1, CCB94892.1, CCB73698.1, YP_001168635.1, YP_004668736.1, YP_004569425.1, YP_003513168.1, YP_004561169.1, ZP_08554945.1, YP_400777.1, ZP_08311476.1, ZP_08310170.1, ZP_08267322.1, ZP_08263846.1, ZP_07898723.1, YP_149301.1, YP_148605.1, YP_004340432.1, EFT09946.1, EFS80513.1, EFS51332.1, EFS42459.1, YP_003060895.1, YP_003059033.1, ZP_03305373.1, YP_847214.1, YP_004095847.1, YP_003338282.1, YP_003337256.1, YP_355846.1, YP_253131.1, ZP_08197563.1, ZP_08196283.1, ADW06447.1, YP_734091.1, NP_372233.1, NP_102173.1, ZP_08170259.1, EGD36706.1, EGD32748.1, ZP_08155540.1, YP_004142849.1, YP_002417649.1, YP_001301040.1, YP_002992892.1, YP_081348.1, YP_080482.1, YP_002476349.1, ZP_08115025.1, ZP_08114403.1, YP_003552869.1, YP_002358112.1, YP_575010.1, YP_477594.1, YP_474564.1, YP_130399.1, YP_129373.1, YP_123314.1, NP_810467.1, NP_646469.1, NP_626044.1, NP_391071.1 (encoded by SEQ ID NO: 11), ZP_08086822.1, ZP_08084776.1, ZP_08083119.1, ZP_08020768.1, ZP_08013590.1, ZP_08011832.1, YP_003783744.1, YP_002781576.1, YP_002780533.1, ZP_02195873.1, NP_797482.1, ZP_07645051.1, ZP_07643260.1, ZP_06611917.1, AAT40119.1, ZP_07864946.1, YP_004068409.1, YP_002796203.1, YP_002774420.1, YP_003600348.1, YP_003599946.1, YP_003565624.1, YP_003565223.1, YP_335198.1, YP_423850.1, YP_155059.1, ZP_07843538.1, ZP_07841226.1, ZP_06928932.1, ZP_05692073.1, ZP_05687006.1, ZP_04867480.1, YP_775531.1, CBE70214.1, ZP_07721182.1, ZP_04302850.1, ZP_04298961.1, ZP_04287684.1, ZP_04277177.1, ZP_04248389.1, ZP_04235899.1, ZP_02159718.1, ZP_02152178.1, YP_003974610.1, YP_003546595.1, YP_002317127.1, ZP_07313778.1, ZP_07302778.1, ZP_07298850.1, CBK69442.1, YP_003413835.1, YP_003595089.1, ZP_06807811.1, YP_003582455.1, YP_003464731.1, YP_003496397.1, YP_003421918.1, CBL07274.1, CBK64956.1, YP_003508515.1,

AAL87460.1, AAC23579.1, AAC23578.1, AAC23577.1, ACU78652.1, YP_003471439.1,
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 5 YP_003387804.1, YP_003058152.1, EFS74272.1, EFS67128.1, ZP_06844564.1,
 YP_826658.1, YP_001195249.1, YP_003095978.1, YP_469292.1, YP_004442054.1,
 YP_004461174.1, YP_004055616.1, YP_003576656.1, YP_003094537.1, YP_001295973.1,
 AEE71143.1, YP_004447480.1, YP_003761844.1, YP_040853.1, YP_003154888.1,
 YP_003142045.1, YP_002280953.1, NP_371963.1, NP_422368.1, EGC98966.1, EGC76398.1,
 10 YP_004263661.1, YP_004252039.1, YP_679036.1, YP_499973.1, ZP_08054972.1,
 ZP_08053009.1, ZP_04067276.1, ZP_03968868.1, ZP_03963857.1, ZP_03933079.1,
 ZP_03497046.1, ZP_06668924.1, ZP_06667106.1, ZP_06324464.1, ZP_06196777.1,
 ZP_05114159.1, ZP_05083968.1, ZP_05070370.1, ZP_05030022.1, ZP_04673064.1,
 ZP_03517011.1, ZP_03505783.1, XP_001310698.1, ABK27691.1 and CAB59281.2 and
 15 variants thereof. For the reaction catalysed by the alanine dehydrogenase, the presence is
 required of not only pyruvate, which is formed as part of the primary metabolism of any cell
 suitable as whole-cell catalyst, but also ammonium. The latter is typically provided in the form of
 inorganic nitrogen salts, for example ammonium salts, nitrates or the like. Preferably, an
 ammonium salt, for example ammonium chloride, is added to the aqueous reaction medium.

20 Furthermore, it is advantageous when the whole-cell catalyst according to the invention
 expresses an alkane hydroxylase and optionally further enzymes essential for the activity of the
 alkane hydroxylase, especially when a fatty acid having only one terminal carboxy function is
 used as substrate for producing a diamine. The alkane hydroxylase and/or an additionally
 25 expressed alcohol dehydrogenase oxidize the terminal carbon atom then up to the aldehyde
 group, which can subsequently be aminated by the transaminase, or up to the carboxyl group,
 which is converted by an α -dioxygenase or the combination of fatty acid reductase and a
 phosphopantetheinyl transferase to a terminal aldehyde group, which can subsequently be
 aminated by the transaminase. In a preferred embodiment, the term "alkane hydroxylase", as
 30 used herein, is understood to mean an enzyme which catalyses the hydroxylation of
 unsubstituted linear alkyl radicals comprising at least six, preferably twelve, carbon atoms.

According to the invention, numerous oxidation systems, as described in, inter alia, PCT/EP
 2008/067447, are suitable as alkane hydroxylases. In a preferred embodiment, the alkane

hydroxylase is a cytochrome P450 monooxygenase of the CYP153 family. In a preferred embodiment, the term "cytochrome P450 monooxygenase of the CYP153 family" is understood to mean a cytosolic oxidase which is part of a 3-component system which further comprises a ferredoxin and a ferredoxin reductase, with an alkane binding site and the ability to hydroxylate alkanes. In a particularly preferred embodiment, it is an enzyme which has to at least 80, preferably 90, most preferably 95 or 99%, sequence identity to the cytochrome P450 monooxygenase of the CYP153 family from *Alcanivorax borkumensis* SK2 (database code YP_691921) or an enzyme which comprises a polypeptide sequence which has at least 80, preferably 90, most preferably 95 or 99%, sequence identity to the cytochrome P450 monooxygenase of the CYP153 family from *Alcanivorax borkumensis* SK2 (database code YP_691921) and moreover has alkane hydroxylase activity. As throughout this application, the stated database codes concern the NCBI (National Center for Biotechnology Information, Bethesda, USA) databases, specifically the version available online on 21 November 2012. In a preferred embodiment, the term "cytochrome P450 monooxygenase of the CYP153 family" is understood to mean a non-membrane-bound oxidase which includes a binding site for alkanes, unsubstituted linear alkyl radicals comprising at least five, preferably twelve, carbon atoms or monohydroxylated alkanes and the polypeptide chain of which comprises the motif LL(I/L)(V/I)GGNDTTRN. In a preferred embodiment, a "cytochrome P450 monooxygenase of the CYP153 family", as used herein, is a cytochrome P450 monooxygenase of the CYP153 family from *Alcanivorax borkumensis* SK2 (database code YP_691921) or a variant thereof which preferably has alkane hydroxylase activity.

For the optimal supply of the cytochrome P450 monooxygenase of the CYP153 family with electrons from the reducing agent, preferably NADH, it is preferred that the cell the alkane hydroxylase is expressed together with ferredoxin reductase that interacts functionally with it and ferredoxin that interacts functionally with it. These may be isolated polypeptides or, in the case of using a whole-cell catalyst, coexpressed polypeptides or polypeptides fused on the N- or C-terminus with the cytochrome P450 monooxygenase of the CYP153 family. Whether a ferredoxin reductase or a ferredoxin interact functionally with a given cytochrome P450 monooxygenase of the CYP153 family with one another can be readily established by the person skilled in the art by whether the reducing agent is oxidized more efficiently in the presence of an alkane substrate and the three polypeptides than when at least one of the three is absent. Alternatively, it is possible to use the enzyme assay described by Scheps, D., Malca, H., Hoffmann, B., Nestl, B. M., and Hauer, B. (2011) *Org. Biomol. Chem.*, 9, 6727, which, in the

case of functionally interacting polypeptides, exhibits a considerable increase in the reaction rate. In a particularly preferred embodiment, the cytochrome P450 monooxygenase of the CYP153 family, the ferredoxin and the ferredoxin reductase originate from the same organism. In a particularly preferred embodiment, they are the ferredoxin reductase from *Alcanivorax*
 5 *borkumensis* SK2 (database code YP_691923) or a variant thereof, the ferredoxin from *Alcanivorax borkumensis* SK2 (database code YP_691920) or a variant thereof and the cytochrome P450 monooxygenase of the CYP153 family from *Alcanivorax borkumensis* SK2 (database code YP_691921) or a variant thereof.

10 In a further preferred embodiment, the alkane hydroxylase is an AlkB monooxygenase. AlkB represents an oxidoreductase which initially became known from the AlkBGT system of *Pseudomonas putida* Gpo1, and which is dependent on two further polypeptides, AlkG and AlkT. AlkT is characterized as a FAD-dependent rubredoxin reductase, which transfers
 15 electrons from NADH to AlkG. AlkG is a rubredoxin, an iron-containing redox protein which functions as a direct electron donor to AlkB. In a preferred embodiment, the term "AlkB monooxygenase" is a polypeptide having a sequence homology of at least 75, 80, 85, 90, 92, 94, 96, 98 or 99% – specified in order of increasing preference – to the sequence of the AlkB of *Pseudomonas putida* Gpo1 (database code: CAB54050.1; this database code originates like all
 20 others from the prior art used in the application, namely from the NCBI database, more precisely the release available online on 15th October 2012) having the capability to oxidize alkanes. In a particularly preferred embodiment, the AlkB monooxygenase is an oxidoreductase which functionally interacts with the AlkG (CAB54052.1) and AlkT (CAB54063.1) polypeptides from *Pseudomonas putida* Gpo1 and oxidizes alkanes. For the optimal supply of the AlkB
 25 alkane hydroxylase with electrons, it is preferred that the cell the alkane hydroxylase is expressed together with auxiliary proteins that interact functionally with it, preferably AlkG and/or AlkT or variants of each, and in a particularly preferred embodiment they are again AlkG (CAB54052.1) and AlkT (CAB54063.1) polypeptides from *Pseudomonas putida* Gpo1.

When using a whole-cell catalyst, the problem can arise that a substrate has to be brought into
 30 contact with an intracellularly localized enzyme so that it results in the desired reaction. In the case of long-chain alkanes and derivatives thereof, it is preferred that the whole-cell catalyst has a polypeptide of the AlkL family. AlkL is a membrane protein from *Pseudomonas putida*, which protein can import long-chain fatty acids and derivatives thereof into bacterial cells. In a preferred embodiment, a "polypeptide of the AlkL family", as used herein is a polypeptide which,

over a length of 230 successive amino acids, has an at least 80, preferably 90, more preferably 90% sequence identity to AlkL from *Pseudomonas putida* (database code CAB69081) or a variant of AlkL from *Pseudomonas putida* and preferably the ability to assist the import of long-chain alkanes into the inside of a cell. In a further embodiment, a "polypeptide of the AlkL family", as used herein, is a polypeptide located in the outer membrane of a Gram-negative bacterium and which has the sequence motif DXWAPAXQ(V/A)GXR, where X is a proteinogenic amino acid, and preferably is additionally AlkL from *Pseudomonas putida* (database code CAB69081) or a variant thereof. Examples of members of the AlkL family include AlkL from *Pseudomonas putida* (database code CAB69081), *Marinobacter aquaeolei* VT8 (database code YP_957722), *Oceanicaulis alexandrii* HTCC2633 (database code ZP_00953584), *Marinobacter manganoxydans* Mnl7-9 (database code ZP_09158756), *Caulobacter* sp. K31 (database code YP_001672217), *Pseudomonas oleovorans* (database code Q00595) and variants thereof.

The teaching of the present invention can be carried out by not only using macromolecules having the exact amino acid or nucleic acid sequence to which reference is made herein or by not only using a cell having reduced activity, relative to the respective wild type, of a polypeptide having the exact amino acid sequence to which reference is made herein, but also by using a variant of such macromolecules or a cell having a reduced activity, relative to the respective wild type of the respective cell, of a variant of the polypeptide, which variant can be obtained by deletion, addition or substitution of one or more than one amino acid or nucleic acid. In a preferred embodiment, the term "variant" of a nucleic acid sequence or amino acid sequence, used hereinafter synonymously and exchangeably with the term "homologue", as used herein, means another nucleic acid or amino acid sequence comprising or representing a sequence which, with respect to the corresponding original wild-type nucleic acid or amino acid sequence, has a homology, used here synonymously with identity, of 70, 75, 80, 85, 90, 92, 94, 96, 98, 99% or greater, wherein preferably amino acids other than the ones forming the catalytically active site or essential for the structure or folding are deleted or substituted or are merely conservatively substituted, for example a glutamate instead of an aspartate or a leucine instead of a valine. The prior art describes algorithms, which may be used to calculate the degree of homology of two sequences, e.g. Arthur Lesk (2008), Introduction to Bioinformatics, 3rd edition. In a further more preferred embodiment of the present invention, the variant of an amino acid or nucleic acid sequence, preferably in addition to the sequence homology mentioned above, has substantially the same enzymatic activity of the wild-type molecule and/or of the original

molecule. For example, a variant of an enzymatically active polypeptide protease has the same, or substantially the same, proteolytic activity as the polypeptide enzyme, i.e. the capability to catalyse the hydrolysis of a peptide bond. In a particular embodiment, the term "substantially the same enzymatic activity" means an activity, with respect to the substrates of the wild-type polypeptide, which clearly lies above the background activity or/and differs from the K_M and/or k_{cat} values by less than 3, more preferably 2, even more preferably one order of magnitude, which the wild-type polypeptide exhibits with respect to the same substrates. In a further preferred embodiment, the term "variant" of a nucleic acid or amino acid sequence includes at least one active part or fragment of the nucleic acid or amino acid sequence. In a further preferred embodiment, the term "active part", as used herein, means an amino acid sequence or a nucleic acid sequence which has a smaller amino acid sequence than the full length of the amino acid sequence or encodes a smaller amino acid sequence than the full length of the amino acid sequence, where the amino acid sequence or the encoded amino acid sequence with the smaller length than the wild-type amino acid sequence has substantially the same enzymatic activity as the wild-type polypeptide or a variant thereof, for example as protease. In a particular embodiment, the term "variant" of a nucleic acid encompasses a nucleic acid, the complementary strand of which, preferably under stringent conditions, binds to the wild-type nucleic acid. The stringency of the hybridization reaction is readily determinable by those skilled in the art and depends in general on the length of the probe, the washing temperatures and the salt concentration. Generally, longer probes require higher temperatures for the hybridization, whereas shorter probes work at lower temperatures. Whether hybridization takes place depends in general on the capability of the denatured DNA to anneal to complementary strands which are present in its environment, specifically below the melting temperature. The stringency of hybridization reaction and corresponding conditions are described in more detail in F M Ausubel (1995), Current Protocols in Molecular Biology. John Wiley & Sons, Inc. Instructions for identifying DNA sequences by means of hybridization can be found by the person skilled in the art inter alia in the handbook "The DIG System Users Guide for Filter Hybridization" from Boehringer Mannheim GmbH (Mannheim, Germany, 1993) and in Liebl *et al.* (International Journal of Systematic Bacteriology 41: 255-260 (1991)). The hybridization takes place in a preferred embodiment under stringent conditions, i.e. only hybrids are formed in which probe and target sequence, i.e. the polynucleotides treated with the probe, are at least 70% identical. It is known that the stringency of the hybridization including the washing steps is influenced and/or determined by varying the buffer composition, the temperature and the salt concentration. The hybridization reaction is generally carried out at a relatively low stringency

compared with the washing steps (Hybaid Hybridisation Guide, Hybaid Limited, Teddington, UK, 1996). For the hybridization reaction, for example, a buffer corresponding to 5x SSC buffer can be used at a temperature of about 50°C - 68°C. In this connection, probes can also hybridize to polynucleotides which have less than 70% identity to the sequence of the probe. Such hybrids

5 are less stable and are removed by washing under stringent conditions. This can be achieved for example by lowering the salt concentration to 2x SSC and optionally subsequently 0.5x SSC (The DIG System User's Guide for Filter Hybridisation, Boehringer Mannheim, Mannheim, Germany, 1995), in which case a temperature of, increasing in order of preference, about 50°C - 68°C, about 52°C - 68°C, about 54°C - 68°C, about 56°C - 68°C, about 58°C - 68°C, about

10 60°C - 68°C, about 62°C - 68°C, about 64°C - 68°C, about 66°C - 68°C is established. Temperature ranges from about 64°C - 68°C or about 66°C - 68°C are preferred. It is optionally possible to reduce the salt concentration down to a concentration corresponding to 0.2 x SSC or 0.1 x SSC. By means of a stepwise increase in the hybridization temperature in steps of about 1 - 2°C from 50°C to 68°C, polynucleotide fragments can be isolated which, for example in the

15 order of increasing preference, have at least 70% or at least 80% or at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identity to the sequence of the nucleic acid molecule used. Further instructions relating to the hybridization are commercially available in the form of so-called kits (e.g. DIG Easy Hyb from Roche Diagnostics GmbH, Mannheim, Germany, Catalogue No. 1603558). In a

20 preferred embodiment, the term "variant" of a nucleic acid, as used herein, encompasses any nucleic acid sequence which encodes the same amino acid sequence as the original nucleic acid or a variant of this amino acid sequence in the context of the degeneracy of the genetic code.

25 In a preferred embodiment, the cell used according to the invention has a reduced activity, with respect to its wild type, of at least one enzyme which catalyses one of the reactions of the β -oxidation of fatty acids, and preferably this is an enzyme from the group comprising fatty acid-CoA ligase, acyl-CoA dehydrogenase, 2,4-dienoyl-CoA reductase, enoyl-CoA hydratase and 3-ketoacyl-CoA thiolase, a fatty acid importer or variants thereof. The β -oxidation of fatty acids is a

30 widespread metabolic pathway which equally permits prokaryotic and eukaryotic organisms to oxidize fatty acids and to make the chemical energy present therein available to metabolism. In the broader sense, it starts with the uptake of a fatty acid into the cell. There, if the conditions require it, the fatty acid is firstly oxidized at the β -position of the CoA-fatty acid ester by an acyl-CoA dehydrogenase, in the case of *E. coli* FadE. A similar molecule can alternatively also be

formed from a double-unsaturated fatty acid by reduction by means of a 2,4-dienoyl-CoA reductase, in the case of *E. coli* FadH. A multifunctional enzyme, the enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase, in the case of *E. coli* FadB, then catalyses the hydration with the formation of the secondary alcohol and its subsequent oxidation to the ketone. In the last

5 step, a 3-ketoacyl-CoA thiolase, in the case of *E. coli* FadA, catalyses the cleavage of the ketoacyl-CoA, with the result that acetyl-CoA and a CoA ester of the fatty acid that is two carbon atoms shorter compared to the starting molecule are released. If the latter is not likewise acetyl-CoA, it can be fed again into the β -oxidation cycle and be shortened via oxidation. Also involved in the regulation of the β -oxidation of fatty acids is FadR, a regulator of the Fad operon, which

10 includes the genes required for the degradation of fatty acids, without FadR appearing to catalyse a reaction of the β -oxidation. In a preferred embodiment, the term "enzyme which catalyses one of the reactions of the β -oxidation of fatty acids" is understood as meaning any enzyme which interacts directly with the fatty acid substrate or a molecule formed therefrom on the pathway to the acetyl-CoA, preferably recognizes it as substrate, and catalyses its

15 conversion to a metabolic product lying closer to the acetyl-CoA on this degradation pathway, preferably including the fatty acid importer, which effects the uptake of the fatty acid into the cell. For example, according to the preceding definition, these enzymes include acyl-CoA dehydrogenase, since it interacts with the fatty acid-CoA ester and catalyses its conversion to the enoyl-CoA, which lies closer to the acetyl-CoA on the metabolic pathway of β -oxidation than

20 the fatty acid-CoA ester. In a particularly preferred embodiment, the term "enzyme which catalyses one of the reactions of the β -oxidation of fatty acids", as used herein, is understood as meaning any enzyme from the group which comprises the gene products FadA, FadB, FadD, FadL and FadE from *E. coli* and/or their variants or homologues from other organisms. The gene products FadA, FadB, FadD, FadL and FadE from *E. coli* as well as variants and

25 homologues from numerous other biotechnologically useful organisms and their nucleic acid and polypeptide sequences are described in the prior art, for example FadA under accession number AP009048.1, FadB under accession number BAE77457.1, FadD under accession number BAA15609.1, and FadE under accession number BAA77891.2. The prior art discloses numerous assays which are suitable specifically for measuring the activity of enzymes which

30 catalyse one of the reactions of the β -oxidation of fatty acids, for example in K Kameda & W D Nunn (1981) *J. Biol. Chem.* 256, 5702-5707, Hi Marrakchi, W E DeWolf, C Quinn, J West, B J Polizzi, C Y So et al. (2003) *Biochem. J.* 370, 1055-1062, Lobo *et al.* (2001) and X Yu, T Liu, F Zhu, and C Khosla (2011) *PNAS*, *electronic publication prior to print*.

For the efficiency of the whole-cell catalyst according to the invention, it is advantageous when the substrate to be converted, preferably the carboxylic acid or dicarboxylic acid or a monoester thereof, can easily enter into contact with the enzymes which are required according to the invention and which are located inside the whole-cell catalyst. Therefore, it is critical that the substrate can reach the interior of the cell. To facilitate this, it is preferred that the whole-cell catalyst expresses a fatty acid importer, in the case of a bacterial, more particularly Gram-negative, whole-cell catalyst, particularly preferably the fatty acid importer FadL (database code: BAA16205.1) or a variant, preferably in a concentration and with an activity which is increased with respect to the activity of the wild type of the corresponding whole-cell catalyst. Increasing the activity of a polypeptide with respect to the wild type of the cell can be achieved via various routes routinely accessible to the person skilled in the art, for example the incorporation of additional copies, functionally linked to a promoter, of the nucleotide sequence encoding the polypeptide or the exchange of the natural promoter for a stronger promoter.

It has been found that, according to the invention, the amines and diamines are produced in a higher yield and purity when the background of enzymes expressed endogenously in the whole-cell catalyst is optimized in such a way to reduce or switch off the activity of endogenous enzymes which degrade reactants, intermediates or products of the process according to the invention or using the cell according to the invention, preferably methyl esters of ω -aminocarboxylic acids, ω -hydroxycarboxylic acids, ω -oxocarboxylic acids and dicarboxylic acids, on metabolic pathways or otherwise modify them, leading away from the development of the desired product. Against this background, it may be advantageous when the whole-cell catalyst according to the invention is a cell having, with respect to its wild type, a reduced activity of the esterase BioH [database code YP_492020.1] or a variant thereof. Such cells having reduced BioH activity, the production thereof and assays to determine activity are described in the European patent application EP 12007663.3.

If the carboxylic acid, dicarboxylic acid or monoester thereof used are mixtures of compounds in which the terminal carboxy groups are present to a high degree, preferably to an extent of at least 50, 60, 70, 80, 90, 95 or 99%, in the form of an ester, for example because of the better availability of these substrates or the toxicity of free carboxylic acids or dicarboxylic acids, the monoester can be provided by partial or complete hydrolysis of completely esterified dicarboxylic acids and the free carboxylic acid by partial or complete hydrolysis of completely

esterified carboxylic acids. In this case, it is advantageous to increase the capacity of the cell in relation to ester hydrolysis by overexpression of a suitable esterase. To this end, in a preferred embodiment, the activity of the ester hydrolase BioH or a variant thereof is increased with respect to the wild type of the whole-cell catalyst used, particularly preferably by overexpression. The corresponding monoester and/or the unesterified carboxylic acid or dicarboxylic acid is then provided *in situ* by ester hydrolysis. The partial or complete hydrolysis of a completely esterified dicarboxylic acid can also be effected by chemically catalysed hydrolysis, for example at low pH levels.

- 10 The whole-cell catalyst according to the invention can be preferably used in a process for converting a carboxylic acid or dicarboxylic acid or a monoester thereof to the corresponding amine or diamine, wherein the carboxylic acid or dicarboxylic acid or the monoester thereof is a compound of the formula (I)



where R^1 is selected from the group comprising -H and $COOR^3$, where R^2 and R^3 are each independently selected from the group comprising H, methyl, ethyl and propyl, with the proviso that at least one of the radicals R^2 and R^3 is H, where A is an unbranched, branched, linear, cyclic, substituted or unsubstituted hydrocarbon group having at least four carbon atoms. In a preferred embodiment, A is a structure of the formula $-(CH_2)_n-$, where n is preferably 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30. In a preferred embodiment, the carboxylic acid or dicarboxylic acid is lauric acid or ω -carboxylauric acid. In a further most preferred embodiment, the carboxylic acid is a carboxylic acid of the formula $CH_3-(CH_2)_n-COOH$, where n is preferably 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30, preferably hexanoic acid or decanoic acid. In a further most preferred embodiment, the carboxylic acid or dicarboxylic acid is a dicarboxylic acid of the formula $HOOC-(CH_2)_n-COOH$, where n is preferably 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30, preferably ω -carboxyhexanoic acid or ω -carboxydecanoic acid. In a further most preferred embodiment, the carboxylic acid or dicarboxylic acid is ω -carboxytetradecanoic acid. Accordingly, the amine or diamine produced according to the invention is preferably a compound of the formula $CH_3-(CH_2)_n-NH_2$ or $NH_2-(CH_2)_n-NH_2$, where n is in each case preferably 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30.

With respect to the carboxylic acid or dicarboxylic acid or the monoester thereof and to any chemical compound described in this application, it is the case that the respective specified formula encompasses all salts, protonated or deprotonated, of the respective compound. For example, the lauric acid encompasses not only the protonated form, but also the salt laureate with all cations, for example sodium laureate.

It is a requirement of the process according to the invention that the enzymes used for the process according to the invention, optionally provided in the form of the whole-cell catalyst according to the invention, be contacted with the carboxylic acid or dicarboxylic acid or the monoester thereof in an aqueous solution. In a preferred embodiment, the term "contacting", as used herein, is understood to mean that the particular enzyme gets into direct contact with its substrate, more particularly without physical barriers such as impermeable membranes or the like being interposed. In the simplest case, contacting occurs by the substrate being added to an aqueous solution in which the enzyme or the whole-cell catalyst is situated.

Suitable for carrying out the teaching according to the invention is a reaction mixture comprising the whole-cell catalyst according to the invention in aqueous solution and a carboxylic acid or dicarboxylic acid or a monoester thereof of the formula (I), where R^1 is selected from the group comprising $-H$ $COOR^3$, where R^2 and R^3 are each independently selected from the group comprising H , methyl, ethyl and propyl, with the proviso that at least one of the radicals R^2 and R^3 is H , where A is an unbranched, branched, linear, cyclic, substituted or unsubstituted hydrocarbon group having at least four carbons, preferably the formula $-(CH_2)_n-$, where n is at least 4, particularly preferably at least 10. The aqueous solution must, for example with respect to composition, pH and temperature, be of such a nature that said solution supports, at least for a certain period, the viability or at least the catalytic capability of the whole-cell catalyst. The person skilled in the art knows numerous aqueous culture media which are suitable as aqueous solution and which are suitable for maintaining or culturing cells, more particularly cells of biotechnological importance. These include, equally, complete media such as LB media, minimal media such as M9 media and also selective media, for example those which have a high salt concentration and therefore only permit the growth of halophilic or at least halotolerant organisms. In a preferred embodiment, the term "aqueous culture medium", as used herein, is understood to mean a water-based reaction medium which, with respect to all relevant factors, in particular pH, salt content and temperature, is of such a nature that it maintains or promotes

the viability of cells present therein, preferably microorganisms, and both aqueous culture medium and hydrophobic organic phase are present in liquid form. The temperature requirements of various biotechnologically important cells can be found in microbiology and molecular biology textbooks, for example Fuchs/Schlegel, 2008. In a preferred embodiment, the pH of the aqueous culture medium at the time point of contacting is between 4 and 9, more preferably between 4.5 and 8.5, most preferably between 6.5 and 7.5. In a further preferred embodiment, the temperature is between 0 and 45°C, more preferably between 15 and 40°C, most preferably between 20 and 37°C. The reaction mixture is typically present in a fermenter. Any reaction vessel which can be sterilized, preferably autoclaved, and allows the culturing of the whole-cell catalyst, aeration and control of the reaction conditions, for example the oxygen content and the temperature, can act as fermenter.

In a preferred embodiment, the reaction mixture comprises, in addition to the aqueous solution, a hydrophobic organic phase. This can comprise an organic solvent and/or a hydrophobic liquid cation exchanger for removing the ω -amino fatty acid from the aqueous solution. Suitable solvents and cation exchangers are described in EP11191520.3.

The present invention is more particularly described by the following figures and non-limiting examples from which further features, embodiments, aspects and advantages of the present invention may be discerned.

Figure 1: Detection of monoamines and diamines in the fermentation broth of the strain *E.coli* W3110 pACYC{Placuv5}[carA_Ms-npt_Noc] / pJ281_alaDH_B.s._TA_C.v.(ct) after a 21.75 h process time.

Example 1

Producing an expression vector for the expression of the genes MSMEG_2956 from *Mycobacterium smegmatis* and *npt* from *Nocardia* sp.

To produce vectors for the coexpression of MSMEG_2956 (carA, SEQ ID No. 1) from *Mycobacterium smegmatis* encoding a fatty acid reductase (YP_887275.1) and with *npt* (SEQ ID No. 2) from *Nocardia* sp. encoding a phosphopantetheinyl transferase (ABI83656.1) which phosphopantetheinylates the fatty acid reductase, both genes were amplified by means of PCR with insertion of homologous regions for recombinant cloning. In this connection, genomic DNA

from the donor organism for the amplification of the gene MSMEG_2956 and a synthesized DNA fragment for the amplification of the gene *npt* served as template. The genes are under the control of a *lacuv5* promoter (SEQ ID No. 3), which was likewise amplified by means of PCR proceeding from an available vector with insertion of homologous regions for the recombinant cloning.

In this connection, the following oligonucleotides were used:

Plac_H1_fw: 5'-TTATGCGACTCCTGCTGGCTATGGTGGGATTTCC-3' (SEQ ID Nr. 4)

Plac_H2_rv: 5'-GATCGTCATATGCCACTCTCCTTGGTTCC-3' (SEQ ID Nr. 5)

10 carA_H2_fw: 5'-TGGCATATGACGATCGAAACGCGCG-3' (SEQ ID Nr. 6)

carA_H3_rv: 5'-TCCTTCTCTTACAGCAATCCGAGCATCT-3' (SEQ ID Nr. 7)

npt_H3_fw: 5'-GCTGTAAGAGAAGGAGTTCTATCATGATCGAG-3' (SEQ ID Nr. 8)

npt_H4_rv: 5'-GCAGCCTAGGTTAATTTATCAGGCGTACGCGATCG-3' (SEQ ID Nr. 9)

15 The following parameters were used for the PCR for the amplification of the P_{lacuv5} and the gene *npt*: 1 x: initial denaturation, 98°C, 0:30 min; 35 x: denaturation, 98°C, 0:10 min, annealing, 55°C, 0:20 min; elongation, 72°C, 0:15 min; 1 x: terminal elongation, 72 °C, 10 min. For the amplification of the gene MSMEG_2956, the following parameters were used: 1 x: initial denaturation, 98°C, 0:30 min; 35 x: denaturation, 98°C, 0:10 min, annealing, 65°C, 0:20 min; elongation, 72°C, 1 min; 1 x: terminal elongation, 72 °C, 10 min. For the amplification, the PhusionTM High-Fidelity Master Mix from New England Biolabs (Frankfurt) was used according to the recommendations from the manufacturer. In each case, 50 µl of the PCR reactions were then resolved on a 1% strength TAE agarose gel. The PCR, the agarose gel electrophoresis, ethidium bromide staining of the DNA and determination of the PCR fragment sizes were performed in the manner known to the person skilled in the art. In all cases, PCR fragments of the expected size could be amplified. These were 325 base pairs for P_{lacuv5} , 3.5 kilobase pairs for MSMEG_2956 and 718 base pairs for *npt*. To isolate the DNA from the agarose gel, the target DNA was cut out of the gel using a scalpel and purified using the *QiaQuick Gel extraction Kit* in accordance with the manufacturer's instructions (Qiagen, Hilden). The purified PCR products were cloned into an *Eco*NI- and *Pac*I-cut pACYCDuet-1 vector (Merck, Darmstadt) by means of recombination using the Geneart® Seamless Cloning and Assembly Kit in accordance with the manufacturer's instructions (Life Technologies, Carlsbad, CA, USA). Chemically competent *E.coli* DH10β (New England Biolabs, Frankfurt) were transformed in the manner

known to the person skilled in the art. The correct insertion of the target genes was checked by restriction analysis and the authenticity of the inserted genes confirmed by DNA sequencing. The finished expression vector was referred to as pACYC{Placuv5}[carA_Ms-npt_Noc] (SEQ ID No. 10).

5

Example 2

Producing an expression vector for the coexpression of the genes *ald* from *Bacillus subtilis* and Cv2025 from *Chromobacterium violaceum*

10 To produce an *E. coli* expression vector for the genes *ald* (SEQ ID No. 11) from *Bacillus subtilis* encoding an alanine dehydrogenase (NP_391071.1) and Cv_2025 (SEQ ID No. 12) from *Chromobacterium violaceum* encoding a transaminase (NP_901695.1), the gene *ald* from *Bacillus subtilis* was, in exchange for the gene *ald* from *Bacillus sphaericus*, cloned into the *E. coli* expression vector pJ281_alaD_Bsp_TA_C.v.(ct) (sequence and production, cf. example 1
15 in WO/2013/024114 and SEQ ID No. 17 listed therein). The gene *ald* from *Bacillus subtilis* was amplified by PCR from chromosomal DNA from the strain *Bacillus subtilis* str. 168. In this connection, the following oligonucleotides were used:

alaDH_pCR22_fw: 5'-ATGATCATAGGGGTTCTAAAGAG-3'
20 (SEQ ID No. 13)
alaDH_pCR22_rev: 5'-TTAAGCACCCGCCACAGATG-3'
(SEQ ID No. 14)

The following parameters were used for the PCR: 1 x: initial denaturation, 98°C, 0:30 min; 35 x:
25 denaturation, 98°C, 0:10 min, annealing, 65°C, 0:30 min; elongation, 72°C, 0:20 min; 1 x:
terminal elongation, 72°C, 10 min. For the amplification, the Phusion™ High-Fidelity Master Mix from New England Biolabs (Frankfurt) was used according to the recommendations from the manufacturer. In each case, 50 µl of the PCR reactions were then resolved on a 1% strength TAE agarose gel. The PCR, the agarose gel electrophoresis, ethidium bromide staining of the
30 DNA and determination of the PCR fragment sizes were performed in the manner known to the person skilled in the art. The PCR fragment showed the expected size of 1137 base pairs and was purified from the PCR volume using the Quick PCR Purification Kit from Qiagen (Hilden) in accordance with the information from the manufacturer. For the ligation of the PCR product to the vector, 5'-phosphates were attached to the PCR product using the polynucleotide kinase

(New England Biolabs, Frankfurt). In this connection, the recommendation from the manufacturer was followed.

The vector was digested with the restriction endonucleases *Hind*III and *Nde*I, and as a result, the gene present, *Bacillus sphaericus ald*, was removed. The restriction digest volume was resolved on a 1% strength TAE agarose gel. It was possible to identify two bands of sizes 5696 bp and 1124 bp. To isolate the vector DNA from the agarose gel, the DNA band of 5696 bp was isolated from the gel using a scalpel and purified using the Quick Gel Extraction Kit from Qiagen (Hilden) in accordance with the information from the manufacturer. To generate blunt ends, the 5'-overhangs of the purified vector DNA were filled using the Klenow fragment of DNA polymerase I (New England Biolabs, Frankfurt). In this connection, the information from the manufacturer was followed. The DNA fragment *Bacillus subtilis ald* with 5'-phosphate residues was ligated into the vector having blunt ends. The finished *E. coli* expression vector was referred to as pJ281_alaDH_B.s._TA_C.v.(Ct) (SEQ ID No. 15).

Example 3

Producing an *E. coli* strain overexpressing the genes MSMEG_2956 from *Mycobacterium smegmatis* and *npt* from *Nocardia sp.*, *ald* from *Bacillus subtilis* and Cv2025 from *Chromobacterium violaceum*

To generate an *E. coli* strain coexpressing the genes MSMEG_2956 from *Mycobacterium smegmatis* encoding a fatty acid reductase (YP_887275.1) and *npt* from *Nocardia sp.* encoding a phosphopantetheinyl transferase (ABI83656.1) which phosphopantetheinylates the fatty acid reductase, in combination with the genes *ald* from *Bacillus subtilis* encoding an alanine dehydrogenase (NP_391071.1) and Cv2025 from *Chromobacterium violaceum* encoding a transaminase (NP_901695.1), the strain *E. coli* W3110 was transformed with the plasmids pACYC{Placuv5}[carA_Ms-npt_Noc] (SEQ ID No. 10) and pJ281_alaDH_B.s._TA_C.v.(ct) (SEQ ID No. 15) by means of electroporation and plated out on LB agar plates containing chloramphenicol (50 µg/ml) and kanamycin (50 µg/ml). Transformants were checked as regards the presence of the correct plasmids by plasmid preparation and analytical restriction analysis.

The strain generated was referred to as *E. coli* W3110 pACYC{Placuv5}[carA_Ms-npt_Noc] / pJ281_alaDH_B.s._TA_C.v.(ct). The strain was used to investigate its capability for the production of dodecanediamine proceeding from dodecanedioic acid and dodecylamine proceeding from dodecanoic acid. The gene product CarA, a fatty acid reductase which is activated by the overexpressed phosphopantetheinyl transferase *npt*, converts the substrate

dodecanoic acid or dodecanedioic acid to the respective aldehyde or dialdehyde. The function of the gene product Cv_2505 is that of converting the (di)aldehyde terminally to the dodecylamine or dodecanediamine. The alanine amino donor required for the amination reaction is provided from pyruvate by the gene product *ald*.

5

Example 4

Production of dodecanediamine and dodecylamine by *E. coli* strains containing an expression vector for the genes MSMEG_2956 from *Mycobacterium smegmatis* and *npt* from *Nocardia* sp. in combination with an expression vector for the genes *ald* from *Bacillus subtilis* and Cv_2025 from *Chromobacterium violaceum*

The strain generated in example 3 was used to investigate its capability in relation to the production of dodecylamine and dodecanediamine. The biotransformation of dodecanoic acid and dodecanedioic acid to dodecylamine and dodecanediamine, respectively, was carried out in the 8-fold parallel fermentation system from DASGIP. The procedure for this was as follows:

For the fermentation, 1 L reactors were used. The pH probes were calibrated by means of a two-point calibration with measurement solutions of pH 4.0 and pH 7.0. The reactors were filled with 300 mL of drinking water and autoclaved for 20 min at 121°C in order to ensure sterility. Then, the pO₂ probes were polarized on the DASGIP system overnight (for at least 6 h). The next morning, the water was removed under the clean bench and replaced with 300 mL of high-cell-density medium containing 50 mg/L chloramphenicol and 50 mg/L kanamycin. Subsequently, the pO₂ probes were calibrated using a single-point calibration (stirrer: 400 rpm / aeration: 10 sL/h air) and the feed, correcting agent and induction agent paths were cleaned by means of clean-in-place. To this end, the hoses were flushed with 70% ethanol, then with 1 M NaOH, then with sterile demineralized water and finally filled with the particular media.

The *E. coli* strain producing dodecanediamine and dodecylamine was firstly cultured from the cryogenic culture in LB medium (25 mL in a 100 mL baffled flask) containing the aforementioned antibiotics overnight at 37°C and 200 rpm, for about 18 h. Then, 2 mL of the culture were inoculated into high-cell-density medium (15 g/L glucose (30 mL/L of a separately autoclaved 500 g/L stock solution containing 1% MgSO₄*7H₂O and 2.2% NH₄Cl), 1.76 g/L (NH₄)₂SO₄, 19.08 g/L K₂HPO₄, 12.5 g/L KH₂PO₄, 6.66 g/L yeast extract, 2.24 g/L trisodium citrate dihydrate, ammonium ferric citrate solution, 17 mL/L of a separately autoclaved 1% strength stock solution, trace element solution, 5 mL/L separately autoclaved stock solution (36.50 g/L HCl (37%), 1.91 g/L MnCl₂*4H₂O, 1.87 g/L ZnSO₄*7H₂O, 0.84 g/L

ethylenediaminetetraacetic acid dihydrate, 0.30 g/L H_3BO_3 , 0.25 g/L $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, 4.70 g/L $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 17.80 g/L $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.15 g/L $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$) (25 mL in a 100 mL baffled flask) containing the aforementioned antibiotics and incubated at 37°C / 200 rpm for a further 5.5 h.

The reactors were inoculated at an optical density of 0.1 by an appropriate volume of the pre-culture being filled into a 5 mL syringe (under sterile conditions) and the reactors being inoculated by means of a needle across a septum covered with 70% ethanol.

The following standard program was used:

DO regulator		pH regulator	
Preset	0%	Preset	0 mL/h
P	0.1	P	5
Ti	300 s	Ti	200 s
Min	0%	Min	0 mL/h
Max	100%	Max	40 mL/h

N (rotation)	from	to	XO ₂ (gas mixture)	from	to	F (gas flow rate)	from	to
Growth and biotransformation	0%	30%	Growth and biotransformation	0%	100%	Growth and biotransformation	15%	80%
	400 rpm	1500 rpm		21 %	21%		6 sL/h	72 sL/h

Script	
Trigger activated	31% DO (1/60 h)
IPTG induction	2 h after feed start
Feed trigger	50% DO
Feed rate	3 [mL/h]

The experiment carried out can be divided into two phases: growth, during which the cells are to reach a certain optical density, and subsequent biotransformation, in which, after addition of the substrates dodecanoic acid, oleic acid and dodecanedioic acid, a conversion to dodecylamine, oleylamine and dodecanediamine, respectively, is to take place by enzymes formed during expression. The pH levels were unilaterally adjusted to pH 6.8 using ammonia (12.5%). During growth and biotransformation, the dissolved oxygen (DO) in the culture was adjusted at 30% by means of stirrer speed and aeration rate. The fermentation was carried out as a fed batch, with the feed start, 5 g/Lh glucose feed (500 g/L glucose containing 1% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and 2.2% NH_4Cl), being triggered via a DO peak. With the feed start, the temperature was also lowered

from previously 37°C to 30°C. The expression of the transaminase, alanine dehydrogenase, carboxylic acid reductase and phosphopantetheinyl transferase was induced 2 h after the feed start by the automated addition of 1 mM IPTG. Before the start of biotransformation, the optical density of the culture broths was determined.

The start of the biotransformation phase took place 1 h or 12 h after the feed start. To this end, 150 mL or 75 mL of a mixture of dodecanoic acid or dodecanedioic acid and oleic acid (technical-grade 90%) were added as a batch to the fermentation broth. To provide an amino group donor for the transaminase, 5 mL of a 3 M ammonium sulphate solution were added to the fermentation broth 30 minutes before the biotransformation start. For sampling, 2 mL of fermentation broth were removed from the tank and a portion thereof was diluted 1/20 in a and in a mixture of 80% acetonitrile, 20% water and 0.1% formic acid and extracted. Samples were taken from all reactors at 1.25 h, 2.75 h, 4.25 h, 18.25 h, and 21.75 h after the start of biotransformation. The conversion rates for oxygen (OTR = oxygen transfer rate) and carbon (CTR = carbon transfer rate) were determined during the fermentation via the waste-gas analyses on the DASGIP systems. The fermentation was ended 21.75 h after the start of biotransformation. The stirrer, the aeration system, the temperature control and pH control were turned off and the tanks were left to stand undisturbed for 5-10 minutes.

HPLC-ESI/MS scan method

The samples were qualitatively assessed by means of HPLC/MS coupling with high-resolution MS detection in the scan mode.

The following instruments were used here:

- Accela HPLC system (Thermo Scientific, Waltham, Massachusetts, USA) with autosampler, quaternary pump, PDA detector and column oven
- LTQ-FT mass spectrometer (Thermo Scientific, Waltham, Massachusetts, USA) with ESI source
- HPLC column: Kinetex C18, 100 x 2.1 mm, particle size: 2.6 µm, pore size 100 Å (Phenomenex; Aschaffenburg)

The samples were prepared by pipetting 1950 µl of solvent (80% (v/v) acetonitrile, 20% double-distilled H₂O (v/v), + 0.1% formic acid) and 50 µl of sample into a 2 ml reaction vessel. The mixture was vortexed for about 10 seconds and then centrifuged at about 13 000 rpm for 5 min. The clear supernatant was removed using a pipette.

The HPLC separation was carried out with the aforementioned HPLC column. The injection volume was 0.5 μ L, the column temperature 40°C, and the flow rate 0.3 mL/min. The mobile phase was composed of eluent A (0.02% strength (v/v) aqueous trifluoroacetic acid) and eluent B (acetonitrile with 0.015% (v/v) trifluoroacetic acid). The following gradient profile was used:

Time [min]	Eluent A [%]	Eluent B [%]
0	98	2
2	98	2
17	2	98
32	2	98

The ESI-MS analysis was carried out in the positive mode with the following parameters of the ESI source:

- ESI voltage: 4kV
- Capillary temperature 300°C
- Sheath gas flow 40
- Aux gas flow 5
- Sweep gas flow 3

The detection was carried out within a mass range of $m/z = 100$ to 1000. The mass spectrometry resolution was $R = 100\,000$.

The results are shown in the table below.

Substrate	Induction	MS intensity [-]		
		Dodecanediamine	Dodecylamine	Oleylamine
LA/oleic acid (150 ml)	1 mM IPTG (12 h)	n.d.	1,160,000	23,700
DDA/oleic acid (75 ml)	1 mM IPTG in H ₂ O/ethanol (12 h)	742,000	n.d.	18,000
DDA/oleic acid (150 ml)	1 mM IPTG (1 h)	30,700	n.d.	n.d.
DDA/oleic acid (150 ml)	1 mM IPTG (12 h)	23,500	n.d.	n.d.

Qualitative detection of monoamines and diamines in the fermentation broth of the strain *E.coli* W3110 pACYC{Placuv5}[carA_Ms-npt_Noc] / pJ281_alaDH_B.s._TA_C.v.(ct) after a 21.75 h process time (n.d. = not detectable, LA = lauric acid, DDA = dodecanedioic acid).

Further data are illustrated in figure 1.

5 1,12-dodecanediamine and dodecylamine are quantitatively determined by means of HPLC/UV measurement, after derivatization by means of ortho-phthaldialdehyde. The methanolic supernatant was measured. The most important chromatographic parameters are summarized in the following table.

Column	Luna 5u C8, 100 Å, 150 x 4.60 mm (Phenomenex; Aschaffenburg)
HPLC system	Agilent 1200
Eluent A	2.5 mL of acetic acid (100%) to 1 L of double-distilled water, pH adjustment with sodium hydroxide solution to pH 6.0
Eluent B	methanol
Column temp.	40 °C
Flow rate	1 mL/min
Gradient	0.0-1 min: 30.0% B, 1.0-17.0 min: 90.0% B, 17-19.5 min: 90.0% B, 19.6-20.5 min: 30.0% B
Detector	DAD, 334 nm
Derivatization/ injection volume	Automatic derivatization by means of injector program, 1 µL of sample is reacted with 9 µL of derivatization reagent; composition of derivatization reagent: 10 g/L o-phthaldialdehyde dissolved in borate buffer (0.4 mol/L), with addition of mercaptoethanol (5 mL/L) and methanol (100 mL/L)
Calibration	External calibration, measurement range 50-1000 mg/L, 5-point calibration, calibration before and after the sample series, averaging via both calibration series, quadratic regression

10 The results are shown in the tables which follow.

Substrate	Induction	Dodecylamine [mg/L]	Dodecanediamine [mg/L]
DDA/oleic acid (75 ml)	1 mM IPTG in H ₂ O/ethanol (12 h)	n.d.	40.5

DDA/oleic acid (150 mL)	1 mM IPTG (1 h)	n.d.	3.1
DDA/oleic acid (150 ml)	1 mM IPTG (12 h)	n.d.	<1 ^{*)}
LA/oleic acid (150 ml)	1 mM IPTG (12 h)	11.6	n.d.

Quantification of monoamines and diamines in the fermentation broth of the strain *E.coli* W3110 pACYC{Placuv5}[carA_Ms-npt_Noc] / pJ281_alaDH_B.s._TA_C.v.(ct) after a 21.75 h process time (n.d. = not detectable, ^{*)} lower than the detection limit, DDA = dodecanedioic acid, LA = lauric acid).

- 5 It was shown that the strains are capable of producing, from dodecanoic acid, dodecanedioic acid and oleic acid, the respective amines dodecylamine, dodecanediamine and oleylamine.

Example 5

10 Producing an expression vector for the expression of the gene α DOX encoding an α -dioxygenase from *Oryza sativa*

To produce a vector for the expression of α DOX (Os12g0448900, SEQ ID No. 16) from *Oryza sativa* encoding an α -dioxygenase (NP_001066718.1), the gene was codon-optimized for expression in *Escherichia coli*, synthesized and, at the same time, an upstream *NdeI* restriction site and a downstream *AvrII* restriction site were introduced. The synthesized DNA fragment was digested with the restriction endonucleases *NdeI* and *AvrII* and ligated into the correspondingly cut vector pACYC{Placuv5}[carA_Ms-npt_Noc] (SEQ ID No. 10) with removal of the genes *carA_Ms* and *npt_Noc*. The *lacuv5* promoter (SEQ ID No. 3) present in the vector was retained. The finished vector was referred to as pACYC{Placuv5}[DOX_Os(co_Ec)] (SEQ ID No. 17). The vector pACYC is an *E. coli* vector which mediates chloramphenicol resistance and also bears a p15A origin of replication and thus has a low copy number (10-15 copies per cell).

25 Example 6

Producing an *E. coli* strain having a deletion in the gene *bioH*, overexpressing the genes α DOX from *Oryza sativa*, *ald* from *Bacillus subtilis* and Cv2025 from *Chromobacterium violaceum*

To generate an *E. coli* strain which coexpresses the gene α DOX from *Oryza sativa* encoding an α -dioxygenase (NP_001066718.1) in combination with the genes *ald* (SEQ ID No. 11) from

Bacillus subtilis encoding an alanine dehydrogenase (NP_391071.1) and Cv2025 (SEQ ID No. 12) from *Chromobacterium violaceum* encoding a transaminase (NP_901695.1), the strain *E. coli* W3110 Δ *bioH* (production: see EP12007663, example 1) was transformed with the plasmids pACYC{Placuv5}[DOX_Os(co_Ec)] (SEQ ID No. 17) and pJ281_alaDH_B.s._TA_C.v.(ct) (SEQ ID No. 15) by means of electroporation and plated out on LB agar plates containing chloramphenicol (50 µg/ml) and kanamycin (50 µg/ml). Transformants were checked as regards the presence of the correct plasmids by plasmid preparation and analytical restriction analysis. The strain generated was referred to as *E. coli* Δ *bioH* pACYC{Placuv5}[DOX_Os(co_Ec)] / pJ281_alaDH_B.s._TA_C.v.(ct).

The strain was used to investigate its capability for the production of methyl aminoundecanoate proceeding from methyl dodecanedioate.

Example 7

Production of methyl aminoundecanoate proceeding from methyl dodecanedioate by an *E. coli* strain containing an expression vector for the gene α DOX from *Oryza sativa* in combination with an expression vector for the genes *ald* from *Bacillus subtilis* and Cv_2025 from *Chromobacterium violaceum*

The strain described in example 8 was used to investigate its capability in relation to the production of methyl aminoundecanoate. The procedure for this was as follows:

The strain under investigation was firstly spread out on an LB agar plate containing 50 µg/ml chloramphenicol and 50 µg/ml kanamycin and incubated overnight at 37°C. As control, the strain *E. coli* W3110 Δ *bioH* was additionally spread out on an LB agar plate not containing antibiotics. The strains were then cultured in Luria-Bertani broth, Miller (Merck, Darmstadt) containing 50 µg/ml chloramphenicol and 50 µg/ml kanamycin (for the plasmid-bearing strain) as a 20 ml pre-culture from a single colony in each case. As main culture, 100 ml of LB broth containing 50 µg/ml chloramphenicol and 50 µg/ml kanamycin were initially charged into a 500 ml Erlenmeyer flask containing baffles and inoculated with 2 ml from the pre-culture. Culturing was firstly carried out at 37°C and 200 rpm in an incubator shaker. Upon attainment of an optical density (600 nm) of 0.5 – 0.7, the gene expression was induced by addition of 1 mM IPTG. Further culturing was carried out overnight at 22°C and 200 rpm. The following day, the cultures were harvested by a 10-minute centrifugation at 4°C and 5525 x g. The supernatant was discarded and the cell pellet washed in 200 mM potassium phosphate buffer (pH 7.5). The cell pellet was lastly taken up in 200 mM potassium phosphate buffer containing 50 mM

ammonium chloride and 0.5% (w/v) glucose, and so an OD (600 nm) of 20 was attained. 12.5 mM methyl dodecanedioate (abcr, Karlsruhe) in ethanol were added to the cell suspension and gently shaken for 4 hours at 30°C and 300 rpm. During the incubation, samples were taken at the times 0 min, 60 min, 120 min, 180 min and 240 min and extracted in a mixture of 80% acetonitrile, 20% water and 0.1% formic acid. The supernatant was analysed by means of HPLC/MS analysis. The results are shown in the table below.

Strain	Time [min]	Peak area Methyl aminoundecanoate
<i>E. coli</i> W3110 Δ bioH	0	n.d.
	120	n.d.
	180	n.d.
	240	n.d.
<i>E. coli</i> W3110 Δ bioH pACYC{Placuv5}[DOX] / pJ281_alaDH_B.s._TA_C.v.(ct)	0	n.d.
	120	57616
	180	371989
	240	1764605

Production of methyl aminoundecanoate with *E. coli* W3110 Δ bioH overexpressing α DOX from *Oryza sativa*, *ald* from *Bacillus subtilis* and Cv_2025 from *Chromobacterium violaceum*. Peak areas are specified (n.d. = not detectable).

5

It was possible to show that the strain *E. coli* W3110 Δ bioH pACYC{Placuv5}[DOX] / pJ281_alaDH_B.s._TA_C.v.(ct) is capable of forming methyl aminoundecanoate proceeding from methyl dodecanedioate.

10

Claims

1. Whole-cell catalyst which expresses a recombinant α -dioxygenase or the combination of a recombinant fatty acid reductase and of a phosphopantetheinyl transferase which phosphopantetheinylates the fatty acid reductase, and which additionally expresses a transaminase, wherein the phosphopantetheinyl transferase and/or transaminase is preferably recombinant.
2. Whole-cell catalyst according to Claim 1, wherein the whole-cell catalyst additionally expresses an amino acid dehydrogenase which is preferably recombinant.
3. Whole-cell catalyst according to either of Claims 1 and 2, wherein the whole-cell catalyst additionally expresses an alkane hydroxylase which is preferably recombinant.
4. Whole-cell catalyst according to any of Claims 1 to 3, wherein the whole-cell catalyst additionally expresses a polypeptide of the AlkL family, which polypeptide is preferably recombinant.
5. Whole-cell catalyst according to any of Claims 1 to 4, which additionally expresses an alcohol dehydrogenase which is preferably recombinant.
6. Whole-cell catalyst according to any of Claims 1 to 5, wherein the activity of at least one enzyme involved in the β -oxidation is reduced with respect to the wild type of the whole-cell catalyst.
7. Whole-cell catalyst according to any of Claims 1 to 6, wherein the activity of BioH or a variant thereof is reduced or elevated with respect to the wild type of the whole-cell catalyst.
8. Whole-cell catalyst according to any of Claims 1 to 7, wherein the activity of FadL or a variant thereof is elevated with respect to the wild type of the whole-cell catalyst.
9. Process for converting a carboxylic acid or dicarboxylic acid or a monoester thereof to an amine or diamine, comprising the steps of

a) providing a carboxylic acid or dicarboxylic acid or a monoester thereof, in the case of a dicarboxylic acid, preferably by contacting a carboxylic acid with an alkane hydroxylase and/or alcohol dehydrogenase,

b) contacting the carboxylic acid or dicarboxylic acid or the monoester thereof with a phosphopantetheinylated fatty acid reductase or an α -dioxygenase to form an aldehyde product, and

c) contacting the aldehyde product from step a) with a transaminase.

10. Process according to Claim 9, wherein an amino acid dehydrogenase is present in step c).

11. Process according to any of Claims 9 to 10, wherein at least one enzyme from the group comprising phosphopantetheinylated fatty acid reductase, α -dioxygenase, transaminase, amino acid dehydrogenase and alkane hydroxylase, preferably all the enzymes used from said group, are provided in the form of a whole-cell catalyst according to any of Claims 1 to 8.

12. Process according to any of Claims 9 to 11, wherein the carboxylic acid or dicarboxylic acid or the monoester thereof is a compound of the formula (I)



where R^1 is selected from the group comprising -H and $COOR^3$,

where R^2 and R^3 are each independently selected from the group comprising H, methyl, ethyl and propyl,

with the proviso that at least one of the radicals R^2 and R^3 is H,

where A is an unbranched, branched, linear, cyclic, substituted or unsubstituted hydrocarbon group having at least four carbons.

13. Process according to any of Claims 9 to 12, wherein A has the formula - (CH₂)_n -, where n is at least 4, preferably at least 10.

14. Use of the whole-cell catalyst according to any of Claims 1 to 8 or of the process according to any of Claims 8 to 12 for aminating a carboxylic acid or dicarboxylic acid or a monoester thereof.

15. Reaction mixture comprising the whole-cell catalyst according to any of Claims 1 to 8 in aqueous solution and also a carboxylic acid or dicarboxylic acid or a monoester thereof of the formula (I)



where R¹ is selected from the group comprising -H and COOR³,

where R² and R³ are each independently selected from the group comprising H, methyl, ethyl and propyl,

with the proviso that at least one of the radicals R² and R³ is H,

where A is an unbranched, branched, linear, cyclic, substituted or unsubstituted hydrocarbon group having at least four carbons, preferably the formula - (CH₂)_n -, where n is at least 4, particularly preferably at least 10.

<110> Evonik Industries AG
<120> Producing amines and diamines from a carboxylic acid
or dicarboxylic acid or a monoester thereof

<130> 2012000206

<160> 17

<170> Patentin version 3.5

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