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Description

[0001] The present invention relates to a method for preparing an amino salt compound comprising: i) providing a carbonyl compound; ii) reacting the carbonyl compound provided according to (i) in the presence of a transaminase with
 5 ii-a) at least one primary amine; and ii-b) at least one carboxylic acid; thereby obtaining a mixture comprising an at least partially crystallized amino salt compound comprising an amino cation and a carboxylate anion based on the at least one carboxylic acid added according to (ii-b).

[0002] Biotransformations became over the past decades a powerful technique for the synthesis of valuable compounds on laboratory and industrial scale. Herein pyridoxal 5'-phosphate (PLP)-dependent transaminases (TAs) and especially
 10 amine transaminases (ATAs) have gained in recent years a significant impact in the synthesis of optically pure amines, which are valuable building blocks for various agrochemicals and active pharmaceutical ingredients, e.g. sitagliptin. These enzymes basically catalyze the deamination of a primary amine (amine donor) with a simultaneous amination of an aldehyde or ketone (amine acceptor). The transamination-reaction can be carried out as a kinetic resolution of a racemic amine or an asymmetric synthesis from the respective prochiral ketone. Due to a maximum yield of 100% the
 15 asymmetric synthesis is in theory preferred, especially if a catalyst with high enantioselectivity is used. Unfortunately thermodynamic limitations and certain product inhibitions tend to limit the applicability of transaminases in asymmetric synthesis, which needs to be overcome for synthetic purposes. Aside using an uneconomic excess of the amine donor, complex (co-)product removal techniques are currently considered, e.g. enzymatic cascades, membrane processes and non-catalytic side reactions. Such techniques unfortunately always increase (in general) overall complexity of the bio-
 20 catalytic reaction systems, require additional or tailor-made co-substrates and generate further by-products.

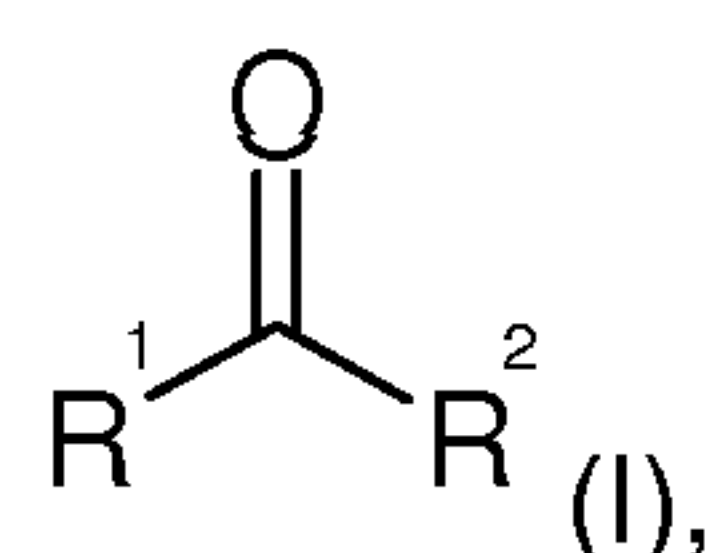
[0003] The object of the present invention was therefore the provision of a method for the preparation of a desired product amine via biotransformation, especially using a transaminase, which overcomes the above-mentioned drawbacks.

[0004] Enzymes referred to in the following are known from e.g. WO 2011/026556 (present SEQ ID NOS 1-4 and
 25 enzymes closely related to SEQ ID NOS 5-6) and UniParc - UPI0009F6A547 (present SEQ 6).

[0005] Further, Buque-Taboada et al, Applied Microbiology and Biotechnology; 2006, pages 1-12, disclose basic principles of in situ product recovery by crystallization.

[0006] The object was solved by a method for preparing an amino salt compound comprising:

30 i) providing a carbonyl compound of general formula (I)



wherein

40 R^1 is selected from the group consisting of branched or unbranched C2-C20-alkyl, branched or unbranched C2-C20-alkenyl, branched or unbranched C2-C20-alkinyl, branched or unbranched C1-C5-alkyl-O-C1-C5-alkyl, branched or unbranched C1-C10-alkoxy, C4-C20-cycloalkyl, C5-C20-cycloalkenyl, C5-C20-cycloalkinyl, C5-C20-aryl, C6-C20-alkylaryl, C6-C20-arylalkyl, C2-C20-heteroalkyl, C3-C20-cyclic heteroalkyl, C4-C20-heteroaryl, C5-C20-alkylheteroaryl and C5-C20-heteroarylalkyl, wherein in case of more
 45 than one (hetero)aliphatic or (hetero)aromatic ring system the ring systems are condensed or separate, wherein each residue R^1 has at least one substituent R^{1a} selected from the group consisting of hydrogen atom, halogen atom (F, Cl, Br, I), hydroxyl, thiol, C1-C3 thioester, C1-C3-thioether, C1-C3-alkyl and C1-C3-alkoxy; and

50 R^2 is selected from the group consisting of hydrogen atom, branched or unbranched C1-C5-alkyl, branched or unbranched C1-C5-alkyl-O-C1-C5-alkyl, branched or unbranched C1-C10-alkoxy, C5-C10-aryl, C6-C10-alkylaryl and C6-C10-arylalkyl, wherein in case of more than one aromatic ring system the ring systems are condensed or separate, wherein each residue R^2 has at least one substituent R^{2a} selected from the group consisting of hydrogen atom, halogen atom (F, Cl, Br, I), hydroxyl, thiol, C1-C3 thioester, C1-C3-thioether, C1-C3-alkyl and C1-C3-alkoxy; or

55 R^1, R^2 together form a C3-C10-cycloalkyl or C3-C10-cycloalkenyl, wherein the C3-C10-cycloalkyl or C3-C10-cycloalkenyl each has at least one substituent R^x selected from the group consisting of hydrogen atom, C1-C5-alkyl, C1-C4-heteroalkyl and C1-C5-alkyl- R^y , wherein R^y is hydroxyl or thiol;

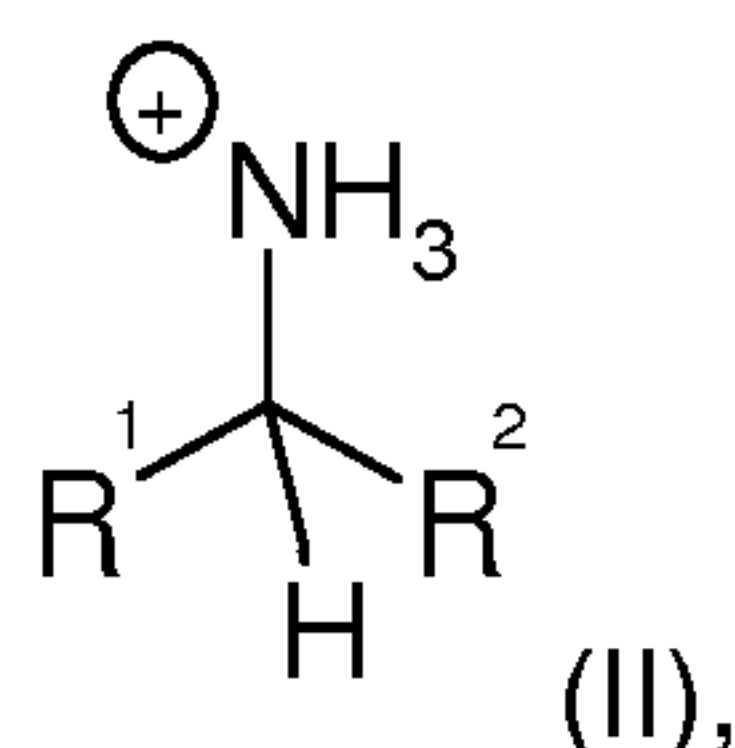
ii) reacting the carbonyl compound provided according to (i) in the presence of a transaminase with

ii-a) at least one primary amine; and

ii-b) at least one carboxylic acid;

thereby obtaining a mixture comprising an at least partially crystallized amino salt compound comprising

- a cation of general formula (II)



wherein R^1 and R^2 are as defined for general formula (I) and

- a carboxylate anion based on the at least one carboxylic acid added according to (ii-b); wherein the at least one carboxylic acid according to (ii-b) is a carboxylic acid of general formula (III) below (see Carboxylic acid).

[0007] The expression "amino salt compound" comprises cation and anion as described above. The amino salt compound can be present as pure salt compound (unsolvated, anhydrous) or as solvate or hydrate or mixture thereof, wherein hydrate includes hemihydrate, monohydrate and polyhydrate and solvate includes hemisolvate, monosolvate and polysolvate. "At least partially crystallized" means that at least 50%, preferably at least 60%, more preferably at least 70%, more preferably at least 80%, more preferably at least 90% of the amine salt compound precipitate in crystalline form.

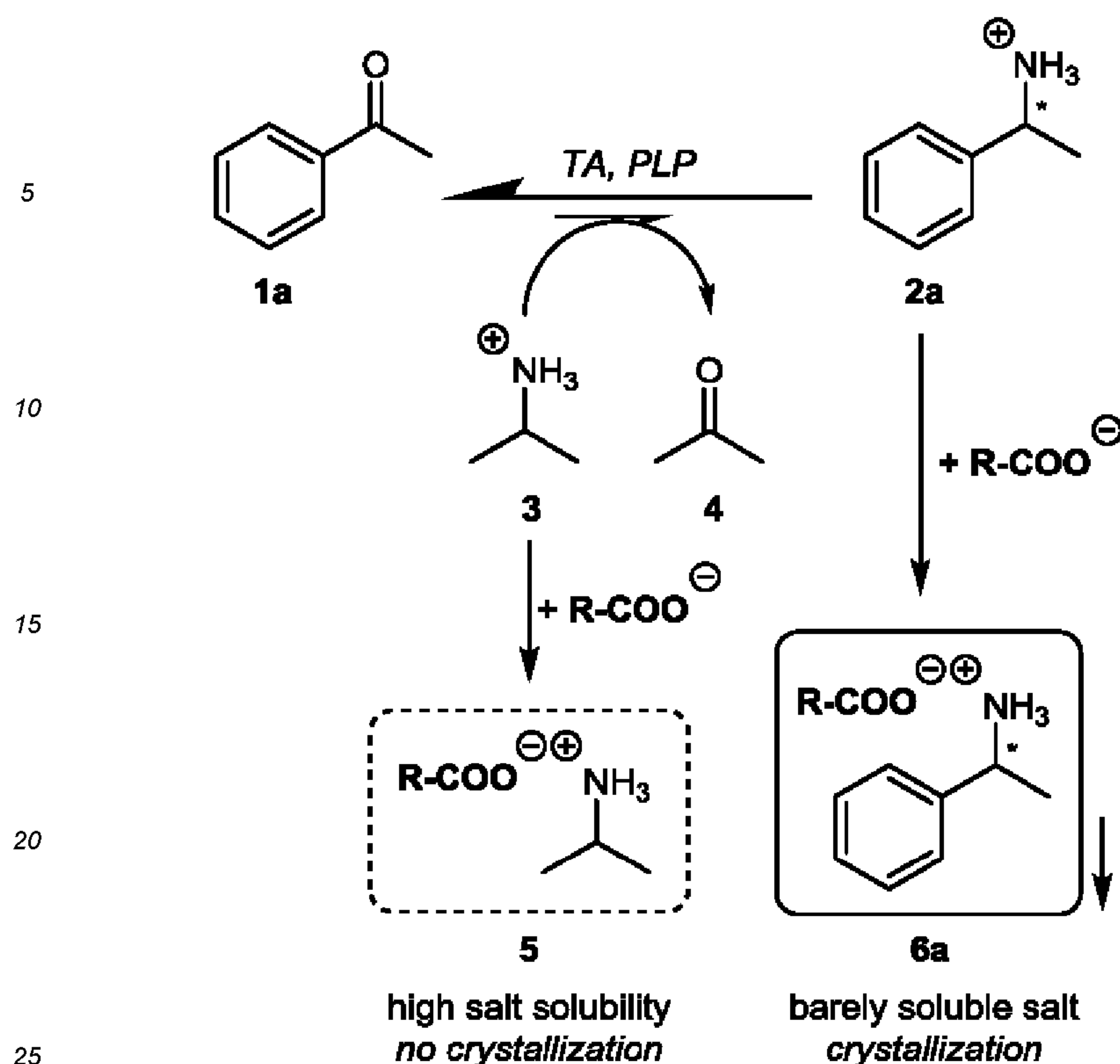
[0008] The wording "hetero" means that heteroatom(s) are present, which are selected from the group consisting of nitrogen atom, sulphur atom and oxygen atom, if not otherwise indicated. The heteroatom(s) is/are present as members of the respective chain or ring structure.

[0009] The residues $\text{R}^{1a, 2a}$ are as defined above. It has to be noted that neither R^{1a} nor R^{2a} is a substituent which comprises a charge, i.e., substituents having a charge are excluded as R^{1a} and as R^{2a} . For example, R^{1a} and also R^{2a} are no carboxyl/carboxylate, no phosphate, no sulfonate.

[0010] In a preferred embodiment, the combination of R^1 , R^{1a} being a perfluorinated alkyl is excluded. The same applies for R^2 and R^{2a} , i.e. the combination of R^2 and R^{2a} being a perfluorinated alkyl is excluded as well. In other words, neither the combination of R^1 and R^{1a} nor the combination of R^2 and R^{2a} is a perfluorinated alkyl.

[0011] Regarding the embodiment wherein R^1 and R^2 together form a C3-C10-cycloalkyl or a C3-C10-cycloalkenyl, the number of C atoms indicated includes the carbonyl C atom situated between R^1 and R^2 .

[0012] With this method as described above, the inventors now present a crystallization-based approach for the direct removal of the desired product amine from a transaminase-catalyzed reaction. Scheme 1 shows the method based on the exemplarily product amine 1-phenylethylamine. The product amine 2 is herein selectively crystallized from solution as a barely soluble amine salt 6, while all other reactants, especially the applied donor amine (isopropyl amine, 3), remains in solution. This in situ-product crystallization (ISPC) continuously removes the desired product amine from solution and thus yields an equilibrium displacement towards the products. The counter ion (here shown as a carboxylate) is added directly to the reaction solution and can be isolated for reuse from the formed solid salt. A stoichiometric use of the carboxylate in comparison to the applied amines is not required (see below).



Scheme 1: Combination of an *in situ*-product crystallization (ISPC) with a transaminase-catalyzed reaction to yield a barely soluble 1-phenylethylamine salt; shown protonation of reactants are based on an aqueous solution at pH 7.5; TA = transaminase, PLP = pyridoxal 5'-phosphate, R = exemplary residue representing a suitable carbon containing residue.

[0013] First, the main requirement of this concept is the choice of a specific counter ion, i.e. a specific carboxylic acid, which generates a barely soluble salt with the target amine for its crystallization, while the donor amine salt is not crystallized. Unfortunately, most commonly used amine salts, especially amine halide salts, show very high solubilities in aqueous solutions and thus are not applicable for such an ISPC concept. Thus, a preferred embodiment of the method is that the amino salt compound obtained according to (ii) has a solubility in water at pH 7 which is smaller than the solubility in water of the at least one primary amine added according to (ii-a). A further preferred embodiment of the method is that the amino salt compound obtained according to (ii) has a solubility in water at pH 7 ≤ 30 mmol/l, preferably ≤ 25 mmol/l, more preferably ≤ 10 mmol/l.

[0014] According to a preferred embodiment, the solubility difference (Δ_{sol}) between the solubility of the salt of the primary amine and the solubility of the amino salt compound is at least 10 mmol/l. As explained above, the solubility of the salt of the primary amine ($\text{sol}_{\text{primary amine salt}}$) is higher than the solubility of the amino salt compound ($\text{sol}_{\text{amino salt compound}}$), i.e.:

$$\text{sol}_{\text{primary amine salt}} > \text{sol}_{\text{amino salt compound}}.$$

[0015] Second, the used transaminase has to tolerate the required concentration of the chosen compound. Third, the formed salt has to be stable under process conditions and should be easily recovered from the reaction mixture.

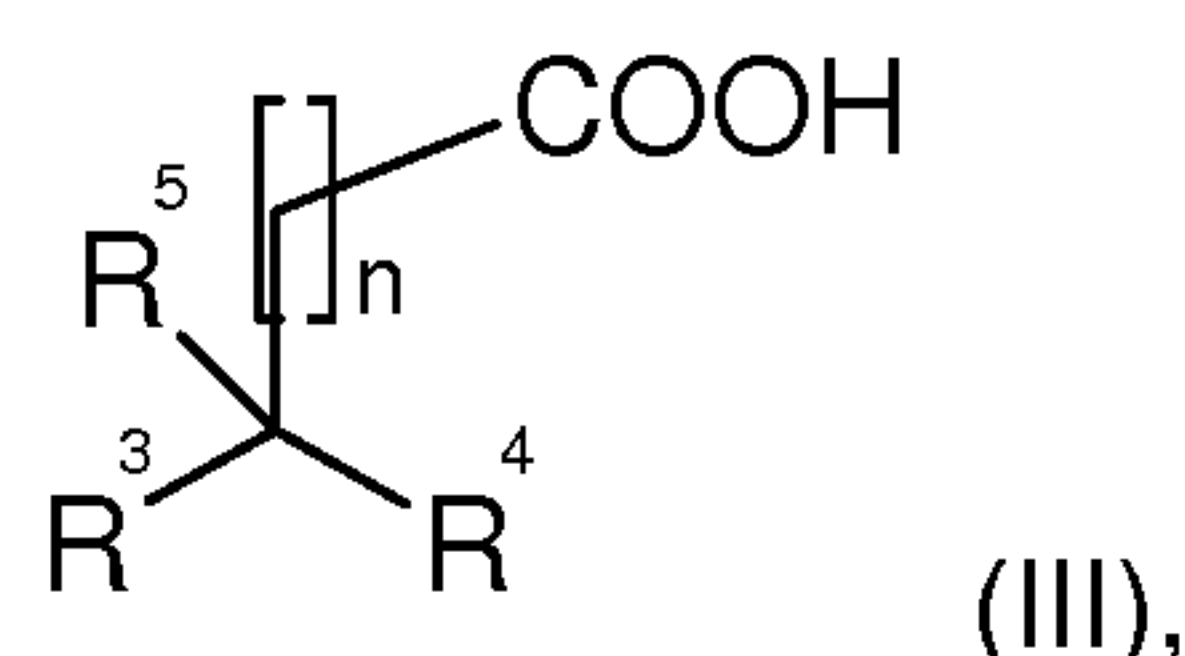
Carboxylic acid

[0016] According to step (ii), the carbonyl compound provided according to (i) is reacted in the presence of a transaminase with ii-a) at least one primary amine; and ii-b) at least one carboxylic acid.

[0017] Regarding the carboxylic acid to be used for (ii-b), commercially readily available aliphatic, aromatic and het-

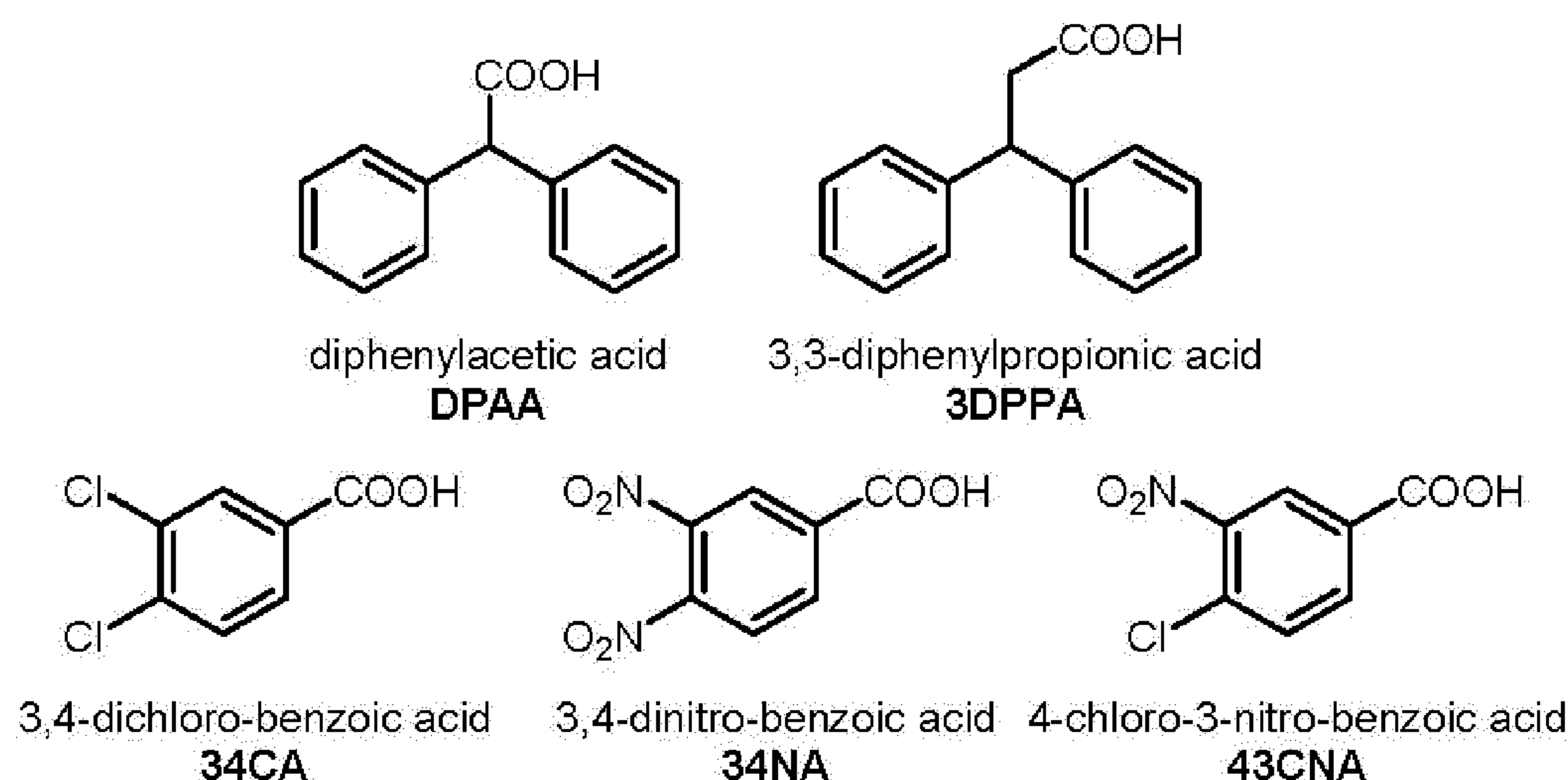
eroaromatic carboxylic acids (R-COOH) were selected and screened as their respective carboxylate salts towards common amines from a transaminase-catalyzed reaction. 1-phenylethylamine and substituted derivatives 2a-f thereof served as model product amines and were compared with typical donor amines such as isopropylamine 3, racemic 2-butylamine, DL-alanine and L-alanine (see Example section for further details). Here the salt of the product amine needs to exhibit a significant lower solubility than its donor amine salt counterpart since the donor amine is still applied with an excess.

[0018] Preferably, the at least one carboxylic acid according to (ii-b) is used in its protonated form or in deprotonated form with a suitable counter cation. The above-described straight-forward screening approach resulted in the finding, that the at least one carboxylic acid according to (ii-b) is a carboxylic acid of general formula (III)



wherein n is zero or 1;

the residues R³ and R⁴ are both phenyl or together form a phenyl ring, wherein each phenyl ring has at least one further substituent selected from the group consisting of hydrogen atom, halogen atom, preferably chloro, and nitro group; and the residue R⁵ is hydrogen atom or methyl or absent if R³ and R⁴ together form a phenyl ring. The straight-forward screening approach especially resulted in two benzylbenzene-based acids and three benzoic acid derivatives that matched the above mentioned criteria (as the respective carboxylate ions at pH 7.5) (Scheme 2).



Scheme 2: Potential carboxylic acids and the abbreviations used for an in situ product crystallization of amines from a transaminase-catalyzed reaction.

[0019] In addition, 2,2-diphenylpropionic acid (2DPPA) was identified as suitable carboxylic acid. Thus, a preferred embodiment of the method relates to the at least one carboxylic acid according to (ii-b) being a carboxylic acid selected from the group consisting of diphenylacetic acid (DPAA), 2,2-diphenylpropionic acid (2DPPA), 3,3-diphenylpropionic acid (3DPPA), 3,4-dichloro-benzoic acid (34CA), 3,4-dinitro-benzoic acid (34NA) and 4-chloro-3-nitro-benzoic acid (43CNA). More preferably, the at least one carboxylic acid according to (ii-b) is selected from the group consisting of diphenylacetic acid (DPAA), 2,2-diphenylpropionic acid (2DPPA) and 3,3-diphenylpropionic acid (3DPPA), more preferably 2,2-diphenylpropionic acid (2DPPA) or 3,3-diphenylpropionic acid (3DPPA), more preferably at least 3DPPA.

Carbonyl compound

[0020] According to step (i), a carbonyl compound of general formula (I) is provided. Preferably,

R¹ is selected from the group consisting of branched or unbranched C2-C20-alkyl, branched or unbranched C2-C20-

alkenyl, branched or unbranched C2-C20-alkinyl, branched or unbranched C1-C5-alkyl-O-C1-C5-alkyl, branched or unbranched C1-C10-alkoxy, C4-C20-cycloalkyl, C5-C20-cycloalkenyl, C5-C20-cycloalkinyl, C5-C20-aryl, C6-C20-alkylaryl, C6-C20-arylalkyl, C2-C20-heteroalkyl, C3-C20-cyclic heteroalkyl, C4-C20-heteroaryl, C5-C20-alkylheteroaryl and C5-C20-heteroarylalkyl, wherein in case of more than one (hetero)aliphatic or (hetero)aromatic ring system the ring systems are condensed or separate, wherein each residue R¹ has at least one substituent R^{1a} selected from the group consisting of hydrogen atom, halogen atom (F, Cl, Br, I), hydroxyl, thiol, C1-C3 thioester, C1-C3-thioether, C1-C3-alkyl and C1-C3-alkoxy; and

R² is selected from the group consisting of hydrogen atom, branched or unbranched C1-C5-alkyl, branched or unbranched C1-C5-alkyl-O-C1-C5-alkyl, branched or unbranched C1-C10-alkoxy, C5-C10-aryl, C6-C10-alkylaryl and C6-C10-arylalkyl, wherein in case of more than one aromatic ring system the ring systems are condensed or separate, wherein each residue R² has at least one substituent R^{2a} selected from the group consisting of hydrogen atom, halogen atom (F, Cl, Br, I), hydroxyl, thiol, C1-C3 thioester, C1-C3-thioether, C1-C3-alkyl and C1-C3-alkoxy.

[0021] More preferably, the residue R¹ is selected from the group consisting of branched or unbranched C2-C20-alkyl, branched or unbranched C2-C20-alkenyl, branched or unbranched C2-C20-alkinyl, branched or unbranched C1-C5-alkyl-O-C1-C5-alkyl, branched or unbranched C1-C10-alkoxy, C4-C20-cycloalkyl, C5-C20-cycloalkenyl, C5-C20-cycloalkinyl, C5-C20-aryl, C6-C20-alkylaryl, C6-C20-arylalkyl, C2-C20-heteroalkyl, C3-C20-cyclic heteroalkyl, C4-C20-heteroaryl, C5-C20-alkylheteroaryl and C5-C20-heteroarylalkyl, wherein the hetero atom(s) in C4-C20-heteroaryl, C5-C20-alkylheteroaryl and C5-C20-heteroarylalkyl is/are oxygen or sulfur and the hetero atom(s) in C2-C20-heteroalkyl and C3-C20-cyclic heteroalkyl, is/are selected from oxygen, sulfur and nitrogen, wherein in case of more than one (hetero)aliphatic or (hetero)aromatic ring system the ring systems are condensed or separate, wherein each residue R¹ has at least one substituent R^{1a} selected from the group consisting of hydrogen atom, halogen atom (F, Cl, Br, I), hydroxyl, thiol, C1-C3 thioester, C1-C3-thioether, C1-C3-alkyl and C1-C3-alkoxy.

[0022] According to a preferred embodiment of the method, the residue R¹ is selected from the group consisting of branched or unbranched C2-C20-alkyl, branched or unbranched C2-C20-alkenyl, branched or unbranched C2-C20-alkinyl, branched or unbranched C1-C5-alkyl-O-C1-C5-alkyl, branched or unbranched C1-C10-alkoxy, C4-C20-cycloalkyl, C5-C20-cycloalkenyl, C5-C20-cycloalkinyl, C5-C20-aryl, C6-C20-alkylaryl, C6-C20-arylalkyl, C2-C20-heteroalkyl, C3-C20-cyclic heteroalkyl, wherein the hetero atom(s) is/are selected from oxygen, sulfur and nitrogen wherein in case of more than one aliphatic or aromatic ring system the ring systems are condensed or separate, wherein each residue R¹ has at least one substituent R^{1a} selected from the group consisting of hydrogen atom, halogen atom (F, Cl, Br, I), hydroxyl, thiol, C1-C3 thioester, C1-C3-thioether, C1-C3-alkyl and C1-C3-alkoxy. According to a further preferred embodiment of the method, the residue R¹ is selected from the group consisting of branched or unbranched C2-C20-alkyl, branched or unbranched C2-C20-alkenyl, branched or unbranched C2-C20-alkinyl, C4-C20-cycloalkyl, C5-C20-cycloalkenyl, C5-C20-cycloalkinyl and C5-C20-aryl, wherein in case of more than one aliphatic or aromatic ring system the ring systems are condensed or separate, wherein each residue R¹ has at least one substituent R^{1a} selected from the group consisting of hydrogen atom, halogen atom, C1-C3-alkyl and C1-C3-alkoxy. According to a more preferred embodiment of the method, the residue R¹ is selected from the group consisting of branched or unbranched C2-C10-alkyl, C5-C10-cycloalkyl and C5-C20-aryl, wherein in case of more than one aliphatic or aromatic ring system the ring systems are condensed or separate, wherein each residue R¹ has at least one substituent R^{1a} selected from the group consisting of hydrogen atom, halogen atom, C1-C3-alkyl and C1-C3-alkoxy; R¹ being preferably selected from the group consisting of methyl, iso-propyl, cyclohexyl and phenyl, wherein phenyl has at least one substituent R^{1a} selected from the group consisting of hydrogen atom, halogen atom, preferably fluoro or chloro, and methoxy, preferably meta- or para-methoxy.

[0023] For R¹ being branched or unbranched C2-C20-alkyl or branched or unbranched C2-C20-alkenyl, a preferred embodiment relates to R¹ being branched or unbranched C4-C20-alkyl or branched or unbranched C4-C20-alkenyl.

[0024] According to a preferred embodiment of the method, the residue R² is selected from the group consisting of hydrogen atom, branched or unbranched C1-C5-alkyl, wherein each residue R² has at least one substituent R^{2a} selected from the group consisting of hydrogen atom, halogen atom, C1-C3-alkyl and C1-C3-alkoxy. According to a further preferred embodiment of the method, the residue R² is selected from the group of branched or unbranched C1-C3-alkyl, R² being preferably methyl.

Transaminase

[0025] According to step (ii), the carbonyl compound provided according to (i) is reacted in the presence of a transaminase. Preferably, the transaminase according to (ii) is selected from the group of transaminases, preferably from the group of amine transaminases, more preferably selected from the group consisting of amine transaminase from *Aspergillus fumigatus* (AfATA) according to SEQ ID NO 1, amine transaminase from *Gibberella zeae* (GzATA) according to SEQ ID NO 2, amine transaminase from *Neosartorya fischeri* (NfATA) according to SEQ ID NO 3, amine transaminase

from *Aspergillus oryzae* (AoATA) according to SEQ ID NO 4, amine transaminase from *Aspergillus terreus* (AtATA) according to SEQ ID NO 5, amine transaminase from *Mycobacterium vanbaalenii* (MvATA) according to SEQ ID NO 6, amine transaminase from *Silicibacter pomeroyi* (SpATA) according to SEQ ID NO 7 and a homologue enzyme having sequence identity of at least 65% with any one of SEQ ID NO 1 to 7 and having the same function as the amine transaminase of SEQ ID NO 1 to 7, more preferably selected from the group consisting of the amine transaminase from *Mycobacterium vanbaalenii* (MvATA) according to SEQ ID NO 6, the amine transaminase from *Silicibacter pomeroyi* (SpATA) according to SEQ ID NO 7 and a homologue enzyme having sequence identity of at least 65% with any one of SEQ ID NO 6 or 7 and having the same function as the amine transaminase of SEQ ID NO 6 or 7. Table 1 shows an overview of the preferred amine transaminases:

Table 1: Amine transaminases of SEQ ID Nos 1 to 7

Name	Abbreviation	SEQ ID NO.	NCBI* accession no.	GenBank* accession no.
amine transaminase from <i>Aspergillus fumigates</i>	AfATA	1	XP_748821.1	
amine transaminase from <i>Gibberella zeae</i>	GzATA	2	XP_011317603.1	
amine transaminase from <i>Neosartorya fischeri</i>	NfATA	3	XP_001261640.1	
amine transaminase from <i>Aspergillus oryzae</i>	AoATA	4		BAE56564.1
amine transaminase from <i>Aspergillus terreus</i>	AtATA	5	XP_001209325.1	
amine transaminase from <i>Mycobacterium vanbaalenii</i>	MvATA	6	WP_083062280.1	
amine transaminase from <i>Silicibacter pomeroyi</i>	SpATA	7	WP_011049154.1	
* Databank of the National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/), status: October 24, 2017				

[0026] The expression "having the same function as the amine transaminase of any one of SEQ ID NO. 1 to 7" means that the homologue enzyme catalyzes an amine transaminase reaction at least with an effectivity of 90% as the transaminase of any one of SEQ ID NO. 1 to 7. Preferably, the homologue enzyme has a sequence identity of at least 75%, preferably at least 85%, more preferably at least 90%, more preferably at least 95%, more preferably at least 98%, more preferably at least 99%, with any one of SEQ ID NO 1 to 7 and has the same function as the amine transaminase of SEQ ID NO 1 to 7.

Primary amine

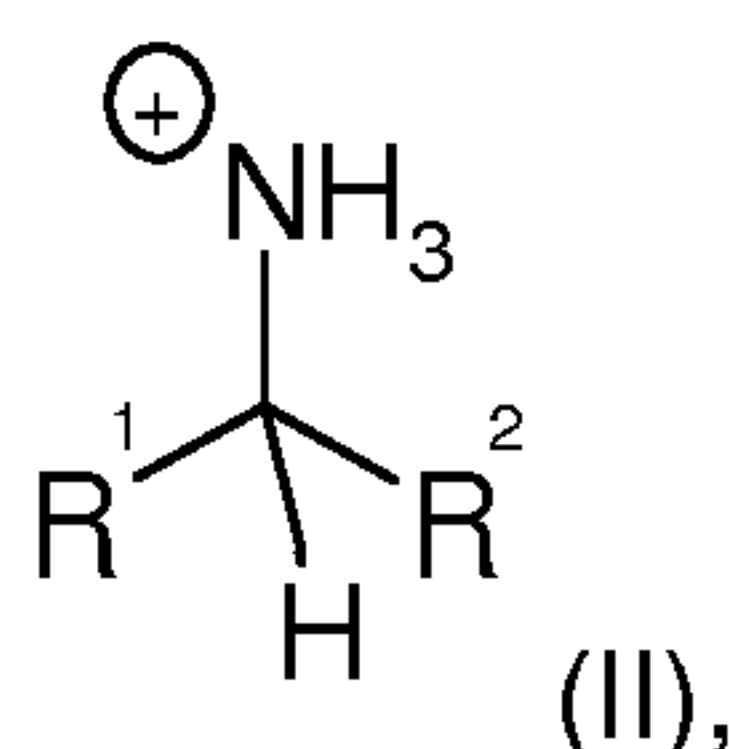
[0027] According to step (ii), the carbonyl compound provided according to (i) is reacted in the presence of a transaminase with ii-a) at least one primary amine; and ii-b) at least one carboxylic acid. Preferably, the at least one primary amine according to (ii-a) is used in non-protonated form or in protonated form with a suitable counter anion.

[0028] According to a preferred embodiment of the method, the at least one primary amine according to (ii-a) is selected from the group of mono- and diamines having one to 10 carbon atoms, preferably from the group consisting of 1,5-diamino-pentane (cadaverine), alanine, 2-aminobutane (sec-butylamine) and 2-amino-propane, and is preferably 2-amino-propane (isopropylamine) in its non-protonated or protonated form, wherein the protonated form is present in combination with a suitable anion.

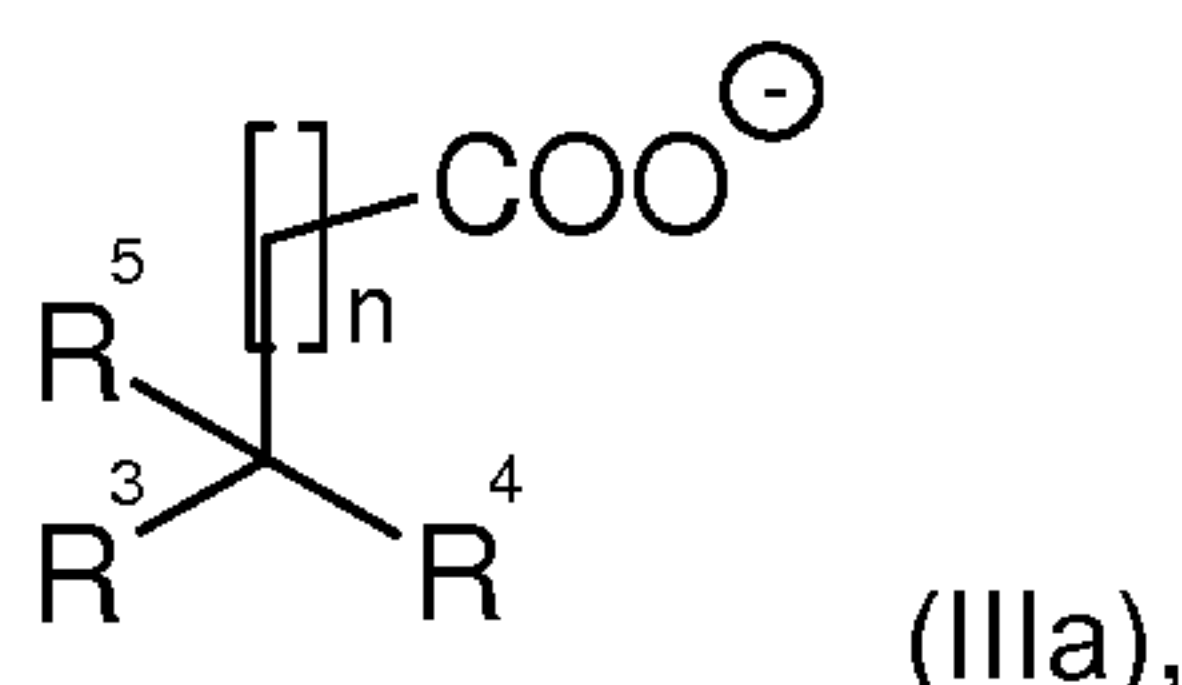
[0029] According to a more preferred embodiment of the method, the at least one primary amine according to (ii-a) and the at least one carboxylic acid according to (ii-b) are used as one or more salt(s) comprising the protonated form of the at least one primary amine and the deprotonated form of the at least one carboxylic acid, preferably as one salt comprising the protonated form of the at least one primary amine and the deprotonated form of the at least one carboxylic acid, more preferably as isopropyl ammonium 3,3-diphenylpropionate.

Amino salt compound

[0030] According to step (ii), an amino salt compound is obtained. The amino salt compound obtained according to (ii) comprises a cation of general formula (II)



wherein R^1 and R^2 are as defined for general formula (I), and an anion based on the at least one carboxylic acid, which is an anion of general formula (IIIa)



wherein n is zero or 1;

the residues R^3 and R^4 are both phenyl or together form a phenyl ring, wherein each phenyl ring has at least one further substituent selected from the group consisting of hydrogen atom, halogen atom, preferably chloro, and nitro group and the residue R^5 is hydrogen atom or methyl or absent if R^3 and R^4 together form a phenyl ring; wherein the anion of general formula (IIIa) is preferably selected from the group consisting of diphenylacetate, 2,2-diphenylpropionate, 3,3-diphenylpropionate, 3,4-dichloro-benzoate, 3,4-dinitro-benzoate and 4-chloro-3-nitro-benzoate, more preferably from the group consisting of diphenylacetate, 2,2-diphenylpropionate, 3,3-diphenylpropionate, more preferably 2,2-diphenylpropionate or 3,3-diphenylpropionate, more preferably 3,3-diphenylpropionate.

Further reaction conditions

[0031] According to a preferred embodiment of the method, the reaction according to (ii) is carried out in an aqueous solution, which preferably comprises at least 80 weight-%, more preferably at least 90 weight-%, more preferably at least 95 weight-%, more preferably at least 98 weight-%, water, based on the overall weight of the aqueous solution. The aqueous solution preferably comprises a buffer, preferably selected from the group of tris(hydroxymethyl)aminomethane buffer (TRIS buffer), 3-(N-morpholino)propanesulfonic acid buffer (MOPS buffer), N,N-Bis(2-hydroxyethyl)-2-aminoethanesulfonic acid buffer (BES buffer), N-(tris(hydroxymethyl)methyl)-glycine buffer (Tricine buffer), Carbonate buffer, N-cyclohexyl-2-aminoethanesulfonic acid buffer (CHES buffer), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer (HEPES buffer) and phosphate buffer, more preferably at least a phosphate buffer.

[0032] According to further a preferred embodiment of the method, the reaction according to (ii) is carried out at a pH value in the range of 6.0 to 9.5, more preferably in the range of from 6.5 to 9.0, more preferably in the range of from 7.0 to 8.0. According to a further preferred embodiment of the method, the reaction according to (ii) is carried out for a time period of at least one hour, more preferably for a time in the range of from 1 to 1,000 hours, more preferably in the range of from 5 to 500 hours, more preferably in the range of from 10 to 200 hours. According to a further preferred embodiment of the method, the reaction according to (ii) is carried out at a temperature in the range of from 10 to 50 °C, more preferably in the range of from 15 to 45 °C, more preferably in the range of from 20 to 40 °C, more preferably in the range of from 25 to 35 °C.

[0033] According to another preferred embodiment of the method, for the at least one carboxylic acid being a carboxylic acid according to general formula (III), wherein the residues R^3 and R^4 together form a phenyl ring, preferably a carboxylic acid selected from the group consisting of 3,4-dichloro-benzoic acid (34CA), 3,4-dinitro-benzoic acid (34NA) and 4-chloro-3-nitro-benzoic acid (43CNA), the concentration of the at least one carboxylic acid is kept in the range of from 0.001 to 50 mM, preferably in the range of from 1 to 45 mM, more preferably in the range of from 5 to 40 mM during step (ii). According to another preferred embodiment of the method, for the at least one carboxylic acid being a carboxylic acid according to general formula (III), wherein the residues R^3 and R^4 are each a phenyl ring, preferably a carboxylic acid selected from the group consisting of diphenylacetic acid (DPAA), 2,2-diphenylpropionic acid (2DPPA) and 3,3-diphenylpropionic acid (3DPPA), the concentration of the at least one carboxylic acid is ≤ 50 mM during step (ii).

Further reaction steps

[0034] As described above, the method comprises reaction steps (i) and (ii). According to a preferred embodiment, the method further comprises:

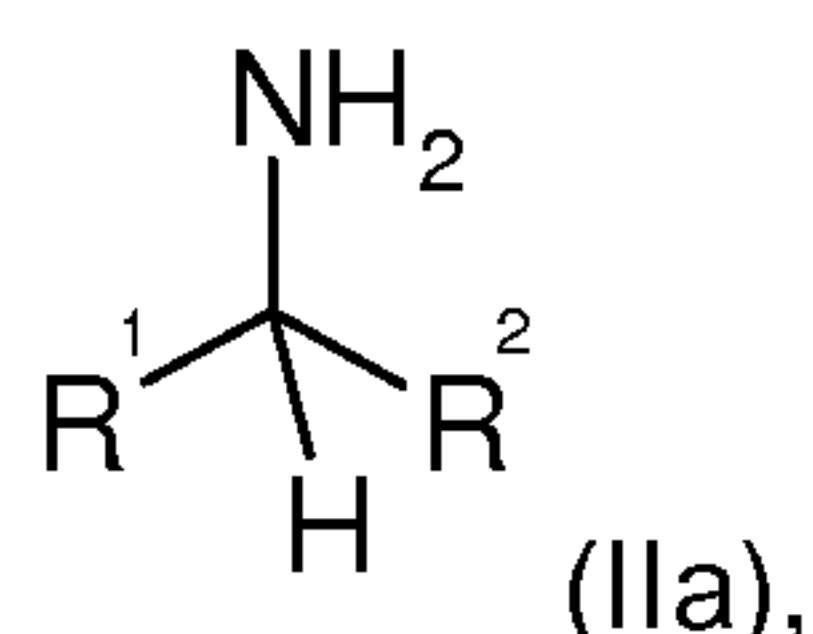
iii) separating the at least partially crystallized amine salt compound obtained according to (ii) from the mixture thereby obtaining the crystallized amine salt compound.

[0035] Preferably, separating the crystallized amine salt compound according to (iii) from the mixture is done by sedimentation, centrifugation or filtration, preferably by filtration.

[0036] According to a further preferred embodiment, the method further comprises, preferably in addition to (i), (ii) and (iii):

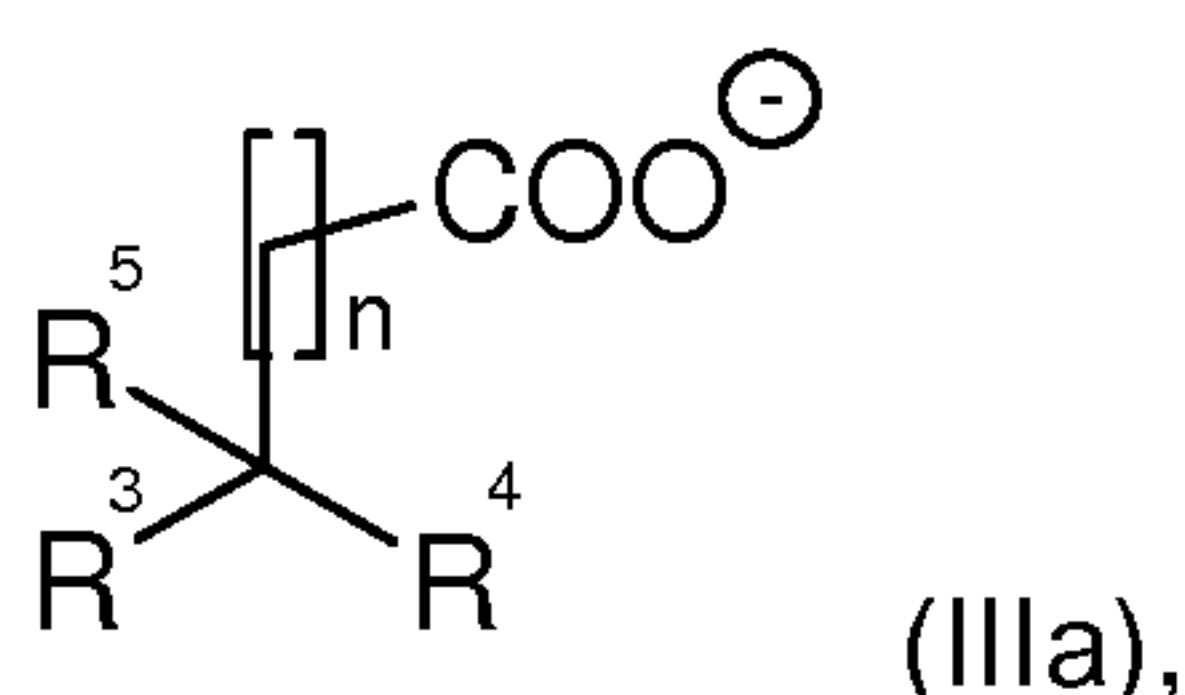
(iv) optionally washing the separated crystallized amino salt compound obtained according to (iii), preferably with water or an organic solvent or a mixture thereof, more preferably with water or methyl tert-butyl ether or a mixture thereof, thereby obtaining a washed crystallized amino salt compound;

(v) dissolving the crystallized amino salt compound obtained according to (iii) or optionally the washed crystallized amino salt compound obtained according to (iv) in an aqueous solution having a pH value in the range of from 10 to 14 comprising at least one base, preferably a base comprising a hydroxide ion, thereby obtaining an aqueous solution comprising an amine of general formula (IIa)



wherein R^1 and R^2 are as defined for general formula (I);

(vi) extracting the aqueous solution obtained according to (v) at least once with a water immiscible organic solvent obtaining an organic phase comprising at least parts of the amine of general formula (IIa); and an aqueous phase comprising at least parts of the anion of general formula (IIIa)

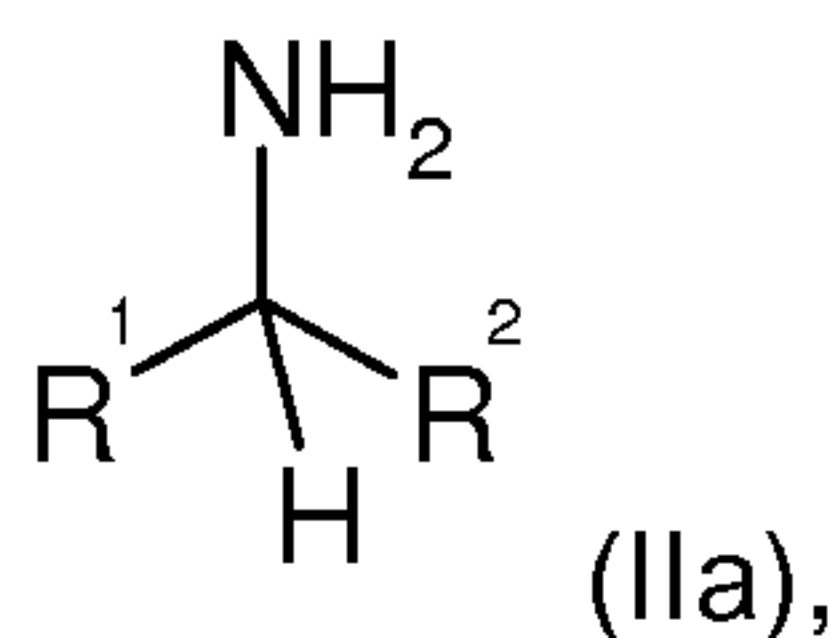


wherein n is zero or 1; and wherein the residues R^3 and R^4 are both phenyl or together form a phenyl ring, wherein each phenyl ring has at least one further substituent selected from the group consisting of hydrogen atom, halogen atom, preferably chloro, and nitro group and the residue R^5 is hydrogen atom or methyl or absent if R^3 and R^4 together form a phenyl ring; wherein the anion of general formula (IIIa) is preferably selected from the group consisting of diphenylacetate, 2,2-diphenylpropionate, 3,3-diphenylpropionate, 3,4-dichloro-benzoate, 3,4-dinitro-benzoate and 4-chloro-3-nitrobenzoate, more preferably from the group consisting of diphenylacetate, 2,2-diphenylpropionate, 3,3-diphenylpropionate, more preferably 2,2-diphenylpropionate or 3,3-diphenylpropionate, more preferably 3,3-diphenylpropionate.

[0037] The water immiscible organic solvent according to (vi) has preferably a K_{ow} value of at least 0.5, more preferably of at least 0.6, more preferably of at least 0.7, more preferably of at least 0.8. According to a preferred embodiment, the water immiscible organic solvent according to (vi) is selected from the group of ethers, more preferably from the group of aliphatic ethers, more preferably MTBE (methyl-tert-butylether).

[0038] According to a further preferred embodiment, the method further comprises, preferably in addition to (i), (ii), (iii), (iv), (v) and (vi):

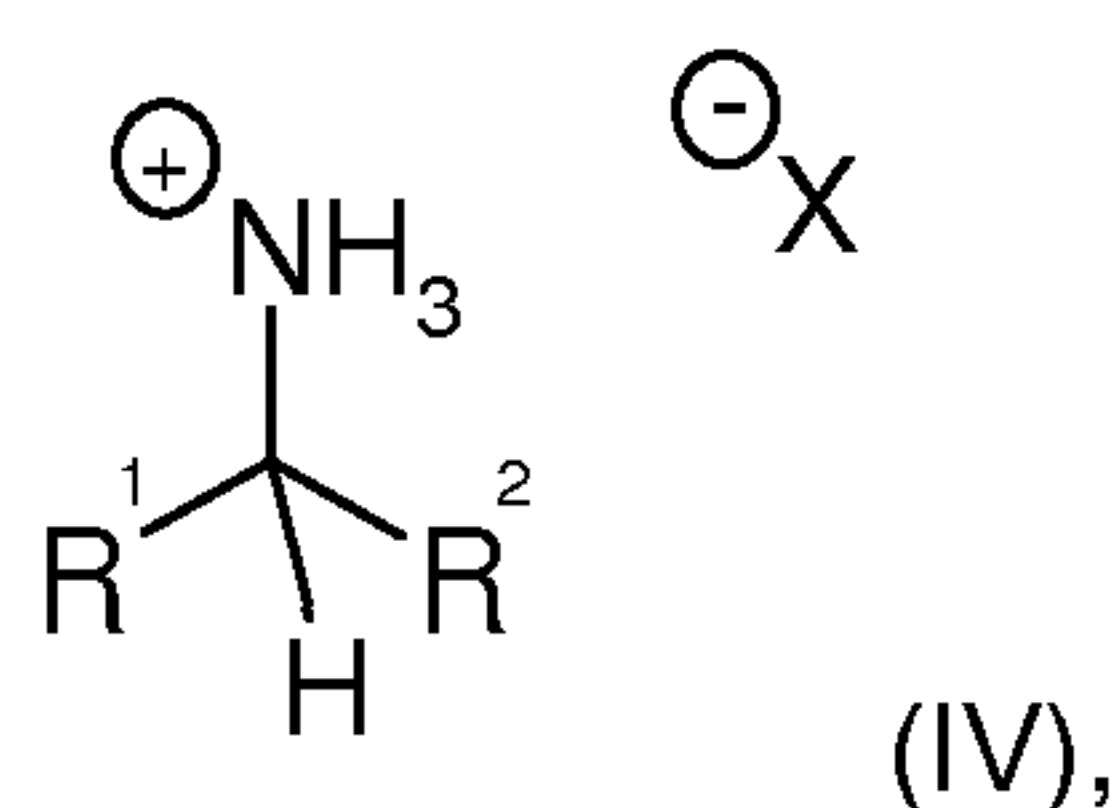
(vii-a) removal of the water immiscible organic solvent from the organic phase obtained in (vi) thereby obtaining the amine of general formula (IIa),



wherein R^1 and R^2 are as defined for general formula (I).

[0039] According to an alternative preferred embodiment, the method further comprises, preferably in addition to (i), (ii), (iii), (iv), (v) and (vi):

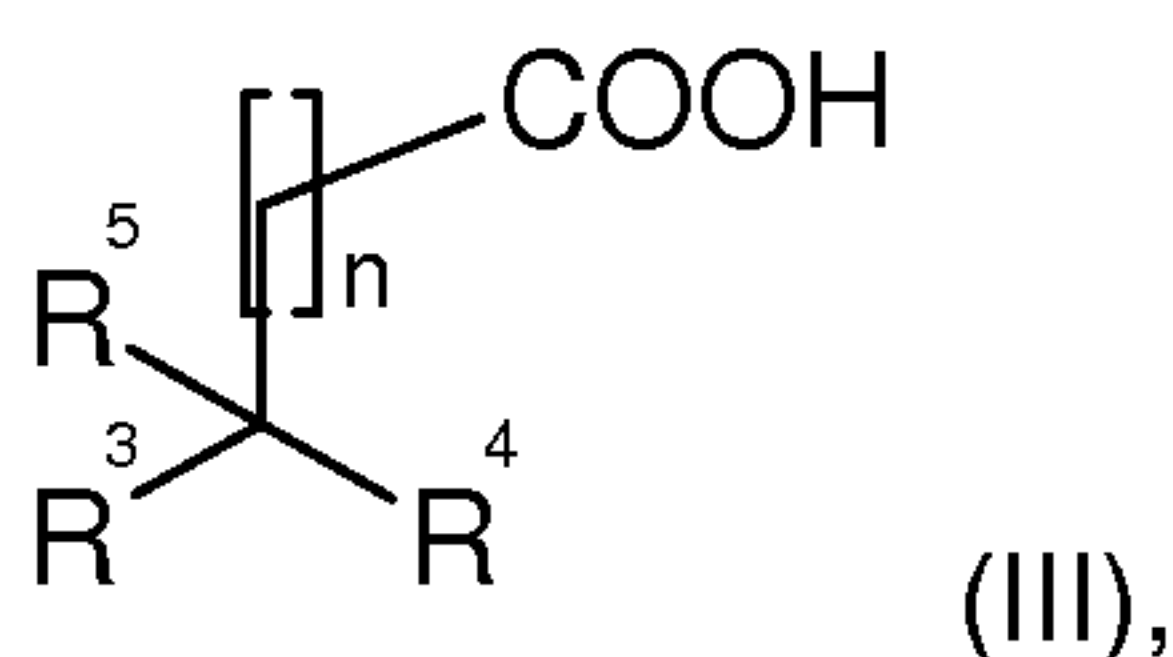
(vii-b) adding at least one acid HX to the organic phase obtained according to (vi), preferably HCl , thereby obtaining a salt of general formula (IV)



wherein R^1 and R^2 are as defined for general formula (I) and (II) and X is an ion based on the at least one acid HX , preferably Cl .

[0040] According to an alternative preferred embodiment, the method further comprises, preferably in addition to (i), (ii), (iii), (iv), (v), (vi) and (vii-a) or in addition to (i), (ii), (iii), (iv), (v), (vi) and (vii-b):

(viii) optionally precipitating the at least one carboxylic acid of general formula (III)



wherein

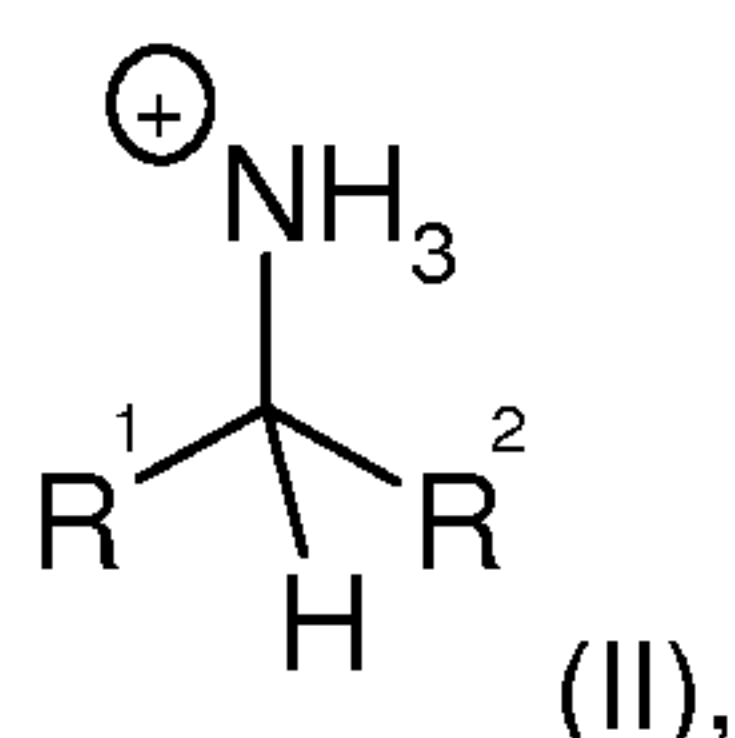
n is zero or 1;

the residues R^3 and R^4 are both phenyl or together form a phenyl ring, wherein each phenyl ring has at least one further substituent selected from the group consisting of hydrogen atom, halogen atom, preferably chloro, and nitro group and the residue R^5 is hydrogen atom or methyl or absent if R^3 and R^4 together form a phenyl ring; preferably the at least one carboxylic acid being selected from the group consisting of diphenylacetic acid (DPAA), 2,2-diphenylpropionic acid (2DPPA), 3,3-diphenylpropionic acid (3DPPA), 3,4-dichloro-benzoic acid (34CA), 3,4-dinitro-benzoic acid (34NA) and 4-chloro-3-nitro-benzoic acid (43CNA), more preferably form the group consisting of diphenylacetic acid (DPAA), 2,2-diphenylpropionic acid (2DPPA) and 3,3-diphenylpropionic acid (3DPPA), more preferably 2,2-diphenylpropionic acid (2DPPA) or 3,3-diphenylpropionic acid (3DPPA), more preferably at least 3DPPA, from the aqueous phase obtained according to (vi) by adjusting the pH to a value in the range of from 0 to 6, preferably by addition of HCl , and

(ix) optionally recycling the at least one carboxylic acid precipitated according to (viii) to the process, preferably to step (ii).

[0041] In another aspect, the present invention relates to an amino salt compound comprising

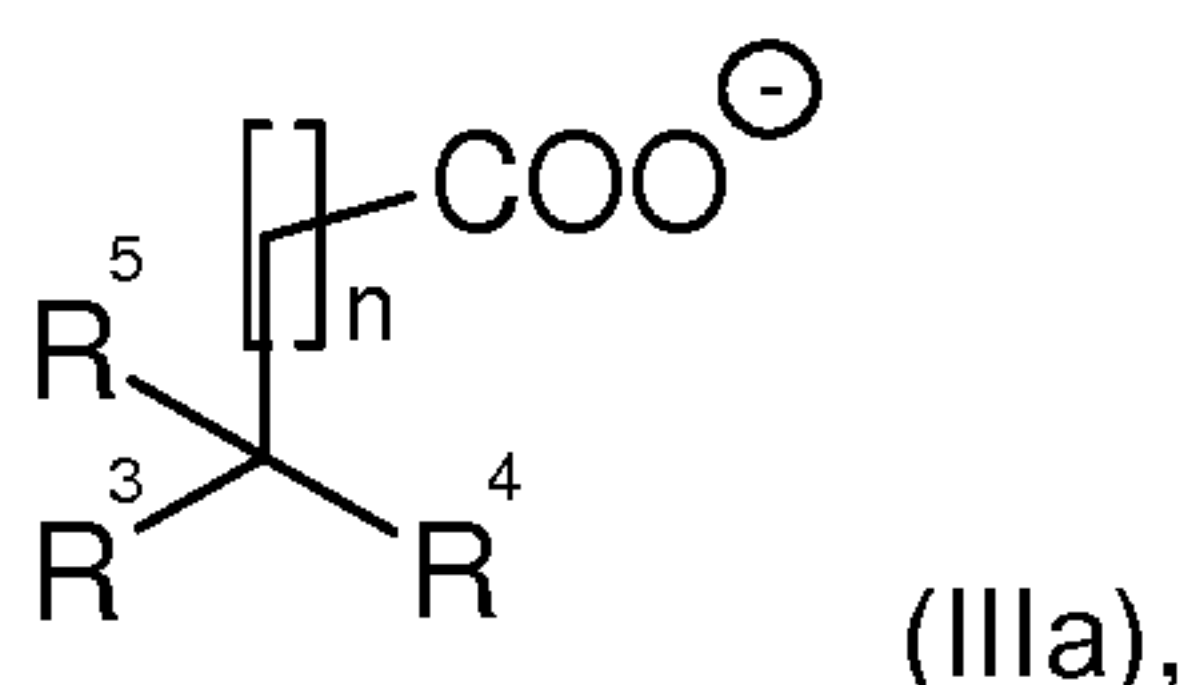
- a cation of general formula (II)



wherein

- R^1 is selected from the group consisting of branched or unbranched C2-C20-alkyl, branched or unbranched C2-C20-alkenyl, branched or unbranched C2-C20-alkinyl, branched or unbranched C1-C5-alkyl-O-C1-C5-alkyl, branched or unbranched C1-C10-alkoxy, C4-C20-cycloalkyl, C5-C20-cycloalkenyl, C5-C20-cycloalkinyl, C5-C20-aryl, C6-C20-alkylaryl, C6-C20-arylalkyl, C2-C20-heteroalkyl, C3-C20-cyclic heteroalkyl, C4-C20-heteroaryl, C5-C20-alkylheteroaryl and C5-C20-heteroarylalkyl, wherein in case of more than one (hetero)aliphatic or (hetero)aromatic ring system the ring systems are condensed or separate, wherein each residue R^1 has at least one substituent R^{1a} selected from the group consisting of hydrogen atom, halogen atom (F, Cl, Br, I), hydroxyl, thiol, C1-C3 thioester, C1-C3-thioether, C1-C3-alkyl and C1-C3-alkoxy; and
- R^2 is selected from the group consisting of hydrogen atom, branched or unbranched C1-C5-alkyl, branched or unbranched C1-C5-alkyl-O-C1-C5-alkyl, branched or unbranched C1-C10-alkoxy, C5-C10-aryl, C6-C10-alkylaryl and C6-C10-arylalkyl, wherein in case of more than one aliphatic or aromatic ring system the ring systems are condensed or separate, wherein each residue R^2 has at least one substituent R^{2a} selected from the group consisting of hydrogen atom, halogen atom (F, Cl, Br, I), hydroxyl, thiol, C1-C3 thioester, C1-C3-thioether, C1-C3-alkyl and C1-C3-alkoxy; or
- R^1, R^2 together form a C3-C10-cycloalkyl or C3-C10-cycloalkenyl, wherein the C3-C10-cycloalkyl or C3-C10-cycloalkenyl each has at least one substituent R^x selected from the group consisting of hydrogen atom, C1-C5-alkyl, C1-C4-heteroalkyl and C1-C5-alkyl- R^y , wherein R^y is hydroxyl or thiol; and

- an anion of general formula (IIIa)



wherein

- n is zero or 1;
the residues R^3 and R^4 are both phenyl or together form a phenyl ring, wherein each phenyl ring has at least one further substituent selected from the group consisting of hydrogen atom, halogen atom, preferably chloro, and nitro group and the residue R^5 is hydrogen atom or methyl or absent if R^3 and R^4 together form a phenyl ring.

[0042] Preferably, the anion of general formula (IIIa) of the amine salt compound is selected from the group consisting of diphenylacetate, 2,2-diphenylpropionate, 3,3-diphenylpropionate, 3,4-dichloro-benzoate, 3,4-dinitro-benzoate and 4-chloro-3-nitro-benzoate, more preferably from the group consisting of diphenylacetate, 2,2-diphenylpropionate, 3,3-diphenylpropionate, more preferably 2,2-diphenylpropionate or 3,3-diphenylpropionate, more preferably 3,3-diphenylpropionate.

[0043] According to a preferred embodiment of the amino salt compound,

- R^1 is selected from the group consisting of branched or unbranched C2-C20-alkyl, branched or unbranched C2-C20-alkenyl, branched or unbranched C2-C20-alkinyl, branched or unbranched C1-C5-alkyl-O-C1-C5-alkyl, branched or unbranched C1-C10-alkoxy, C4-C20-cycloalkyl, C5-C20-cycloalkenyl, C5-C20-cycloalkinyl, C5-C20-aryl, C6-C20-alkylaryl, C6-C20-arylalkyl, C2-C20-heteroalkyl, C3-C20-cyclic heteroalkyl, C4-C20-heteroaryl, C5-C20-alkylheteroaryl and C5-C20-heteroarylalkyl, wherein in case of more than one (hetero)aliphatic or (hetero)aromatic ring system the ring systems are condensed or separate, wherein each residue R^1 has at least one substituent R^{1a} selected from the group consisting of hydrogen atom, halogen atom (F, Cl, Br, I), hydroxyl, thiol, C1-C3

thioester, C1-C3-thioether, C1-C3-alkyl and C1-C3-alkoxy; and

R^2 is selected from the group consisting of hydrogen atom, branched or unbranched C1-C5-alkyl, branched or unbranched C1-C5-alkyl-O-C1-C5-alkyl, branched or unbranched C1-C10-alkoxy, C5-C10-aryl, C6-C10-alkylaryl and C6-C10-arylalkyl, wherein in case of more than one aliphatic or aromatic ring system the ring systems are condensed or separate, wherein each residue R^2 has at least one substituent R^{2a} selected from the group consisting of hydrogen atom, halogen atom (F, Cl, Br, I), hydroxyl, thiol, C1-C3 thioester, C1-C3-thioether, C1-C3-alkyl and C1-C3-alkoxy.

[0044] More preferably, the residue R^1 is selected from the group consisting of branched or unbranched C2-C20-alkyl, branched or unbranched C2-C20-alkenyl, branched or unbranched C2-C20-alkinyl, branched or unbranched C1-C5-alkyl-O-C1-C5-alkyl, branched or unbranched C1-C10-alkoxy, C4-C20-cycloalkyl, C5-C20-cycloalkenyl, C5-C20-cycloalkinyl, C5-C20-aryl, C6-C20-alkylaryl, C6-C20-arylalkyl, C2-C20-heteroalkyl, C3-C20-cyclic heteroalkyl, C4-C20-heteroaryl, C5-C20-alkylheteroaryl and C5-C20-heteroarylalkyl, wherein the hetero atom(s) in C4-C20-heteroaryl, C5-C20-alkylheteroaryl and C5-C20-heteroarylalkyl is/are oxygen or sulfur and the hetero atom(s) in C2-C20-heteroalkyl and C3-C20-cyclic heteroalkyl, is/are selected from oxygen, sulfur and nitrogen, wherein in case of more than one (hetero)aliphatic or (hetero)aromatic ring system the ring systems are condensed or separate, wherein each residue R^1 has at least one substituent R^{1a} selected from the group consisting of hydrogen atom, halogen atom (F, Cl, Br, I), hydroxyl, thiol, C1-C3 thioester, C1-C3-thioether, C1-C3-alkyl and C1-C3-alkoxy. According to a further preferred embodiment of the amino salt compound, the residue R^1 is selected from the group consisting of branched or unbranched C2-C20-alkyl, branched or unbranched C2-C20-alkenyl, branched or unbranched C2-C20-alkinyl, branched or unbranched C1-C5-alkyl-O-C1-C5-alkyl, branched or unbranched C1-C10-alkoxy, C4-C20-cycloalkyl, C5-C20-cycloalkenyl, C5-C20-cycloalkinyl, C5-C20-aryl, C6-C20-alkylaryl, C6-C20-arylalkyl, C2-C20-heteroalkyl, C3-C20-cyclic heteroalkyl, wherein in case of more than one aliphatic or aromatic ring system the ring systems are condensed or separate, wherein each residue R^1 has at least one substituent R^{1a} selected from the group consisting of hydrogen atom, halogen atom (F, Cl, Br, I), hydroxyl, thiol, C1-C3 thioester, C1-C3-thioether, C1-C3-alkyl and C1-C3-alkoxy. According to a further preferred embodiment of the amino salt compound, the residue R^1 is selected from the group consisting of branched or unbranched C2-C20-alkyl, branched or unbranched C2-C20-alkenyl, branched or unbranched C2-C20-alkinyl, C4-C20-cycloalkyl, C5-C20-cycloalkenyl, C5-C20-cycloalkinyl, C5-C20-aryl, wherein in case of more than one aliphatic or aromatic ring system the ring systems are condensed or separate, wherein each residue R^1 has at least one substituent R^{1a} selected from the group consisting of hydrogen atom, halogen atom, C1-C3-alkyl and C1-C3-alkoxy. According to a further preferred embodiment of the amino salt compound, the residue R^1 is selected from the group consisting of branched or unbranched C2-C10-alkyl, C5-C10-cycloalkyl and C5-C20-aryl, wherein in case of more than one aliphatic or aromatic ring system the ring systems are condensed or separate, wherein each residue R^1 has at least one substituent R^{1a} selected from the group consisting of hydrogen atom, halogen atom, C1-C3-alkyl and C1-C3-alkoxy; R^1 being preferably selected from the group consisting of methyl, iso-propyl, cyclohexyl and phenyl, wherein phenyl has at least one substituent selected from the group consisting of hydrogen atom, halogen atom, preferably fluoro or chloro, and methoxy, preferably meta- or para-methoxy.

[0045] For R^1 being branched or unbranched C2-C20-alkyl or branched or unbranched C2-C20-alkenyl, a preferred embodiment relates to R^1 being branched or unbranched C4-C20-alkyl or branched or unbranched C4-C20-alkenyl.

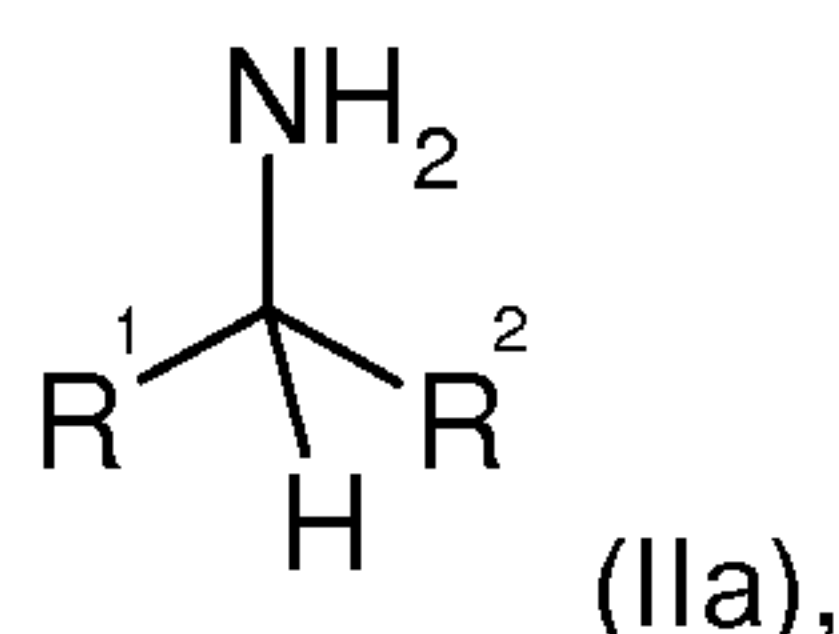
[0046] According to a preferred embodiment of the amino salt compound, the residue R^2 is selected from the group consisting of hydrogen atom, branched or unbranched C1-C5-alkyl, wherein each residue R^2 has at least one substituent R^{2a} selected from the group consisting of hydrogen atom, halogen atom, C1-C3-alkyl and C1-C3-alkoxy. Preferably, the residue R^2 is selected from the group consisting of branched or unbranched C1-C3-alkyl, R^2 being preferably methyl.

[0047] In a preferred embodiment, the combination of R^1 , R^{1a} being a perfluorinated alkyl is excluded. The same applies for R^2 and R^{2a} , i.e. the combination of R^2 and R^{2a} being a perfluorinated alkyl is excluded as well. In other words, neither the combination of R^1 and R^{1a} nor the combination of R^2 and R^{2a} is a perfluorinated alkyl.

[0048] Regarding the embodiment wherein R^1 and R^2 together form a C3-C10-cycloalkyl or a C3-C10-cycloalkenyl, the number of C atoms indicated includes the carbonyl C atom situated between R^1 and R^2 .

[0049] The present invention in another aspect relates to a composition comprising

a) an amine of general formula (IIa)



wherein

R^1 is selected from the group consisting of branched or unbranched C2-C20-alkyl, branched or unbranched C2-C20-alkenyl, branched or unbranched C2-C20-alkinyl, branched or unbranched C1-C5-alkyl-O-C1-C5-alkyl, branched or unbranched C1-C10-alkoxy, C4-C20-cycloalkyl, C5-C20-cycloalkenyl, C5-C20-cycloalkinyl, C5-C20-aryl, C6-C20-alkylaryl, C6-C20-arylalkyl, C2-C20-heteroalkyl, C3-C20-cyclic heteroalkyl, C4-C20-heteroaryl, C5-C20-alkylheteroaryl and C5-C20-heteroarylalkyl, wherein in case of more than one (hetero)aliphatic or (hetero)aromatic ring system the ring systems are condensed or separate, wherein each residue R^1 has at least one substituent R^{1a} selected from the group consisting of hydrogen atom, halogen atom (F, Cl, Br, I), hydroxyl, thiol, C1-C3 thioester, C1-C3-thioether, C1-C3-alkyl and C1-C3-alkoxy; and

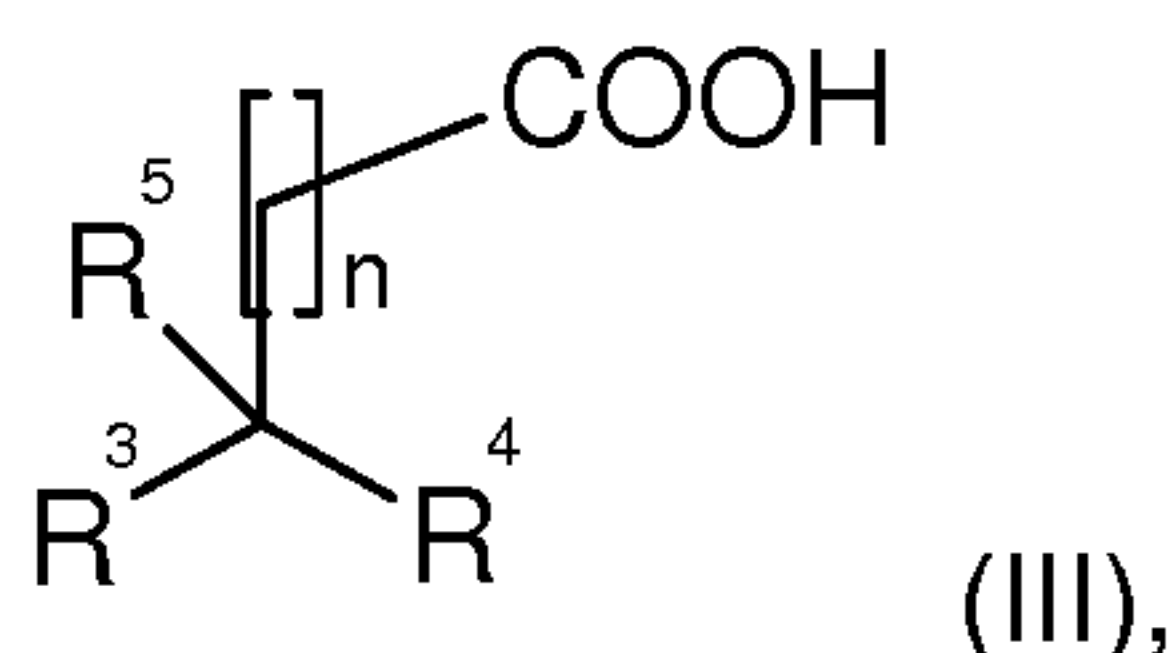
R^2 is selected from the group consisting of hydrogen atom, branched or unbranched C1-C5-alkyl, branched or unbranched C1-C5-alkyl-O-C1-C5-alkyl, branched or unbranched C1-C10-alkoxy, C5-C10-aryl, C6-C10-alkylaryl and C6-C10-arylalkyl, wherein in case of more than one aromatic ring system the ring systems are condensed or separate, wherein each residue R^2 has at least one substituent R^{2a} selected from the group consisting of hydrogen atom, halogen atom (F, Cl, Br, I), hydroxyl, thiol, C1-C3 thioester, C1-C3-thioether, C1-C3-alkyl and C1-C3-alkoxy;

or

R^1, R^2 together form a C3-C10-cycloalkyl or C3-C10-cycloalkenyl, wherein the C3-C10-cycloalkyl or C3-C10-cycloalkenyl each has at least one substituent R^x selected from the group consisting of hydrogen atom, C1-C5-alkyl, C1-C4-heteroalkyl and C1-C5-alkyl- R^y , wherein R^y is hydroxyl or thiol;

and

b) at least one carboxylic acid of general formula (III)



wherein

n is zero or 1;

the residues R^3 and R^4 are both phenyl or together form a phenyl ring, wherein each phenyl ring has at least one further substituent selected from the group consisting of hydrogen atom, halogen atom, preferably chloro, and nitro group; and the residue R^5 is hydrogen atom or methyl or absent if R^3 and R^4 together form a phenyl ring, wherein the at least one carboxylic acid is present in its protonated form or as carboxylate with a suitable counter ion.

[0050] According to a preferred embodiment of the composition,

R^1 is selected from the group consisting of branched or unbranched C2-C20-alkyl, branched or unbranched C2-C20-alkenyl, branched or unbranched C2-C20-alkinyl, branched or unbranched C1-C5-alkyl-O-C1-C5-alkyl, branched or unbranched C1-C10-alkoxy, C4-C20-cycloalkyl, C5-C20-cycloalkenyl, C5-C20-cycloalkinyl, C5-C20-aryl, C6-C20-alkylaryl, C6-C20-arylalkyl, C2-C20-heteroalkyl, C3-C20-cyclic heteroalkyl, C4-C20-heteroaryl, C5-C20-alkylheteroaryl and C5-C20-heteroarylalkyl, wherein in case of more than one (hetero)aliphatic or (hetero)aromatic ring system the ring systems are condensed or separate, wherein each residue R^1 has at least one substituent R^{1a} selected from the group consisting of hydrogen atom, halogen atom (F, Cl, Br, I), hydroxyl, thiol, C1-C3 thioester, C1-C3-thioether, C1-C3-alkyl and C1-C3-alkoxy; and

R^2 is selected from the group consisting of hydrogen atom, branched or unbranched C1-C5-alkyl, branched or unbranched C1-C5-alkyl-O-C1-C5-alkyl, branched or unbranched C1-C10-alkoxy, C5-C10-aryl, C6-C10-alkylaryl and C6-C10-arylalkyl, wherein in case of more than one aromatic ring system the ring systems are condensed or separate, wherein each residue R^2 has at least one substituent R^{2a} selected from the group consisting of hydrogen atom, halogen atom (F, Cl, Br, I), hydroxyl, thiol, C1-C3 thioester, C1-C3-thioether, C1-C3-alkyl and C1-C3-alkoxy.

[0051] More preferably, the at least one carboxylic acid according to (b) is a carboxylic acid selected from the group consisting of diphenylacetic acid (DPAA), 2,2-diphenylpropionic acid (2DPPA), 3,3-diphenylpropionic acid (3DPPA), 3,4-dichloro-benzoic acid (34CA), 3,4-dinitro-benzoic acid (34NA) and 4-chloro-3-nitro-benzoic acid (43CNA), more preferably from the group consisting of diphenylacetic acid (DPAA), 2,2-diphenylpropionic acid (2DPPA) and 3,3-diphenylpropionic acid (3DPPA), more preferably 2,2-diphenylpropionic acid (2DPPA) or 3,3-diphenylpropionic acid (3DPPA), more preferably at least 3DPPA.

[0052] According to a further preferred embodiment of the composition, the residue R^1 of the amine according to (a) is selected from the group consisting of branched or unbranched C2-C20-alkyl, branched or unbranched C2-C20-alkenyl, branched or unbranched C2-C20-alkinyl, branched or unbranched C1-C5-alkyl-O-C1-C5-alkyl, branched or unbranched C1-C10-alkoxy, C4-C20-cycloalkyl, C5-C20-cycloalkenyl, C5-C20-cycloalkinyl, C5-C20-aryl, C6-C20-alkylaryl, C6-C20-arylalkyl, C2-C20-heteroalkyl, C3-C20-cyclic heteroalkyl, C4-C20-heteroaryl, C5-C20-alkylheteroaryl and C5-C20-heteroarylalkyl, wherein the hetero atom(s) in C4-C20-heteroaryl, C5-C20-alkylheteroaryl and C5-C20-heteroarylalkyl is/are oxygen or sulfur and the hetero atom(s) in C2-C20-heteroalkyl and C3-C20-cyclic heteroalkyl, is/are selected from oxygen, sulfur and nitrogen, wherein in case of more than one (hetero)aliphatic or (hetero)aromatic ring system the ring systems are condensed or separate, wherein each residue R^1 has at least one substituent R^{1a} selected from the group consisting of hydrogen atom, halogen atom (F, Cl, Br, I), hydroxyl, thiol, C1-C3 thioester, C1-C3-thioether, C1-C3-alkyl and C1-C3-alkoxy. Preferably, the residue R^1 of the amine according to (a) is selected from the group consisting of branched or unbranched C2-C20-alkyl, branched or unbranched C2-C20-alkenyl, branched or unbranched C2-C20-alkinyl, branched or unbranched C1-C5-alkyl-O-C1-C5-alkyl, branched or unbranched C1-C10-alkoxy, C4-C20-cycloalkyl, C5-C20-cycloalkenyl, C5-C20-cycloalkinyl, C5-C20-aryl, C6-C20-alkylaryl, C6-C20-arylalkyl, C2-C20-heteroalkyl, C3-C20-cyclic heteroalkyl, wherein in case of more than one aliphatic or aromatic ring system the ring systems are condensed or separate, wherein each residue R^1 has at least one substituent R^{1a} selected from the group consisting of hydrogen atom, halogen atom (F, Cl, Br, I), hydroxyl, thiol, C1-C3 thioester, C1-C3-thioether, C1-C3-alkyl and C1-C3-alkoxy. More preferably, the residue R^1 of the amine according to (a) is selected from the group consisting of branched or unbranched C2-C20-alkyl, branched or unbranched C2-C20-alkenyl, branched or unbranched C2-C20-alkinyl, C4-C20-cycloalkyl, C5-C20-cycloalkenyl, and C5-C20-cycloalkinyl, C5-C20-aryl, wherein in case of more than one aliphatic or aromatic ring system the ring systems are condensed or separate, wherein each residue R^1 has at least one substituent R^{1a} selected from the group consisting of hydrogen atom, halogen atom, C1-C3-alkyl and C1-C3-alkoxy. More preferably, the residue R^1 of the amine according to (a) is selected from the group consisting of branched or unbranched C2-C10-alkyl, C5-C10-cycloalkyl and C5-C20-aryl, wherein in case of more than one aliphatic or aromatic ring system the ring systems are condensed or separate, wherein each residue R^1 has at least one substituent R^{1a} selected from the group consisting of hydrogen atom, halogen atom, C1-C3-alkyl and C1-C3-alkoxy; R^1 being preferably selected from the group consisting of methyl, iso-propyl, cyclohexyl and phenyl, wherein phenyl has at least one substituent selected from the group consisting of hydrogen atom, halogen atom, preferably fluoro or chloro, and methoxy, preferably meta- or para-methoxy.

[0053] For R^1 being branched or unbranched C2-C20-alkyl or branched or unbranched C2-C20-alkenyl, a preferred embodiment relates to R^1 being branched or unbranched C4-C20-alkyl or branched or unbranched C4-C20-alkenyl.

[0054] According to a further preferred embodiment of the composition, the residue R^2 of the amine according to (a) is selected from the group consisting of hydrogen atom, branched or unbranched C1-C5-alkyl, wherein each residue R^2 has at least one substituent R^{2a} selected from the group consisting of hydrogen atom, halogen atom, C1-C3-alkyl and C1-C3-alkoxy. Preferably, the residue R^2 of the amine according to (a) is selected from the group consisting of branched or unbranched C1-C3-alkyl, R^2 being preferably methyl.

[0055] In a preferred embodiment of the composition, the combination of R^1 , R^{1a} being a perfluorinated alkyl is excluded. The same applies for R^2 and R^{2a} , i.e. the combination of R^2 and R^{2a} being a perfluorinated alkyl is excluded as well. In other words, neither the combination of R^1 and R^{1a} nor the combination of R^2 and R^{2a} is a perfluorinated alkyl.

[0056] Regarding the embodiment of the composition, wherein R^1 and R^2 together form a C3-C10-cycloalkyl or a C3-C10-cycloalkenyl, the number of C atoms indicated includes the carbonyl C atom situated between R^1 and R^2 .

[0057] According to a preferred embodiment of the composition, the composition comprises the amine according to (a) in an amount in the range of from 90 to 99.9 weight-%, preferably in an amount in the range of from 95 to 99.9 weight-%, more preferably in an amount in the range of from 98 to 99.9 weight-%. According to a further preferred embodiment of the composition, the composition comprises the at least one carboxylic acid according to (b) (protonated form or carboxylate with a suitable counter ion) in an amount of at least 0.003 weight-%, preferably in an amount in the range of from 0.003 to 5 weight-%, more preferably in an amount in the range of from 0.003 to 3 weight-%.

[0058] Below, the investigations carried out by the inventors are described in more detail:

As described above, commercially readily available aliphatic, aromatic and heteroaromatic carboxylic acids (R -COOH) were selected and screened as their respective carboxylate salts towards common amines from a transaminase-catalyzed reaction. 1-phenylethylamine and substituted derivatives 2a-f thereof served as model product amines and were compared with typical donor amines such as isopropylamine 3, racemic 2-butylamine, DL-alanine and L-alanine. The straightforward screening approach especially resulted in the identification of two benzylbenzene-based acids and three benzoic

acid derivatives that matched the required criteria: DPAA, 3DPPA, 34CA, 34NA and 43CNA. In addition, 2DPPA was identified as suitable carboxylic acid.

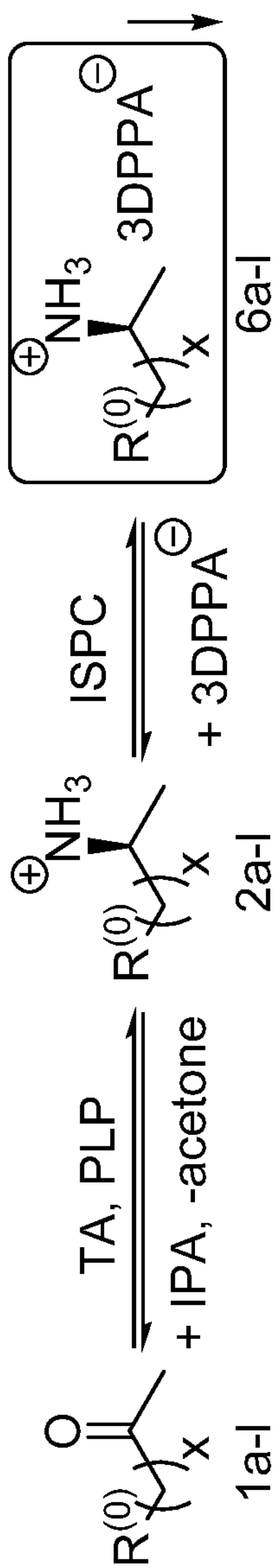
[0059] For example, the isopropylamine salt of 4-chloro-3-nitrobenzoic acid (43CNA) **5** shows a very high solubility of 993 mmol·l⁻¹, while the 1-phenylethylamine salt of 43CNA **6a** is considerably less soluble with 22 mmol·l⁻¹. As stated by Le Chatelier's principle, the solubility of amine salts can be further reduced if an over-stoichiometric amount of carboxylate is added to the mother liquor, which pushes the equilibrium from the dissociated forms (present in solution) towards its non-dissociated solid salt form.

[0060] Aside the general solubility difference, the applicability of these 3 acids - 43CNA, DPAA and 3DPPA - was tested with 7 exemplary amine transaminases from *Aspergillus fumigates* (AfATA), *Gibberella zeae* (GzATA), *Neosartorya fischeri* (NfATA), *Aspergillus oryzae* (AoATA), *Aspergillus terreus* (AtATA), *Mycobacterium vanbaalenii* (MvATA) and *Silicibacter pomeroyi* (SpATA) (Fig. 1). The results show that almost all investigated amine transaminases are already strongly inhibited by 50 mM of the more soluble acids 43CNA and DPAA. Herein MvATA and SpATA were identified as the most stable enzymes. A noticeable exception from these results is acid 3DPPA, which is only sparingly soluble in buffered solutions. This effectively limits the carboxylate concentration in aqueous solution to a maximum of ≤25 mM, depending on temperature and pH, while the excess of solid 3DPPA remains in the reaction mixture. This low 3DPPA-concentration also does not significantly inhibit the investigated ATAs. Fortunately, the investigated 3DPPA-salts exhibit the lowest solubility of product amine salt **6**, which fits perfectly into the above mentioned ISPC-requirements (see Example section, Tables 4 and 5). Consequently, 3DPPA was identified as the most valuable acid for the application in a crystallization-based *in situ*-product removal (ISPR) of amine **2** from an amine transaminase-catalyzed reaction.

[0061] The use of 3DPPA in combination with an exemplary SpATA-catalyzed conversion of 100 mM acetophenone **1a** to (S)-1-phenylethylamine shows clearly the synthetic advantage of an acid-based ISPC (Fig. 2). The classical reaction approach with a low donor amine concentration of only 250 mM isopropylamine yields a non-sufficient conversion of 19%. A simple addition of 1.25 eq. solid 3DPPA improved the overall conversion directly to ca. 75%, regardless of the use as a whole cell biocatalyst or partially purified cell extract. The majority of product **2** is afterwards present as solid salt **6**, which can be almost quantitatively recovered by filtration after cooling the reaction mixture to 0 °C. Consequently, this ISPC-concept with 3DPPA translates to a more atom-efficient synthesis since less donor amine is required and a simplified downstream processing-approach is facilitated (see below). Noteworthy, the low solubility of 3DPPA does not limit the crystallization of product amine salt since the constant removal of 3DPPA from aqueous solution is continuously compensated back to its original solubility limit by a simultaneous dissolution of 3DPPA (from excess solid 3DPPA).

[0062] The shown ISPC-concept with acid 3DPPA was also successfully used for the SpATA-catalyzed conversion of selected acetophenone derivatives **1b-g** and further non-aromatic substrates **1h-k** (Table 2).

Table 2: |SPC-supported SpATA-catalyzed synthesis of chiral amines



substrate	R ⁽⁰⁾	x	conversion reference / %	conversion ISPC / %	e.e. / % ^[a]
1a	Ph	0	19	75	>99.5
1b	m-F-Ph	0	21	69	>99.5
1c	p-F-Ph	0	11	61	>99.5
1d	m-Cl-Ph	0	8	46	>99.5
1e	p-Cl-Ph	0	8	65	>99.5
1f	m-MeO-Ph	0	10	37	>99.5
1g	p-MeO-Ph	0	4	8	>99.5
1h	Cy	0	0	8	n.d.
1i	Me	3	37	72	>99.5
1j	Me	4	20	78	98.7
1k	<i>i</i> Pr	1	36	96	n.d.

[0063] [a] Values are given for the ISPC-supported reaction; Conditions: 200 mM phosphate buffer pH 7.5, 100 mM substrate, 250 mM isopropylamine, 15 mg·mL⁻¹ lyophilized whole cells, 30 °C; 125 mM 3DPPA for ISPC

[0064] As expected, for all substrates low conversions were obtained without ISPC due to the low, but still over-stoichiometric, use of 250 mM isopropylamine. A simple addition of 3DPPA increases product formation significantly for almost all investigated substrates. Improvements range between 2 and 8.1-fold with yields of up to 96 % for **1k**, while the products are selectively crystallized as its 3DPPA-salts. The remaining mother liquor, including excess isopropylamine, can be directly reused for a further increase of atom efficiency of the transaminase-catalyzed reaction. Even higher donor amine concentrations will yield a further increase in conversion, but include the risk of an undesired crystallization of donor amine salt, which then eventually yields a decrease in product formation.

[0065] The isolation of product amine **2** is easily realized by dissolving product salt **6** in an aqueous solution at high pH, followed by an extraction and evaporation of the solvent, e.g. MTBE. Alternatively, the respective hydrochloride salt can be directly crystallized from the ether phase by a careful addition of concentrated HCl. In addition, the spent acid 3DPPA can also be precipitated from the remaining aqueous phase by acidification with concentrated HCl, due to its low solubility at low pH.

[0066] Summarizing, the presented *in situ*-product crystallization of an amine from a transaminase-catalyzed reaction by the addition of a selected acid/carboxylate presents a powerful synthetic alternative to the use of tailor-made donor amines and complex cascade reaction systems. The main advantages of this ISPC are a more atom-efficient use of classical, cheap donor amines and a simplified downstream processing-approach by simple filtration. The targeted product amine can be afterwards extracted from its salt and the applied 3DPPA acid easily recycled.

Description of Figures

[0067]

Fig. 1 Effects of 43CNA, DPAA and 3DPPA on selected amine transaminases; Conditions: 200 mM phosphate buffer pH 7.5, 10 mM acetophenone **1**, 500 mM (43CNA and DPAA) or 250 mM (3DDPA) isopropylamine, 5 mg·mL⁻¹ lyophilized cell extract, 30 °C, 22 h; the obtained concentrations of **2** were intentionally held below the solubility limit of product salt **6** to prevent an undesired *in situ*-product crystallization; 43CNA was chosen as a representative of the identified benzoic acid derivatives;

Fig. 2 Time progression curves for the SpTA-catalyzed formation of (S)-1-phenylethyl-amine with and without *in situ*-product crystallization (ISPC); □-whole cells or ■-cell extract with 125 mM 3DPPA, ○-whole cells or ●-cell extract without 3DPPA; Conditions: 200 mM phosphate buffer pH 7.5, 100 mM acetophenone, 250 mM isopropylamine, 15 mg·mL⁻¹ lyophilized cell extract or whole cells, 30 °C;

Fig. 3: Exemplary result for the precipitation of (S)-4-Chloro-1-phenylethylamine salts.

Experimental Section

1. General information

[0068] Materials: All chemicals were obtained from commercial suppliers and used as received. Deionized water was used throughout this study. The investigated enzymes (raw extract and whole cells) were received from Enzymicals AG (Greifswald, Germany) as lyophilizates; ECS-ATA01 (TA from *Aspergillus fumigates*, SEQ ID NO. 1), ECS-ATA02 (TA from *Gibberella zeae*, SEQ ID NO. 2), ECS-ATA03 (TA from *Neosartorya fischeri*, SEQ ID NO. 3), ECS-ATA04 (TA from *Aspergillus oryzae*, SEQ ID NO. 4), ECS-ATA05 (TA from *Aspergillus terreus*, SEQ ID NO. 5), ECS-ATA07 (TA from *Mycobacterium vanbaalenii*, SEQ ID NO. 6) and ECS-ATA08 (TA from *Silicibacter pomeroyi*, SEQ ID NO. 7).

[0069] Gas chromatography: Conversion was measured with a Trace 1310 gaschromatograph by Thermo Scientific (Dreieich, Germany), equipped with a 1300 flame ionization detector and a Chirasil-Dex-CB-column (25 m x 0.25 mm x 0.25 µm). n-Decane was used as internal standard in all measurements. Temperatures of injector and detector were set to 250 °C.

[0070] Temperature programs:

Compounds 1a/2a, 1b/2b and 1c/2c: start at 90 °C, followed by a heating rate of 2 K/min to 114 °C and 20 K/min to 150 °C

Compounds 1d/2d, 1e/2e, 1f/2f and 1g/2g: Start at 90 °C, followed by a heating rate of 2 K/min to 100 °C, 20 K/min to 130 °C, 2 K/min to 150 °C and 20 K/min to 160 °C

Compounds 1i/2i, 1j/2j, 1k/2k: Start at 90 °C, followed by a heating rate of 2 K/min to 96 °C and 20 K/min to 110 °C.

[0071] HPLC: Enantiomeric excess was measured with a 1100 Series HPLC by Agilent (Santa Clara, California, United

States), equipped with a diode array detector, with a Chiralcel OD-H (250 mm length, 4.6 mm internal diameter, particle size: 5 μm) and a flow of 1 mL/min at 25 °C. Eluent composition for the respective amine: 99% n-heptane/ 1% ethanol for 2f; 98% n-heptane/ 2% ethanol for 2b, 2c and 2g; 95% n-heptane/ 5% ethanol for 2a, 2d and 2e

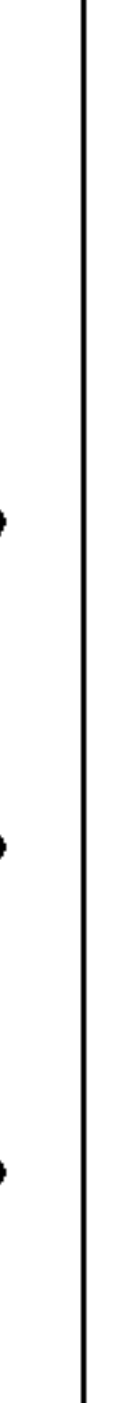
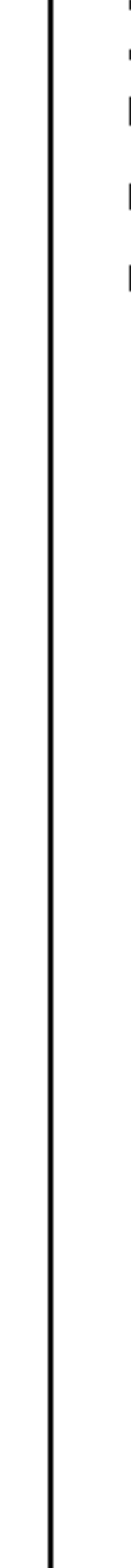
[0072] Enzyme Activity Assay: Enzyme activity was measured at a wave length of 245 nm with the spectrophotometer Specord 200 from Analytik Jena (Jena, Germany). Extinction coefficient of acetophenone: 11.852 (mM $\cdot$ cm)⁻¹.

[0073] Composition of the assay: 250 μL buffer solution, 250 μL 10 mM (S)-1-phenylethylamine in buffer solution, 250 μL 10 mM sodium pyruvate in buffer solution and 250 μL enzyme sample in buffer solution with 0.1 mM pyridoxal phosphate. All measurements were measured against a reference solution, whereas the enzyme solution was replaced with 200 μL buffer solution and 50 μL of 10 mM pyridoxal phosphate in buffer solution. Buffer solution: 50 mM phosphate buffer pH 8 with 0.25% DMSO.

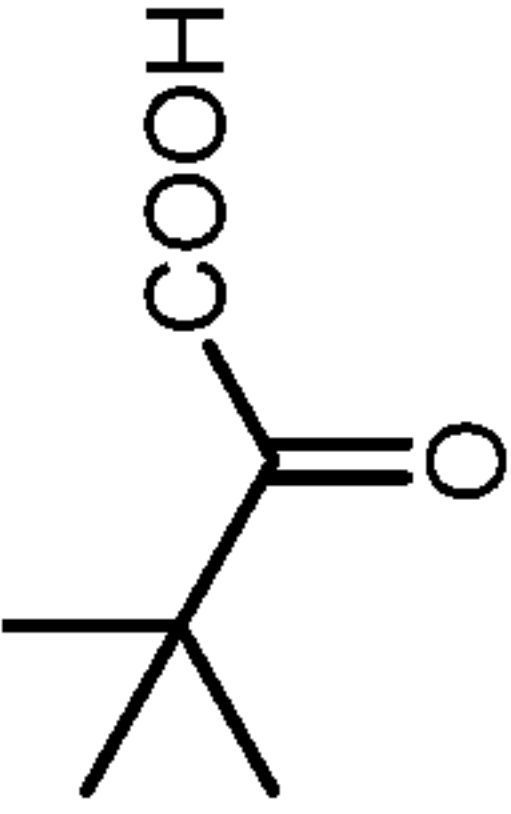
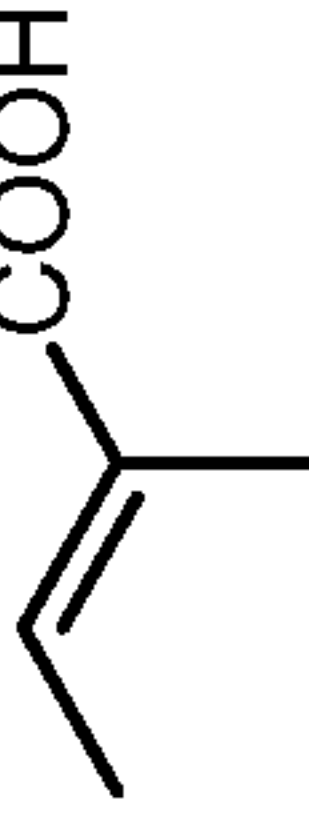
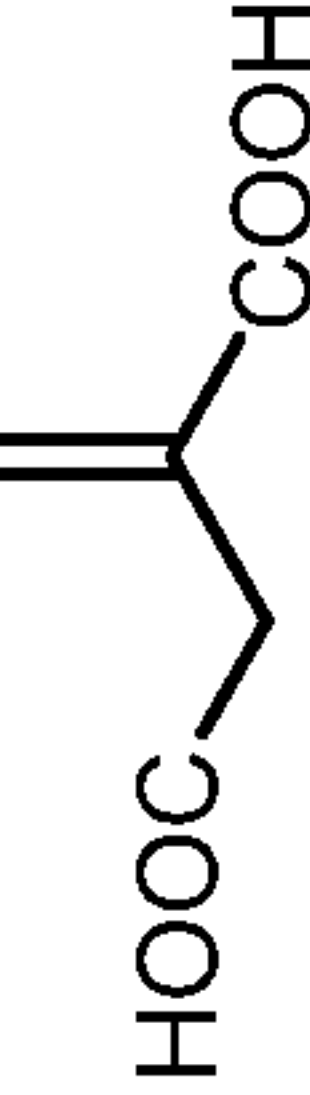
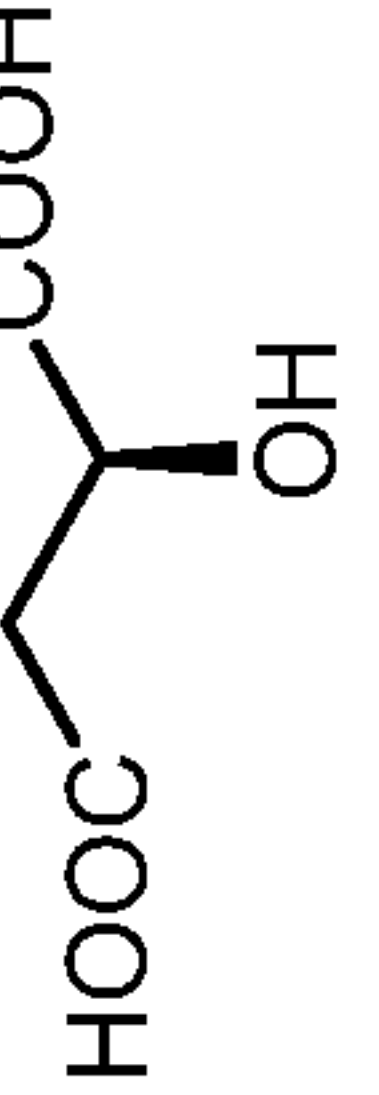
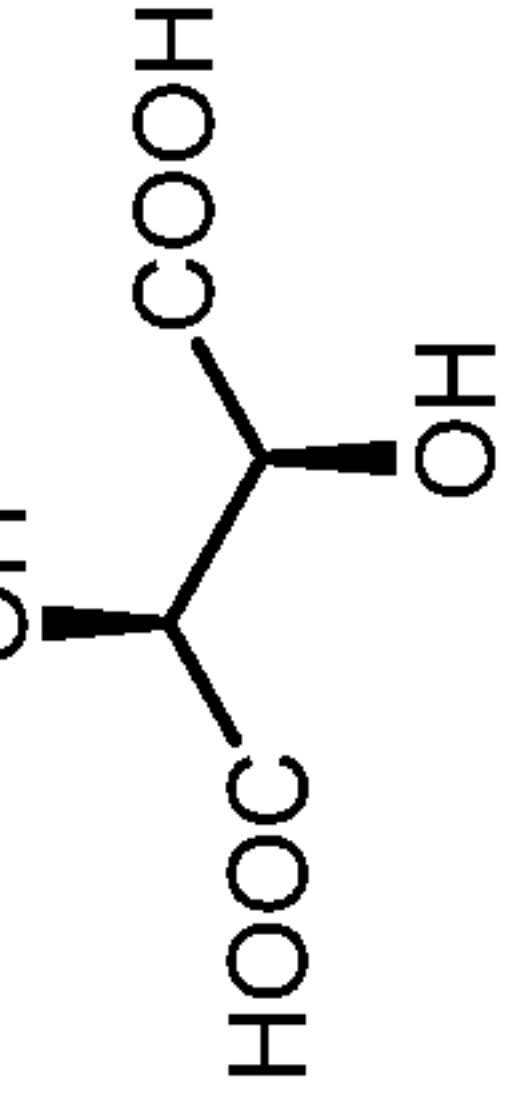
2. Investigated acids for the *in situ*-product crystallization of amines

[0074] A total of 79 acids were chosen for the screening procedure of relevant amines (table 3). The selection is mostly based on commercial availability and stability in aqueous solution.

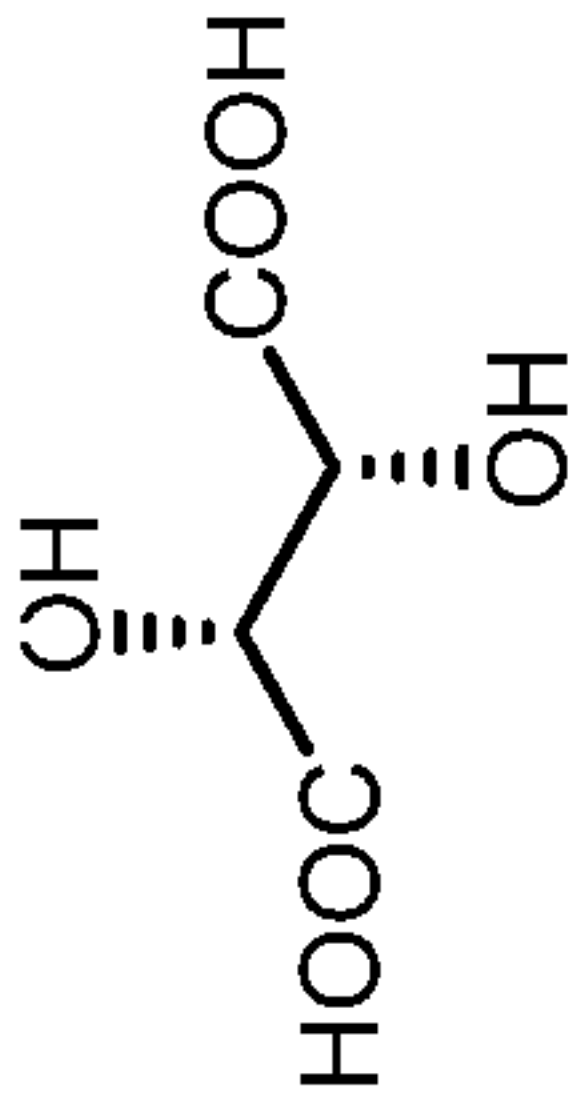
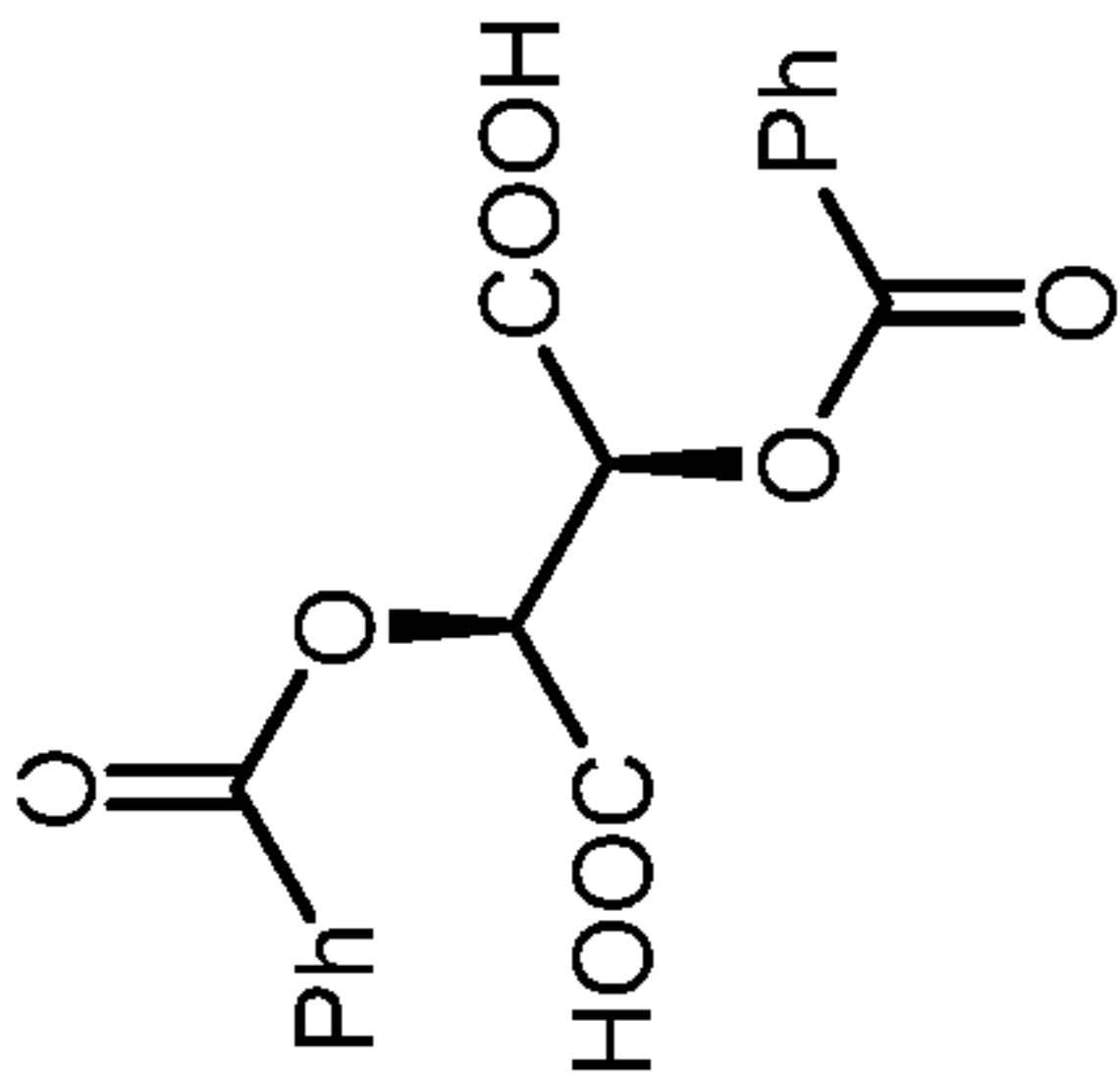
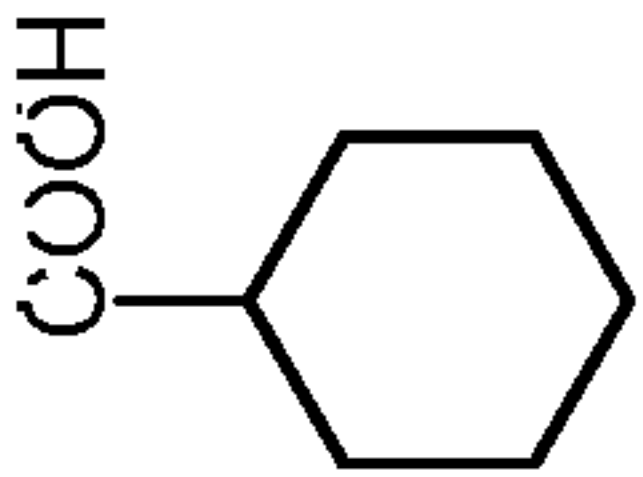
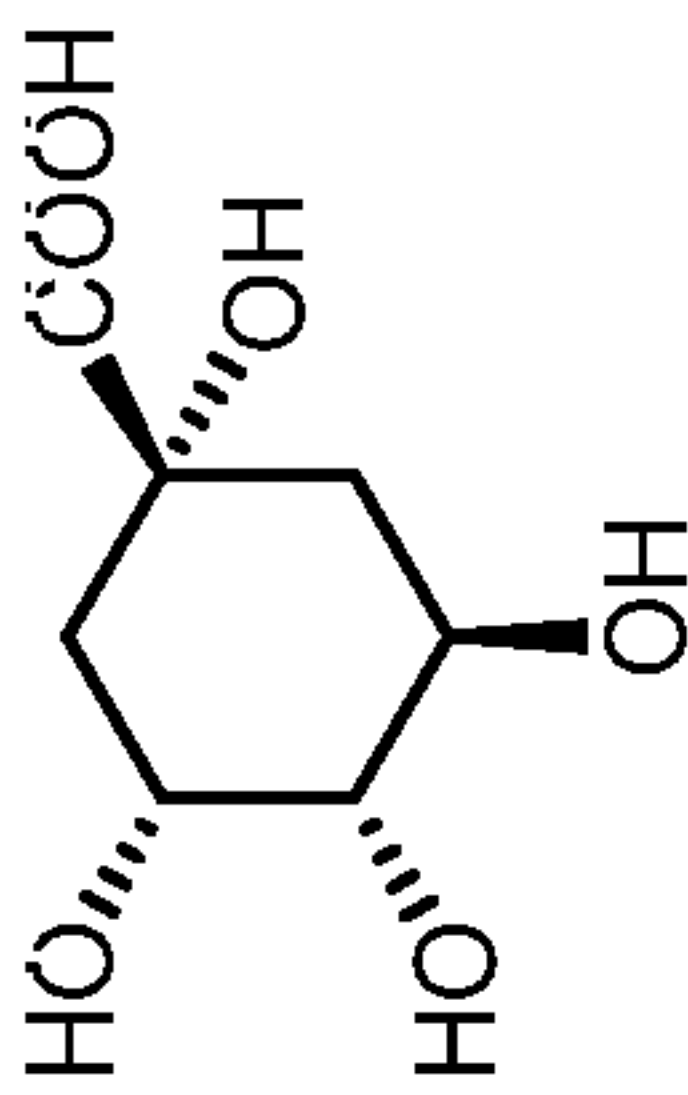
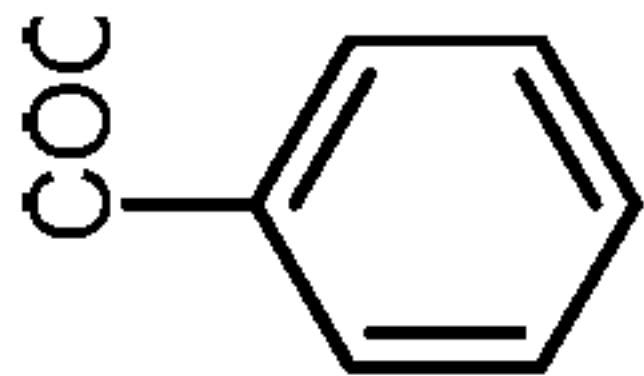
Table 3: List of investigated acids for the ISPC-concept

entry	abbreviation	full name	chemical structure	molar mass [g/mol]
1	FCC	Formic acid	HCOOH	46.03
2	ACC	Acetic acid		60.05
3	PPA	Propionic acid		74.08
4	BCA	Butyric acid		88.11
5	VCA	Valeric acid		102.13
6	HXCA	Hexanoic acid		116.16
7	HA	Heptanoic acid		130.19
8	CCA	Caprylic acid		144.21
9	nNA	Nonanoic acid		158.23
10	DCC	Decanoic acid		172.27
11	OA	Oxalic acid	HOOC-COOH	90.03
12	MA	Malonic acid	HOOC-CH2-COOH	104.06
13	SCCA	Succinic acid	HOOC-CH2-CH2-COOH	118.09
14	GA	Glutaric acid	HOOC-CH2-CH2-CH2-COOH	132.12
15	APS	Adipic acid	HOOC-CH2-CH2-CH2-CH2-COOH	146.14
16	PIA	Pimelic acid	HOOC-CH2-CH2-CH2-CH2-CH2-COOH	160.17

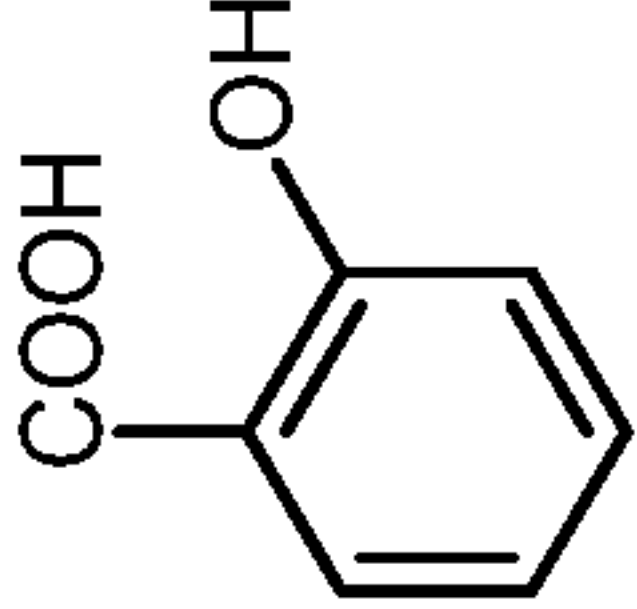
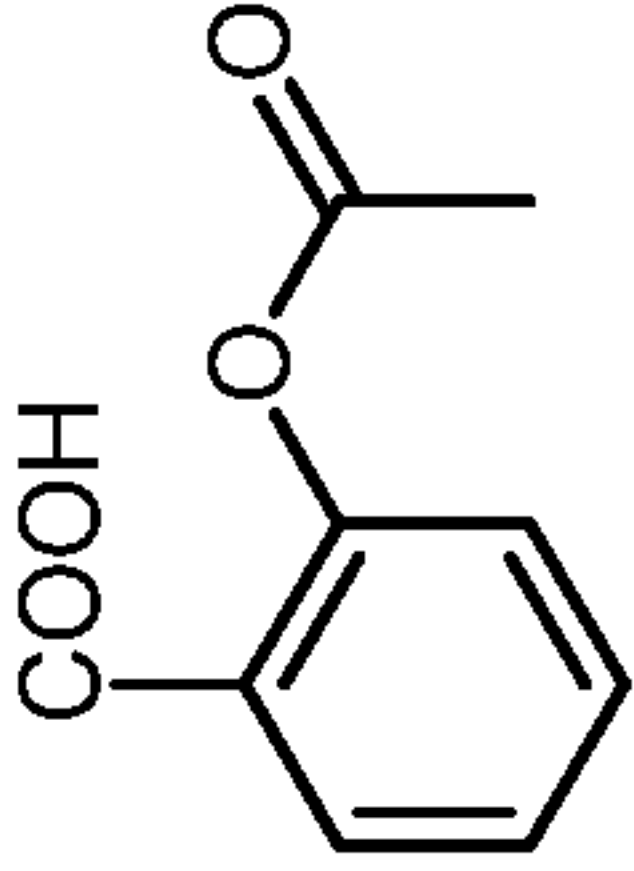
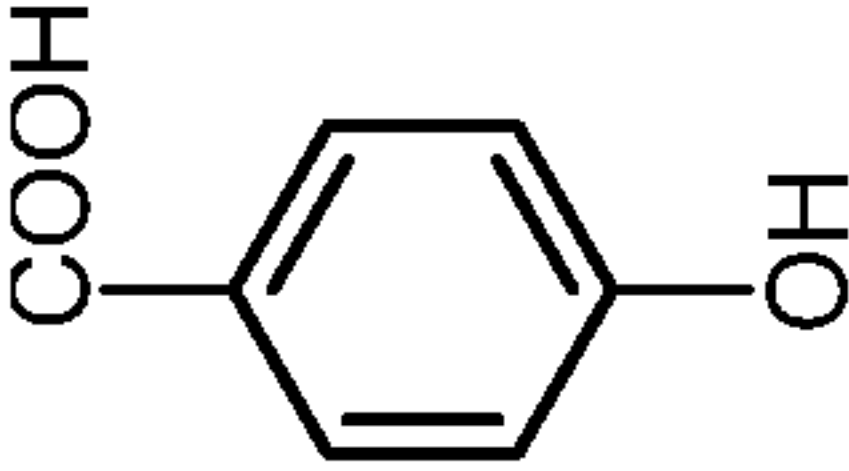
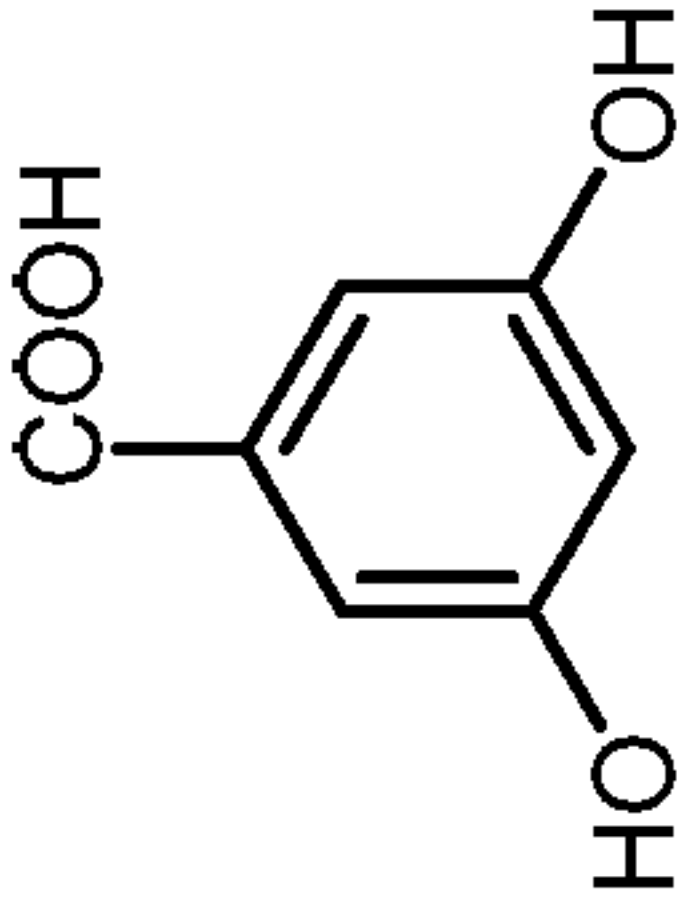
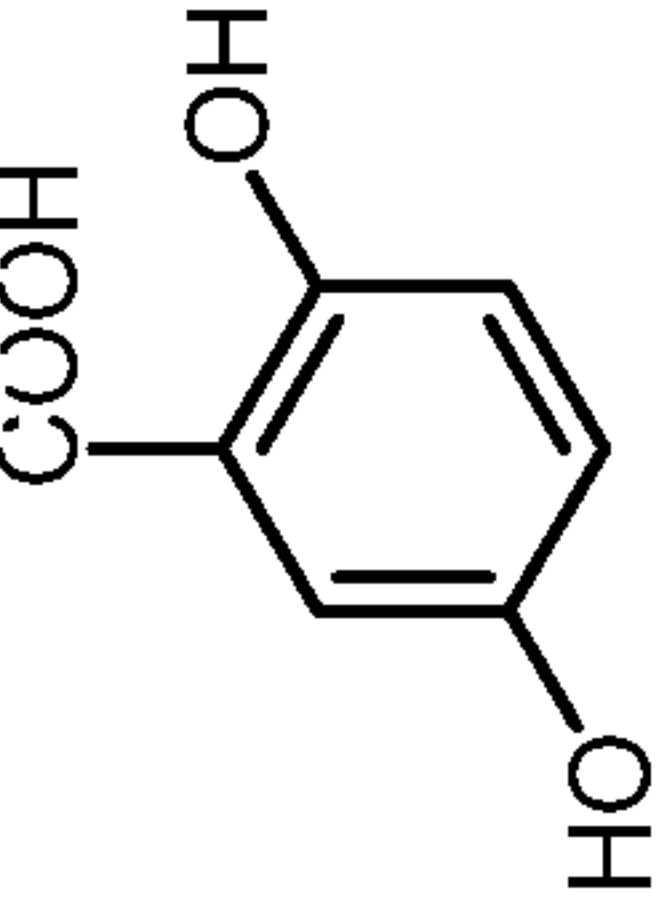
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entry	abbreviation	full name	chemical structure	molar mass [g/mol]
17	SBCA	Suberic acid		174.20
18	AA	Azelaic acid		188.22
19	SCA	Sebacic acid		202.25
20	TMPA	Trimethyl-pyruvic acid		130.14
21	GCA	Glycolic acid		76.05
22	TG	Tiglic acid		100.116
23	IS	Itaconic acid		130.10
24	FA	Fumaric acid		116.07
25	LMA	L-Malic acid		134.09
26	DTA	D-(-)-Tartaric acid		150.09

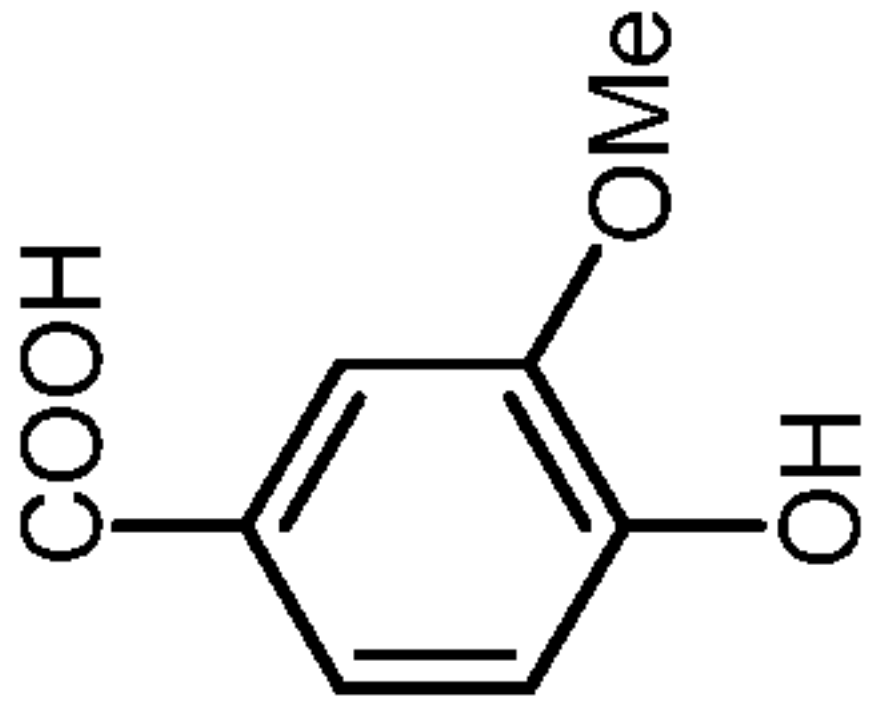
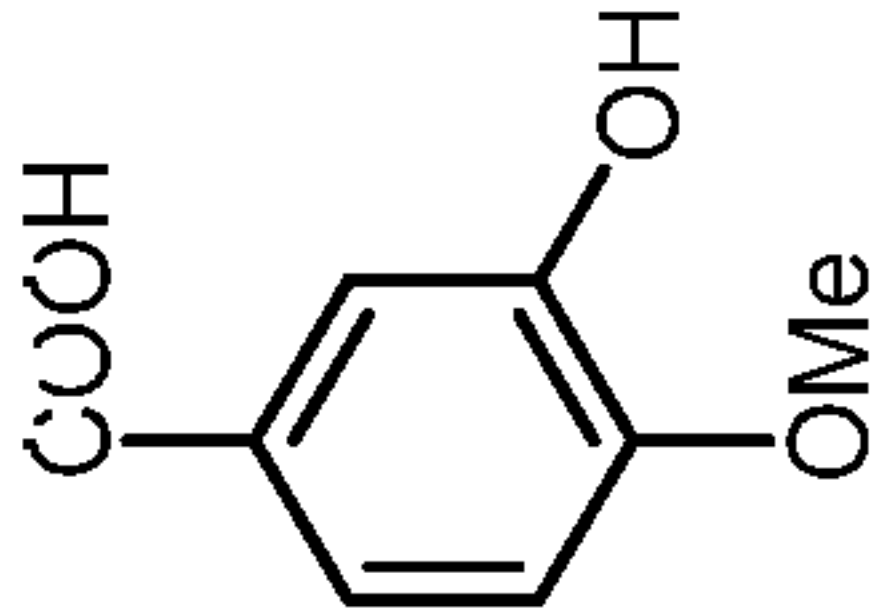
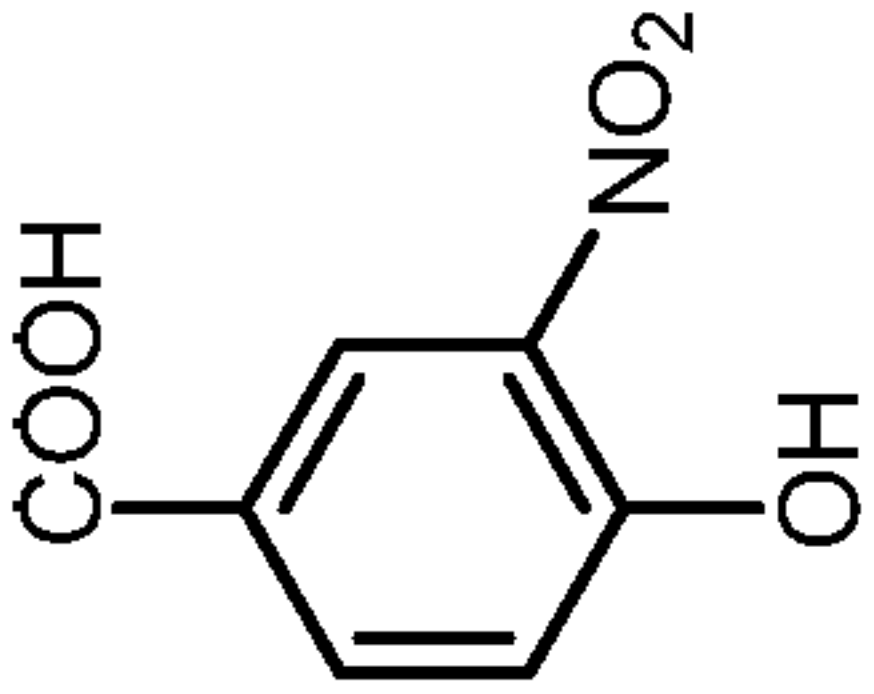
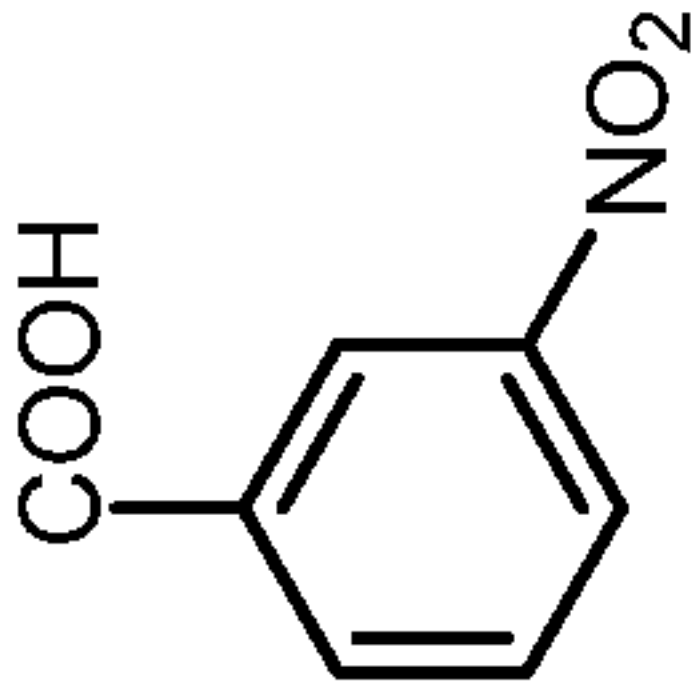
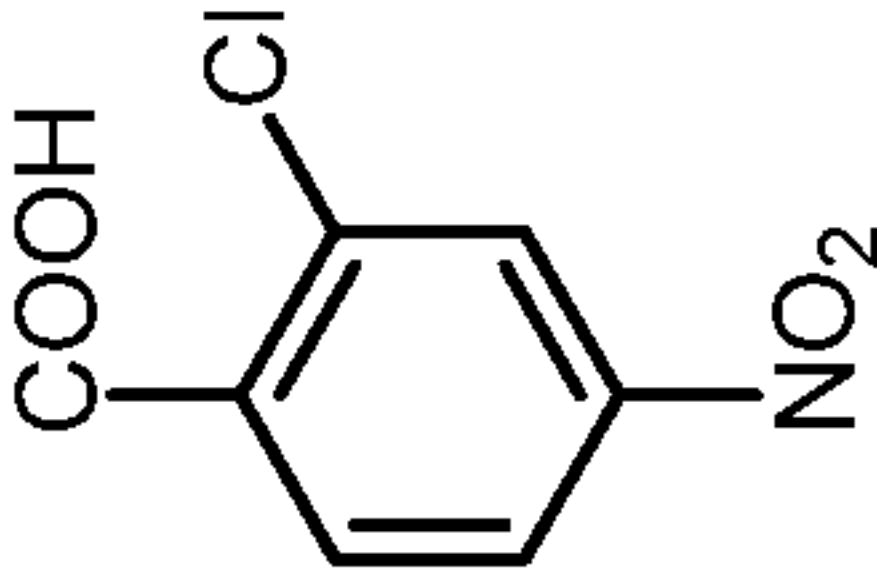
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entry	abbreviation	full name	chemical structure	molar mass [g/mol]
27	LTA	L-(-)-Tartaric acid		150.09
28	DBDT	Dibenzoyl-D-tartaric acid		358.30
29	CHCA	Cyclohexane carboxylic acid		128.17
30	DQA	D-(-)-Quinic acid		192.17
31	BZA	Benzoic acid		122.12

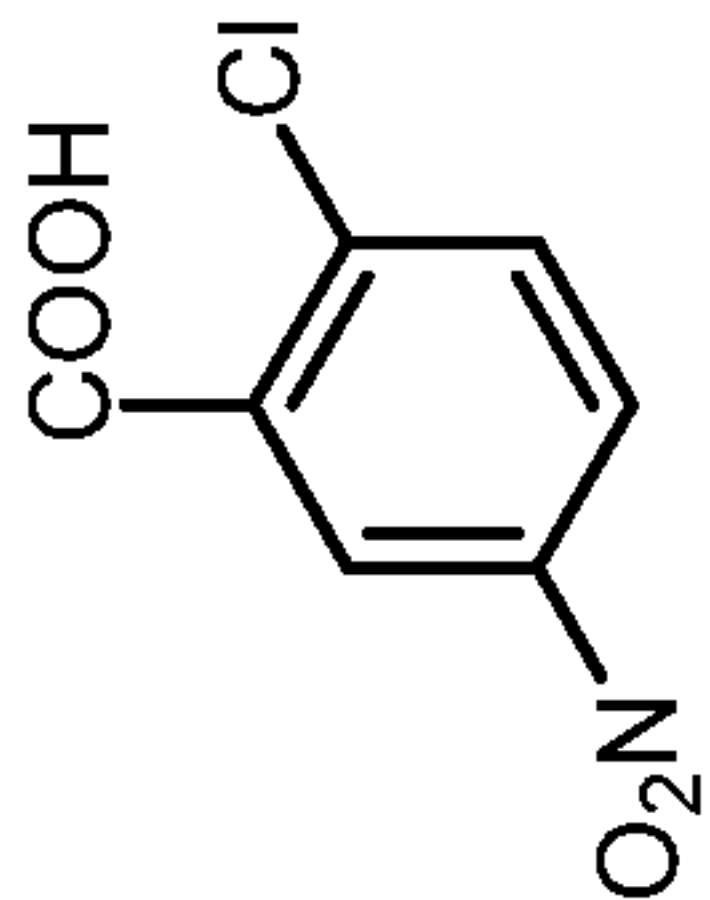
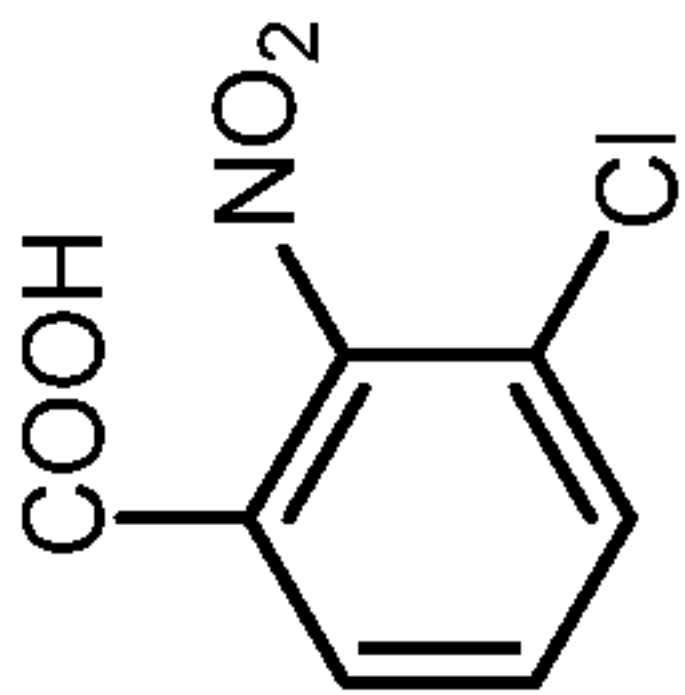
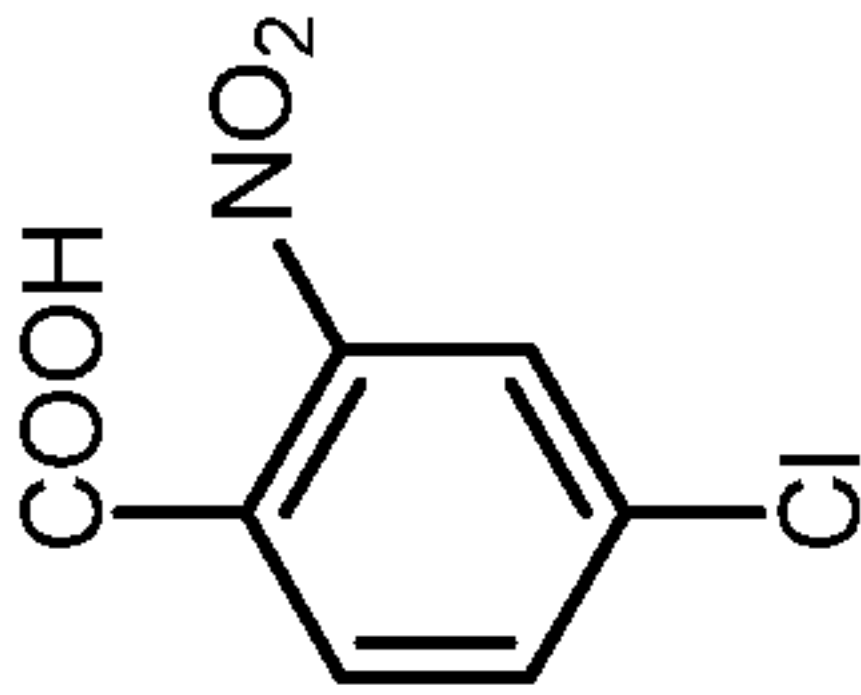
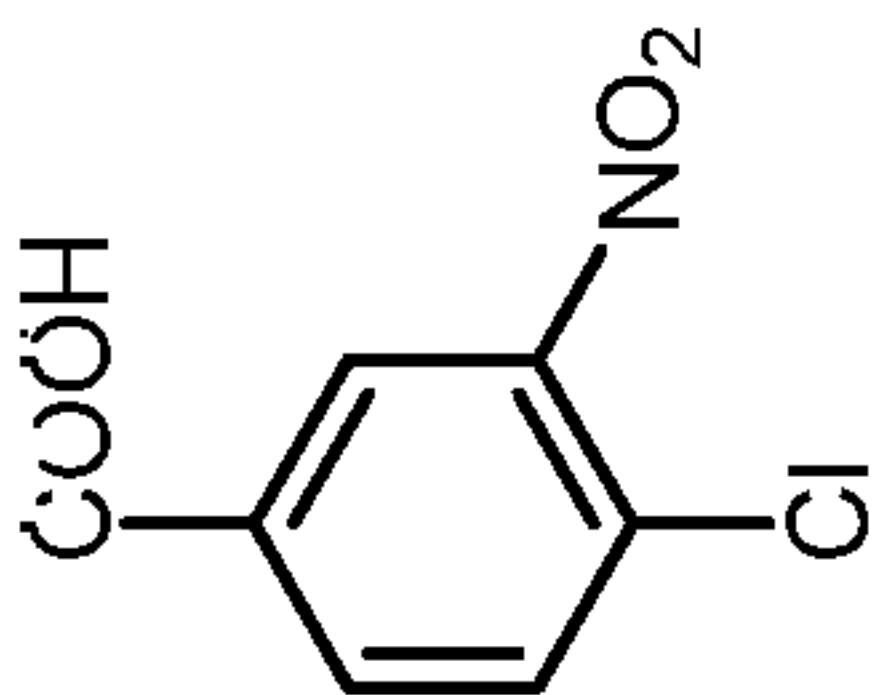
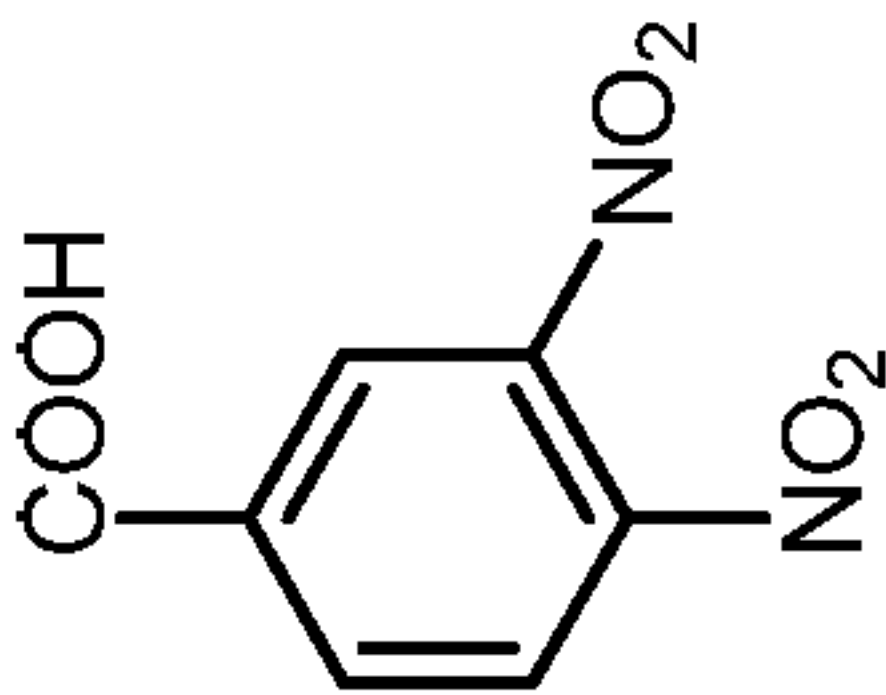
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entry	abbreviation	full name	chemical structure	molar mass [g/mol]
32	SA	Salicylic acid		138.12
33	ASA	Acetylsalicylic acid		180.16
34	4HBA	4-Hydroxybenzoic acid		138.12
35	35HBA	3,5-Dihydroxybenzoic acid		154.12
36	25DHBA	2,5-Dihydroxybenzoic acid		154.12

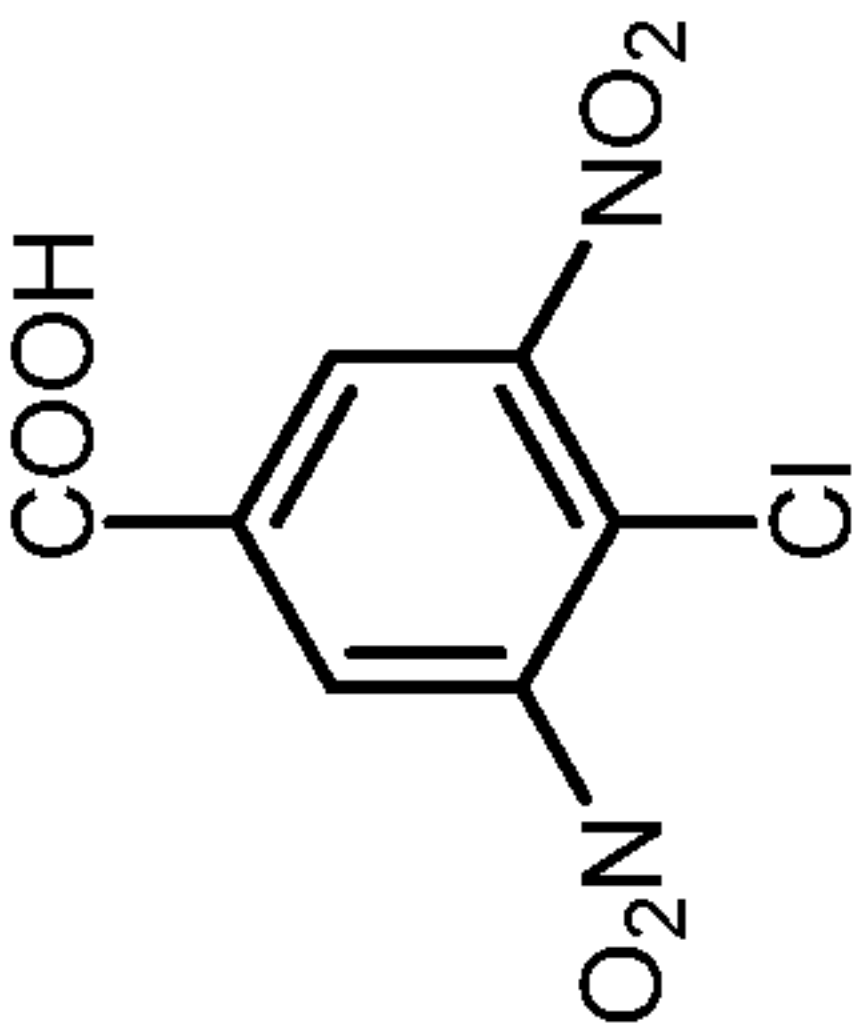
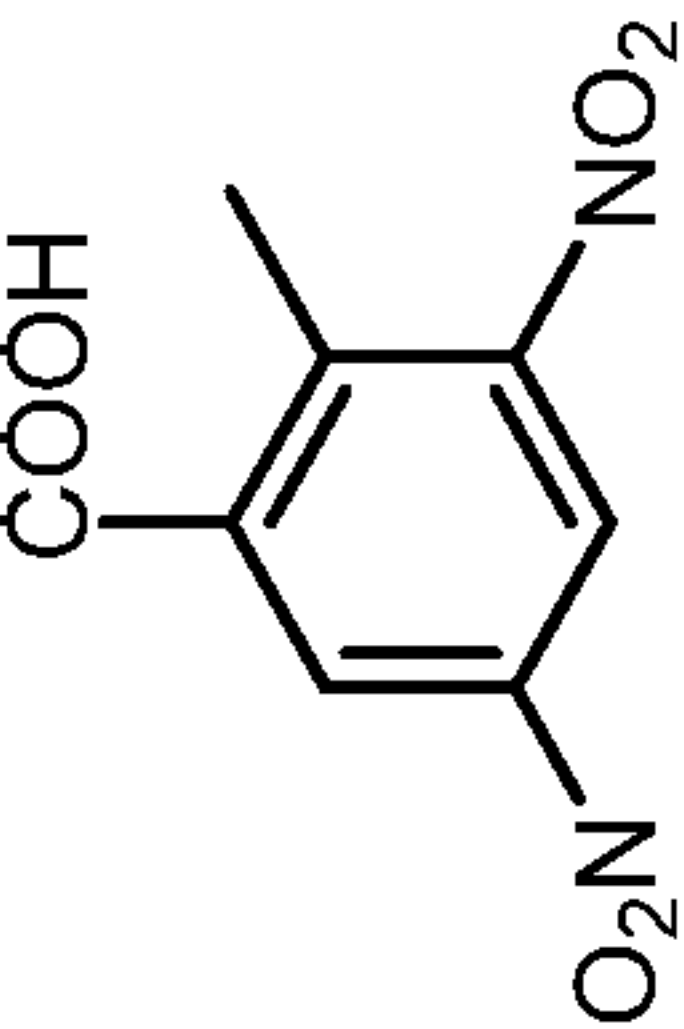
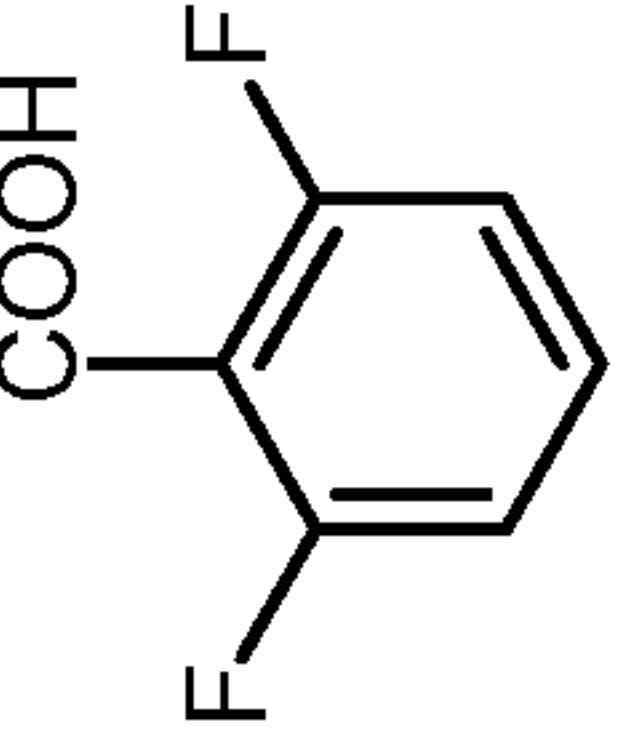
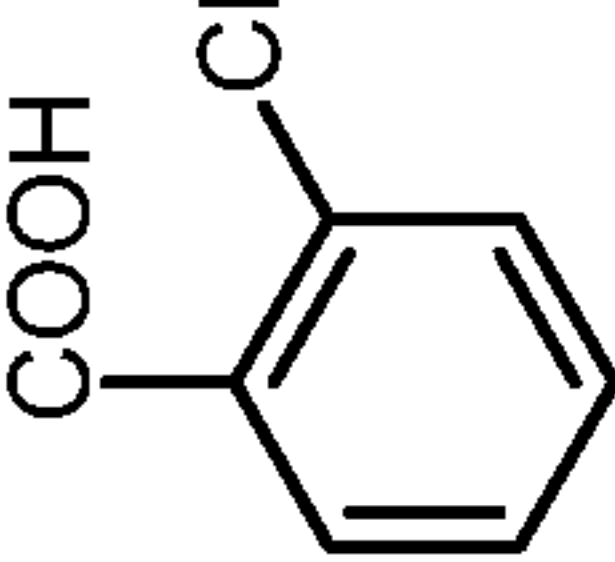
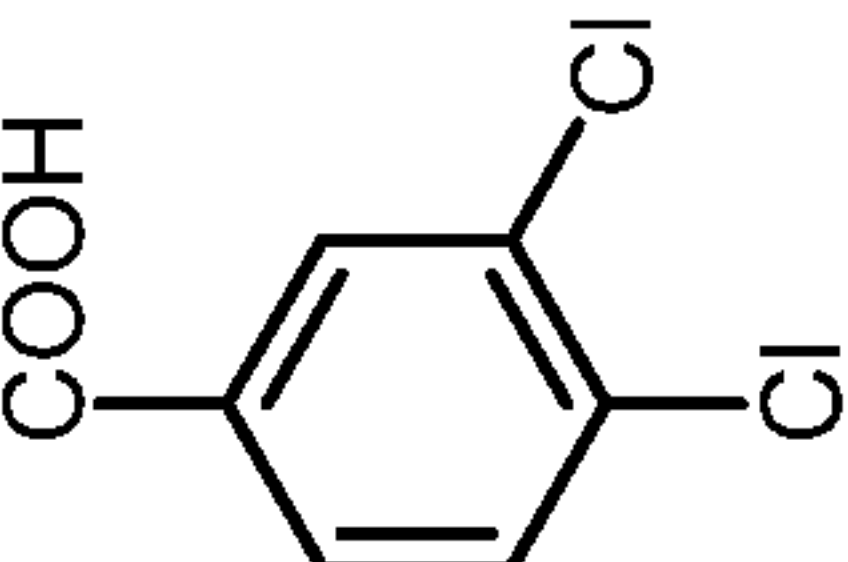
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entry	abbreviation	full name	chemical structure	molar mass [g/mol]
37	VA	Vanillic acid		168.15
38	IVA	Isovanillic acid		168.15
39	43HNBA	4-Hydroxy-3-nitrobenzoic acid		183.12
40	3NA	3-Nitrobenzoic acid		167.12
41	24CNA	2-Chloro-4-nitrobenzoic acid		201.56

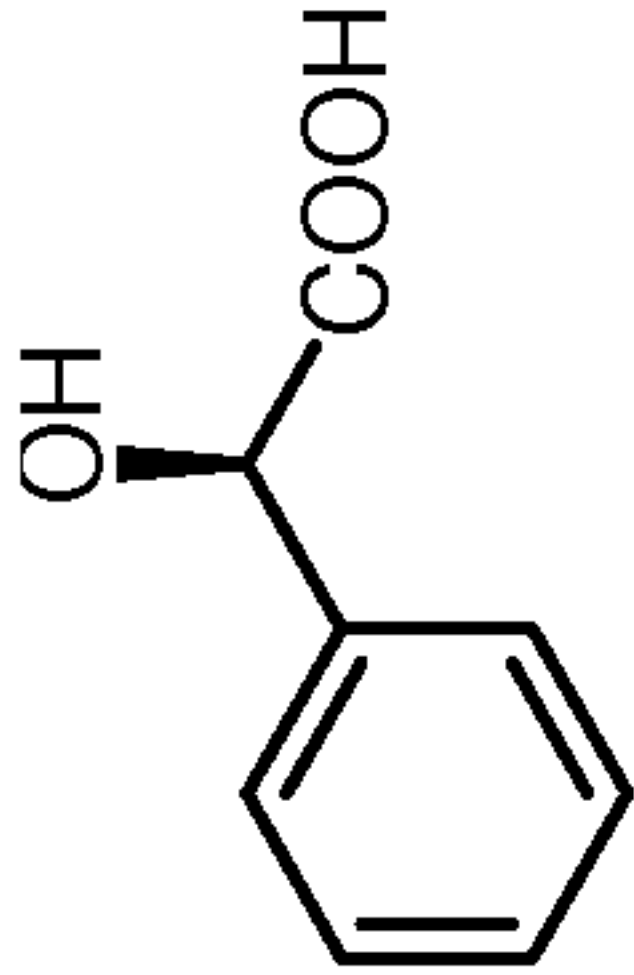
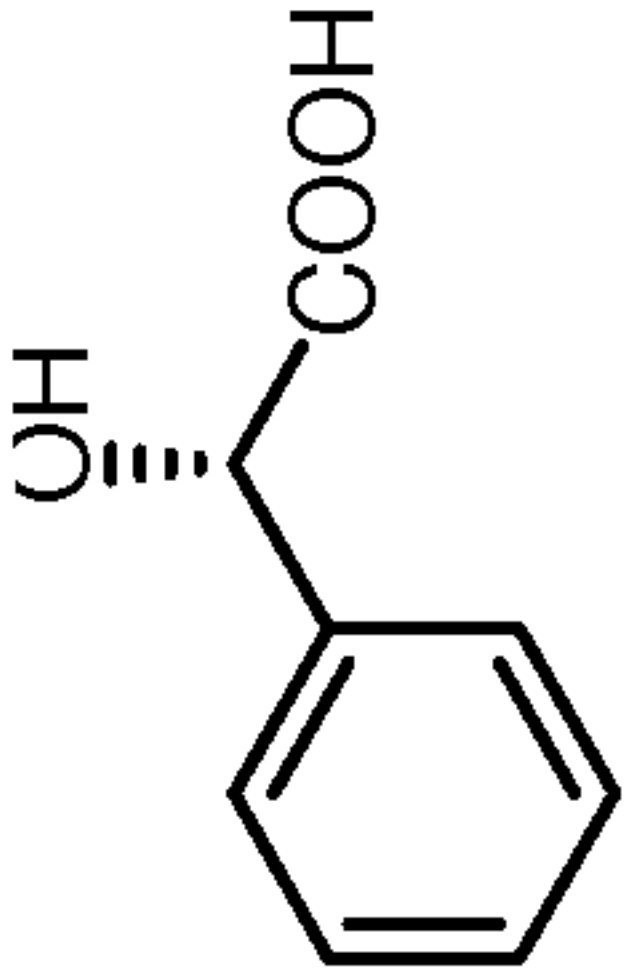
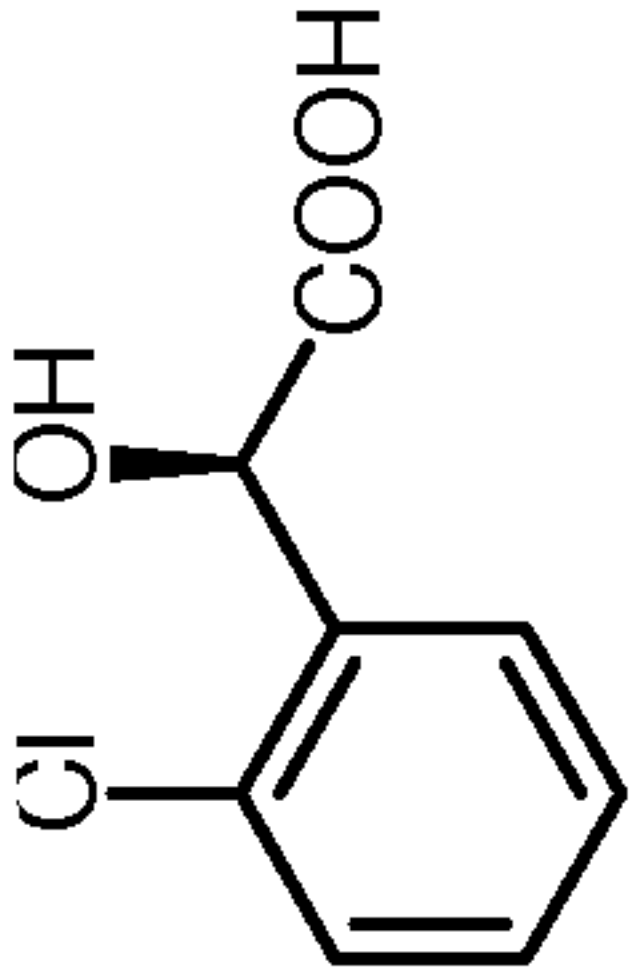
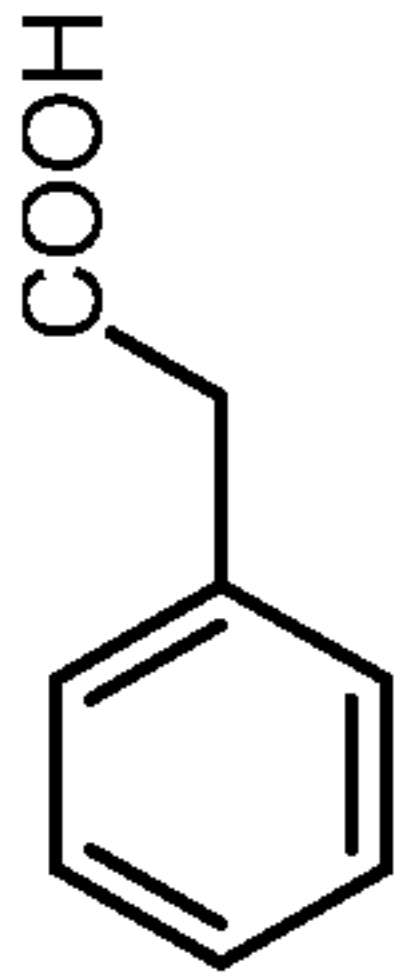
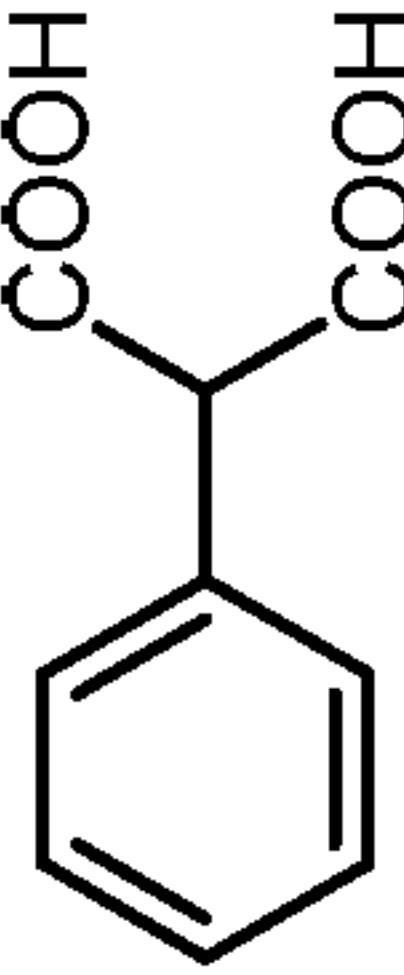
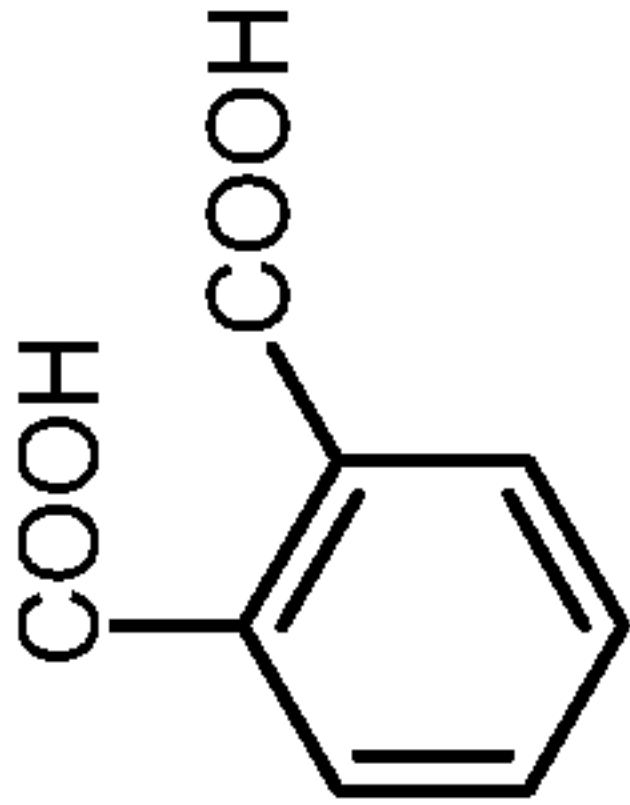
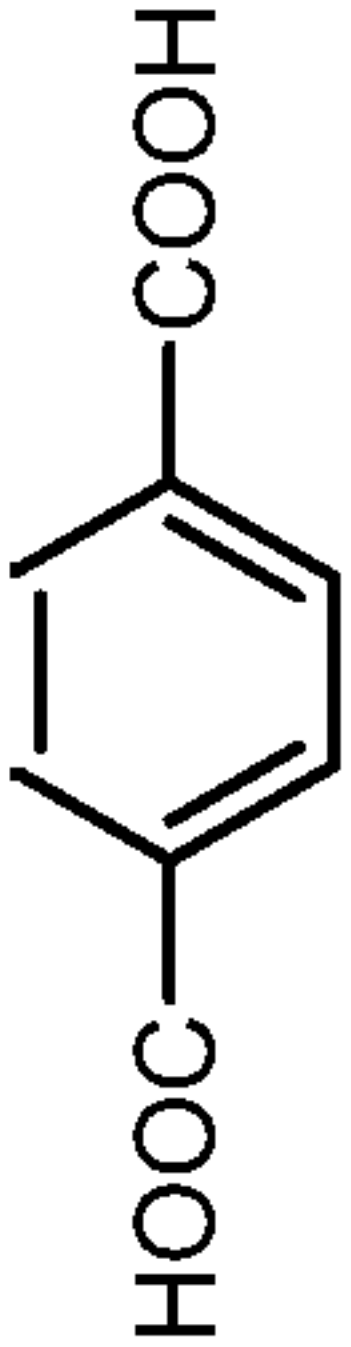
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entry	abbreviation	full name	chemical structure	molar mass [g/mol]
42	25CNA	2-Chloro-5-nitrobenzoic acid		201.56
43	32CNA	3-Chloro-2-nitrobenzoic acid		201.56
44	42CNA	4-Chloro-2-nitrobenzoic acid		201.56
45	43CNA	4-Chloro-3-nitrobenzoic acid		201.56
46	34NA	3,4-Dinitro-benzoic acid		212.12

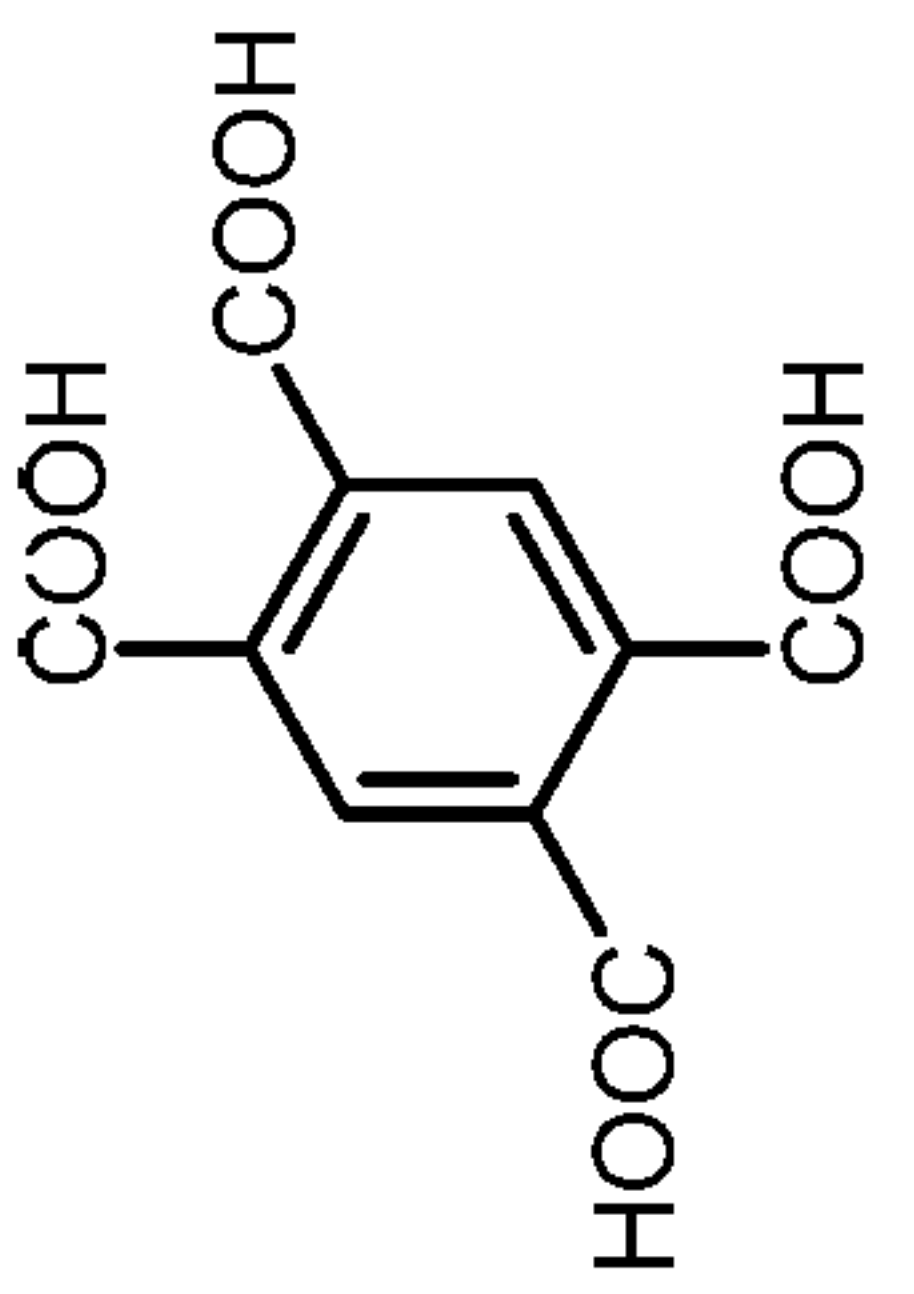
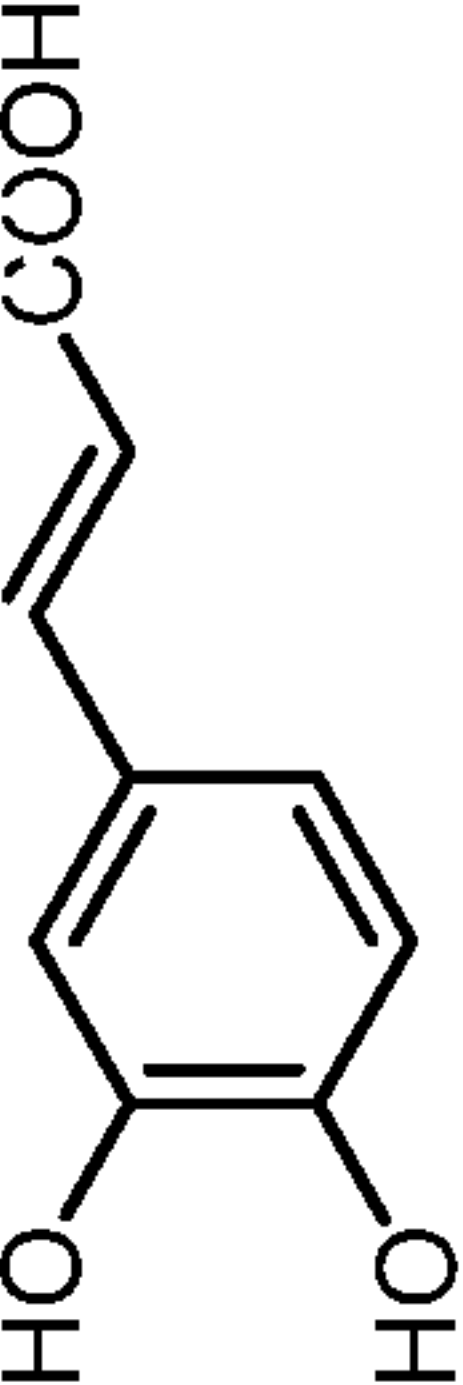
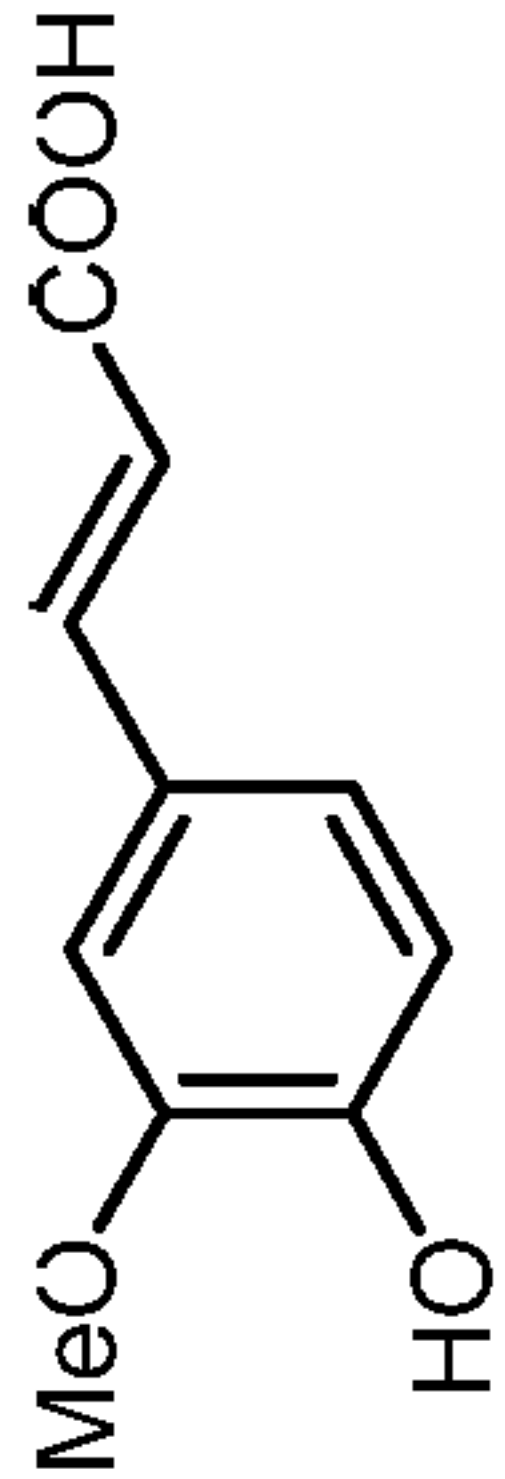
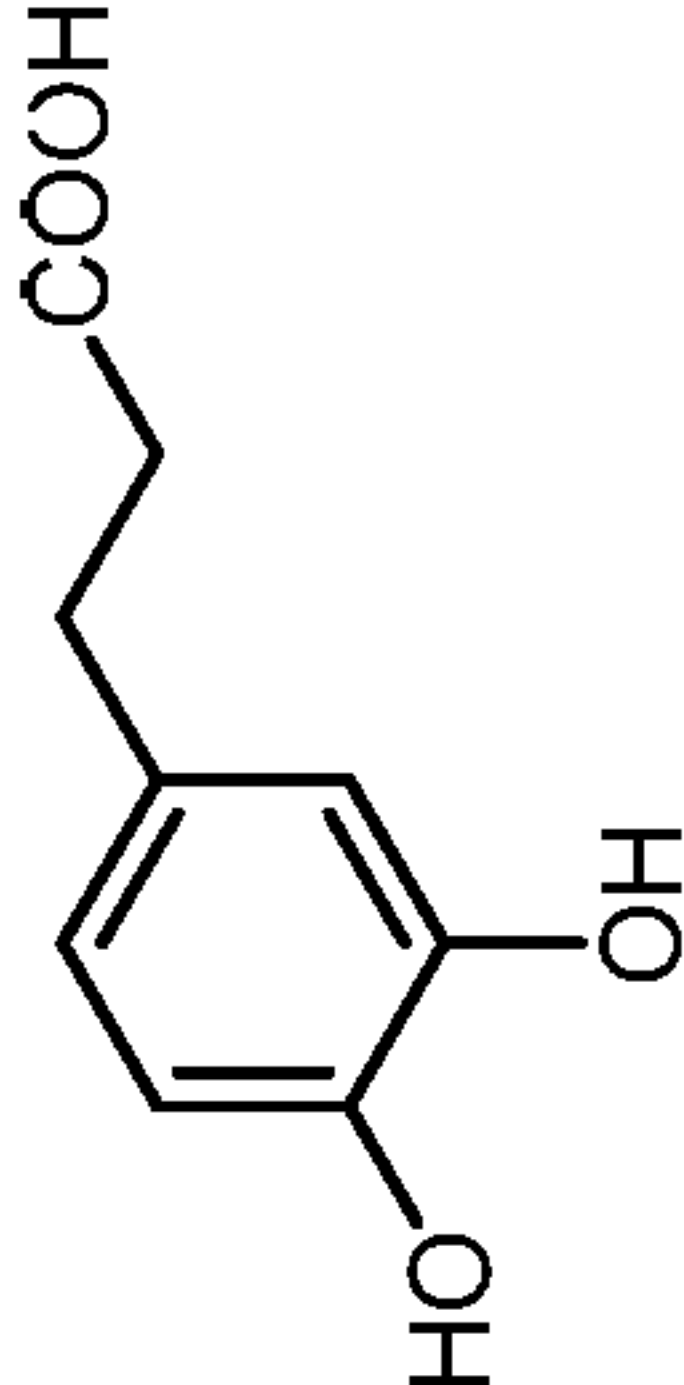
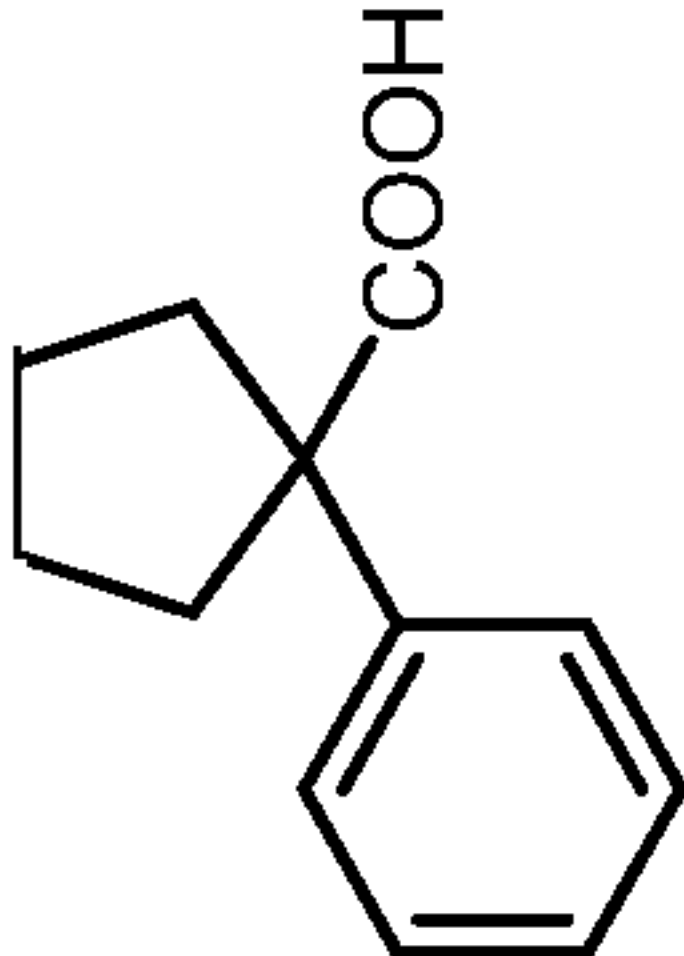
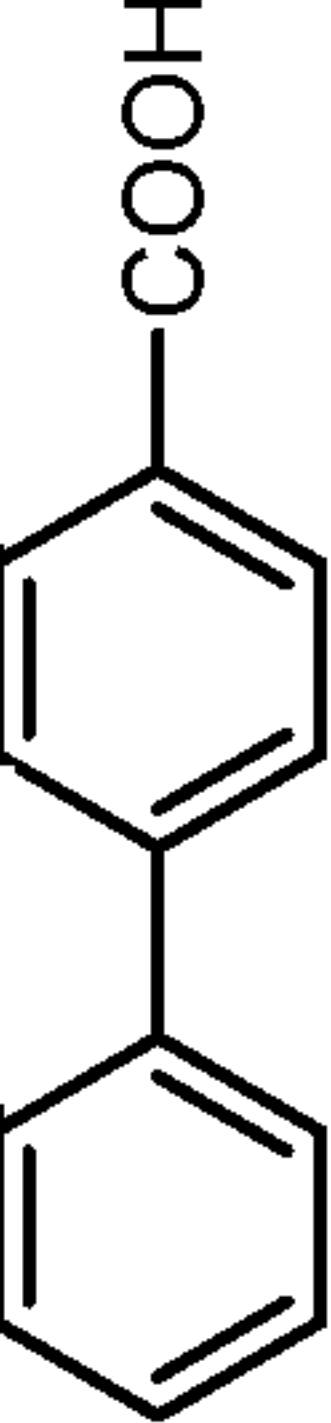
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47	435CNBA	4-Chloro-3,5-dinitrobenzoic acid		246.56
48	35DNOT	3,5-Dinitro- <i>o</i> -toluic acid		226.14
49	26FBA	2,6-Difluorobenzoic acid		158.10
50	2CA	2-Chlorobenzoic acid		156.57
51	34 CA	3,4-Dichloro-benzoic acid		191.01

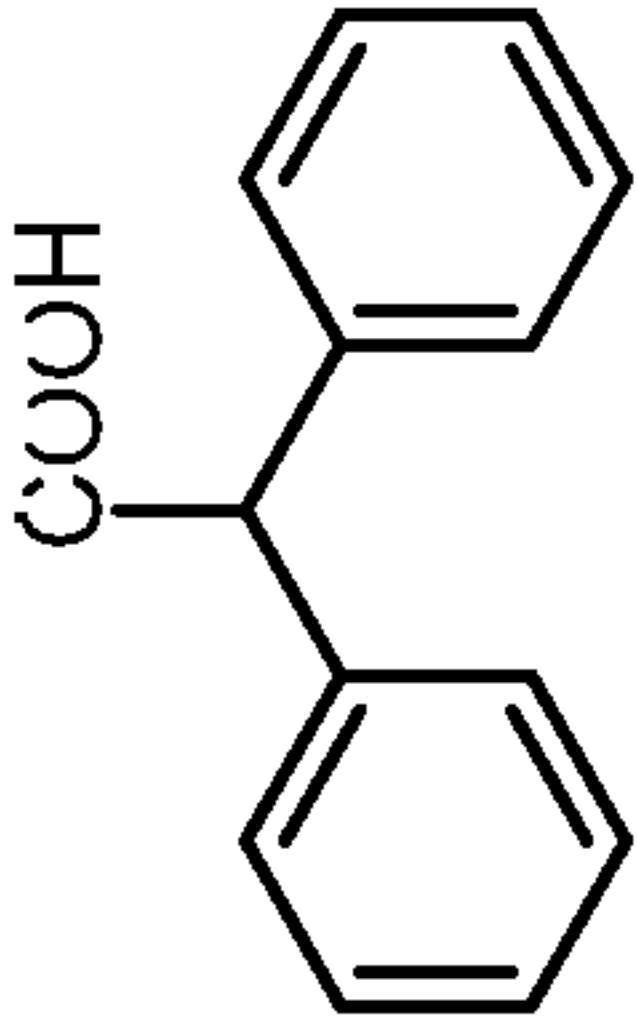
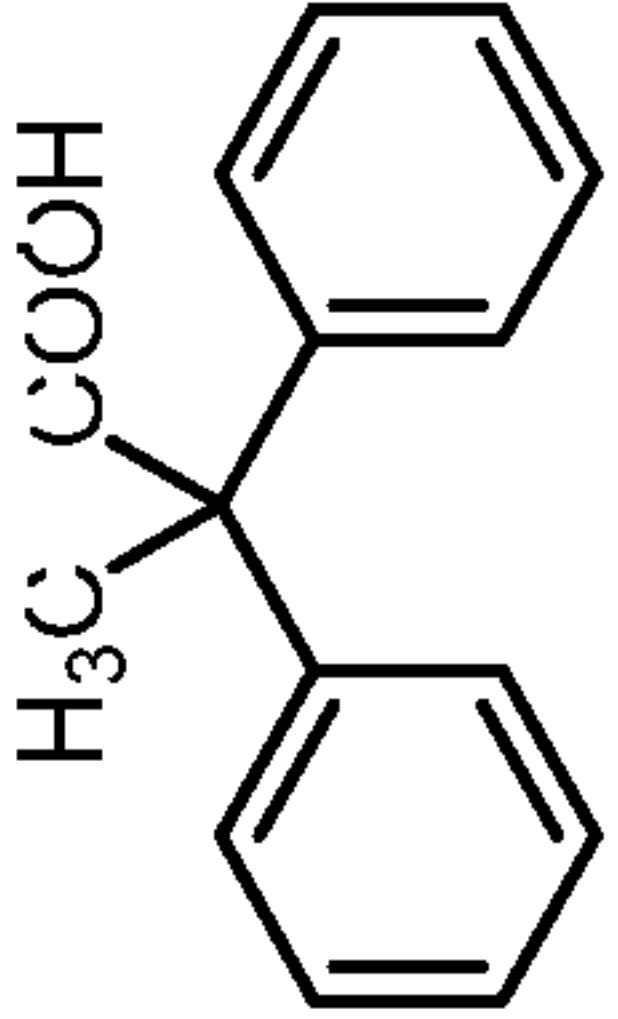
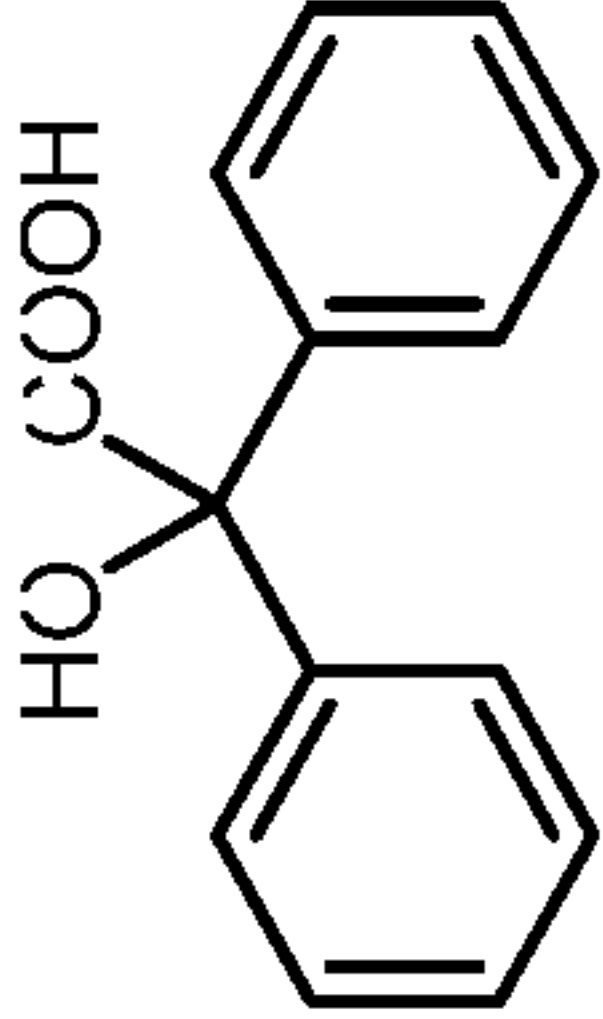
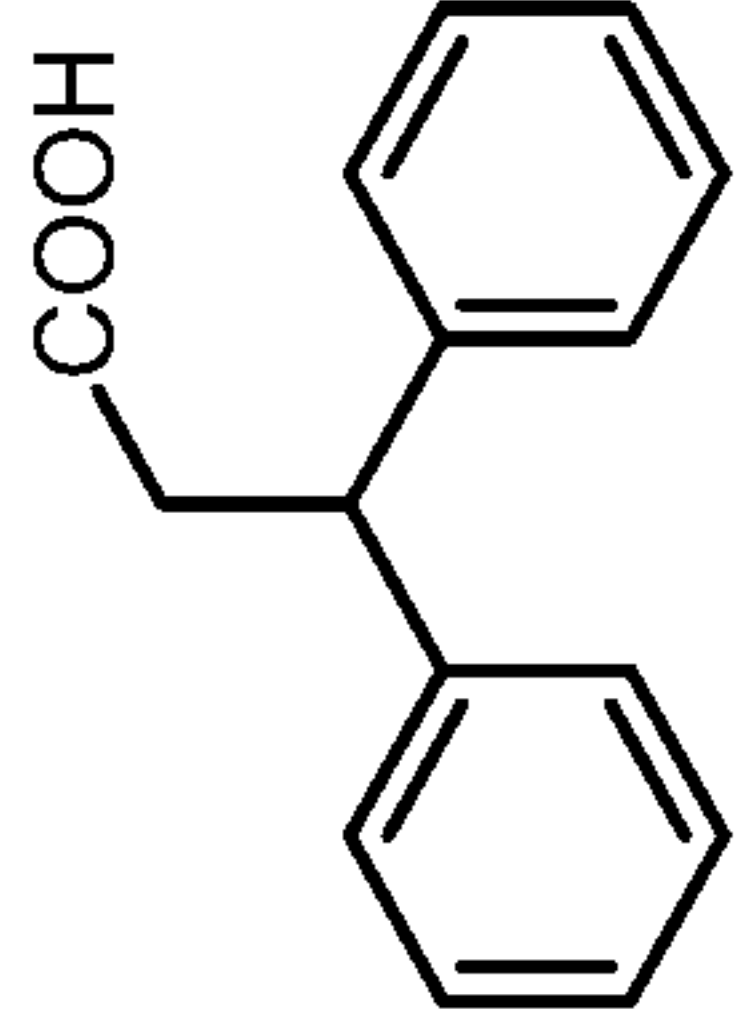
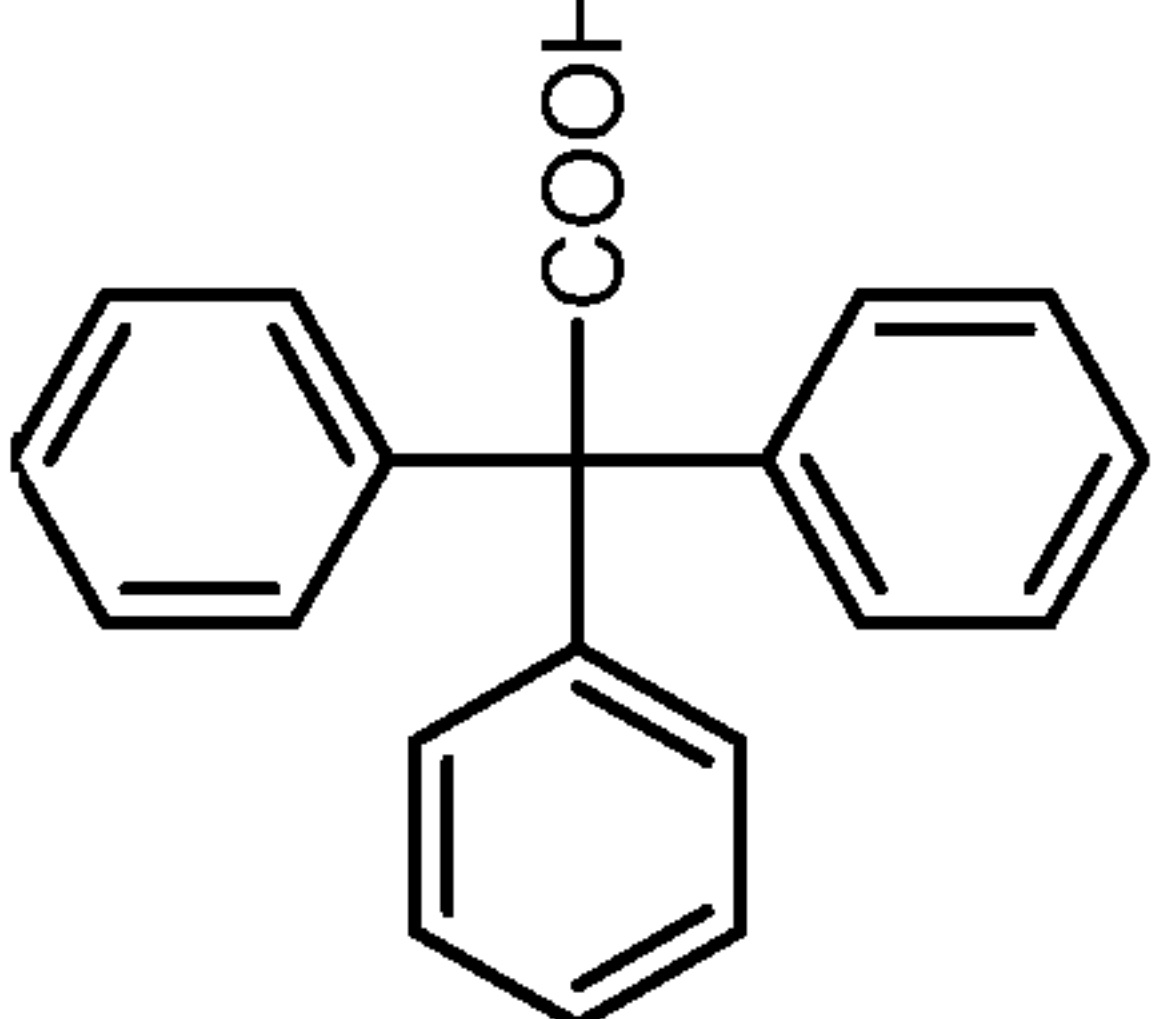
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entry	abbreviation	full name	chemical structure	molar mass [g/mol]
52	RMA	(R)-Mandelic acid		152.15
53	SMA	(S)-Mandelic acid		152.15
54	CMA	R-(-)-2-Chloromandelic acid		186.59
55	PAA	Phenylacetic acid		136.15
56	PMA	Phenyl-malonic acid		180.16
57	PA	Phthalic acid		166.13
58	TPA	Terephthalic acid		166.13

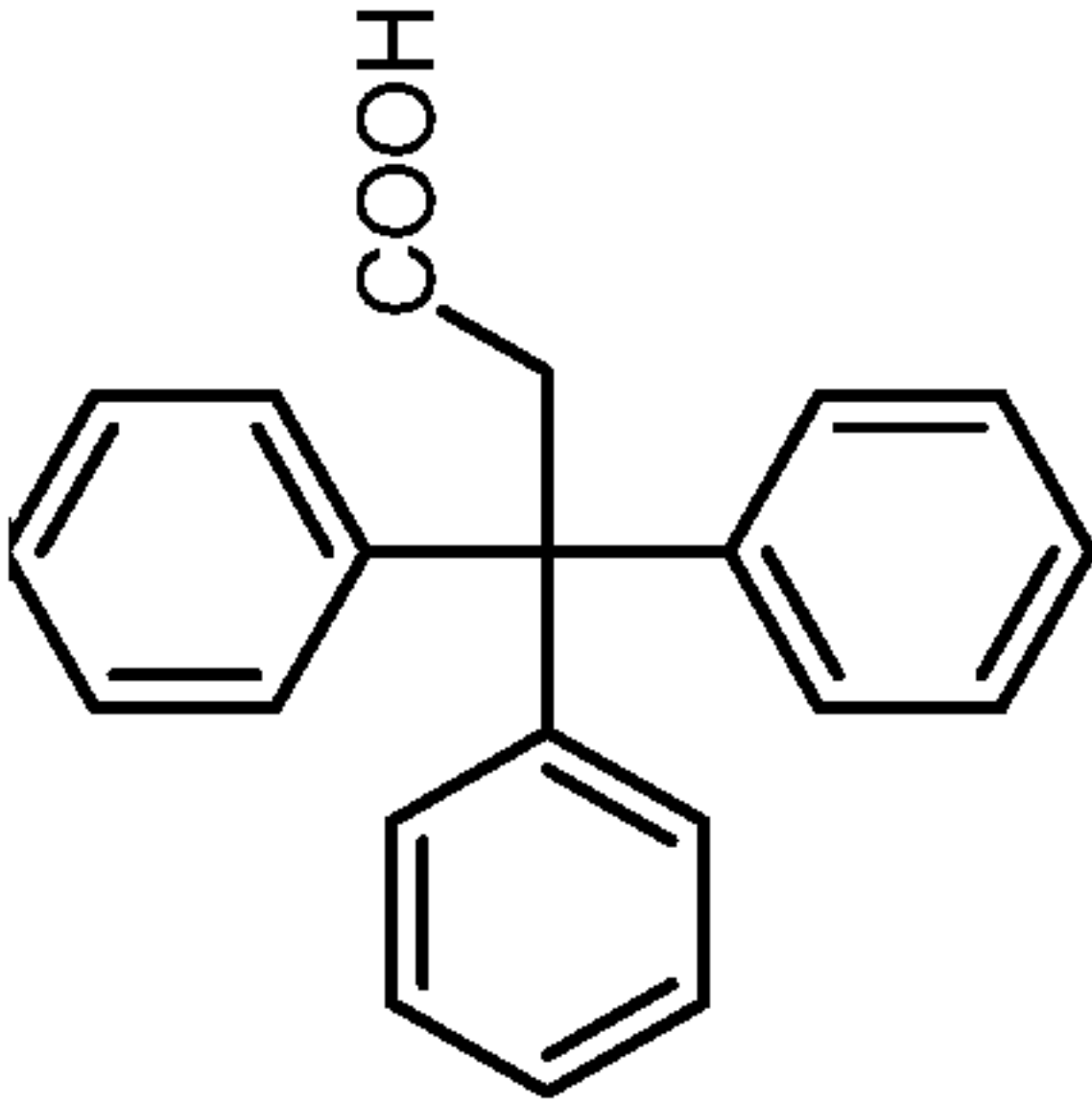
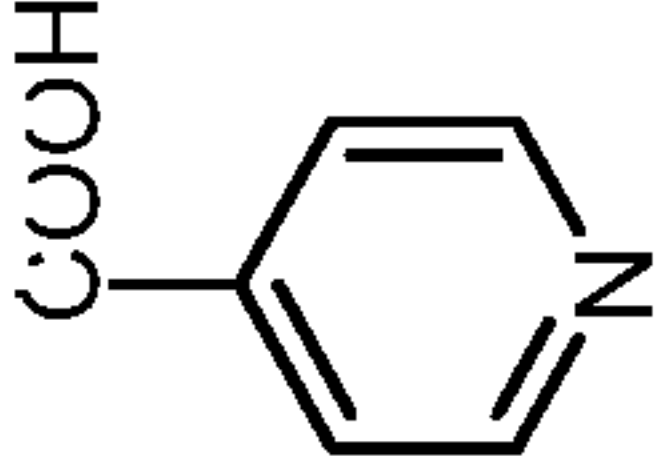
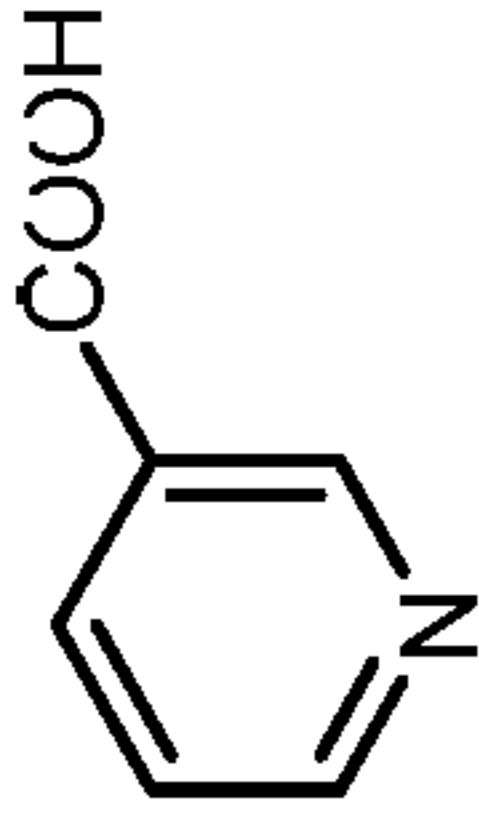
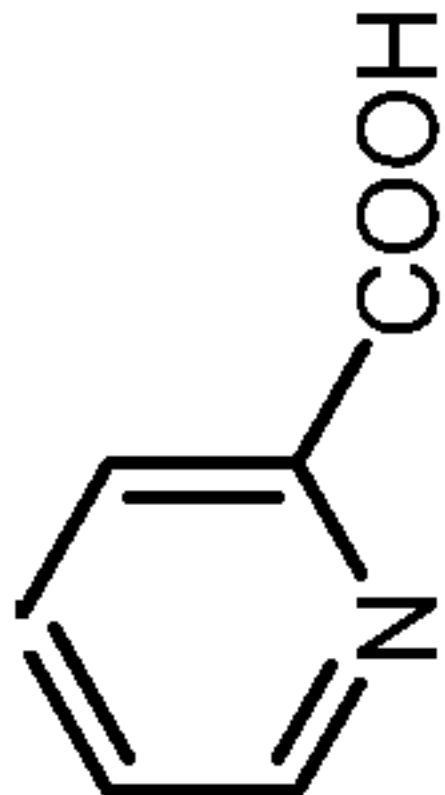
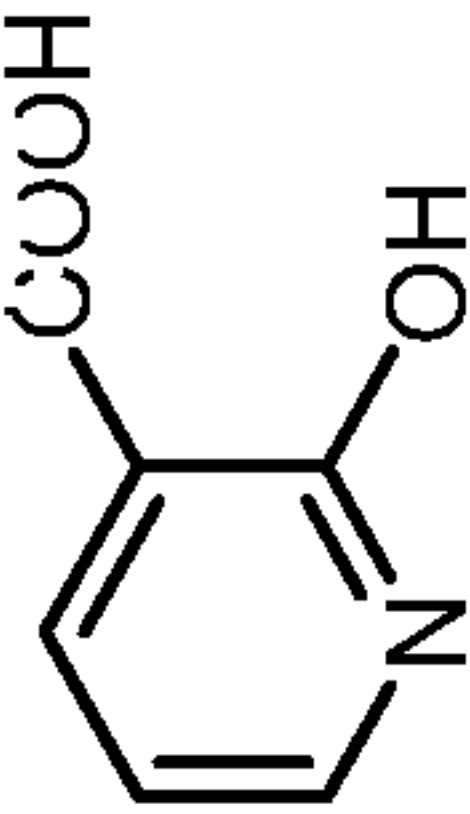
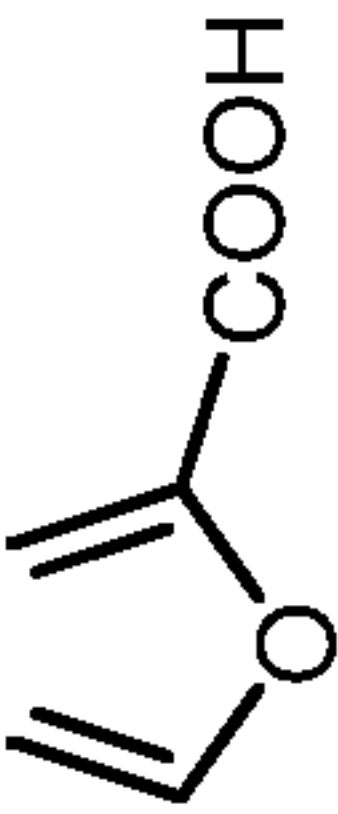
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entry	abbreviation	full name	chemical structure	molar mass [g/mol]
59	BTA	1,2,4,5-Benzene-tetracarboxylic acid		254.15
60	CA	Caffeic acid		180.16
61	FCA	Ferulic acid		194.18
62	34HHCAA	3,4-Dihydroxyhydrocinnamic acid		182.17
63	PCPA	1-Phenylcyclopentane-carboxylic acid		190.24
64	BPA	4-Biphenyl-carboxylic acid		198.22

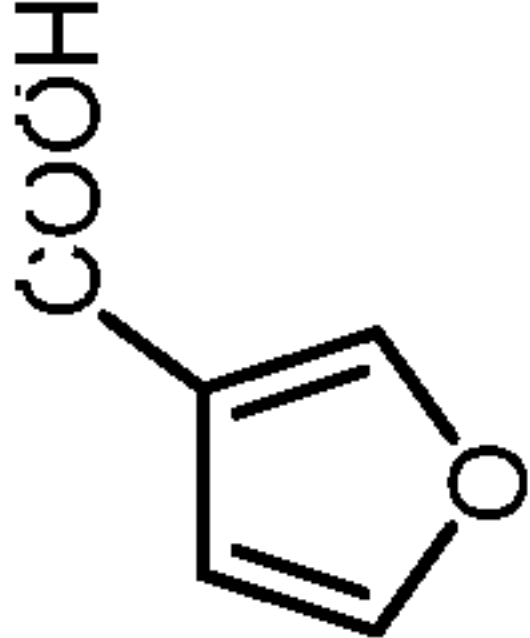
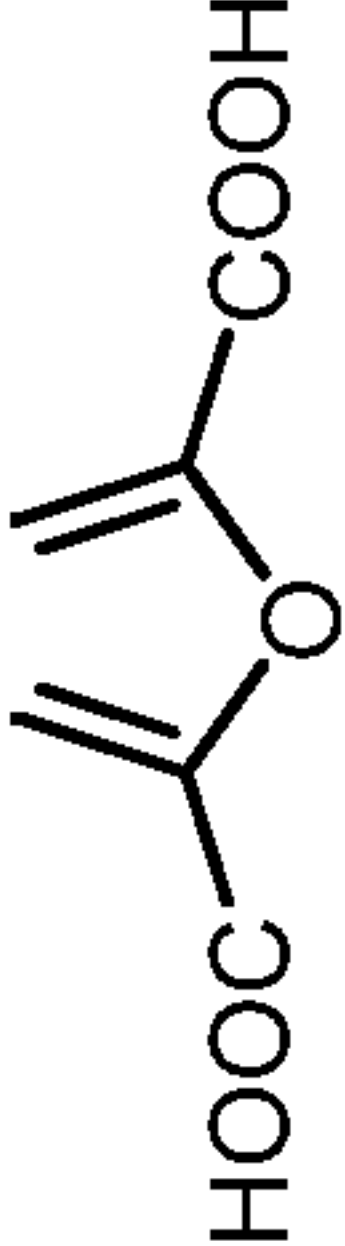
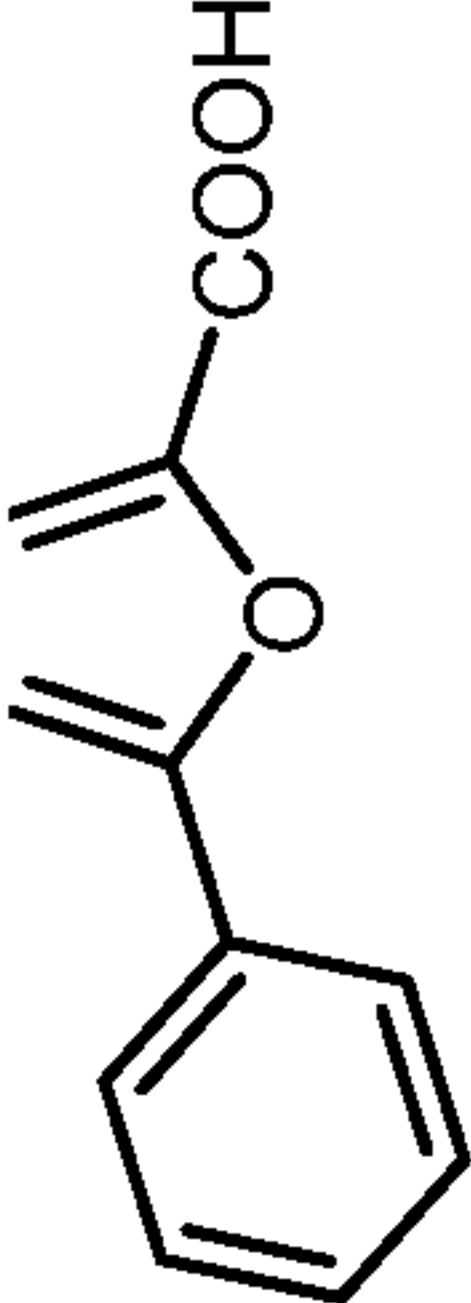
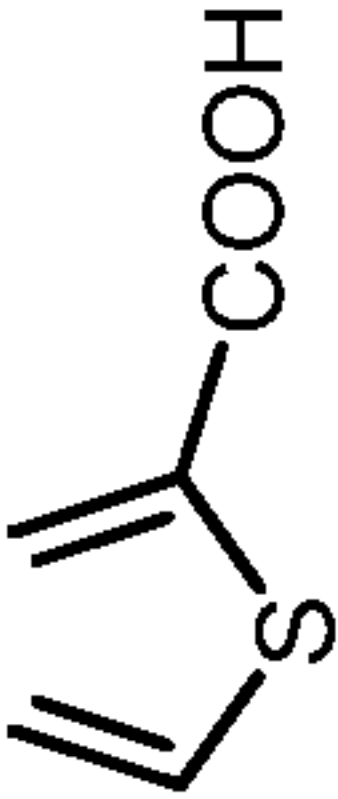
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entry	abbreviation	full name	chemical structure	molar mass [g/mol]
65	DPAA	Diphenylacetic acid		212.24
66	2DPPA	2,2'-Diphenylpropionic acid		226.27
67	BA	Benzilic acid		228.25
68	3DPPA	3,3-Diphenylpropionic acid		226.27
69	TPAA	Triphenyl-acetic acid		288.34

(continued)

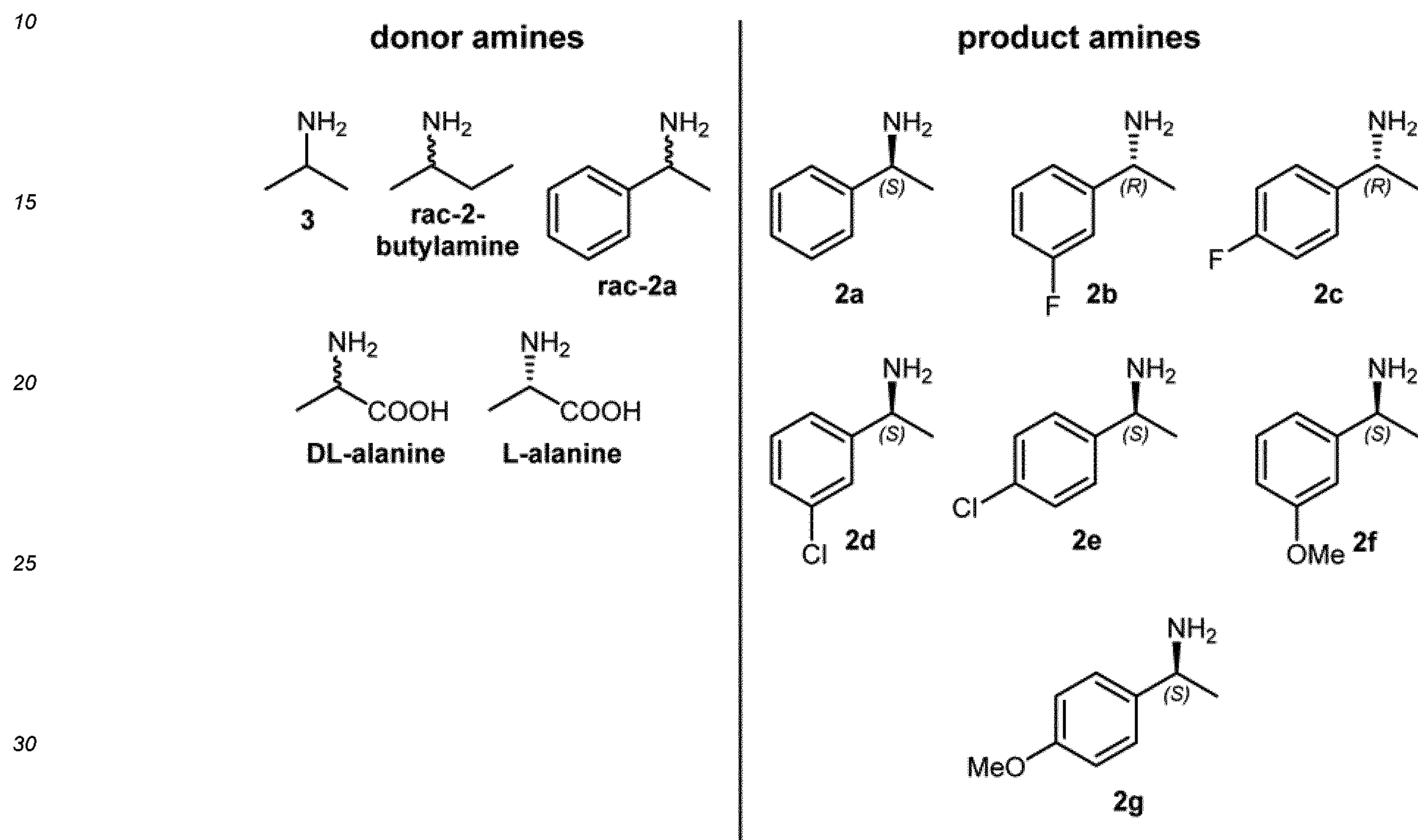
entry	abbreviation	full name	chemical structure	molar mass [g/mol]
70	TPPA	3,3,3-Triphenyl-propionic acid		302.37
71	INA	Isonicotinic acid		123.11
72	NCC	Nicotinic acid		123.11
73	PCC	Picolinic acid		123.11
74	2HNA	2-Hydroxy-nicotinic acid		139.11
75	2FCA	2-Furoic acid		112.08

(continued)

entry	abbreviation	full name	chemical structure	molar mass [g/mol]
76	3FCA	3-Furoic acid		112.08
77	25FDCA	2,5-Furandicarboxylic acid		156.09
78	PFA	5-Phenyl-2-furoic acid		188.18
79	2TPCA	2-Thiophenecarboxylic acid		128.15

3. Precipitation screening for suitable acids

[0075] Screening of suitable acids was conducted with enantiomerically pure 1-phenylethylamine **2a** and 6 derivatives thereof as model product amines at 50 mM. As typical donor amines racemic 2-butylamine, racemic alanine & L-alanine (at 100 mM) and isopropylamine **3** & racemic 1-phenylethylamine **rac-2a** (at 250 mM & 1000 mM) were chosen (scheme 3). The choice of the respective product amine enantiomer is not relevant for solubility screening since enantiomers have identical physical-chemical properties (incl. solubility), except its rotation of plane-polarized light in opposite directions. Results with racemic amines may differ significantly.



Scheme 3: Exemplary product and donor amines for acid screening

Neutralized acid solutions:

[0076] All 79 separate 400 mM acid solutions (typically 20 mL) were prepared by dissolving the respective acid in 50 mM phosphate buffer pH 7.5. Afterwards within all resulting solutions the pH was carefully adjusted back to 7.5. Please note that acids with low aqueous solubility may require multiple pH adjustments due to an additional dissolution process after its previous pH-adjustment. Acid solution with a remaining solid fraction were filtered and used in their resulting (unknown) concentration.

Neutralized amine solutions:

[0077] The selected exemplary product amines were dissolved in 50 mM phosphate buffer pH 7.5 with a concentration of 100 mM each and the pH carefully adjusted back to 7.5. Similarly solutions of the donor amines were prepared (200 mM for racemic 2 butylamine, racemic alanine and L-alanine and 500 mM & 2000 mM for isopropylamine and racemic 1-phenylethylamine).

Precipitation screening procedure

[0078] 200 μ l fractions of the neutralized acid solutions (79 in total) were given each into a 96 well plate and their position documented. Afterwards 200 μ l of one neutralized amine solution was added into each filled well, which led to a clear solution or an almost instantaneous precipitation. The result was documented by visual observation and photography against a black background after 1 and 24 h. Fig. 3 shows an exemplary result for (S)-4-Chloro-1-phenylethylamine salts after 1 hour. The procedure was repeated for all amine solutions and the results compared against each

other (table 4). The intensity of precipitation, as shown by shade code, serves as a qualitative approximation of salt solubilities and their differences.

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Table 4: graphical presentation of the screening results

exemplary donor amines										exemplary product amines					
	isopropyl-amine	rac-1-phenylethylamine		2-butylamine	LD-alanine	L-alanine	(S)-1-PEA 2a	(S)-3Cl-1-PEA	(S)-4Cl-1-PEA	(R)-3F-1-PEA	(R)-4F-1-PEA	(S)-3MeO-1-PEA	(R)-4MeO-1-PEA		
		rac-2a						2b	2c	2d	2e	2f	2g		
	3	1000 mM	250 mM	1000 mM	250 mM	each 100 mM									
conc.				each 100 mM											
24CNA															
25CNA															
25DHBA															
25FDCA															
26FBA															
2CA															
2DPPA															
2FCA															
2HNA															
2TPCA															
32CNA															
34CA															
34HHCAA															
34NA															
35DNOT															
35HBA															
3DPPA															
3FCA															
3NA															
42CNA															

(continued)

	exemplary donor amines					exemplary product amines							
	isopropyl-amine	rac-1-phenylethylamine		2-butylamine	LD-alanine	L-alanine	(S)-1-PEA 2a	(S)-3Cl-1-PEA	(S)-4Cl-1-PEA	(R)-3F-1-PEA	(R)-4F-1-PEA	(S)-3MeO-1-PEA	(R)-4MeO-1-PEA
	3	rac-2a						2b	2c	2d	2e	2f	2g
	1000 mM	250 mM	1000 mM	250 mM	each 100 mM								
conc.													
435CNBA													
43CNA													
43HNBA													
4HBA													
AA													
ACC													
APS													
ASA													
BA													
BCA													
BPA													
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CCA													
CHCA													
CMA													
DBDT													
DCC													
DPAA													

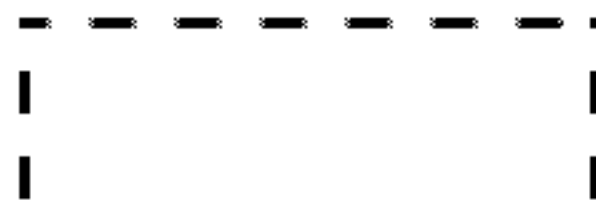
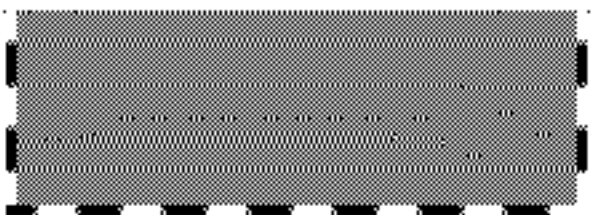
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	exemplary donor amines						exemplary product amines						
	isopropyl-amine	rac-1-phenylethylamine		2-butylamine	LD-alanine	L-alanine	(S)-1-PEA 2a	(S)-3Cl-1-PEA	(S)-4Cl-1-PEA	(R)-3F-1-PEA	(R)-4F-1-PEA	(S)-3MeO-1-PEA	(R)-4MeO-1-PEA
	3	rac-2a	2b					2c	2d	2e	2f	2g	
	1000 mM	250 mM	1000 mM	250 mM	each 100 mM								
conc.				each 100 mM									
DQA													
DTA													
FA													
FCA													
FCC													
GA													
GCA													
HA													
HXCA													
INA													
IS													
IVA													
LMA													
LTA													
MA													
NCC													
nNA													
OA													
PA													
PAA													

(continued)

exemplary donor amines														exemplary product amines						
	isopropyl-amine		rac-1-phenylethylamine		2-butylamine	LD-alanine	L-alanine	(S)-1-PEA 2a	(S)-3Cl-1-PEA	(S)-4Cl-1-PEA	(R)-3F-1-PEA	(R)-4F-1-PEA	(S)-3MeO-1-PEA	(R)-4MeO-1-PEA						
	3																			
	1000 mM	250 mM	1000 mM	250 mM	each 100 mM				2b	2c	2d	2e	2f	2g						
conc.					each 100 mM				each 100 mM											
PCC																				
PCPA																				
PFA																				
PIA																				
PMA																				
PPA																				
RMA																				
SA																				
SBCA																				
SCA																				
SCCA																				
SMA																				
TG																				
TMPA																				
TPA																				
TPAA																				
TPPA																				
VA																				
VCA																				
24CNA																				

[0079] description of symbols and grey shade code for Table 3

5		
	no precipitation	
10		
	few crystals	
15		
	medium precipitation	
		
	significant precipitation	

[0080] The results of table 3 show clearly that certain acids allow significant differences in solubility between investigated salts. In this screening especially differences between product amines and the commonly used donor amines isopropylamine/alanine were targeted. Based on this consideration the following acids appear most applicable: 34CA, 34NA, 43CNA and 3DPPA. In addition, 2DPPA was identified as suitable acid. Acids with less strong differences are PCPA, 24CNA, 25CNA, 35DNOT, 3NA and 435CNBA, which might be useful for other product amines. PCPA was later excluded from the list of potential acids due to an unknown decomposition reaction of certain salt solutions (strong discoloration).

4. transaminase-catalyzed synthesis of product amines in combination with an *in situ*-product crystallization

[0081] General semi-preparative procedure for the amine transaminase-catalyzed synthesis of **2a-k** in combination with an *in situ*-product crystallization of the product amine **6a-k** via 3DPPA: To **25** ml 200 mM phosphate buffer pH 7.5 532 μ L isopropylamine ($\approx$ 250 mM) and 707 mg 3DPPA ($\approx$ 125 mM) were given and the resulting suspension adjusted to pH 7.5 with aqueous H_3PO_4 -solution. Afterwards PLP, substrate ($\approx$ 100 mM) and biocatalyst were added and the resulting mixture shaken at 200 rpm. After completion of the reaction the resulting mixture was filtered to obtain the formed product amine salt. (This solid will contain remaining biocatalyst and excess 3DPPA.) Afterwards the solid fraction was washed with 10 mL MTBE to remove remaining substrate and parts of excess 3DPPA. The solid was then given into 5 mL water, 0,5 ml conc. NaOH was added to increase pH and formed product **2** extracted with 5 mL MTBE. After phase separation the product was obtained as its hydrochloride by a slow addition of conc. HCl to the ether phase. 3DPPA can be precipitated from the remaining aqueous solution by adding conc. HCl, e.g. for recycling (isolated yield 71 %).

[0082] General reaction control procedure: Samples (500 μ L) were taken periodically and thoroughly mixed by a vortex mixer with 50 μ l conc. NaOH to quench the reaction and increase pH. Afterwards 500 μ L MTBE were added, mixed again by a vortex mixer and centrifuged (2 min, 3000 rpm) to improve phase separation. 200 μ l were taken from the organic layer, combined with 50 μ l of a 25 mM *n*-decane-solution in MTBE (internal standard) and subsequently analyzed by gas chromatography (column: CP-Chirasil-Dex CB; 25 m, 0.25 mm, 0.25 μ m by Agilent, USA)

SEQUENCE LISTING**[0083]**

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Universität Rostock

<120> Method for preparing amines from carbonyl compounds by transaminase reaction under salt precipitation

<130> PVA1235EP

<160> 7

<170> PatentIn version 3.3

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25

Asp Gly Arg Phe Phe Arg Leu Asp Asp His Leu Gln Arg Ile Leu Glu
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30

Ser Cys Asp Lys Met Arg Leu Lys Phe Pro Leu Ala Leu Ser Ser Val
85 90 95

35

Lys Asn Ile Leu Ala Glu Met Val Ala Lys Ser Gly Ile Arg Asp Ala
100 105 110

Phe Val Glu Val Ile Val Thr Arg Gly Leu Thr Gly Val Arg Gly Ser
115 120 125

40

Lys Pro Glu Asp Leu Tyr Asn Asn Asn Ile Tyr Leu Leu Val Leu Pro
130 135 140

45

Tyr Ile Trp Val Met Ala Pro Glu Asn Gln Leu His Gly Gly Glu Ala
145 150 155 160

Ile Ile Thr Arg Thr Val Arg Arg Thr Pro Pro Gly Ala Phe Asp Pro

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	Ala	Met	Asp	Arg	Gly	Ala	Thr	Tyr	Pro	Phe	Leu	Thr	Asp	Gly	Asp	Thr	
10			195					200					205				
	Asn	Leu	Thr	Glu	Gly	Ser	Gly	Phe	Asn	Ile	Val	Leu	Val	Lys	Asn	Gly	
		210					215					220					
15	Ile	Ile	Tyr	Thr	Pro	Asp	Arg	Gly	Val	Leu	Arg	Gly	Ile	Thr	Arg	Lys	
	225					230					235					240	
	Ser	Val	Ile	Asp	Val	Ala	Arg	Ala	Asn	Ser	Ile	Asp	Ile	Arg	Leu	Glu	
20					245					250					255		
	Val	Val	Pro	Val	Glu	Gln	Ala	Tyr	His	Ser	Asp	Glu	Ile	Phe	Met	Cys	
25				260					265					270			
	Thr	Thr	Ala	Gly	Gly	Ile	Met	Pro	Ile	Thr	Leu	Leu	Asp	Gly	Gln	Pro	
			275					280					285				
30	Val	Asn	Asp	Gly	Gln	Val	Gly	Pro	Ile	Thr	Lys	Lys	Ile	Trp	Asp	Gly	
		290					295					300					
	Tyr	Trp	Glu	Met	His	Tyr	Asn	Pro	Ala	Tyr	Ser	Phe	Pro	Val	Asp	Tyr	
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	Gly	Ser	Gly														

- 40<210> 2<211> 325<212> PRT<213> Gibberella zeae
- 45<400> 2

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				20					25					30		
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			35					40					45			
	Gln	Gly	Phe	Met	His	Gly	Asp	Leu	Thr	Tyr	Asp	Val	Pro	Ala	Val	Trp
15																
20																
25																
30																
35																
40																
45																
50																
55																

	50					55					60					
5	Asp 65	Gly	Arg	Phe	Phe	Arg 70	Leu	Asp	Asp	His	Leu 75	Asp	Arg	Leu	Glu	Ala 80
10	Ser	Val	Lys	Lys	Met 85	Arg	Met	Gln	Phe	Pro 90	Ile	Pro	Arg	Asp	Glu 95	Ile
	Arg	Met	Thr	Leu 100	Leu	Asp	Met	Leu	Ala 105	Lys	Ser	Gly	Ile	Lys 110	Asp	Ala
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REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

- WO 2011026556 A [0004]

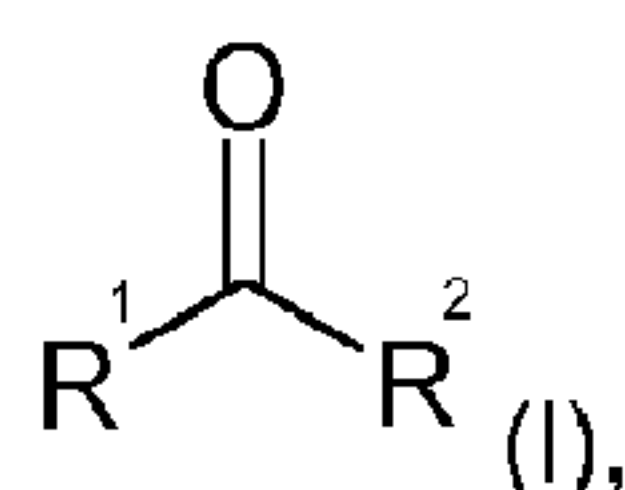
Non-patent literature cited in the description

- **BUQUE-TABOADA et al.** *Applied Microbiology and Biotechnology*, 2006, 1-12 [0005]

KRAV

1. Fremgangsmåde til fremstilling af en aminosaltforbindelse omfattende:

- 5 i) tilvejebringelse af en carbonylforbindelse med den generelle formel (I)



10 hvor

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R¹ er valgt fra gruppen bestående af forgrenet eller uforgrenet C2-C20-alkyl, forgrenet eller uforgrenet C2-C20-alkenyl, forgrenet eller uforgrenet C2-C20-alkinyl, forgrenet eller uforgrenet C1-C5-alkyl-O-C1-C5-alkyl, forgrenet eller uforgrenet C1-C10-alkoxy, C4-C20-cycloalkyl, C5-C20-cycloalkenyl, C5-C20-cycloalkinyl, C5-C20-aryl, C6-C20-alkylaryl, C6-C20-arylalkyl, C2-C20-heteroalkyl, C3-C20-cyklisk heteroalkyl, C4-C20-heteroaryl, C5-C20-alkylheteroaryl og C5-C20-heteroarylalkyl, hvori ringsystemerne, i tilfælde af mere end ét (hetero)-alifatisk eller (hetero)-aromatisk ringsystem, er kondenserede eller separate, idet hver rest R¹ har mindst én substituent R^{1a} valgt fra gruppen bestående af hydrogenatom, halogenatom (F, Cl, Br, I), hydroxyl, thiol, C1-C3 thioester, C1-C3-thioether, C1-C3-alkyl og C1-C3-alkoxy; og

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R² er valgt fra gruppen bestående af hydrogenatom, forgrenet eller uforgrenet C1-C5-alkyl, forgrenet eller uforgrenet C1-C5-alkyl-O-C1-C5-alkyl, forgrenet eller uforgrenet C1-C10-alkoxy, C5-C10-aryl C6-C10-alkylaryl og C6-C10-arylalkyl, idet ringsystemerne i tilfælde af mere end ét aromatisk ringsystem er kondenserede eller separate, idet hver rest R² har mindst én substituent R^{2a} valgt fra gruppen bestående af hydrogenatom, halogenatom (F, Cl, Br, I), hydroxyl, thiol, C1-C3 thioester, C1-C3-thioether, C1-C3-alkyl og C1-C3-alkoxy; eller

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R¹, R² sammen danner en C3-C10-cycloalkyl eller C3-C10-cycloalkenyl, hvor C3-C10-cycloalkyl eller C3-C10-cycloalkenyl hver har mindst én substituent R^x valgt fra gruppen bestående af hydrogenatom, C1-C5-alkyl, C1-C4-heteroalkyl og C1-C5-alkyl-R^y, hvor R^y er hydroxyl eller thiol;

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ii) omsætning af carbonylforbindelsen tilvejebragt ifølge (i) i tilstedeværelse af en transaminase med

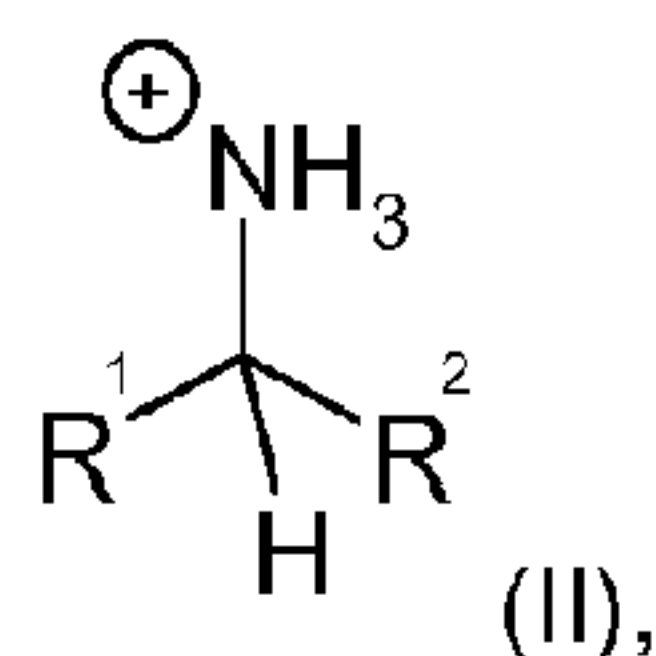
ii-a) mindst en primær amin; og

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ii-b) mindst en carboxylsyre;

hvorved der opnås en blanding omfattende en mindst delvis krystalliseret aminosaltforbindelse omfattende

- en kation med den generelle formel (II)



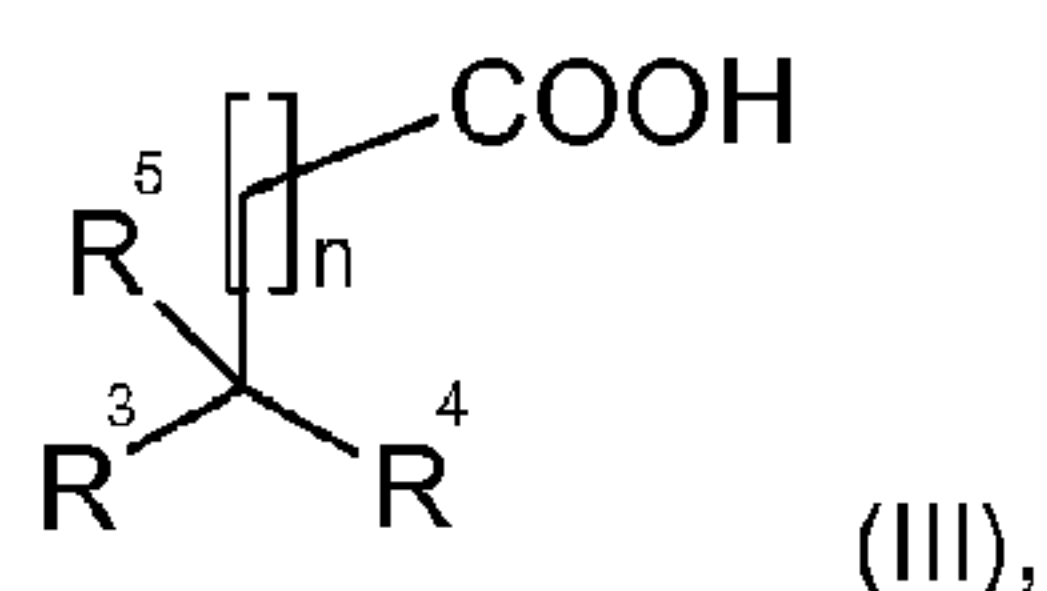
5

hvor R^1 og R^2 er som defineret for den generelle formel (I) og

- en carboxylat-anion baseret på den mindst ene carboxylsyre tilsat ifølge (ii-b);

hvor den mindst ene carboxylsyre ifølge (ii-b) er én carboxylsyre med den generelle formel (III)

10



hvor

15

n er nul eller 1;

resterne R^3 og R^4 begge er phenyl eller sammen danner en phenylring, hvor hver phenylring har mindst én yderligere substituent valgt fra gruppen bestående af hydrogenatom, halogenatom og nitrogruppe; og resten R^5 er hydrogenatom eller methyl eller fraværende, hvis R^3 og R^4 sammen danner en phenylring.

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2. Fremgangsmåde ifølge krav 1, hvor aminosaltforbindelsen opnået ifølge (ii) har en opløselighed i vand ved pH 7, som er mindre end opløseligheden i vand af mindst en primær amin tilsat ifølge (ii-a).

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3. Fremgangsmåde ifølge krav 1 eller 2, hvor resten R^1 er valgt fra gruppen bestående af forgrenet eller uforgrenet C2-C10-alkyl, C5-C10-cycloalkyl og C5-C20-aryl, idet ringsystemerne i tilfælde af mere end et alifatisk eller aromatisk ringsystem er kondenserede eller separate, idet hver rest R^1 har mindst én substituent R^{1a} valgt fra gruppen bestående af hydrogenatom, halogenatom, C1-C3-alkyl og C1-C3-alkoxy.

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4. Fremgangsmåde ifølge et hvilket som helst af kravene 1 til 3, hvor resten R^2 er valgt fra gruppen bestående af hydrogenatom, forgrenet eller uforgrenet C1-C5-alkyl, idet hver rest R^2 har mindst én substituent R^{2a} valgt fra gruppen bestående af hydrogenatom, halogenatom, C1-C3-alkyl og C1-C3-alkoxy.

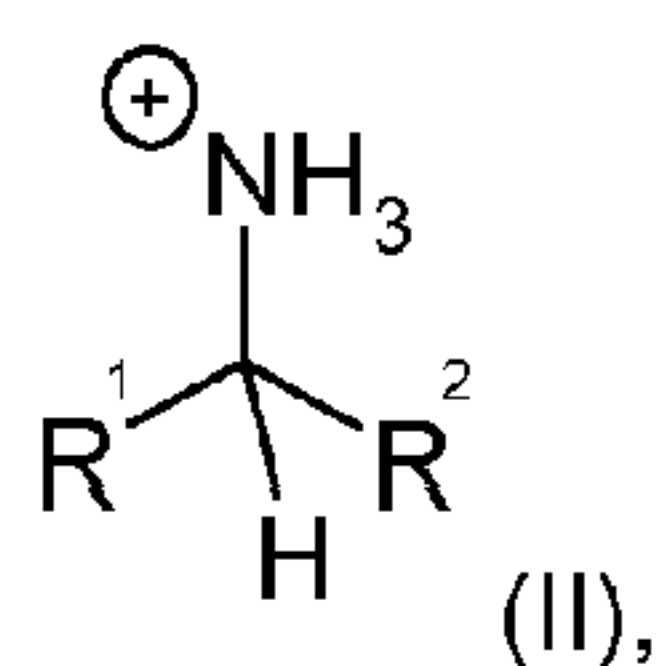
5. Fremgangsmåde ifølge et hvilket som helst af kravene 1 til 4, hvor transaminasen ifølge (ii) er valgt fra gruppen af amintransaminaser.

6. Fremgangsmåde ifølge et hvilket som helst af kravene 1 til 5, hvor den mindst ene primære amin ifølge (ii-a) er valgt fra gruppen af mono- og diaminer med ét til 10 carbonatomer.

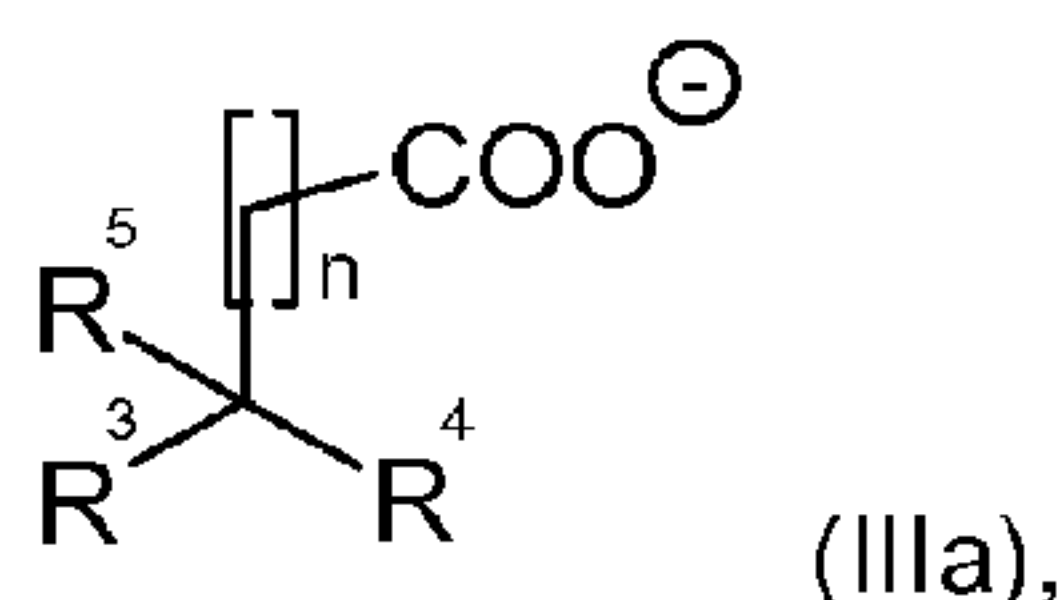
7. Fremgangsmåde ifølge et hvilket som helst af kravene 1 til 6, hvor den mindst ene carboxylsyre ifølge (ii-b) er valgt fra gruppen bestående af diphenyleddikesyre (DPAA), 2,2-diphenylpropionsyre (2DPPA), 3,3-diphenylpropionsyre (3DPPA), 3,4-dichlorbenzosyre (34CA), 3,4-dinitrobenzosyre (34NA) og 4-chlor-3-nitrobenzosyre (43CNA).

8. Fremgangsmåde ifølge et hvilket som helst af kravene 1 til 7, hvor den mindst ene primære amin ifølge (ii-a) og den mindst ene carboxylsyre ifølge (ii-b) anvendes som ét eller flere salte omfattende den protonerede form af den mindst ene primære amin og den deprotonerede form af den mindst ene carboxylsyre.

9. Fremgangsmåde ifølge et hvilket som helst af kravene 1 til 8, hvor aminosaltforbindelsen opnået ifølge (ii) omfatter en kation med den generelle formel (II)



hvor R^1 og R^2 er som defineret for den generelle formel (I), og en anion baseret på den mindst ene carboxylsyre, som er en anion med den generelle formel (IIIa)



hvor

n er nul eller 1;

resterne R^3 og R^4 begge er phenyl eller sammen danner en phenylring, hvor hver phenylring har mindst én yderligere substituent valgt fra gruppen bestående af hydrogenatom, halogenatom, fortrinsvis chlor og nitrogruppe, og resten R^5 er hydrogenatom eller methyl eller fraværende, hvis R^3 og R^4 tilsammen danner en phenylring.

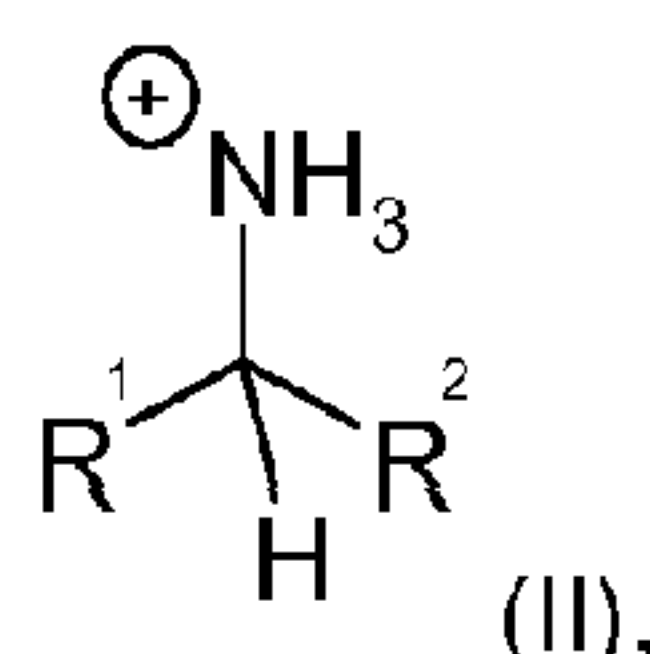
10. Fremgangsmåde ifølge et hvilket som helst af kravene 1 til 9, yderligere omfattende:

- 5 iii) adskillelse af den i det mindste delvist krystalliserede aminsaltforbindelse opnået ifølge (ii) fra blandingen, hvorved den krystalliserede aminsaltforbindelse opnås.

11. En aminosaltforbindelse omfattende

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- en kation med den generelle formel (II)



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hvor

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R^1 er valgt fra gruppen bestående af forgrenet eller uforgrenet C2-C20-alkyl, forgrenet eller uforgrenet C2-C20-alkenyl, forgrenet eller uforgrenet C2-C20-alkinyl, forgrenet eller uforgrenet C1-C5-alkyl-O-C1-C5-alkyl, forgrenet eller uforgrenet C1-C10-alkoxy, C4-C20-cycloalkyl, C5-C20-cycloalkenyl, C5-C20-cycloalkinyl, C5-C20-aryl, C6-C20-alkylaryl, C6-C20-arylalkyl, C2-C20-heteroalkyl, C3-C20-cyklisk heteroalkyl, C4-C20-heteroaryl, C5-C20-alkylheteroaryl og C5-C20-heteroarylalkyl, hvori ringsystemerne, i tilfælde af mere end ét (hetero)-alifatisk eller (hetero)-aromatisk ringsystem er kondenserede eller separate, idet hver rest R^1 har mindst én substituent R^{1a} valgt fra gruppen bestående af hydrogenatom, halogenatom (F, Cl, Br, I), hydroxyl, thiol, C1-C3 thioester, C1-C3-thioether, C1-C3-alkyl og C1-C3-alkoxy; og

25

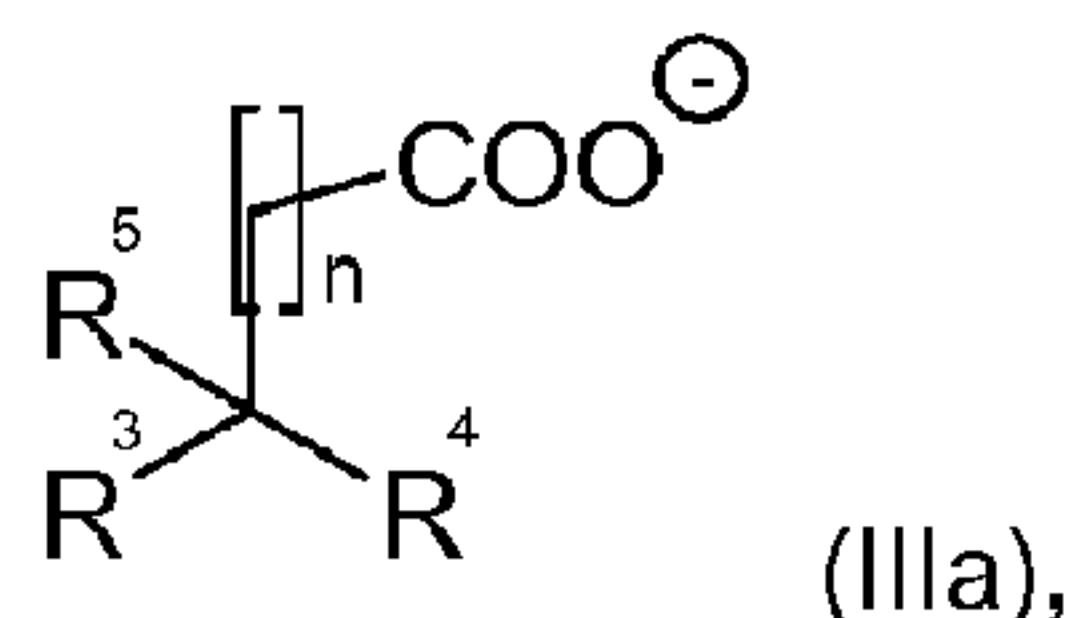
R^2 er valgt fra gruppen bestående af hydrogenatom, forgrenet eller uforgrenet C1-C5-alkyl, forgrenet eller uforgrenet C1-C5-alkyl-O-C1-C5-alkyl, forgrenet eller uforgrenet C1-C10-alkoxy, C5-C10-aryl, C6-C10-alkylaryl og C6-C10-arylalkyl, hvori ringsystemerne, i tilfælde af mere end ét alifatisk eller aromatisk ringsystem, er kondenserede eller separate, idet hver rest R^2 har mindst én substituent R^{2a} valgt fra gruppen bestående af hydrogenatom, halogenatom (F, Cl, Br, I), hydroxyl, thiol, C1-C3 thioester, C1-C3-thioether, C1-C3-alkyl og C1-C3-alkoxy; eller R^1 , R^2 tilsammen danner en C3-C10-cycloalkyl eller C3-C10-cycloalkenyl, hvor C3-C10-cycloalkyl eller C3-C10-cycloalkenyl hver har mindst én substituent R^x valgt fra gruppen bestående af hydrogenatom, C1-C5-alkyl, C1-C4-heteroalkyl og C1-C5-alkyl- R^y , hvor R^y er hydroxyl eller thiol;

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og

- en anion med den almene formel (IIIa)



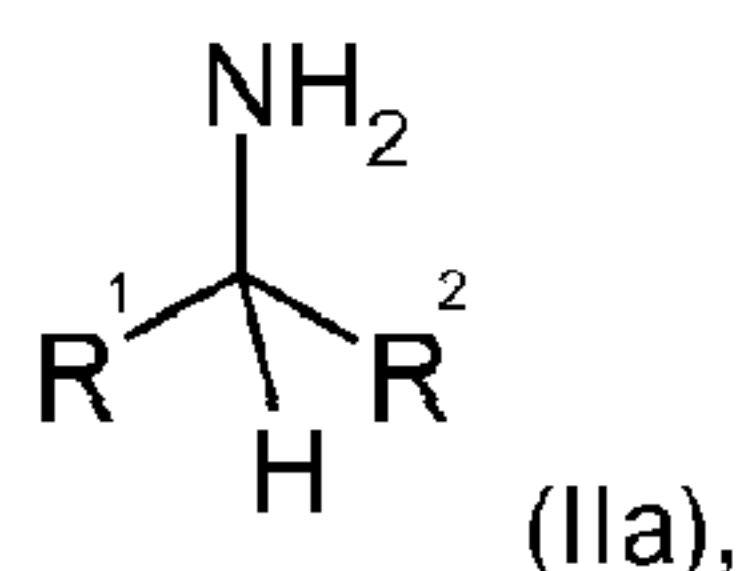
5 hvor

n er nul eller 1;

resterne R^3 og R^4 begge er phenyl eller sammen danner en phenylring, hvor hver phenylring har mindst én yderligere substituent valgt fra gruppen bestående af hydrogenatom, halogenatom og nitrogruppe, og resten R^5 er hydrogenatom eller methyl eller fraværende, hvis R^3 og R^4 sammen danner en phenylring.

12. En sammensætning omfattende

15 a) en amin med den generelle formel (IIa)



hvor

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R^1 er valgt fra gruppen bestående af forgrenet eller uforgrenet C2-C20-alkyl, forgrenet eller uforgrenet C2-C20-alkenyl, forgrenet eller uforgrenet C2-C20-alkinyl, forgrenet eller uforgrenet C1-C5-alkyl-O-C1-C5-alkyl forgrenet eller uforgrenet C1-C10-alkoxy, C4-C20-cycloalkyl, C5-C20-cycloalkenyl, C5-C20-cycloalkinyl, C5-C20-aryl, C6-C20-alkylaryl, C6-C20-arylalkyl, C2-C20-heteroalkyl, C3-C20-cyklisk heteroalkyl, C4-C20-heteroaryl, C5-C20-alkylheteroaryl og C5-C20-heteroarylalkyl, hvori ringsystemerne, i tilfælde af mere end ét (hetero)-alifatisk eller (hetero)-aromatisk ringsystem, er kondenserede eller separate, idet hver rest R^1 har mindst én substituent R^{1a} valgt fra gruppen bestående af hydrogenatom, halogenatom (F, Cl, Br, I), hydroxyl, thiol, C1-C3 thioester, C1-C3-thioether, C1-C3-alkyl og C1-C3-alkoxy; og

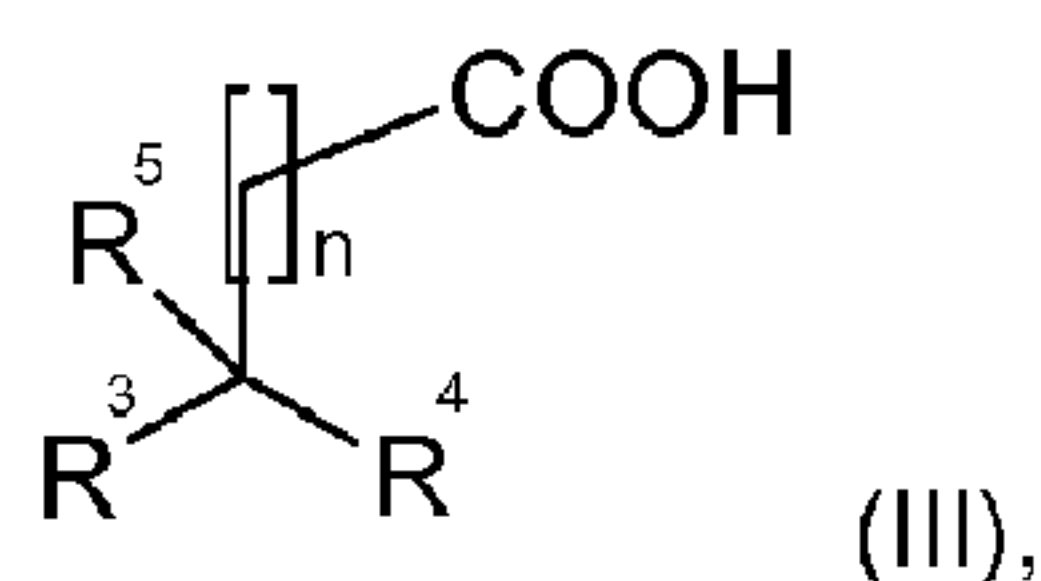
30

R^2 er valgt fra gruppen bestående af hydrogenatom, forgrenet eller uforgrenet C1-C5-alkyl, forgrenet eller uforgrenet C1-C5-alkyl-O-C1-C5-alkyl, forgrenet eller uforgrenet C1-C10-alkoxy, C5-C10-aryl, C6-C10-alkylaryl og C6-C10-arylalkyl, hvor ringsystemerne, i tilfælde af mere end et aromatisk ringsystem, er kondenserede eller separate, idet hver rest R^2 har mindst én

substituent R^{2a} valgt fra gruppen bestående af hydrogenatom, halogenatom (F, Cl, Br, I), hydroxyl, thiol, C1-C3 thioester, C1-C3-thioether, C1-C3-alkyl og C1-C3-alkoxy; eller R^1 , R^2 sammen danner en C3-C10-cycloalkyl eller C3-C10-cycloalkenyl, hvor C3-C10-cycloalkyl eller C3-C10-cycloalkenyl hver har mindst én substituent R^x valgt fra gruppen bestående af hydrogenatom, C1-C5-alkyl, C1-C4-heteroalkyl og C1-C5-alkyl- R^y , hvor R^y er hydroxyl eller thiol;

og

b) mindst én carboxylsyre med den almene formel (III)



hvor

n er nul eller 1;

resterne R^3 og R^4 begge er phenyl eller sammen danner en phenylring, hvor hver phenylring har mindst én yderligere substituent valgt fra gruppen bestående af hydrogenatom, halogenatom og nitrogruppe; og resten R^5 er hydrogenatom eller methyl eller fraværende, hvis R^3 og R^4 sammen danner en phenylring, hvor den mindst ene carboxylsyre er til stede i sin protonerede form eller som carboxylat med en egnet modion.

13. Sammensætning ifølge krav 12, hvor sammensætningen omfatter aminen ifølge (a) i en størrelsesorden fra 90 til 99,9 vægt%.

14. Sammensætning ifølge krav 12 eller 13, hvor sammensætningen omfatter den mindst ene carboxylsyre ifølge (b) i en størrelsesorden på mindst 0,003 vægt%.

DRAWINGS

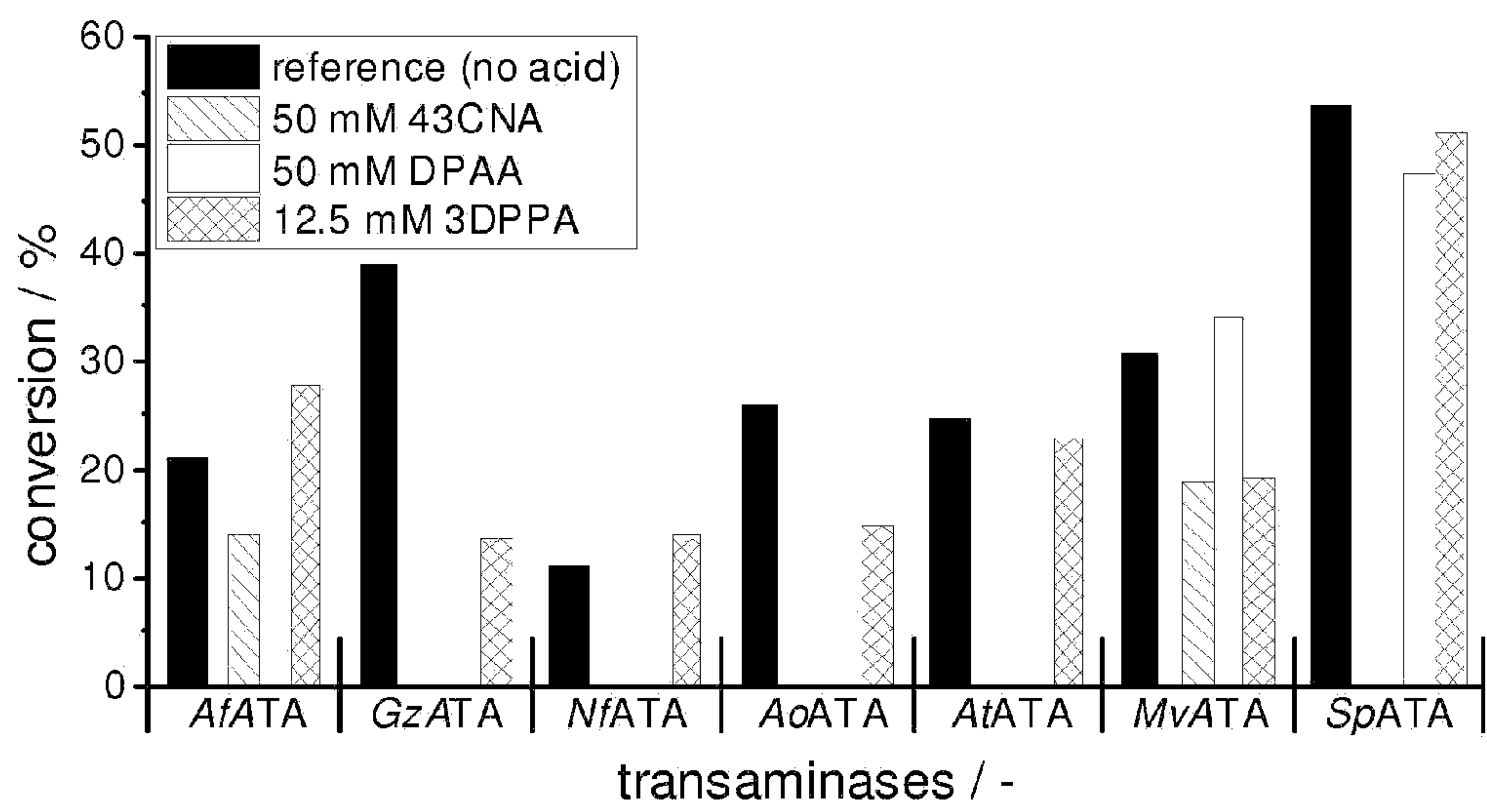


Fig. 1

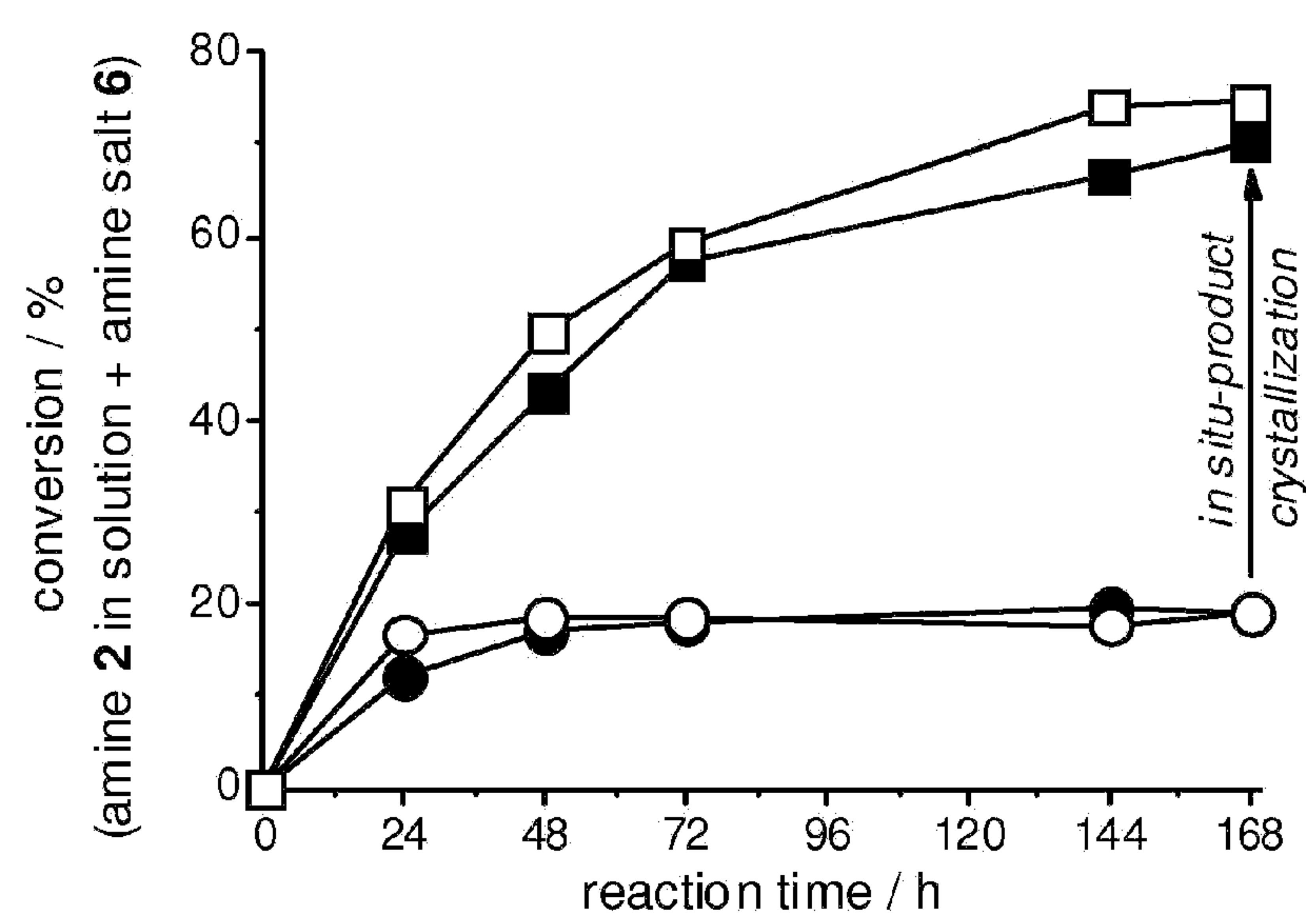


Fig. 2

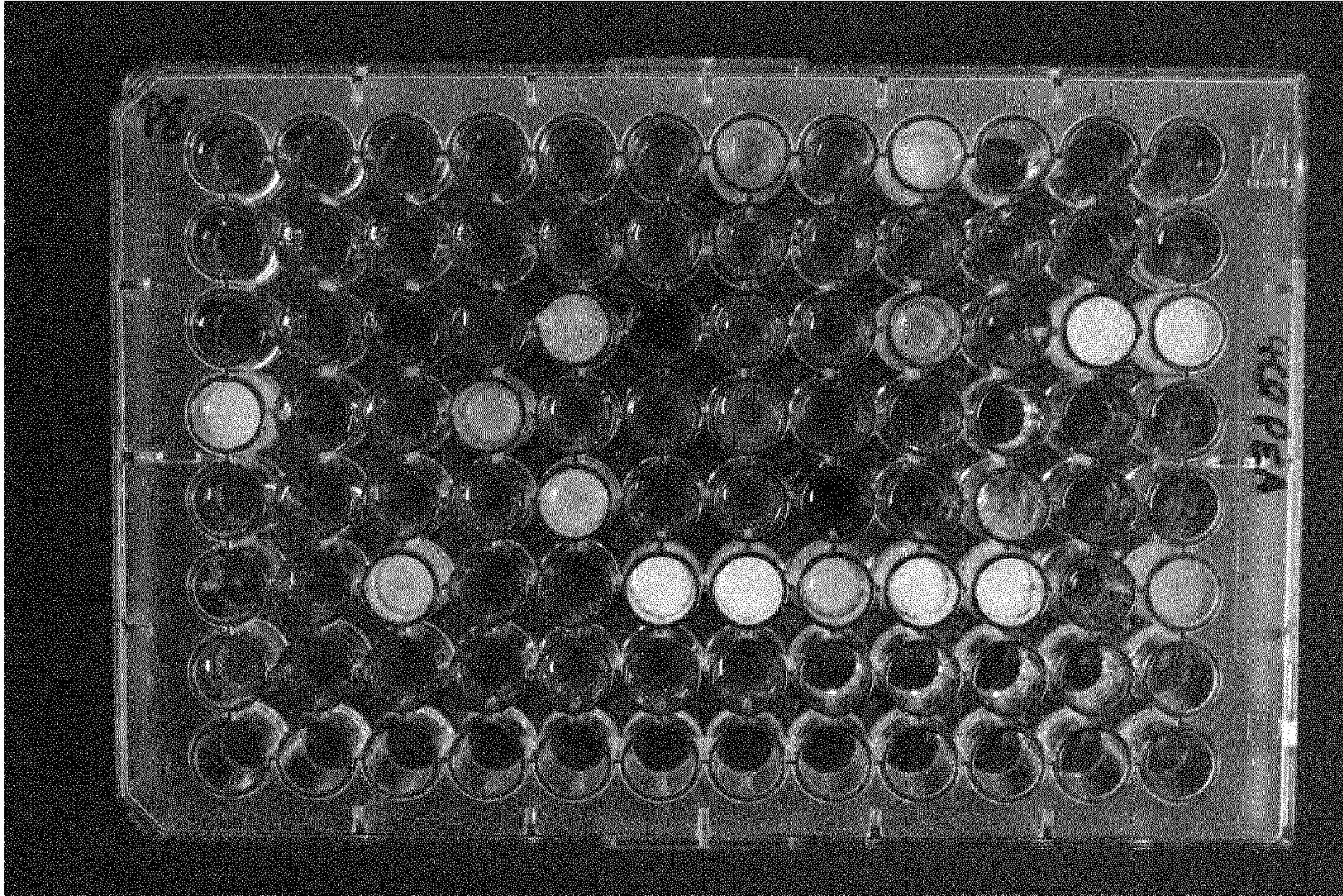


Fig. 3