(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
21 January 2016 (21.01.2016)

WIPO | PCT

(10) International Publication Number
WO 2016/009195 A1

(51) International Patent Classification:
C12Q 1/52 (2006.01) *C12P 13/00* (2006.01)

(21) International Application Number:
PCT/GB2015/052037

(22) International Filing Date:
15 July 2015 (15.07.2015)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
1412545.4 15 July 2014 (15.07.2014) GB

(71) Applicants: **THE UNIVERSITY OF MANCHESTER** [GB/GB]; Oxford Road, Manchester M13 9PL (GB). **MANCHESTER METROPOLITAN UNIVERSITY** [GB/GB]; All Saints Building, All Saints, Manchester M15 6BH (GB).

(72) Inventors: **O'REILLY, Elaine**; School of Chemistry, University of Nottingham, University Park, Nottingham NG7 2RD (GB). **GREEN, Anthony Peter**; 36 Burnside Avenue, Salford M6 8WR (GB). **TURNER, Nicholas J.**; Manchester Institute of Biotechnology, University of Manchester, 131 Princess Street, Manchester M1 7DN (GB).

(74) Agents: **HINKS, Nathan Joel** et al.; HGF Limited, 4th Floor, Merchant Exchange, 17-19 Whitworth Street West, Manchester M1 5WG (GB).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *of inventorship (Rule 4.17(iv))*

Published:

— *with international search report (Art. 21(3))*

[Continued on next page]

(54) Title: ENZYMATIC PROCESSES AND USES

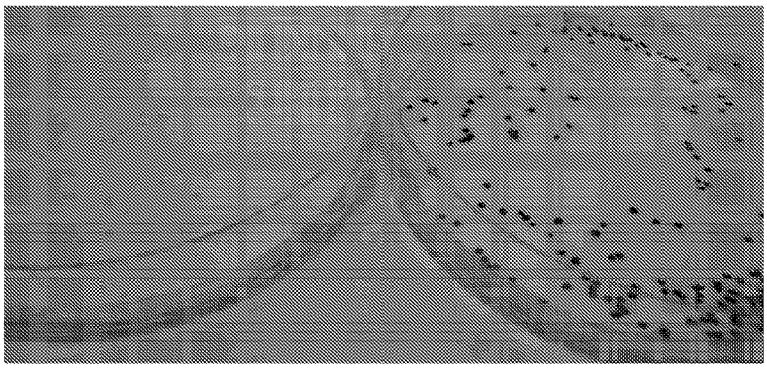


FIG. 3

(57) Abstract: Improved processes for performing transaminase or aminotransferase-mediated amination reactions are disclosed. Also disclosed are processes for determining the compatibility of a transaminase or aminotransferase with a particular substrate and amine donating compound. Also disclosed are processes for determining whether a sample exhibits transaminase or aminotransferase activity.

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— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

ENZYMATIC PROCESSES AND USES

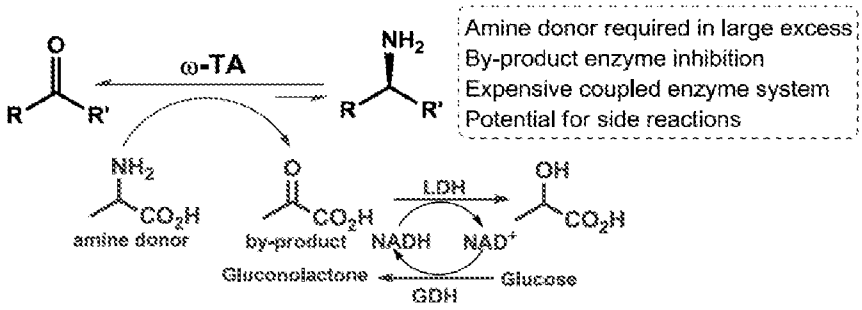
INTRODUCTION

[0001] The present invention relates to methods of preparing chiral amines using transaminases or aminotransferases. The invention also relates to methods of screening transaminases or aminotransferases for their usefulness as catalysts in the preparation of chiral amines. The invention also relates to methods of preparing polymers formed from by-products of the transaminase- or aminotransferase-mediated reactions.

BACKGROUND OF THE INVENTION

[0002] The development of broadly applicable and sustainable catalytic methods for the production of chiral amines has been highlighted as a key research priority by the pharmaceutical industry [1]. The importance of this challenge is driven by the large number of active pharmaceutical ingredients (APIs), bioactive natural products and pharmaceutical building blocks containing chiral amine functionality [2-4]. Biocatalytic routes for the production of chiral amines has received considerable attention [5,6], including methods employing ω-Transaminases (ω-TAs) [7,8], ammonia lyases [9,10], imine reductases [11], amine dehydrogenases [12], and monoamine oxidases [13-16]. The structural simplicity and broad availability of prochiral ketones makes these structures attractive starting materials for the synthesis of chiral building blocks.

[0003] ω-TAs are a family of pyridoxal-5'-phosphate (PLP) dependent enzymes which require a sacrificial amine donor to mediate the reversible conversion of prochiral ketones to the corresponding optically pure amines [7,8]. Despite the potential of ω-TAs, fundamental challenges associated with severe by-product inhibition and with displacing unfavorable equilibrium positions towards product formation have prevented the widespread application of these biocatalysts. The use of amine donors in large excess combined with *in situ* removal of ketone by-products are generally required to achieve high yields of the desired amines. As a possible solution to these problems, much emphasis has been placed on multi-step enzyme cascade reactions, wherein the equilibrium is shifted towards the product side by removing the ketone by-product via one or more enzyme-catalysed reactions, thereby allowing for a theoretical yield of 100% [17-21]. Within this approach, perhaps the most widely employed reaction uses alanine as the amine donor and relies on combinations of expensive, co-factor dependent enzymes for pyruvate removal [22-24]. This reaction is illustrated below in Scheme 1.

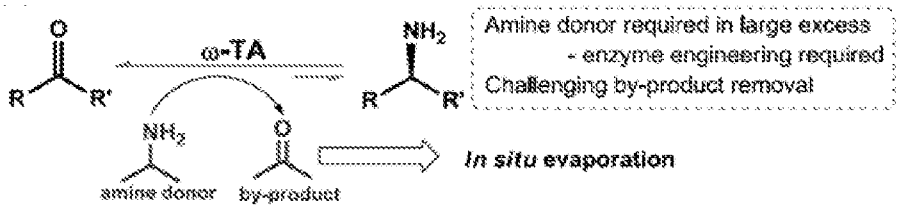


Scheme 1

[0004] Multi-enzyme cascade reactions of the type described in Scheme 1 are, however, hampered by their complexity, which usually involves reaction conditions that have been compromised in an attempt to ensure that all enzymes co-existing in the same system are active. However, generally, the compromised conditions are at best optimal for only one of the enzymes in the system. Therefore, under such conditions, the performance of the various enzymes, in terms of both activity and enantioselectivity, is notably stunted. Hence, whilst multi-step enzyme cascade reactions have the potential to overcome problematic reaction equilibria, their efficiency is compromised by the diverse requirements of the individual enzymes involved.

[0005] An alternative strategy to multi-step enzyme cascade reactions has been described by Wang et al. [25], in which the ketone by-product is effectively removed by spontaneous conversion to a more stable tautomer. However, the application of this technique to scalable chiral amine synthesis is hampered by the relatively high costs involved.

[0006] Currently, the preferred method for large scale production of chiral amines involves using a significant excess of isopropyl amine donor, which is not only costly, but also necessarily results in the formation of considerable quantities of acetone by-product, which acts as a competitive inhibitor for ω-TA. The technically challenging *in-situ* evaporation of acetone in order to drive the reaction to completion is not possible without specialised, and therefore costly, equipment. This reaction is illustrated below in Scheme 2.



Scheme 2

[0007] In addition to the shortcomings identified above, yet a further challenge which complicates the development of efficient ω-TA-mediated transformations is the limited availability of screening methods for the purpose of either identifying potentially new enzymes, or evaluating existing genomic libraries of enzymes, engineered variants and culture collections for those having suitable levels of activity, selectivity and stability under the required reaction conditions.

[0008] Having further regard to the existing ω-TA-mediated transformations discussed above, an additional drawback is the formation of waste by-products having little to no commercial value. There is therefore a need to develop optimised ω-TA-mediated transformations, which, in addition to affording the desired chiral amine, also provide potentially valuable by-products by comparatively simpler synthetic processes.

[0009] The present invention was devised with the foregoing in mind.

SUMMARY OF THE INVENTION

[0010] According to a first aspect of the present invention there is provided a process for the preparation of an amine, the process comprising the step of reacting a ketone or aldehyde substrate with an amine donating compound of formula (I) or (II) defined herein in the presence of a transaminase or aminotransferase.

[0011] According to a second aspect of the present invention, there is provided a use of an amine donating compound of formula (I) or (II) defined herein in a process of preparing an amine from a ketone or aldehyde substrate using a transaminase or aminotransferase.

[0012] According to a third aspect of the present invention, there is provided a process for assessing the ability of a transaminase or aminotransferase to catalyse the conversion of a ketone or aldehyde substrate into an amine, the process comprising the steps of:

a) contacting one or more ketone or aldehyde substrate with an amine donating compound of formula (I) or (II) defined herein in the presence of one or more transaminase or aminotransferase, and
b) analysing the reaction medium for the presence of a by-product,

wherein the presence of a by-product indicates a successful conversion of the ketone or aldehyde substrate into the amine.

[0013] According to a fourth aspect of the present invention, there is provided a use of an amine donating compound of formula (I) or (II) defined herein in a process for assessing the

ability of a transaminase or aminotransferase to catalyse the conversion of a ketone or aldehyde substrate into an amine, the process comprising the steps of:

a) contacting one or more ketone substrate with an amine donating compound of formula (I) or (II) defined herein in the presence of one or more transaminase or aminotransferase, and
b) analysing the reaction medium for the presence of a by-product,

wherein the presence of a by-product indicates a successful conversion of the ketone or aldehyde substrate into the chiral amine.

[0014] According to a fifth aspect of the present invention, there is provided a kit of parts suitable for assessing the ability of a transaminase or aminotransferase to catalyse the conversion of a ketone or aldehyde substrate into an amine in accordance with a screening process or use defined herein, the kit comprising:

a) one or more amine donating compounds of formula (I) or (II) defined herein, and one or both of:
    i. one or more means transaminases or aminotransferases
    ii. one or more ketone or aldehyde substrates.

[0015] According to a sixth aspect of the present invention, there is provided a process for assessing the ability of an amine oxidase to catalyse the oxidation of an amine donating compound of formula (I) or (II) defined herein, the process comprising the steps of:

a) contacting one or more amine donating compound of formula (I) or (II) defined herein with an oxidizing agent, in the presence of one or more amine oxidase, and
b) analysing the reaction medium for the presence of a by-product,

wherein the presence of a by-product indicates a successful oxidation of the amine donating compound.

[0016] According to a seventh aspect of the present invention, there is provided a process for determining whether a sample exhibits transaminase or aminotransferase activity, the process comprising the steps of:

a) providing one or more sample having putative transaminase or aminotransferase activity;

b) contacting the sample of step a) with one or more amine-donating compound of formula (I) or (II) defined herein; and

c) analysing the reaction medium for the presence of a by-product,

wherein the presence of a by-product indicates that the sample exhibits transaminase or aminotransferase activity.

[0017] According to an eighth aspect of the present invention, there is provided a use of an amine donating compound of formula (I) or (II) defined herein in a process for determining whether a sample exhibits transaminase or aminotransferase activity, the process comprising the steps of:

a) providing one or more sample having putative transaminase or aminotransferase activity;

b) contacting the sample of step a) with one or more amine-donating compound of formula (I) or (II) defined herein; and

c) analysing the reaction medium for the presence of a by-product,

wherein the presence of a by-product indicates that the sample exhibits transaminase or aminotransferase activity.

[0018] According to a ninth aspect of the present invention, there is provided a kit of parts suitable for determining whether a sample exhibits transaminase or aminotransferase activity in accordance with a screening process or use defined herein, the kit comprising:

a) One or more amine donating compound of formula (I) or (II) defined herein, and

b) Instructions for performing a screening process defined herein.

[0019] According to a tenth aspect of the present invention, there is provided a process for determining whether a sample exhibits amino oxidase activity, the process comprising the steps of:

a) providing one or more sample having putative amino oxidase activity;

b) contacting the one or more samples of step a) with one or more amine-donating compound of formula (I) or (II) defined herein and a suitable oxidizing agent; and

c) analysing the reaction medium for the presence of a by-product,

wherein the presence of a by-product indicates that the sample exhibits amino oxidase activity.

[0020] According to an eleventh aspect of the present invention, there is provided a process for preparing a polymer, the process comprising the steps of:

a) reacting a ketone or aldehyde substrate with an amine donating compound of formula (I) as defined herein in the presence of a transaminase or aminotransferase, such that a reaction by-product is formed, and
b) subjecting the reaction by-product to conditions suitable for causing polymerisation of the reaction by-product.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

[0021] Unless otherwise stated, the following terms used in the specification and claims have the following meanings set out below

[0022] The term “alkyl” includes both straight and branched chain alkyl groups. References to individual alkyl groups such as “propyl” are specific for the straight chain version only and references to individual branched chain alkyl groups such as “isopropyl” are specific for the branched chain version only. For example, “(1-6C)alkyl” includes (1-4C)alkyl, (1-3C)alkyl, propyl, isopropyl and *t*-butyl. A similar convention applies to other radicals, for example “phenyl(1-6C)alkyl” includes phenyl(1-4C)alkyl, benzyl, 1-phenylethyl and 2-phenylethyl.

[0023] The terms “alkenyl” and “alkynyl” include both straight and branched chain alkenyl and alkynyl groups.

[0024] The terms “alkylene”, “alkenylene” and “alkynylene” respectively relate to alkyl, alkenyl and alkynyl groups in which one of the terminal hydrogen atoms has been replaced by another atom.

[0025] The term "(m-nC)" or "(m-nC) group" used alone or as a prefix, refers to any group having m to n carbon atoms.

[0026] The term “halo” refers to fluoro, chloro, bromo and iodo.

[0027] The term “fluoroalkyl” means an alkyl group as defined herein which is substituted with one or more fluoro atoms, e.g. $-CF_3$, or $-CH_2CF_3$ and the like.

[0028] The term “heterocyclyl”, “heterocyclic” or “heterocycle” means a non-aromatic saturated or partially saturated monocyclic, fused, bridged, or spiro bicyclic heterocyclic ring system(s). The term heterocyclyl includes both monovalent species and divalent species. Monocyclic heterocyclic rings contain from about 3 to 12 (suitably from 3 to 7) ring atoms, with from 1 to 5 (suitably 1, 2 or 3) heteroatoms selected from nitrogen, oxygen or sulfur in the ring. Bicyclic heterocycles contain from 7 to 17 member atoms, suitably 7 to 12 member

atoms, in the ring. Bicyclic heterocycles contain from about 7 to about 17 ring atoms, suitably from 7 to 12 ring atoms. Bicyclic heterocyclic(s) rings may be fused, spiro, or bridged ring systems. As the skilled person would appreciate, any heterocycle may be linked to another group via any suitable atom, such as via a carbon or nitrogen atom.

[0029] The term “carbocyclyl”, “carbocyclic” or “carbocycle” means a non-aromatic saturated or partially saturated monocyclic, fused, bridged, or spiro bicyclic carbocyclic ring system(s). Monocyclic carbocyclic rings contain from about 3 to 12 (suitably from 3 to 7) ring atoms. Bicyclic carbocycles contain from 7 to 17 member atoms, suitably 7 to 12 member atoms, in the ring. Bicyclic carbocycles contain from about 7 to about 17 ring atoms, suitably from 7 to 12 ring atoms. Bicyclic carbocyclic rings may be fused, spiro, or bridged ring systems.

[0030] The term “aryl” is used herein to denote phenyl, naphthalene or anthracene ring. In an embodiment, an “aryl” is phenyl or naphthalene, and particularly is phenyl.

[0031] The term “heteroaryl” or “heteroaromatic” means an aromatic mono-, bi-, or tri-cyclic ring incorporating one or more (for example 1-4, particularly 1, 2 or 3) heteroatoms selected from N, O, S, Si or Se. Examples of heteroaryl groups are monocyclic, bicyclic and tricyclic groups containing from five to eighteen ring members. The heteroaryl group can be, for example, a 5- or 6-membered monocyclic ring, a 8-, 9- or 10-membered bicyclic ring or a 15-, 16-, 17- or 18-membered tricyclic ring. Suitably each ring in a bicyclic or tricyclic ring system comprises five or six ring atoms.

[0032] The term "optionally substituted" refers to either groups, structures, or molecules that are substituted and those that are not substituted.

[0033] Where optional substituents are chosen from “one or more” groups it is to be understood that this definition includes all substituents being chosen from one of the specified groups or the substituents being chosen from two or more of the specified groups.

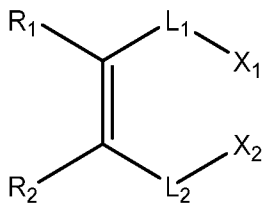
[0034] The term “salt” will be understood to include an acid-addition salt of an amine donating compound forming part of the invention, for example, an acid-addition salt with, for example, an inorganic or organic acid, for example hydrochloric, hydrobromic, sulfuric, phosphoric, trifluoroacetic, formic, citric or maleic acid. Also encompassed are alkali metal salts, for example a sodium or potassium salt, an alkaline earth metal salt, for example a calcium or magnesium salt, an ammonium salt or a salt with an organic base, for example a salt with methylamine, dimethylamine, trimethylamine, piperidine, morpholine or tris-(2-hydroxyethyl)amine.

[0035] A “biological sample” for the purposes of the present disclosure may be taken as encompassing any sample of biological origin that may include transaminase or

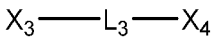
aminotransferase enzyme activity. In a suitable embodiment a biological sample may comprise biological cells. Biological cells may be in the form of a tissue sample including cells, cultured cells, or microorganisms. Alternatively a biological sample may comprise an extract, such as a cell extract or lysate that may include a putative transaminase or aminotransferase enzyme. It will be appreciated that biological samples should be treated in a manner that is able to preserve any transaminase or aminotransferase enzyme activity that may be present in the sample in question. Biological samples may be of animal (including human or non-human animals), plant, or microorganism origin.

Preparation of amines

[0036] As discussed hereinbefore, the present invention provides a process for the preparation of an amine, the process comprising the step of reacting a ketone or aldehyde substrate with an amine donating compound of formula (I) or (II) in the presence of a transaminase or aminotransferase



(I)



(II)

wherein

L_1 and L_2 are selected from one of the following options:

L_1 is absent and L_2 is $–[CR_aR_b]_2–$, or
L_1 is $–[CR_aR_b]_2–$ and L_2 is absent, or
L_1 is $–CR_aR_b–$ and L_2 is $–CR_aR_b–$;

L_3 is a straight or branched alkylene, alkenylene or alkynylene linker optionally comprising one or more substituted or unsubstituted heteroatoms disposed within the alkylene, alkenylene or alkynylene linker, wherein L_3 is optionally substituted with one or more R_c;

each group R_a and R_b is independently absent or selected from hydrogen, oxo, halo, trifluoromethyl, trifluoromethoxy, cyano, nitro, hydroxy, mercapto, amino, formyl, carboxy, carbamoyl, ureido, (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl,

(1-4C)alkoxy, aryl, (1-4C)alkylthio, (1-4C)alkylsulphinyl, (1-4C)alkylsulphonyl, (1-4C)alkylamino, di-[(1-4C)alkyl]amino, (1-4C)alkoxycarbonyl, *N*-(1-4C)alkylcarbamoyl, *N*,*N*-di-[(1-4C)alkyl]carbamoyl, (2-4C)alkanoyl, (2-4C)alkanoyloxy, (2-4C)alkanoylamino, sulphamoyl, *N*-(1-4C)alkylsulphamoyl and *N*,*N*-di-[(1-4C)alkyl]sulphamoyl;

each group R_c is independently absent or selected from hydrogen, oxo, halo, trifluoromethyl, trifluoromethoxy, cyano, nitro, hydroxy, mercapto, amino, formyl, carbamoyl, ureido, (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl, (1-4C)alkoxy, aryl, (1-4C)alkylthio, (1-4C)alkylsulphinyl, (1-4C)alkylsulphonyl, (1-4C)alkylamino, di-[(1-4C)alkyl]amino, (1-4C)alkoxycarbonyl, *N*-(1-4C)alkylcarbamoyl, *N*,*N*-di-[(1-4C)alkyl]carbamoyl, (2-4C)alkanoyl, (2-4C)alkanoyloxy, (2-4C)alkanoylamino, sulphamoyl, *N*-(1-4C)alkylsulphamoyl and *N*,*N*-di-[(1-4C)alkyl]sulphamoyl

X_1, X_2, X_3 and X_4 are each selected from NH_2, NHR_d, NR_dR_e, SH and OH, with the proviso that at least one of X_1 and X_2, and at least one of X_3 and X_4, is NH_2;

R_d and R_e are independently selected from (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl and (1-4C)alkoxy; and

R_1 and R_2 are

(i) independently selected from hydrogen halo, trifluoromethyl, trifluoromethoxy, cyano, nitro, hydroxy, mercapto, amino, formyl, carboxy, carbamoyl, ureido, (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl, (1-4C)alkoxy, (1-4C)alkylthio, (1-4C)alkylsulphinyl, (1-4C)alkylsulphonyl, (1-4C)alkylamino, di-[(1-4C)alkyl]amino, (1-4C)alkoxycarbonyl, *N*-(1-4C)alkylcarbamoyl, *N*,*N*-di-[(1-4C)alkyl]carbamoyl, (2-4C)alkanoyl, (2-4C)alkanoyloxy, (2-4C)alkanoylamino, sulphamoyl, *N*-(1-4C)alkylsulphamoyl and *N*,*N*-di-[(1-4C)alkyl]sulphamoyl, or

(ii) linked, so that when R_1 and R_2 are taken together with the carbon atom to which they are each attached, they collectively form a carbocyclic, heterocyclic, aryl or heteroaryl ring, optionally substituted with one or more substituents selected from halo, trifluoromethyl, trifluoromethoxy, cyano, nitro, hydroxy, mercapto, amino, formyl, carboxy, carbamoyl, ureido, (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl, (1-4C)alkoxy, (1-4C)alkylthio, (1-4C)alkylsulphinyl, (1-4C)alkylsulphonyl, (1-4C)alkylamino, di-[(1-4C)alkyl]amino, (1-4C)alkoxycarbonyl, *N*-(1-

4C)alkylcarbamoyl, *N*,*N*-di-[(1-4C)alkyl]carbamoyl, (2-4C)alkanoyl, (2-4C)alkanoyloxy, (2-4C)alkanoylamino, sulphamoyl, *N*-(1-4C)alkylsulphamoyl and *N*,*N*-di-[(1-4C)alkyl]sulphamoyl,

or a salt thereof.

[0037] The present invention also provides a use of an amine donating compound of formula (I) or (II) defined herein in a process of preparing an amine from a ketone or aldehyde substrate using a transaminase or aminotransferase.

[0038] When compared with currently-available techniques, the process and use described above offer a number of advantages. Perhaps most notably, the process and use described above make use of low-cost amine donating compounds that efficiently displace unfavourable reaction equilibria towards product formation, without the need for complex or costly removal of reaction by-products, thereby providing an improved synthetic means of accessing amines or chiral amines in both high yield and high enantiomeric excess.

[0039] In certain embodiments, the reaction of the amine donating compound with the ketone or aldehyde substrate in the presence of a transaminase or aminotransferase results in the generation of reaction by-products that are either themselves insoluble, or can be readily converted into insoluble derivatives. The ability of the precipitated reaction by-product to compromise reaction equilibria by competitive inhibition the transaminase or aminotransferase is therefore either markedly reduced or entirely eliminated.

[0040] Additionally, certain amine donating compounds used in conjunction with the above-described process and use afford reaction by-products that lend themselves being readily detectable and/or quantifiable, thereby providing an inherent means of indicating when a transaminase or aminotransferase-mediated transformation has proceeded to completion. In certain embodiments, the amine donating compounds used in conjunction with the above-described process and use afford reaction by-products (soluble or insoluble) that are coloured.

[0041] In one embodiment, the substrate is a ketone substrate. The skilled person will readily appreciate that reductive amination of a ketone results in the formation of a chiral amine. Chiral amines are highly useful functionalities to organic, and particular pharmaceutical, chemistry. In another embodiment, the substrate is an aldehyde, in which case the resulting amine may not necessarily be chiral.

[0042] In another embodiment, when X_3 is OH and X_4 is NH_2, the carbon atom to which X_3 is attached is not substituted with oxo,

and when X_4 is OH and X_3 is NH_2, the carbon atom to which X_4 is attached is not substituted with oxo.

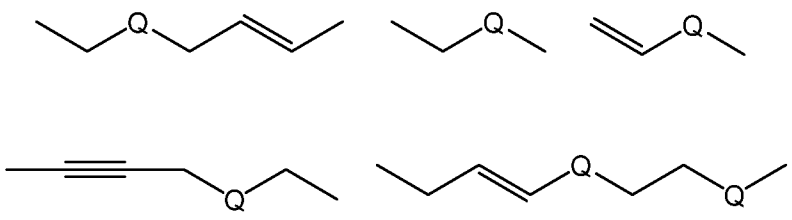
[0043] In another embodiment, the amine donating compound of formula (II) is not an amino acid.

[0044] In another embodiment, R_c is not oxo.

[0045] In another embodiment, each group R_c is independently absent or selected from hydrogen, halo, trifluoromethyl, trifluoromethoxy, cyano, nitro, hydroxy, mercapto, amino, formyl, carbamoyl, ureido, (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl, (1-4C)alkoxy, aryl, (1-4C)alkylthio, (1-4C)alkylsulphinyl, (1-4C)alkylsulphonyl, (1-4C)alkylamino, di-[(1-4C)alkyl]amino, (1-4C)alkoxycarbonyl, *N*-(1-4C)alkylcarbamoyl, *N*,*N*-di-[(1-4C)alkyl]carbamoyl, (2-4C)alkanoyl, (2-4C)alkanoyloxy, (2-4C)alkanoylamino, sulphamoyl, *N*-(1-4C)alkylsulphamoyl and *N*,*N*-di-[(1-4C)alkyl]sulphamoyl.

[0046] In another embodiment, the transaminase or aminotransferase is selected from a group consisting of polyamine aminotransferases (including diamine aminotransferases) and ω-TAs. Suitably, the transaminase or aminotransferase is a ω-TAs.

[0047] Having regard to formula (II) defined above, it will be understood that the phrase "L_3 is a straight or branched alkylene, alkenylene or alkynylene linker optionally comprising one or more substituted or unsubstituted heteroatoms disposed within the alkylene, alkenylene or alkynylene linker" means an alkylene, alkenylene or alkynylene chain that is optionally broken by one or more substituted or unsubstituted heteroatoms. In this regard, it will be understood that the one or more substituted or unsubstituted heteroatoms, when present, each replace a carbon atom in the alkylene, alkenylene or alkynylene chain. Examples of such moieties are shown below, wherein the one or more substituted or unsubstituted heteroatoms are denoted as Q:



[0048] Having regard to formulae (I) and (II), each of the groups L_1, L_2, L_3, X_1, X_2, X_3, X_4, R_1, R_2, R_a, R_b, R_c, R_d and R_e may be as defined in either of the numbered paragraphs (1) to (29) below:

1) L_1 is absent and L_2 is $-[CR_aR_b]_2-$; or L_1 is $-[CR_aR_b]_2-$ and L_2 is absent; or L_1 is $-CR_aR_b-$ and L_2 is $-CR_aR_b-$.
2) L_1 is $-CR_aR_b-$ and L_2 is $-CR_aR_b-$.

3) L_3 is a straight or branched alkylene, alkenylene or alkynylene linker optionally comprising one or more substituted or unsubstituted heteroatoms disposed within the alkylene, alkenylene or alkynylene linker, wherein L_3 is optionally substituted with one or more R_c.
4) L_3 is a straight or branched (2-12C)alkylene, (2-12C)alkenylene or (2-12C)alkynylene linker optionally comprising one or more substituted or unsubstituted heteroatoms disposed within the alkylene, alkenylene or alkynylene linker, wherein L_3 is optionally substituted with one or more R_c.
5) L_3 is a straight or branched (2-12C)alkylene, (2-12C)alkenylene or (2-12C)alkynylene linker optionally comprising one or more substituted or unsubstituted heteroatoms selected from N, S and O disposed within the alkylene, alkenylene or alkynylene linker, wherein L_3 is optionally substituted with one or more R_c.
6) L_3 is a straight or branched (2-12C)alkylene, (2-12C)alkenylene or (2-12C)alkynylene linker optionally comprising one or more substituted or unsubstituted heteroatoms selected from N, S and O disposed within the alkylene, alkenylene or alkynylene linker, wherein L_3 is optionally substituted with one or more R_c.
7) L_3 is a straight or branched (2-12C)alkylene, (2-12C)alkenylene or (2-12C)alkynylene linker optionally comprising one or more heteroatoms selected from N, S and O disposed within the alkylene, alkenylene or alkynylene linker, the one or more N-heteroatoms being optionally substituted with one or more groups selected from (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl, (1-4C)alkoxy or aryl, wherein L_3 is optionally substituted with one or more R_c.
8) L_3 is a straight or branched (2-12C)alkylene linker optionally comprising one or more heteroatoms selected from N, S and O disposed within the alkylene linker, the one or more heteroatoms being optionally substituted with one or more groups selected from (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl, (1-4C)alkoxy or aryl, wherein L_3 is optionally substituted with one or more R_c.
9) X_1, X_2, X_3 and X_4 are each selected from NH_2, NHR_d, NR_dR_e, SH and OH, with the proviso that at least one of X_1 and X_2, and at least one of X_3 and X_4, is NH_2.
10) X_1, X_2, X_3 and X_4 are each selected from NH_2, NHR_d, NR_dR_e, SH and OH, with the proviso that at least one of X_1 and X_2, and at least one of X_3 and X_4, is NH_2.
11) X_1, X_2, X_3 and X_4 are each selected from NH_2, NHR_d and NR_dR_e, with the proviso that at least one of X_1 and X_2, and at least one of X_3 and X_4, is NH_2.
12) R_1 and R_2 are independently selected from hydrogen halo, trifluoromethyl, trifluoromethoxy, cyano, nitro, hydroxy, mercapto, amino, formyl, carboxy, carbamoyl, ureido, (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl, (1-4C)alkoxy, (1-4C)alkylthio, (1-4C)alkylsulphinyl, (1-4C)alkylsulphonyl, (1-4C)alkylamino, di-[(1-4C)alkyl]amino, (1-

4C)alkoxycarbonyl, *N*-(1-4C)alkylcarbamoyl, *N*,*N*-di-[(1-4C)alkyl]carbamoyl, (2-4C)alkanoyl, (2-4C)alkanoyloxy, (2-4C)alkanoylamino, sulphamoyl, *N*-(1-4C)alkylsulphamoyl and *N*,*N*-di-[(1-4C)alkyl]sulphamoyl.

13) R_1 and R_2 are independently selected from hydrogen halo, trifluoromethyl, trifluoromethoxy, hydroxy, amino, formyl, carboxy, carbamoyl, ureido, (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl, (1-4C)alkoxy, (1-4C)alkylthio, (1-4C)alkylamino, di-[(1-4C)alkyl]amino, (1-4C)alkoxycarbonyl, *N*-(1-4C)alkylcarbamoyl, *N*,*N*-di-[(1-4C)alkyl]carbamoyl, (2-4C)alkanoyl, (2-4C)alkanoyloxy and (2-4C)alkanoylamino.

14) R_1 and R_2 are independently selected from hydrogen halo, trifluoromethyl, trifluoromethoxy, hydroxy, amino, carboxy, carbamoyl, (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl and (1-4C)alkoxy.

15) R_1 and R_2 are linked, so that when R_1 and R_2 are taken together with the carbon atom to which they are each attached, they collectively form a carbocyclic, heterocyclic, aryl or heteroaryl ring, optionally substituted with one or more substituents selected from halo, trifluoromethyl, trifluoromethoxy, cyano, nitro, hydroxy, mercapto, amino, formyl, carboxy, carbamoyl, ureido, (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl, (1-4C)alkoxy, (1-4C)alkylthio, (1-4C)alkylsulphinyl, (1-4C)alkylsulphonyl, (1-4C)alkylamino, di-[(1-4C)alkyl]amino, (1-4C)alkoxycarbonyl, *N*-(1-4C)alkylcarbamoyl, *N*,*N*-di-[(1-4C)alkyl]carbamoyl, (2-4C)alkanoyl, (2-4C)alkanoyloxy, (2-4C)alkanoylamino, sulphamoyl, *N*-(1-4C)alkylsulphamoyl and *N*,*N*-di-[(1-4C)alkyl]sulphamoyl.

16) R_1 and R_2 are linked, so that when R_1 and R_2 are taken together with the carbon atom to which they are each attached, they collectively form a phenyl or naphthalenyl group, or a 5-10 membered heteroaryl ring, optionally substituted with one or more substituents selected from halo, trifluoromethyl, trifluoromethoxy, cyano, nitro, hydroxy, mercapto, amino, formyl, carboxy, carbamoyl, ureido, (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl, (1-4C)alkoxy, (1-4C)alkylthio, (1-4C)alkylsulphinyl, (1-4C)alkylsulphonyl, (1-4C)alkylamino, di-[(1-4C)alkyl]amino, (1-4C)alkoxycarbonyl, *N*-(1-4C)alkylcarbamoyl, *N*,*N*-di-[(1-4C)alkyl]carbamoyl, (2-4C)alkanoyl, (2-4C)alkanoyloxy, (2-4C)alkanoylamino, sulphamoyl, *N*-(1-4C)alkylsulphamoyl and *N*,*N*-di-[(1-4C)alkyl]sulphamoyl.

17) R_1 and R_2 are linked, so that when R_1 and R_2 are taken together with the carbon atom to which they are each attached, they collectively form a phenyl group, or 5-6 membered heteroaryl ring, optionally substituted with one or more substituents selected from halo, trifluoromethyl, trifluoromethoxy, cyano, nitro, hydroxy, mercapto, amino, formyl, carboxy, carbamoyl, ureido, (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl, (1-4C)alkoxy, (1-4C)alkylthio, (1-4C)alkylsulphinyl, (1-4C)alkylsulphonyl, (1-4C)alkylamino, di-[(1-4C)alkyl]amino, (1-4C)alkoxycarbonyl, *N*-(1-4C)alkylcarbamoyl,

N,*N*-di-[(1-4C)alkyl]carbamoyl, (2-4C)alkanoyl, (2-4C)alkanoyloxy, (2-4C)alkanoylamino, sulphamoyl, *N*-(1-4C)alkylsulphamoyl and *N*,*N*-di-[(1-4C)alkyl]sulphamoyl.

18) R_1 and R_2 are linked, so that when R_1 and R_2 are taken together with the carbon atom to which they are each attached, they collectively form a phenyl group, or 6 membered heteroaryl ring containing 1-3 nitrogen atoms, optionally substituted with one or more substituents selected from halo, trifluoromethyl, trifluoromethoxy, cyano, nitro, hydroxy, mercapto, amino, formyl, carboxy, carbamoyl, ureido, (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl, (1-4C)alkoxy, (1-4C)alkylthio, (1-4C)alkylsulphinyl, (1-4C)alkylsulphonyl, (1-4C)alkylamino, di-[(1-4C)alkyl]amino, (1-4C)alkoxycarbonyl, *N*-(1-4C)alkylcarbamoyl, *N*,*N*-di-[(1-4C)alkyl]carbamoyl, (2-4C)alkanoyl, (2-4C)alkanoyloxy, (2-4C)alkanoylamino, sulphamoyl, *N*-(1-4C)alkylsulphamoyl and *N*,*N*-di-[(1-4C)alkyl]sulphamoyl.

19) R_1 and R_2 are linked, so that when R_1 and R_2 are taken together with the carbon atom to which they are each attached, they collectively form a phenyl group, or 6 membered heteroaryl ring containing 1-3 nitrogen atoms, optionally substituted with one or more substituents selected from halo, trifluoromethyl, trifluoromethoxy, hydroxy, amino, carboxy, carbamoyl, (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl, (1-4C)alkoxy, (1-4C)alkylthio, (1-4C)alkylsulphinyl, (1-4C)alkylsulphonyl, (1-4C)alkylamino, di-[(1-4C)alkyl]amino, (1-4C)alkoxycarbonyl, *N*-(1-4C)alkylcarbamoyl, *N*,*N*-di-[(1-4C)alkyl]carbamoyl, (2-4C)alkanoyl, (2-4C)alkanoyloxy, (2-4C)alkanoylamino, sulphamoyl, *N*-(1-4C)alkylsulphamoyl and *N*,*N*-di-[(1-4C)alkyl]sulphamoyl.

20) R_1 and R_2 are linked, so that when R_1 and R_2 are taken together with the carbon atom to which they are each attached, they collectively form a phenyl group, or 6 membered heteroaryl ring containing 1-3 nitrogen atoms, optionally substituted with one or more substituents selected from halo, trifluoromethyl, trifluoromethoxy, hydroxy, amino, carboxy, carbamoyl, (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl, (1-4C)alkoxy, (1-4C)alkylthio, (1-4C)alkylamino and di-[(1-4C)alkyl]amino.

21) Each group R_a and R_b is independently absent or selected from hydrogen, oxo, halo, trifluoromethyl, trifluoromethoxy, cyano, nitro, hydroxy, mercapto, amino, formyl, carboxy, carbamoyl, ureido, (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl, (1-4C)alkoxy, aryl, (1-4C)alkylthio, (1-4C)alkylsulphinyl, (1-4C)alkylsulphonyl, (1-4C)alkylamino, di-[(1-4C)alkyl]amino, (1-4C)alkoxycarbonyl, *N*-(1-4C)alkylcarbamoyl, *N*,*N*-di-[(1-4C)alkyl]carbamoyl, (2-4C)alkanoyl, (2-4C)alkanoyloxy, (2-4C)alkanoylamino, sulphamoyl, *N*-(1-4C)alkylsulphamoyl and *N*,*N*-di-[(1-4C)alkyl]sulphamoyl.

22) Each group R_a and R_b is independently absent or selected from hydrogen, oxo, halo, trifluoromethyl, trifluoromethoxy, hydroxy, amino, carboxy, carbamoyl, (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl, (1-4C)alkoxy, aryl, (1-4C)alkylthio, (1-4C)alkylamino, di-[(1-4C)alkyl]amino, (1-4C)alkoxycarbonyl, *N*-(1-4C)alkylcarbamoyl, *N*,*N*-di-[(1-4C)alkyl]carbamoyl, (2-4C)alkanoyl, (2-4C)alkanoyloxy and (2-4C)alkanoylamino.

23) Each group R_a and R_b is independently absent or selected from hydrogen, oxo, halo, trifluoromethyl, trifluoromethoxy, hydroxy, amino, carboxy, carbamoyl, (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl, (1-4C)alkoxy and aryl.

24) Each group R_c is independently absent or selected from hydrogen, oxo, halo, trifluoromethyl, trifluoromethoxy, cyano, nitro, hydroxy, mercapto, amino, formyl, carbamoyl, ureido, (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl, (1-4C)alkoxy, aryl, (1-4C)alkylthio, (1-4C)alkylsulphinyl, (1-4C)alkylsulphonyl, (1-4C)alkylamino, di-[(1-4C)alkyl]amino, (1-4C)alkoxycarbonyl, *N*-(1-4C)alkylcarbamoyl, *N*,*N*-di-[(1-4C)alkyl]carbamoyl, (2-4C)alkanoyl, (2-4C)alkanoyloxy, (2-4C)alkanoylamino, sulphamoyl, *N*-(1-4C)alkylsulphamoyl and *N*,*N*-di-[(1-4C)alkyl]sulphamoyl.

25) Each group R_c is independently absent or selected from hydrogen, oxo, halo, trifluoromethyl, trifluoromethoxy, hydroxy, amino, carbamoyl, (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl, (1-4C)alkoxy, aryl, (1-4C)alkylthio, (1-4C)alkylamino, di-[(1-4C)alkyl]amino, (1-4C)alkoxycarbonyl, *N*-(1-4C)alkylcarbamoyl, *N*,*N*-di-[(1-4C)alkyl]carbamoyl, (2-4C)alkanoyl, (2-4C)alkanoyloxy and (2-4C)alkanoylamino.

26) Each group R_c is independently absent or selected from hydrogen, oxo, halo, trifluoromethyl, trifluoromethoxy, hydroxy, amino, carbamoyl, (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl, (1-4C)alkoxy and aryl.

27) Each group R_c is independently absent or selected from hydrogen, oxo, halo, trifluoromethyl, trifluoromethoxy, hydroxy, amino, carbamoyl, (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl, (1-4C)alkoxy and phenyl.

28) Each group R_c is independently absent or selected from hydrogen, halo, trifluoromethyl, trifluoromethoxy, hydroxy, amino, carbamoyl, (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl, (1-4C)alkoxy and phenyl.

29) R_d and R_e are independently selected from (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl and (1-4C)alkoxy.

[0049] In an embodiment, R_1 and R_2 are as defined in numbered paragraph (12) or any one of numbered paragraphs (15) to (20).

[0050] In another embodiment, R_1 and R_2 are as defined in numbered paragraph (13) or any one of numbered paragraphs (15) to (20).

[0051] In another embodiment, R_1 and R_2 are as defined in numbered paragraph (14) or any one of numbered paragraphs (15) to (20).

[0052] In a particular embodiment, formulae (I) and (II) are defined as follows:

L_1 and L_2 are selected from one of the following options:

L_1 is absent and L_2 is $–[CR_aR_b]_2–$, or
L_1 is $–[CR_aR_b]_2–$ and L_2 is absent, or
L_1 is $–CR_aR_b–$ and L_2 is $–CR_aR_b–$;

L_3 is a straight or branched (2-12C)alkylene, (2-12C)alkenylene or (2-12C)alkynylene linker optionally comprising one or more substituted or unsubstituted heteroatoms disposed within the alkylene, alkenylene or alkynylene linker, wherein L_3 is optionally substituted with one or more R_c;

each group R_a and R_b is independently absent or selected from hydrogen, oxo, halo, trifluoromethyl, trifluoromethoxy, cyano, nitro, hydroxy, mercapto, amino, formyl, carboxy, carbamoyl, ureido, (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl, (1-4C)alkoxy, aryl, (1-4C)alkylthio, (1-4C)alkylsulphinyl, (1-4C)alkylsulphonyl, (1-4C)alkylamino, di-[(1-4C)alkyl]amino, (1-4C)alkoxycarbonyl, *N*-(1-4C)alkylcarbamoyl, *N*,*N*-di-[(1-4C)alkyl]carbamoyl, (2-4C)alkanoyl, (2-4C)alkanoyloxy, (2-4C)alkanoylamino, sulphamoyl, *N*-(1-4C)alkylsulphamoyl and *N*,*N*-di-[(1-4C)alkyl]sulphamoyl;
each group R_c is independently absent or selected from hydrogen, oxo, halo, trifluoromethyl, trifluoromethoxy, cyano, nitro, hydroxy, mercapto, amino, formyl, carbamoyl, ureido, (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl, (1-4C)alkoxy, aryl, (1-4C)alkylthio, (1-4C)alkylsulphinyl, (1-4C)alkylsulphonyl, (1-4C)alkylamino, di-[(1-4C)alkyl]amino, (1-4C)alkoxycarbonyl, *N*-(1-4C)alkylcarbamoyl, *N*,*N*-di-[(1-4C)alkyl]carbamoyl, (2-4C)alkanoyl, (2-4C)alkanoyloxy, (2-4C)alkanoylamino, sulphamoyl, *N*-(1-4C)alkylsulphamoyl and *N*,*N*-di-[(1-4C)alkyl]sulphamoyl;

X_1, X_2, X_3 and X_4 are each selected from NH_2, NHR_d, NR_dR_e, SH and OH, with the proviso that at least one of X_1 and X_2, and at least one of X_3 and X_4, is NH_2;

R_d and R_e are independently selected from (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl and (1-4C)alkoxy; and

R_1 and R_2 are

(i) independently selected from hydrogen halo, trifluoromethyl, trifluoromethoxy, hydroxy, amino, formyl, carboxy, carbamoyl, ureido,

(1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl, (1-4C)alkoxy, (1-4C)alkylthio, (1-4C)alkylamino, di-[(1-4C)alkyl]amino, (1-4C)alkoxycarbonyl, *N*-(1-4C)alkylcarbamoyl, *N*,*N*-di-[(1-4C)alkyl]carbamoyl, (2-4C)alkanoyl, (2-4C)alkanoyloxy and (2-4C)alkanoylamino, or

(ii) linked, so that when R_1 and R_2 are taken together with the carbon atom to which they are each attached, they collectively form a phenyl or naphthalenyl group, or a 5-10 membered heteroaryl ring, optionally substituted with one or more substituents selected from halo, trifluoromethyl, trifluoromethoxy, cyano, nitro, hydroxy, mercapto, amino, formyl, carboxy, carbamoyl, ureido, (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl, (1-4C)alkoxy, (1-4C)alkylthio, (1-4C)alkylsulphinyl, (1-4C)alkylsulphonyl, (1-4C)alkylamino, di-[(1-4C)alkyl]amino, (1-4C)alkoxycarbonyl, *N*-(1-4C)alkylcarbamoyl, *N*,*N*-di-[(1-4C)alkyl]carbamoyl, (2-4C)alkanoyl, (2-4C)alkanoyloxy, (2-4C)alkanoylamino, sulphamoyl, *N*-(1-4C)alkylsulphamoyl and *N*,*N*-di-[(1-4C)alkyl]sulphamoyl

or a salt thereof.

[0053] In another particular embodiment, formulae (I) and (II) are defined as follows:

L_1 and L_2 are selected from one of the following options:

L_1 is absent and L_2 is $–[CR_aR_b]_2–$, or
L_1 is $–[CR_aR_b]_2–$ and L_2 is absent, or
L_1 is $–CR_aR_b–$ and L_2 is $–CR_aR_b–$;

L_3 is a straight or branched (2-12C)alkylene, (2-12C)alkenylene or (2-12C)alkynylene linker optionally comprising one or more substituted or unsubstituted heteroatoms selected from N, S and O disposed within the alkylene, alkenylene or alkynylene linker, wherein L_3 is optionally substituted with one or more R_c;

each group R_a and R_b is independently absent or selected from hydrogen, oxo, halo, trifluoromethyl, trifluoromethoxy, hydroxy, amino, carboxy, carbamoyl, (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl, (1-4C)alkoxy, aryl, (1-4C)alkylthio, (1-4C)alkylamino, di-[(1-4C)alkyl]amino, (1-4C)alkoxycarbonyl, *N*-(1-4C)alkylcarbamoyl, *N*,*N*-di-[(1-4C)alkyl]carbamoyl, (2-4C)alkanoyl, (2-4C)alkanoyloxy and (2-4C)alkanoylamino;

each group R_c is independently absent or selected from hydrogen, oxo, halo, trifluoromethyl, trifluoromethoxy, hydroxy, amino, carbamoyl, (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl, (1-4C)alkoxy, aryl, (1-4C)alkylthio, (1-

4C)alkylamino, di-[(1-4C)alkyl]amino, (1-4C)alkoxycarbonyl, *N*-(1-4C)alkylcarbamoyl, *N*,*N*-di-[(1-4C)alkyl]carbamoyl, (2-4C)alkanoyl, (2-4C)alkanoyloxy and (2-4C)alkanoylamino;

X_1, X_2, X_3 and X_4 are each selected from NH_2, NHR_d, NR_dR_e, SH and OH, with the proviso that at least one of X_1 and X_2, and at least one of X_3 and X_4, is NH_2;

R_d and R_e are independently selected from (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl and (1-4C)alkoxy; and

R_1 and R_2 are

(i) independently selected from hydrogen halo, trifluoromethyl, trifluoromethoxy, hydroxy, amino, formyl, carboxy, carbamoyl, ureido, (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl, (1-4C)alkoxy, (1-4C)alkylthio, (1-4C)alkylamino, di-[(1-4C)alkyl]amino, (1-4C)alkoxycarbonyl, *N*-(1-4C)alkylcarbamoyl, *N*,*N*-di-[(1-4C)alkyl]carbamoyl, (2-4C)alkanoyl, (2-4C)alkanoyloxy and (2-4C)alkanoylamino, or

(ii) linked, so that when R_1 and R_2 are taken together with the carbon atom to which they are each attached, they collectively form a phenyl group, or 5-6 membered heteroaryl ring, optionally substituted with one or more substituents selected from halo, trifluoromethyl, trifluoromethoxy, cyano, nitro, hydroxy, mercapto, amino, formyl, carboxy, carbamoyl, ureido, (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl, (1-4C)alkoxy, (1-4C)alkylthio, (1-4C)alkylsulphinyl, (1-4C)alkylsulphonyl, (1-4C)alkylamino, di-[(1-4C)alkyl]amino, (1-4C)alkoxycarbonyl, *N*-(1-4C)alkylcarbamoyl, *N*,*N*-di-[(1-4C)alkyl]carbamoyl, (2-4C)alkanoyl, (2-4C)alkanoyloxy, (2-4C)alkanoylamino, sulphamoyl, *N*-(1-4C)alkylsulphamoyl and *N*,*N*-di-[(1-4C)alkyl]sulphamoyl

or a salt thereof.

[0054] In another particular embodiment, formulae (I) and (II) are defined as follows:

L_1 and L_2 are selected from one of the following options:

L_1 is absent and L_2 is $–[CR_aR_b]_2–$, or
L_1 is $–[CR_aR_b]_2–$ and L_2 is absent, or
L_1 is $–CR_aR_b–$ and L_2 is $–CR_aR_b–$;

L_3 is a straight or branched (2-12C)alkylene, (2-12C)alkenylene or (2-12C)alkynylene linker optionally comprising one or more heteroatoms selected from N, S and O disposed within the alkylene, alkenylene or alkynylene linker, the one or more N heteroatoms being optionally

substituted with one or more groups selected from (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl, (1-4C)alkoxy or aryl, wherein L_3 is optionally substituted with one or more R_c;

each group R_a and R_b is independently absent or selected from hydrogen, oxo, halo, trifluoromethyl, trifluoromethoxy, hydroxy, amino, carboxy, carbamoyl, (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl, (1-4C)alkoxy and aryl;

each group R_c is independently absent or selected from hydrogen, oxo, halo, trifluoromethyl, trifluoromethoxy, hydroxy, amino, carbamoyl, (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl, (1-4C)alkoxy and phenyl;

X_1, X_2, X_3 and X_4 are each selected from NH_2, NHR_d, NR_dR_e, SH and OH, with the proviso that at least one of X_1 and X_2, and at least one of X_3 and X_4, is NH_2;

R_d and R_e are independently selected from (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl and (1-4C)alkoxy; and

R_1 and R_2 are

(i) independently selected from hydrogen halo, trifluoromethyl, trifluoromethoxy, hydroxy, amino, carboxy, carbamoyl, (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl and (1-4C)alkoxy, or

(ii) linked, so that when R_1 and R_2 are taken together with the carbon atom to which they are each attached, they collectively form a phenyl group, or 6 membered heteroaryl ring containing 1-3 nitrogen atoms, optionally substituted with one or more substituents selected from halo, trifluoromethyl, trifluoromethoxy, hydroxy, amino, carboxy, carbamoyl, (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl, (1-4C)alkoxy, (1-4C)alkylthio, (1-4C)alkylsulphinyl, (1-4C)alkylsulphonyl, (1-4C)alkylamino, di-[(1-4C)alkyl]amino, (1-4C)alkoxycarbonyl, *N*-(1-4C)alkylcarbamoyl, *N,N*-di-[(1-4C)alkyl]carbamoyl, (2-4C)alkanoyl, (2-4C)alkanoyloxy, (2-4C)alkanoylamino, sulphamoyl, *N*-(1-4C)alkylsulphamoyl and *N,N*-di-[(1-4C)alkyl]sulphamoyl

or a salt thereof.

[0055] In another particular embodiment, formulae (I) and (II) are defined as follows:

L_1 and L_2 are selected from one of the following options:

L_1 is absent and L_2 is $–[CR_aR_b]_2–$, or

L_1 is $–[CR_aR_b]_2–$ and L_2 is absent, or

L_1 is $–CR_aR_b–$ and L_2 is $–CR_aR_b–$;

L_3 is a straight or branched (2-12C)alkylene linker optionally comprising one or more heteroatoms selected from N, S and O disposed within the alkylene linker, the one or more N heteroatoms being optionally substituted with one or more groups selected from (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl, (1-4C)alkoxy or aryl, wherein L_3 is optionally substituted with one or more R_c;

each group R_a and R_b is independently absent or selected from hydrogen, oxo, halo, trifluoromethyl, trifluoromethoxy, hydroxy, amino, carboxy, carbamoyl, (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl, (1-4C)alkoxy and phenyl;

each group R_c is independently absent or selected from hydrogen, oxo, halo, trifluoromethyl, trifluoromethoxy, hydroxy, amino, carbamoyl, (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl, (1-4C)alkoxy and phenyl;

X_1, X_2, X_3 and X_4 are each selected from NH_2, NHR_d, NR_dR_e, SH and OH, with the proviso that at least one of X_1 and X_2, and at least one of X_3 and X_4, is NH_2;

R_d and R_e are independently selected from (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl and (1-4C)alkoxy; and

R_1 and R_2 are

(i) independently selected from hydrogen halo, trifluoromethyl, trifluoromethoxy, hydroxy, amino, carboxy, carbamoyl, (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl and (1-4C)alkoxy, or

(ii) linked, so that when R_1 and R_2 are taken together with the carbon atom to which they are each attached, they collectively form a phenyl group, or 6 membered heteroaryl ring containing 1-3 nitrogen atoms, optionally substituted with one or more substituents selected from halo, trifluoromethyl, trifluoromethoxy, hydroxy, amino, carboxy, carbamoyl, (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl, (1-4C)alkoxy, (1-4C)alkylthio, (1-4C)alkylsulphinyl, (1-4C)alkylsulphonyl, (1-4C)alkylamino, di-[(1-4C)alkyl]amino, (1-4C)alkoxycarbonyl, *N*-(1-4C)alkylcarbamoyl, *N,N*-di-[(1-4C)alkyl]carbamoyl, (2-4C)alkanoyl, (2-4C)alkanoyloxy, (2-4C)alkanoylamino, sulphamoyl, *N*-(1-4C)alkylsulphamoyl and *N,N*-di-[(1-4C)alkyl]sulphamoyl

or a salt thereof.

[0056] In another particular embodiment, formulae (I) and (II) are defined as follows:

L_1 and L_2 are selected from one of the following options:

L_1 is absent and L_2 is $–[CR_aR_b]_2–$, or

L_1 is $–[CR_aR_b]_2–$ and L_2 is absent, or

L_1 is $–CR_aR_b–$ and L_2 is $–CR_aR_b–$;

L_3 is a straight or branched (2-12C)alkylene linker optionally comprising one or more heteroatoms selected from N, S and O disposed within the alkylene linker, the one or more N heteroatoms being optionally substituted with one or more groups selected from (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl, (1-4C)alkoxy or aryl, wherein L_3 is optionally substituted with one or more R_c;

each group R_a and R_b is independently absent or selected from hydrogen, oxo, halo, trifluoromethyl, trifluoromethoxy, hydroxy, amino, carboxy, carbamoyl, (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl, (1-4C)alkoxy and phenyl;

each group R_c is independently absent or selected from hydrogen, oxo, halo, trifluoromethyl, trifluoromethoxy, hydroxy, amino, carbamoyl, (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl, (1-4C)alkoxy and phenyl;

X_1, X_2, X_3 and X_4 are each selected from NH_2, NHR_d and NR_dR_e, with the proviso that at least one of X_1 and X_2, and at least one of X_3 and X_4, is NH_2;

R_d and R_e are independently selected from (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl and (1-4C)alkoxy; and

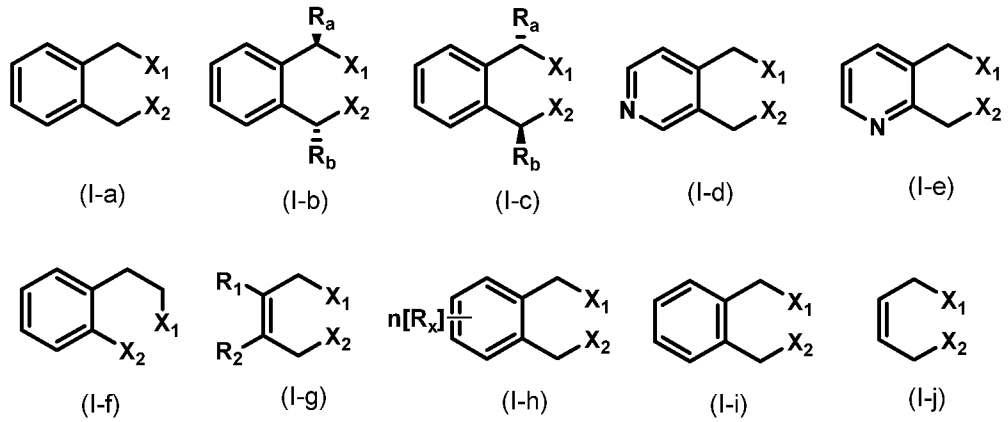
R_1 and R_2 are

(i) independently selected from hydrogen halo, trifluoromethyl, trifluoromethoxy, hydroxy, amino, carboxy, carbamoyl, (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl and (1-4C)alkoxy, or

(ii) linked, so that when R_1 and R_2 are taken together with the carbon atom to which they are each attached, they collectively form a phenyl group, or 6 membered heteroaryl ring containing 1-3 nitrogen atoms, optionally substituted with one or more substituents selected from halo, trifluoromethyl, trifluoromethoxy, hydroxy, amino, carboxy, carbamoyl, (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl, (1-4C)alkoxy, (1-4C)alkylthio, (1-4C)alkylamino and di-[(1-4C)alkyl]amino

or a salt thereof.

[0057] In another embodiment, formula (I) may be represented by any of formulae (I-a) – (I-j) shown below:



wherein

R_a and R_b have any of the definitions listed in numbered paragraphs (21) – (23) above;

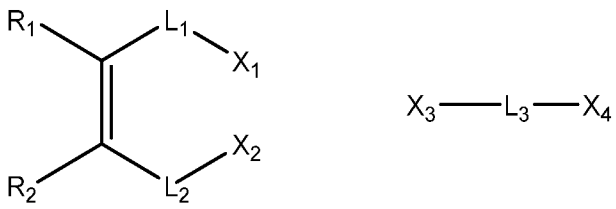
X_1 and X_2 have any of the definitions listed in numbered paragraphs (9) – (11) above;

each group R_x is independently selected from any of the ring substituents listed in numbered paragraphs (15) – (20) above; and

n is a whole integer 1 – 4

or a salt thereof.

[0058] In another embodiment, the amine donating compound has a structure according to either of formulae (I) or (II) shown below:



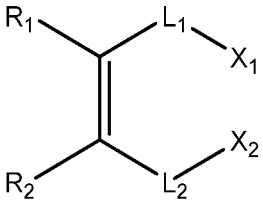
(I) (II)

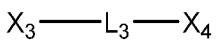
wherein

R_1, R_2, L_1, L_2, L_3, X_1 and X_2 have any of the definitions discussed herein; and

X_3 and X_4 are both NH_2.

[0059] In another embodiment, the amine donating compound has a structure according to either of formulae (I) or (II) shown below:





(I) (II)

wherein

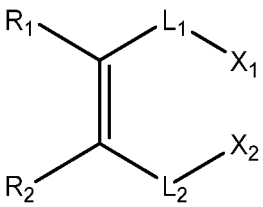
R_1, R_2, L_1, L_2, X_1 and X_2 have any of the definitions discussed herein;

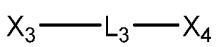
L_3 is as defined in any of numbered paragraphs (6)-(8) above;

each group R_c is independently as defined in numbered paragraph (28) above; and

X_3 and X_4 are both NH_2.

[0060] In another embodiment, the amine donating compound has a structure according to either of formulae (I) or (II) shown below:





(I) (II)

wherein

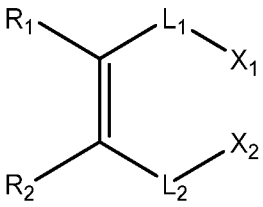
R_1, R_2, L_1, L_2, X_1 and X_2 have any of the definitions discussed herein;

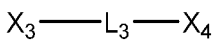
L_3 is as defined in numbered paragraph (8) above;

each group R_c is independently as defined in numbered paragraph (28) above; and

X_3 and X_4 are both NH_2.

[0061] In another embodiment, the amine donating compound has a structure according to either of formulae (I) or (II) shown below:





(I) (II)

wherein

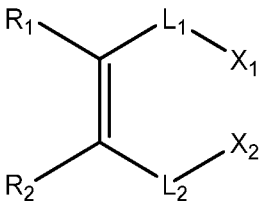
R_1, R_2, L_1, L_2, X_1 and X_2 have any of the definitions discussed herein;

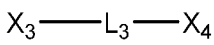
L_3 is a straight or branched (2-12C)alkylene linker optionally comprising one or more heteroatoms selected from N, S and O disposed within the alkylene linker, the one or more heteroatoms being optionally substituted with one or more groups selected from (1-4C)alkyl or (2-4C)alkenyl, wherein L_3 is optionally substituted with one or more R_c;

each group R_c is independently (1-4C)alkyl (e.g. methyl) or (2-4C)alkenyl; and

X_3 and X_4 are both NH_2.

[0062] In another embodiment, the amine donating compound has a structure according to either of formulae (I) or (II) shown below:





(I) (II)

wherein

R_1, R_2, L_1, L_2, X_1 and X_2 have any of the definitions discussed herein;

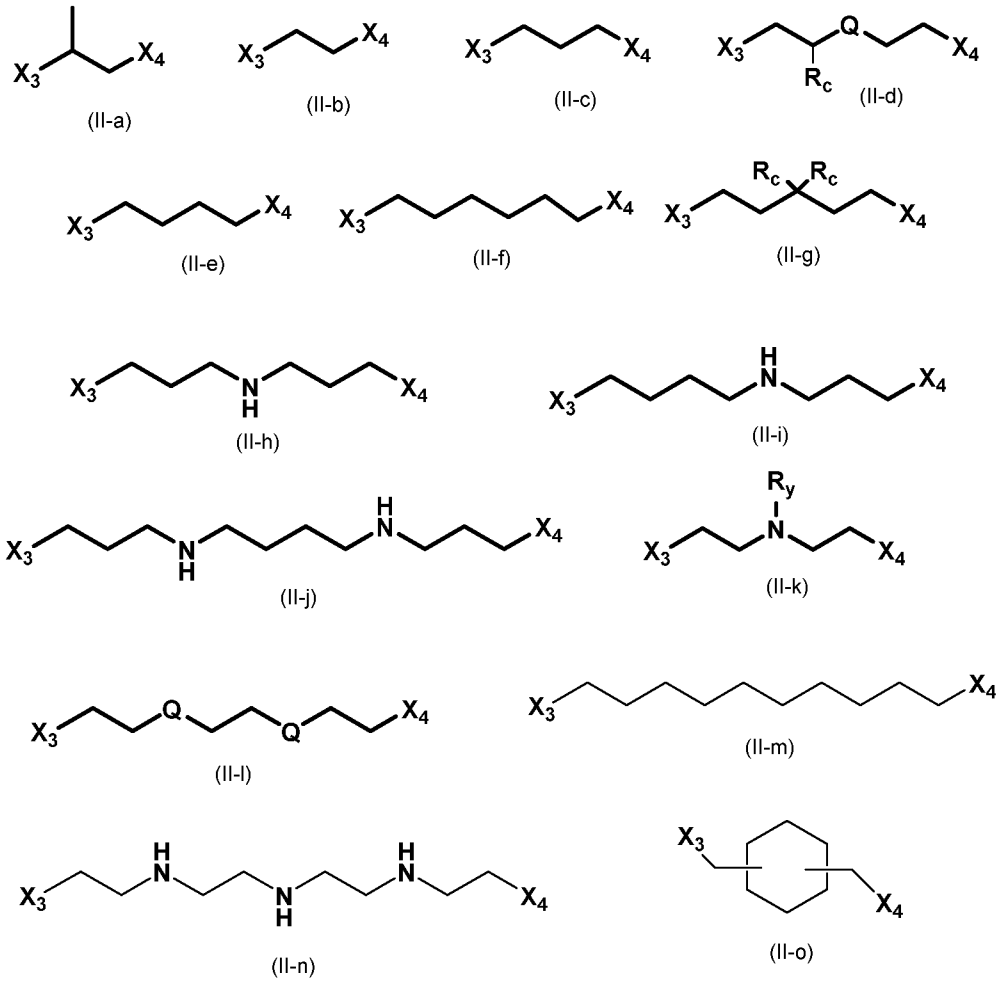
L_3 is a straight or branched (2-12C)alkylene linker optionally comprising one or more heteroatoms selected from N or O disposed within the alkylene linker, the one or more heteroatoms being optionally substituted with one or more groups selected from (1-4C)alkyl or (2-4C)alkenyl, wherein L_3 is optionally substituted with one or more R_c;

each group R_c is independently (1-4C)alkyl (e.g. methyl) or (2-4C)alkenyl; and

X_3 and X_4 are both NH_2.

[0063] In another embodiment, the amine donating compound has a structure according to either of formulae (I) or (II), wherein R_1, R_2, L_1, L_2 and L_3 have any of the definitions discussed herein, and X_1, X_2, X_3 and X_4 are all NH_2.

[0064] In another embodiment, formula (II) may be represented by any of formulae (II-a) – (II-o) shown below:



wherein

X_3 and X_4 have any of the definitions listed in numbered paragraphs (9) – (11) above. Suitably X_3 and X_4 have any of the definitions listed in numbered paragraph (11) above. More suitably X_3 and X_4 are both NH_2;

each group R_c is independently as defined in any of numbered paragraphs (24) – (28) above; suitably, each group R_c is independently as defined in numbered paragraph (28) above;

R_y is selected from any of the heteroatom substituents listed in paragraphs (7) – (8) above; and

Q is either carbon or oxygen;

or a salt thereof.

In another embodiment, in formulae (II-a) – (II-o) shown above, the alkylene linker L_3 may be substituted at any feasible position with one or more groups R_c, wherein each group R_c is independently as defined in numbered paragraph (28) above. Suitably, each group R_c is methyl.

[0065] In another embodiment, formula (II) may be represented by any of formulae (II-a) – (II-o) shown above, wherein

X_3 and X_4 are both NH_2;

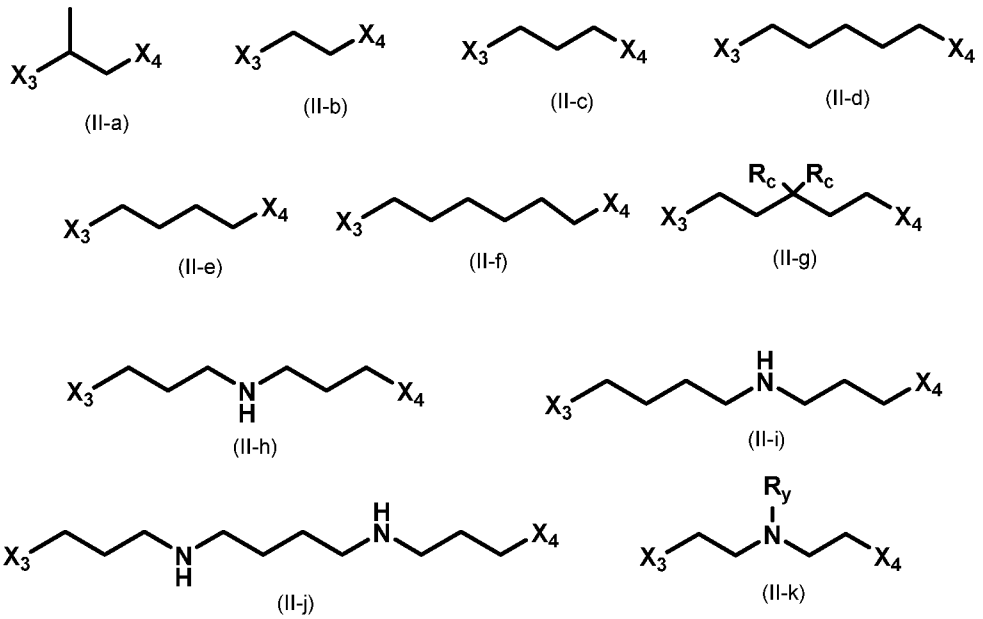
each group R_c is independently selected from (1-4C)alkyl or oxo;

R_y is selected from any of the heteroatom substituents listed in paragraphs (7) – (8) above; and

Q is carbon or oxygen;

or a salt thereof.

[0066] In another embodiment, formula (II) may be represented by any of formulae (II-a) – (II-k) shown below:



wherein

X_3 and X_4 have any of the definitions listed in numbered paragraphs (9) – (11) above. Suitably X_3 and X_4 have any of the definitions listed in numbered paragraph (11) above. More suitably X_3 and X_4 are both NH_2;

each group R_c is independently as defined in any of numbered paragraphs (24) – (27) above; and

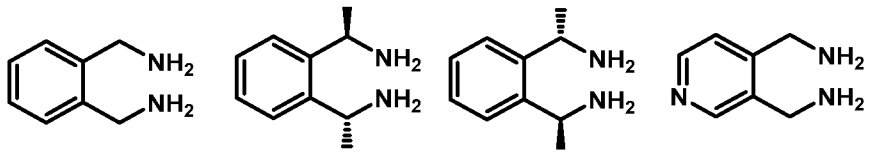
R_y is selected from any of the heteroatom substituents listed in paragraphs (7) – (8) above

or a salt thereof.

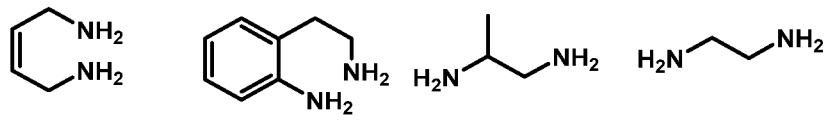
In another embodiment, in formulae (II-a) – (II-k) shown above, the alkylene linker L_3 may be substituted at any feasible position with one or more groups R_c, wherein each group R_c is independently as defined in numbered paragraph (28) above. Suitably, each group R_c is methyl.

[0067] In another embodiment, formula (II) may be represented by any of formulae (II-a) – (II-k) shown above, wherein each group R_c is independently as defined in numbered paragraph (28) above.

[0068] In another embodiment, the amine donating compound of formula (I) or (II) has any of the structures (AD1 – AD8) shown below:



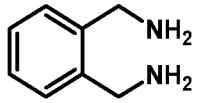
AD1 AD2 AD3 AD4



AD5 AD6 AD7 AD8

The skilled person will appreciate that any of the compounds AD1 – AD8 shown above may be provided as a salt. Suitably, the compounds may be provided as a hydrochloride salt.

[0069] In a particularly preferred embodiment, the amine donating compound of formula (I) has the following structure:



AD1

The skilled person will appreciate that the above compound AD1 may be provided as a salt. Suitably, the compound may be provided as a hydrochloride salt.

[0070] In another embodiment, the amine donating compound may be any one of AD1-AD11 defined herein (e.g. any one of AD1-AD8), or may be:

1,3-diaminopropane, 1,4-diaminobutane, 1,5-diaminopentane, 1,6-diaminohexane, 1,8-diaminooctane, 1,10-diaminodecane, 2-methyl-1,5-diaminopentane, diethylenetriamine, bis-(2-aminoethyl)-ether, spermidine, spermine, cyclohexane-bismethylenediamine, 1,8-diamino-3,6-dioxooctane, or tetraethylenepentamine.

Screening transaminases or aminotransferases

Identification of suitable substrates

[0071] As discussed hereinbefore, the present invention provides a process for assessing the ability of a transaminase or aminotransferase to catalyse the conversion of a ketone or aldehyde substrate into an amine, the process comprising the steps of:

a) contacting one or more ketone or aldehyde substrates with an amine donating compound of formula (I) or (II) defined herein, in the presence of one or more transaminase or aminotransferase, and
b) analysing the reaction medium for the presence of a by-product,

wherein the presence of a by-product indicates a successful conversion of the ketone or aldehyde substrate into the amine.

[0072] The present invention also provides a use of an amine donating compound of formula (I) or (II) defined herein in a process for assessing the ability of a transaminase or aminotransferase to catalyse the conversion of a ketone or aldehyde substrate into an amine, the process comprising the steps of:

a) contacting one or more ketone or aldehyde substrates with an amine donating compound of formula (I) or (II) defined herein, in the presence of one or more transaminase or aminotransferase, and
b) analysing the reaction medium for the presence of a by-product,

wherein the presence of a by-product indicates a successful conversion of the ketone or aldehyde substrate into the amine.

[0073] Having regard to the challenges that presently complicate the development of efficient transaminase or aminotransferase-mediated transformations, the above-described method and use provide an ideal high-throughput platform for evaluating panels of

commercially-available transaminase or aminotransferase biocatalysts for activity towards large libraries of ketone or aldehyde substrates using the improved amine donating compounds of formulae (I) and (II).

[0074] In one embodiment, the one or more transaminase or aminotransferase is selected from a group consisting of polyamine aminotransferases (including diamine aminotransferases) and ω-TAs. Suitably, the transaminase or aminotransferase is a ω-TAs.

[0075] In another embodiment, step a) comprises contacting a plurality of different ketone or aldehyde substrates each with an amine donating compound of formula (I) or (II) defined herein, each in the presence of a single transaminase or aminotransferase. The process or use described above can therefore be used to screen a plurality of different substrates for their compatibility with a given transaminase or aminotransferase and an amine donating compound of formula (I) or (II).

[0076] In another embodiment, step a) comprises contacting a plurality of identical ketone or aldehyde substrates each with an amine donating compound of formula (I) or (II) defined herein, each in the presence of a different transaminase or aminotransferase. The process or use described above can therefore be used to screen a plurality of different enzymes for their ability to reductively aminate a ketone or aldehyde substrate using an amine donating compound of formula (I) or (II).

[0077] In another embodiment, step a) comprises contacting a plurality of different ketone or aldehyde substrates each with an amine donating compounds of formula (I) or (II) defined herein, each in the presence of a different transaminase or aminotransferase. The process or use described above can therefore be used to simultaneously screen a range of substrates and a range of enzymes for their compatibility with an amine donating compound of formula (I) or (II).

[0078] In another embodiment, the substrate is a ketone substrate.

[0079] The by-products generated by the method and use described above can be detected by numerous techniques. In one embodiment, step b) involves observing the reaction medium with the naked eye for the presence of a coloured by-product. Where the reaction of the ketone or aldehyde substrate with the amine donating compound of formula (I) or (II) results in the formation of an aromatic species, said species may undergo spontaneous polymerisation (e.g. under oxidative conditions) to yield a highly-coloured insoluble polymeric precipitate. In such instances, the ability of a transaminase or aminotransferase to catalyse the conversion of a ketone or aldehyde substrate into an amine using an amine donating compound of formula (I) or (II) can be determined simply by analysing the reaction medium with the naked eye, thereby enabling high-throughput qualitative screening. Optionally, the

reaction medium may be analysed by colorimetric analytical techniques in order to provide quantitative data on the ability of a transaminase or aminotransferase to catalyse the conversion of a ketone or aldehyde substrate into an amine (e.g. rate of conversion).

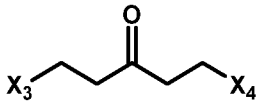
[0080] In another embodiment, where the reaction of the ketone or aldehyde substrate with the amine donating compound of formula (I) or (II) results in the formation of an aromatic species, the process or use may additionally comprise, prior to step b), a step of subjecting the aromatic species to conditions sufficient to cause it to polymerise. The aromatic species may be induced to polymerise spontaneously by oxidizing the aromatic species. The oxidization may be achieved by any chemical, electrochemical or enzymatic approach known to the skilled person. The resulting by-product may be a highly-coloured insoluble polymeric precipitate. In such instances, step b) may therefore involve observing the reaction medium with the naked eye for the presence of a coloured by-product. Optionally, the reaction medium may be analysed by colorimetric analytical techniques in order to provide quantitative data on the ability of a transaminase or aminotransferase to catalyse the conversion of a ketone or aldehyde substrate into an amine (e.g. rate of conversion).

[0081] In another embodiment, where the reaction of the ketone or aldehyde substrate with the amine donating compound of formula (I) or (II) results in the formation of a soluble by-product (aromatic or otherwise), step b) may comprise analysing the reaction medium for the presence of the by-product by one or more techniques selected from spectrophotometry, chromatography (including HPLC and GPC), IR spectroscopy, mass spectroscopy and NMR spectroscopy. Such analyses may be quantitative in nature. Suitably, the reaction medium is analysed by spectrophotometry.

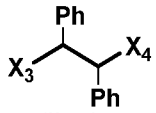
[0082] Having regard to step b), the skilled person will have an awareness of the type of by-product generated by each of the amine donating compounds of formulae (I) and (II) defined herein, and will appreciate which analytical technique will be required in order to detect them.

[0083] In one embodiment, step a) comprises contacting one or more ketone or aldehyde substrate with an amine donating compound of formula (I) or (II) defined in any of the preceding paragraphs, in the presence of one or more transaminase or aminotransferase.

[0084] In another embodiment, step a) comprises contacting one or more ketone or aldehyde substrate with an amine donating compound (a) of formula (I) as defined in any of the preceding paragraphs, or (b) of formula (II-l) – (II-m) defined below, in the presence of one or more transaminase or aminotransferase:



(II-l)

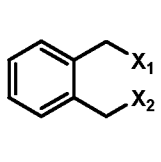


(II-m)

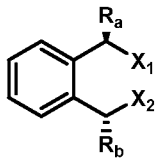
wherein

X_3 and X_4 have any of the definitions listed in numbered paragraphs (9) – (11) above.

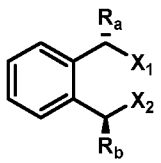
[0085] In another embodiment, step a) comprises contacting one or more ketone or aldehyde substrate with an amine donating compound of formula (I-a) – (I-j) or (II-l) – (II-m) defined below, in the presence of one or more transaminase or aminotransferase:



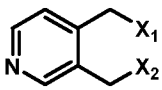
(I-a)



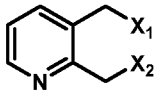
(I-b)



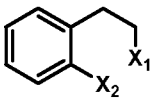
(I-c)



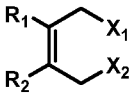
(I-d)



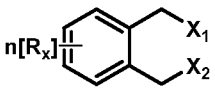
(I-e)



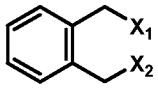
(I-f)



(I-g)



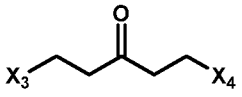
(I-h)



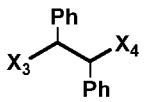
(I-i)



(I-j)



(II-l)



(II-m)

wherein

R_a and R_b have any of the definitions listed in numbered paragraphs (21) – (23) above;

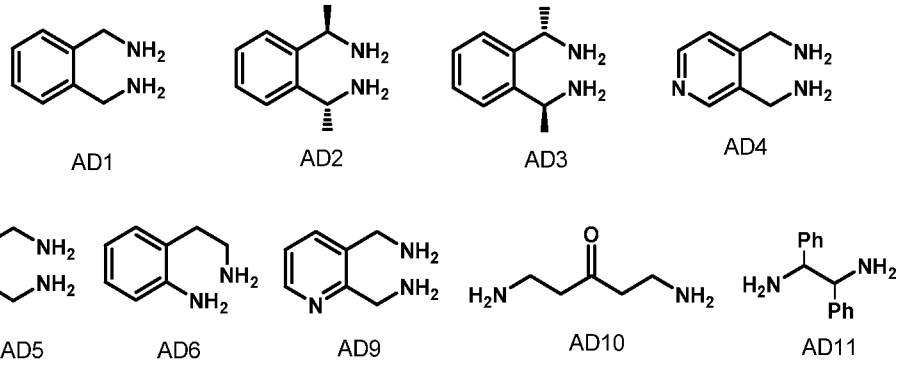
X_1 and X_2 have any of the definitions listed in numbered paragraphs (9) – (11) above;

X_3 and X_4 have any of the definitions listed in numbered paragraphs (9) – (11) above;

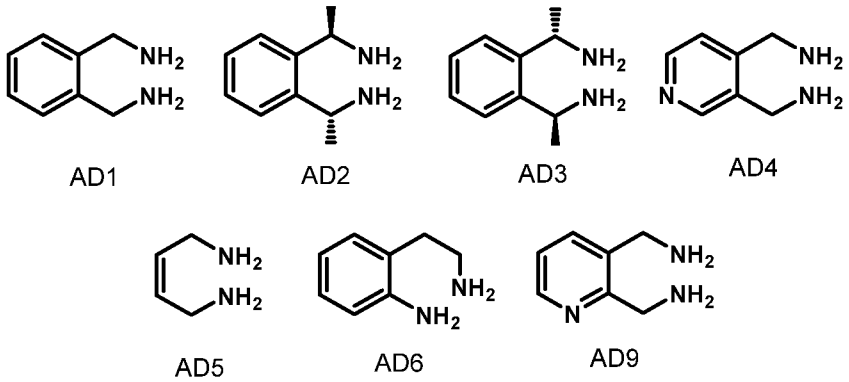
each group R_x is independently selected from any of the ring substituents listed in numbered paragraphs (15) – (20) above; and

n is a whole integer 1 – 4.

[0086] In another embodiment, step a) comprises contacting one or more ketone or aldehyde substrate with an amine donating compound of formula (I) or (II) having any of the structures (AD1-AD6, AD9-AD11) shown below, in the presence of one or more transaminase or aminotransferase:



[0087] In another embodiment, step a) comprises contacting one or more ketone or aldehyde substrate with an amine donating compound of formula (I) having any of the structures (AD1-AD6, AD9) shown below, in the presence of one or more transaminase or aminotransferase:



[0088] In a preferred embodiment, step a) comprises contacting one or more ketone or aldehyde substrate with an amine donating compound of formula (I) having any of the structures AD1-AD6, and AD9 shown above, in the presence of one or more transaminase or aminotransferase, and step b) comprises observing the reaction medium with the naked eye for the presence of a coloured by-product.

[0089] The present invention also provides a kit of parts suitable for assessing the ability of a transaminase or aminotransferase to catalyse the conversion of a ketone or aldehyde substrate into an amine in accordance with a screening process or use defined herein, the kit comprising:

a) one or more amine donating compound of formula (I) or (II) defined herein, and either or both of:

   i. one or more ketone or aldehyde substrates, and

ii. one or more transaminase or aminotransferase.

[0090] It will be understood that the operator of the kit of parts will have a bearing on the specific contents of the kit. For example, where an operator's business activities are constrained by the use of a particular enzyme, he may wish to determine which ketone or aldehyde substrates and which amine donating compounds are compatible with that enzyme. In such cases, it will be understood that the kit will comprise one or more amine donating compound of formula (I) or (II) defined herein, and one or more ketone or aldehyde substrates. Alternatively, where an operator's business activities are constrained by the use of a particular substrate, he may wish to determine which enzymes and amine donating compounds are compatible with that substrate. In such cases, it will be understood that the kit will comprise one or more amine donating compound of formula (I) or (II) defined herein, and one or more transaminase or aminotransferase. In situation where an operator is constrained neither by a particular substrate nor a particular enzyme, it will be understood that the kit may comprise one or more amine donating compound of formula (I) or (II) defined herein, one or more ketone or aldehyde substrates, and one or more transaminase or aminotransferase.

[0091] In an embodiment, the kit may additionally comprise a set of instructions for performing a screening process defined herein.

[0092] In another embodiment, the kit may additionally comprise means for analysing for the presence of a by-product. As discussed hereinbefore, the skilled person will have an awareness of the type of by-product generated by reaction of each of the amine donating compounds of formulae (I) and (II) defined herein with a ketone or aldehyde substrate, and will therefore appreciate which analytical technique (and therefore which analytical means) will be required in order to detect them. For amine donating compounds that generate a coloured by-product, the means for analysing may comprise a reference result (e.g. a colour chart) informing an operator of the particular colour expected in the case of a positive result. For amine donating compounds that do not generate coloured by-products, the means for analysing may comprise one or more analytical tests commensurate with the specific type of by-product being detected. Suitable analytical tests will be apparent to the skilled person.

[0093] The present invention also provides a process for assessing the ability of an amine oxidase to catalyse the oxidation of an amine donating compound of formula (I) or (II) defined herein, the process comprising the steps of:

a) contacting one or more amine donating compound of formula (I) or (II) defined herein with an oxidizing agent, in the presence of one or more amine oxidase, and
b) analysing the reaction medium for the presence of a by-product,

wherein the presence of a by-product indicates a successful oxidation of the amine donating compound.

[0094] Owing to the similarities in by-products formed, the amine donating compound may also be used as a substrate in methods of screening for amine oxidases (for example monoamine oxidases and diamine oxidases).

[0095] In one embodiment, step a) involves contacting one or more amine donating compound of formula (I) defined herein with an oxidizing agent, in the presence of one or more amine oxidase. Suitably, step a) involves contacting an amine donating compound AD1 defined herein with an oxidizing agent, in the presence of one or more amine oxidase.

Identification and development of new enzymes

[0096] As described hereinbefore, the present invention also provides a process for determining whether a sample exhibits transaminase or aminotransferase activity, the process comprising the steps of:

a) providing one or more sample having putative transaminase or aminotransferase activity;

b) contacting the one or more samples of step a) with one or more amine-donating compound of formula (I) or (II) defined herein; and

c) analysing the reaction medium for the presence of a by-product,

wherein the presence of a by-product indicates that the sample exhibits transaminase or aminotransferase activity.

[0097] The present invention also provides a use of an amine donating compound of formula (I) or (II) defined herein in a process for determining whether a sample exhibits transaminase or aminotransferase activity, the process comprising the steps of:

a) providing one or more sample having putative transaminase or aminotransferase activity;

b) contacting the one or more sample of step a) with one or more amine-donating compound of formula (I) or (II) defined herein; and

c) analysing the reaction medium for the presence of a by-product,

wherein the presence of a by-product indicates that the sample exhibits transaminase or aminotransferase activity.

[0098] Having regard to the challenges that presently complicate the development of efficient transaminase or aminotransferase-mediated transformations, the above-described method and use provide an ideal platform for screening genomic libraries and culture collections in order to identify a new generation of so far undiscovered transaminases or aminotransferases, thereby greatly facilitating the process of engineering enzymes with high activity towards selected amine donors and with enhanced properties for biocatalytic applications.

[0099] Depending on a user's objectives, the above-described process or use may involve a) screening for transaminase or aminotransferase activity in a single sample using a range of amine donating compounds of formulae (I) and (II), b) screening for transaminase or aminotransferase activity in a plurality of samples using a single amine donating compound of formula (I) or (II), or c) a combination of both aforementioned screening methods.

[00100] In one embodiment, the sample(s) being tested for transaminase or aminotransferase activity may be an enzyme, protein or other biological sample.

[00101] In an embodiment, the biological sample may be any biological sample may comprise biological cells (in the form of a tissue sample including cells, cultured cells, or microorganisms), or an extract (such as a cell extract or lysate).

[00102] In another embodiment, the above described process and use may be conducted a part of a colony-based assay.

[00103] The skilled person will appreciate that in the majority of cases, the sample itself contains quantities of ketone or aldehyde substrate sufficient for reacting with the amine-donating compound of formula (I) or (II) defined herein, so as to produce a detectable quantity of by-product. The skilled person will also appreciate which samples are themselves unlikely to contain quantities of ketone or aldehyde substrate. In these embodiments, step b) comprises contacting the one or more sample of step a) with one or more amine-donating compound of formula (I) or (II) defined herein and one or more ketone or aldehyde substrate.

[00104] In one embodiment, the transaminase or aminotransferase is selected from a group consisting of polyamine aminotransferases (including diamine aminotransferases) and ω-TAs. Suitably, the transaminase or aminotransferase is a ω-TAs.

[00105] In another embodiment, step b) comprises contacting the one or more sample with one or more amine donating compound of formula (I) or (II) as defined in any of the preceding paragraphs.

[00106] Step c) may involve one or more of the analytical techniques described hereinbefore. The skilled person will have an awareness of the type of by-product generated

by each of the amine donating compounds of formulae (I) and (II) defined herein, and will appreciate which analytical technique will be required in order to detect them. Where, depending on the particular amine donating compound being used, step b) does not lead to the generation of a detectable by-product (e.g. an aromatic product that does not spontaneously polymerise), the process or use may additionally comprise, prior to step c), a step of subjecting the product of step b) to conditions sufficient to cause it to polymerise. In these cases, the product of step b) may be induced to polymerise spontaneously by oxidization. The oxidization may be achieved by any chemical, electrochemical or enzymatic approach known to the skilled person.

[00107] The present invention also provides a kit of parts for determining whether a sample exhibits transaminase or aminotransferase activity in accordance with a screening process or use defined herein, the kit comprising:

a) one or more amine donating compound of formula (I) or (II) defined herein, and
b) instructions for performing a screening process defined herein

[00108] Where the skilled person that the sample being tested is unlikely to contain quantities of ketone or aldehyde substrate, the kit additionally comprises one or more ketone or aldehyde substrate.

[00109] In an embodiment, the kit may additionally comprise means for analysing for the presence of a by-product. As discussed hereinbefore, the skilled person will have an awareness of the type of by-product generated by reaction of each of the amine donating compounds of formulae (I) and (II) defined herein with a ketone or aldehyde substrate, and will therefore appreciate which analytical technique (and therefore which analytical means) will be required in order to detect them. For amine donating compounds that generate a coloured by-product, the means for analysing may comprise a reference result (e.g. a colour chart) informing an operator of the particular colour expected in the case of a positive result. For amine donating compounds that do not generate coloured by-products, the means for analysing may comprise one or more analytical tests commensurate with the specific type of by-product being detected. Suitable analytical tests will be apparent to the skilled person.

[00110] The amine donating compound may have any of the definitions outlined herein. In a preferred embodiment, the one or more amine donating compound is selected from any of compounds AD1-AD6 and AD9 defined herein.

[00111] The present invention also provides a process for determining whether a sample exhibits amino oxidase activity, the process comprising the steps of:

a) providing one or more sample having putative amino oxidase activity;

b) contacting the one or more samples of step a) with one or more amine-donating compound of formula (I) or (II) defined herein and a suitable oxidizing agent; and

c) analysing the reaction medium for the presence of a by-product,

wherein the presence of a by-product indicates that the sample exhibits amino oxidase activity.

[00112] Owing to the similarities in by-products formed, the amine donating compound may also be used as a substrate in methods of screening samples for new amine oxidases (for example monoamine oxidases and diamine oxidases).

[00113] In one embodiment, step b) involves contacting the one or more samples of step a) with one or more amine-donating compound of formula (I) defined herein, and a suitable oxidizing agent. Suitably, step a) involves contacting the one or more samples of step a) with an amine-donating compound AD1 defined herein, and a suitable oxidizing agent.

Preparation of polymers

[00114] As discussed hereinbefore, the present invention provides a process for preparing a polymer, the process comprising the steps of:

a) reacting a ketone or aldehyde substrate with an amine donating compound of formula (I) as defined herein in the presence of a transaminase or aminotransferase, such that a reaction by-product is formed, and

b) subjecting the reaction by-product to conditions suitable for causing polymerisation of the reaction by-product.

[00115] In one embodiment, the amine donating compound is AD1 or a salt thereof, and the polymer is polyisoindole.

EXAMPLES

[00116] One or more examples of the invention will now be described, for the purpose of reference and illustration only, with reference to the accompanying figures, in which:

Fig. 1 shows ^{1}H and ^{13}C NMR spectra for (*S*)-1-(4-fluorophenyl)propan-2-amine.

Fig. 2 shows conversion of **S6** (5 mM) to the (*S*) enantiomer of the corresponding amine using commercially available ω-TAs and diamine **AD1** (5 mM). L1, L3 and L5 A-F contain the

(*R*)-selective Codexis enzymes ATA025, 303, 013, 301, 415 and 117 respectively. L2, L4 and L6 contain the (*S*)-selective Codexis enzymes ATA254, G05, 260, 256, 234 and 113 respectively. L1-2 = diamine **AD1** only, 3h; L3-4 = 15min after addition of substrate **S6;** L5-6 = 24h after addition of substrate **S6**; L7 A-F = Almac TAm106, 107, 115, 121, 125 and 140 respectively, substrate **S6**, diamine **AD1**, 24h.

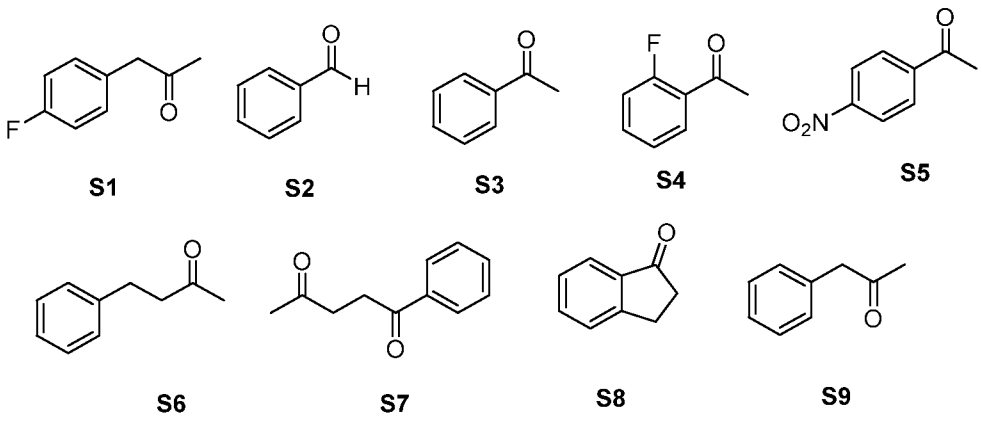
Fig. 3 shows a colony-based screen with *o*-xylylenediamine **AD1**. Cells expressing the *pf*-ATA gene turn dark in colour after 30 min (right). Cells lacking the *pf*-ATA gene remain colourless (left).

General Methods and Materials

[00117] General: All biotransformations were carried out in HEPES buffer (100mM, pH 7.5) at 30 ºC. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 400 spectrometer (400.1 MHz for ^{1}H and and 100.6 MHz for ^{13}C) in $CDCl_3$. The chemical shifts were recorded in ppm with the residual solvent signal referenced to 7.26 ppm and 77.23 ppm for ^{1}H and ^{13}C respectively. Coupling constants (*J*) are reported in Hz and refer to the observed peak multiplicities. GC-FID analysis was performed on an Agilent 5890 series II equipped with an autosampler and a CYCLOSIL-B (J & W scientific), 30m x 0.32mm x 0.25DF column.

[00118] Materials: Commercially available reagents were used throughout without further purification. Reagents were purchased from Sigma Aldrich, Acros or Formedium. Hybond membranes were purchased from GE Healthcare. *Escherichia coli* BL21(DE3) were purchased from Invitrogen (Carlsbad, CA). Expression vector pET-16b (69662-3) was purchased from Novagen (Darmstadt, Germany). Commercially available transaminases were kindly supplied by Almac and AstraZeneca in the form of lyophilised cell extract and as immobilized enzymes from Purolite. The transaminase gene from *Pseudogulbenkiania ferrooxidans* (*pf*-ATA) was expressed and used as described in O'Reilly *et al.*[26]

Substrates **S1-S9** used in the biotransformations are provided in Scheme 3 below:



Scheme 3

Example 1 - *Analytical scale biotransformations of substrates **S1-S8** using ATA113 as ω-TA, and **AD1**, L-alanine or benzylamine as amine donor*

[00119] Commercially available (*S*)-selective ATA113 (available from Codexis) (1.5 mg/mL) was rehydrated in HEPES buffer (1mL, 100 mM, pH 7.5) containing PLP (2.02 mM) and xylylenediamine dihydrochloride (**AD1**) (5 mM, 5.5 mM, 6 mM, 6.5 mM or 7.5 mM). For comparison, benzylamine (7.5 mM, 1.5 equiv) and L-alanine (50 mM, 10 equiv or 500 mM, 100 equiv) were also tested as amine donors under the same reaction conditions (where necessary, the pH of the solutions were adjusted to 7.5). The substrate (5 mM from a 200 mM stock in DMSO) was added and the mixture incubated at 30 ºC, 150 rpm in a shaking incubator. The reactions were analysed by GC-FID after 24 and 48 hours following extraction of the basified (pH 12 adjusted with 5 M NaOH) solution (100 uL) with EtOAc (1 x 300 uL).

Example 2 - *Analytical scale biotransformations of substrates **S1** and **S8** using ATA113 as ω-TA, and L-alanine as amine donor, in combination with the LDH/GDH pyruvate removal system*

[00120] Commercially available (*S*)-selective ATA113 (1.5 mg/mL) was rehydrated in HEPES buffer (1mL, 100 mM, pH 7.5) containing PLP (2.02 mM), NAD^+ (1.5 mM), glucose (10 mg/mL, 55.5 mM), GDH (50 U), LDH (113 U) and L-alanine (50 mM, 10 equiv). The pH of the mixture was adjusted to 7.5. The substrate (5 mM from a 200 mM stock in DMSO) was added and the mixture incubated at 30 ºC, 150 rpm in a shaking incubator. The reactions were analysed by GC-FID after 24 hours following extraction of the basified (pH 12 adjusted with 5 M NaOH) solution (100 uL) with EtOAc (1 x 300 uL).

Example 3 - *Biotransformation of substrate S1 with pf-ATA as ω-TA and AD1 as amine donor*

[00121] *pf*-ATA was expressed as described previously. The 'clarified cell extract' (100 uL/mL) was added to HEPES buffer (100 mM, pH 7.5) containing PLP (2.02 mM) and xylylenediamine dihydrochloride (**AD1**) (5 mM). Substrate **S1** (5 mM from a 200 mM stock in DMSO) was added and the mixture incubated at 30 ºC, 150 rpm in a shaking incubator. The reaction was analysed by GC-FID after 24 hours following extraction of the basified (pH 12 adjusted with 5 M NaOH) solution (100 uL) with EtOAc (1 x 300 uL). The conversion after 24 hours was low, but measureable (<5%).

Example 4 – *Analysis of amine donor performance*

[00122] Table 1 below shows the conversion and enantiomeric excess data for the reactions of Examples 1 and 2.

Table 1 - Conversion and enantiomeric excess data for reactions of Examples 1 and 2.

Substrate	Amine donor	Equiv.	Conv.	*ee* [%]
S1	**AD1**	1.0	> 99	> 99 (*S*)
S2	**AD1**	1.1	98	n.a.
S3	**AD1**	1.5	> 99	> 99 (*S*)
S4	**AD1**	1.1	96	> 99 (*S*)
S5	**AD1**	1.2	97	> 99 (*S*)
S6	**AD1**	1.0	> 99	78 (*S*)
S7	**AD1**	1.0	> 99	> 99 (*S*)
S8	**AD1**	1.5	73	> 99 (*S*)
S1	L-Ala (no p.r.)	10	< 5	> 99 (*S*)
S1	Benzylamine	1.5	< 5	> 99 (*S*)
S8	L-Ala (LDH/GDH)	10	21	> 99 (*S*)
S8	L-Ala (no p.r.)	100	n.d.	n.a.

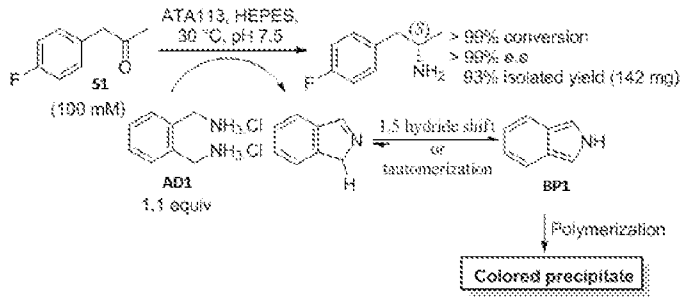
Conversions are reported after 48 hours. p.r. = pyruvate removal system; LDH = lactate dehydrogenase; GDH = Glucose dehydrogenase; n.d. = not detected; n.a. = not applicable

[00123] Commercially available o-xylylenediamine dihydrochloride **AD1** was initially evaluated as an amine donor for the biocatalytic amination of (4-fluorophenyl)acetone **S1** using commercial ω-TA (ATA113 from Codexis). Table 1 shows that complete conversion of **S1** (5 mM) to the corresponding chiral amine (>99%, >99% *e.e.*) was achieved using 1.0 equivalent of amine donor and there was no effect on conversion when the reaction was carried out at 100mM substrate concentration (see Example 5, Scheme 4, below). For comparison, benzylamine (1.5 equiv) or L-alanine (10 equiv, no pyruvate removal) were

tested as the amine donor resulting in poor conversion (<5%) to the desired product (Table 1).

The results shown in Table 1 also demonstrate the broad utility of the methodology described herein. Substrates **S2-S8** were efficiently converted to the corresponding amines with excellent conversion using <1.5 equiv of diamine **AD1**, and with the exception of **S6** the reactions proceeded with high selectivity (>99% *e.e.*). Benzylacetone (**S6**) was converted to (*S*)-(+)-1-methyl-3-phenylpropylamine with 78% *e.e.* using either diamine **AD1** or L-alanine (10 equiv) in combination with the lactate dehydrogenase (LDH)/glucose dehydrogenase (GDH) pyruvate removal system, demonstrating that **AD1** has does not influence the reaction enantioselectivity. The efficient biocatalytic amination of 1-indanone **S8** is challenging using traditional amine donors due to the highly unfavourable positions of equilibria [25]. 1-indanone **S8** was converted to (*S*)-1-aminoindane (73%) using 1.5 equivalents of diamine **AD1**, representing significant improvements to L-alanine (10 equiv) in combination with the (LDH)/(GDH) pyruvate removal system.

Example 5 - *Preparative-scale biotransformation of substrate S1 using ATA113 as ω-TA*



Scheme 4

[00124] Having regard to Scheme 4 above, commercially available (*S*)-selective ATA113 (5 mg/mL) was rehydrated in HEPES buffer (10mL, 100 mM, pH 7.5) containing PLP (2.02 mM) and xylylenediamine dihydrochloride (**AD1**) (230 mg, 110 mM) and the pH of the mixture was adjusted to 7.5. Substrate **S1** (152.2 mg/133.6 uL, 100 mM, from a 1M stock in DMSO) was added and the mixture incubated at 30 ºC, 150 rpm in a shaking incubator for 24h. Having confirmed no starting material remained by GC-FID analysis, the solution was centrifuged at 6000 rpm for 10 minutes. The supernatant was adjusted to pH 2 and extracted with diethyl ether (1 x 30 mL) to remove any residual ketone starting material. The solution was then adjusted to pH 12 and extracted with diethyl ether (3 x 40 mL). The organic layer was dried over $MgSO_4$, filtered and the solvent was removed *in vacuo* providing (*S*)-**3** (142mg, 93% yield, >99% *e.e.*) as a red oil which was not purified further. ^1H NMR (400 MHz, $CDCl_3$) δ 7.16

– 7.09 (m, 2H), 7.01 – 6.94 (m, 2H), 3.17 – 3.07 (m, 1H), 2.66 (dd, *J* = 13.4, 5.4 Hz, 1H), 2.48 (dd, *J* = 13.4, 8.0 Hz, 1H), 1.68 (br.s, 2H), 1.09 (d, *J* = 6.3 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.6 (d, *J* = 242 Hz), 135.4 (d, *J* = 3.2 Hz), 130.7 (d, *J* = 7.8 Hz), 115.3 (d, *J* = 21.0 Hz), 48.6, 45.8, 23.6.

[00125] Fig. 1 shows H and C NMR spectra for the (*S*)-1-(4-fluorophenyl)propan-2-amine product afforded in Scheme 4

Example 6 – *Screening ω-TA biocatalysts – Liquid phase screen with commercial biocatalysts*

[00126] Using **AD1** as the amine donor results in the formation of intensely coloured by-products, which presumably arise due to spontaneous polymerization of isoindole **BP1** (see Scheme 4) and offer a simple high-throughput screening strategy to screen for the desired transaminase activity.

[00127] A panel of commercially available ω-TAs were evaluated for their ability to utilize **AD1** as an amine donor. This panel included a series of six (*S*)-selective (see Fig. 2, L2, L4 and L6 A-F) and six (*R*)-selective (see Fig. 2, L1, L3 and L5 A-F) ω-TAs from the Codex® ATA screening kit v2. The panel also included six (*S*)-selective and six (*R*)-selective ω-TAs were recently commercialised by Purolite®/Codexis®.

[00128] The commercially available Codexis® and Almac® ω-TAs (1.5 mg/mL) and immobilized Codexis®/Purolite® ω-TAs (40mg/mL or 8mg/mL, see manufacturers screening protocol) were rehydrated in HEPES buffer (1mL, 100 mM, pH 7.5) containing PLP (2.02 mM) and xylylenediamine dihydrochloride **AD1** (5 mM). The biotransformations were incubated at 30 ºC, 150 rpm in a shaking incubator for 3h. Benzylacetone **S6** (5 mM) was added to the wells and the reactions were incubated for a further 24h. The reactions were monitored by colour change and activity was confirmed by GC-FID after 24 and 48 hours following extraction of the basified (pH 12 adjusted with 5 M NaOH) solution (100 uL) with EtOAc (1 x 300 uL). The conversions are reported in Tables 2 and 3 below.

Table 2 - Conversion of benzylacetone **S6** to 1-methyl-3-phenylpropylamine using *o*-xylylenediamine dihydrochloride **AD1** and commercially available (*S*)-selective ω-TAs

Transaminase	Biocatalyst Selectivity	Amine donor concentration	Amine acceptor concentration	Conversion
ATA254	(*S*)	5mM	5mM	98%
ATAGO5	(*S*)	5mM	5mM	>99%
ATA260	(*S*)	5mM	5mM	>99%
ATA256	(*S*)	5mM	5mM	>99%
ATA234	(*S*)	5mM	5mM	76%
ATA113	(*S*)	5mM	5mM	>99%
Purolite254	(*S*)	5mM	5mM	97%
PuroliteGO5	(*S*)	5mM	5mM	89%
Purolite260	(*S*)	5mM	5mM	99%
Purolite256	(*S*)	5mM	5mM	99%
Purolite234	(*S*)	5mM	5mM	37%

ω-TAs labelled ATA are non-immobilized Codexis® enzymes. Those labelled Purolite are the corresponding immobilized enzymes (immobilized ATA113 is not included). Only the conversions and not the enantioselectivity have been determined by GC-FID.

Table 3 - Conversion of benzylacetone **S6** to 1-methyl-3-phenylpropylamine using *o*-xylylenediamine dihydrocholride **AD1** and commercially available (*R*)-selective ω-TAs

Transaminase	Biocatalyst Selectivity	Amine donor concentration	Amine acceptor concentration	Conversion
ATA025	(*R*)	5mM	5mM	40%
ATA303	(*R*)	5mM	5mM	65%
ATA013	(*R*)	5mM	5mM	28%
ATA301	(*R*)	5mM	5mM	21%
ATA415	(*R*)	5mM	5mM	41%
ATA117	(*R*)	5mM	5mM	26%
Purolite025	(*R*)	5mM	5mM	35%
Purolite303	(*R*)	5mM	5mM	30%
Purolite013	(*R*)	5mM	5mM	8%
Purolite301	(*R*)	5mM	5mM	20%
Purolite415	(*R*)	5mM	5mM	20%

ω-TAs labelled ATA are non-immobilized Codexis® enzymes. Those labelled Purolite are the corresponding immobilized enzymes (immobilized ATA117 is not included). Only the conversions and not the enantioselectivity have been determined by GC-FID.

[00129] Having regard to Fig. 2, in the absence of an amine acceptor, low levels of colour change were observed with a number of the Codexis ATA biocatalysts following incubation with diamine **AD1** (5 mM) for 3 hours (Fig. 2, L1 and L2). The addition of benzylacetone **S6** (5 mM, 1.0 equiv) resulted in significant colour changes in a number of the biotransformations after only 15 minutes (Fig. 2, L3 and L4). After 12 hours, reactions with all Codex biocatalysts resulted in the formation of intensely coloured solutions and significant quantities of dark precipitate (Fig. 2, L5 and L6). In all cases, significant conversion of **S6** to the corresponding amine was confirmed by GC-FID analysis (see Tables 2 and 3). Comparable levels of conversion of **S6** were achieved using the corresponding immobilized ω-TA biocatalysts that were recently commercialized by Purolite/Codexis (see Tables 2 and 3).

[00130] Further evidence of the reliability of this colorimetric screening method is demonstrated using commercial ω-TAs supplied by Almac (see Fig. 2, L7 A-F). Whilst biotransformations with TAm106/TAm107 (L7A and L7B respectively) proceeded with moderate conversion and became intensely coloured, reactions with TAm121 (L7D) and TAm140 (L7F) gave no conversion (<1%) and wells remained pale yellow. Significantly, low but detectable levels of colour change were observed in reactions with TAm115 (L7C) and TAm125 (L7E), which were shown to proceed with <5% and 5% conversion respectively, as shown in Table 4 below.

Table 4 - Conversion of benzylacetone **S6** to 1-methyl-3-phenylpropylamine using *o*-xylylenediamine dihydrochloride **AD1** and commercially available Almac® ω-TAs

Transaminase	Amine donor concentration	Amine acceptor concentration	Conversion
TAm106	5mM	5mM	18%
TAm107	5mM	5mM	35%
TAm115	5mM	5mM	<5%
TAm121	5mM	5mM	<1%
TAm125	5mM	5mM	5%
TAm140	5mM	5mM	n.d.

ω-TAs labelled TAm are enzymes which have been supplied by Almac®. Only the conversions and not the enantioselectivity have been determined by GC-FID. n.d. = not detected.

Example 7 – *Screening ω-TA biocatalysts – Colony-based screen with pf-ATA*

[00131] In addition to providing a high-throughput method to evaluate the activity of commercial ω-TAs, the colorimetric screening strategy described in Example 6 offers an ideal platform for the development of a new generation of ω-TA biocatalysts which are engineered to efficiently utilize **AD1** as an amine donor. O'Reilly *et al.* [26] previously reported the use of a transaminase from *Pseudogulbenkiania ferrooxidans* (*pf*-ATA) for the regio- and stereoselective amination of diketones using L-alanine as the amine donor. Example 3 now demonstrates that this wild-type biocatalyst displays modest activity towards diamine **AD1**, allowing the development of a single enzyme, colony based assay. The assay was prepared using a glycerol stock (made from an overnight 10mL culture in LB) containing *E. coli* BL21 (DE3) cells transformed with pET16b-*pf*-ATA was diluted 1/10,000 in sterile H_2O and plated onto a Hybond-N membrane on the surface of LB-agar supplemented with Ampicillin (100 µg/mL). Plates were incubated at 30 ºC overnight. As a control, untransformed *E. coli* BL21 (DE3) cells were grown in parallel. The recombinant protein expression was induced by adding IPTG (2 mM) to the surface of the LB-agar plates and replacing the membrane followed by incubated for a further 6 h at 30°C. Blotting paper was soaked in a solution of xylylenediamine dihydrochloride **AD1** (5 mM) (or benzylamine (5 mM) as a control) in potassium phosphate buffer (100 mM, pH 7.5) and the excess liquid was drained. The membranes were placed on top of the blotting paper and incubated at 30°C for 30 min.

[00132] Having regard to Fig. 3, in the presence of diamine **AD1**, *E. coli* colonies expressing the *pf*-ATA gene rapidly became dark (see Fig. 3). Control experiments using benzylamine, or diamine **AD1** in combination with cells lacking the *pf*-ATA gene, did not lead to a colour change in the colonies. The success of this assay relies on the formation of insoluble polymeric isoindole by-products which prevent undesired diffusion.

Example 8 – Activity of commercial ATAs towards bulk diamine donors

[00133] Racemic 1,2-diaminopropane (**AD7**) was employed as the amine donor in the conversion of (4-fluorophenyl)acetone **S1** to the corresponding amine using commercially available transaminases from Codexis.

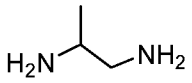
[00134] Commercially available (*S*)-selective ATA113 (1.5 mg/mL) was rehydrated in HEPES buffer (1mL, 100 mM, pH 7.5) containing PLP (2.02 mM) and racemic **AD7** or isopropylamine (20 mM). The substrate, **S1** (5 mM from a 200 mM stock in DMSO) was added and the mixture incubated at 30 ºC, 150 rpm in a shaking incubator. The reactions were analysed by GC-FID after 24 hours following extraction of the basified (pH 12 adjusted with 5 M NaOH) solution (100 uL) with EtOAc (1 x 300 uL) and derivatization (acetylation).

[00135] Table 5 shows that conversion of **S1** (5 mM) to the corresponding chiral amine (80%, >99% *e.e.*) was achieved using 4.0 equivalents of this low cost racemic donor. For comparison, the commonly used donor, isopropylamine (IPA), was also employed under identical conditions and resulted in a lower conversion of **S1** to the desired chiral amine (60%, >99% *e.e.*). These results are extremely significant as these commercial enzymes have been heavily engineered to tolerate high concentrations of IPA, yet display increased efficiency using diamine **AD7** as the amine donor. Additionally, IPA is currently the amine donor employed in manufacturing processes involving transaminase biocatalysts.

Table 5 - Conversion of (4-fluorophenyl)acetone **S1** to the corresponding amine using 1,2-diaminopropane **AD7** and commercially available transaminases from Codexis

Substrate	Amine donor	Equiv.	Conv.	*ee* [%]*
S1	**AD7**	4	80	> 99 (*R*)
S1	**Isopropyl amine**	4	60	> 99 (*R*)

*ATA117 from Codexis.



AD7



Isopropylamine

[00136] While the amine donor (**AD7**) by-product has not yet been characterized, it is likely that dimerization or polymerization is responsible for the increased efficiency compared with isopropylamine. Additionally, the 1,2-diamines such as **AD7** have a significantly reduced pKa of the second amine (compared with simple amines). This may offer advantages in terms of both the kinetics and thermodynamics of transaminase processes. The reduced pKa means that under reaction conditions a greater proportion of the amine is present in the reactive unprotonated state, leading to enhanced reaction rates. With respect to the thermodynamics, using **AD7** as the amine donor the total sum of protonated species before the TA reaction is one (mono-protonated diamine). Following transamination, the number of protonated species is two (amine product and α-amino carbonyl by-product). In contrast, transformations with standard mono-amine donors (e.g. IPA) have an equal number of protonated species before and after reaction. Note that amine protonation is a thermodynamically favorable process.

Example 9 – Activity of wild-type ATAs towards bulk diamine donors

[00137] Further to the work done with commercially available TAs from Codexis, it was sought to identify wild-type amino transferases which display activity towards a diverse panel of amine donors. Although diaminotransferases have been described previously using pyruvate as the amine acceptor, no activity towards standard ketones has been reported (i.e. they have not been exploited as biocatalysts for chiral amine synthesis, and the diamine substrates have not been used as amine donors for chiral amine synthesis).

[00138] Using a bioinformatics approach, transaminases were identified which display the required dual activity towards low cost diamine donors and towards standard ketones. Following expression of one such wild-type ATA, the purified enzyme was initially screened against a panel of structurally diverse diamine donors (Table 6), using pyruvate as the amine acceptor. A previously described coupled enzyme assay was used to measure the specific activity of the enzyme towards these donors:

L-amino acid oxidase (L-AAO) activity assay screen

A stock solution of substrate and reagents was made up of the following; 4.95 mM 2,4,6-Tribromo-3-hydroxybenzoic acid (TBHBA), 1.65 mM 4-aminoantipyrine, 11 mM sodium pyruvate, 6 mg mL^{-1} HRP, 10 mg mL^{-1} PLP and 11 mM of the amine donor in MeOH was added to 50 mM Tris-HCl and adjusted to pH 8.0. The stock solution (90 µL) was dispensed to individual wells in a 96 well plate. 5 µL of L-AAO (4.5 U mL^{-1}) was added to each well and the assays were initiated upon addition of the wild-type ATA 5 µL, 20 µg). Experiments were run on a spectrophotometer at 30 °C with the measured activity at a wavelength of 510 nm and the absorbance taken every 30 seconds. One unit of enzyme activity (U) was defined as the amount of enzyme required to produce one µmole of H_2O_2 produced per min.

[00139] The results are shown in Table 6 below:

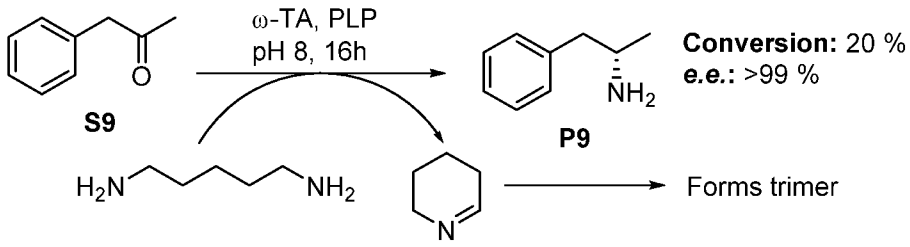
Table 6 - Activity of a wild-type transaminase towards bulk diamine donors

Entry	Amine Donor	Specific activity µmol min^{-1} mg^{-1}
1	**1,2-diaminoethane**	0.002
2	**1,3-diaminopropane**	0.0008
3	**1,4-diaminobutane**	0.4154
4	**1,5-diaminopentane**	0.6231
5	**1,6-diaminohexane**	0.4154
6	**1,8-diaminooctane**	1.3294
7	**1,10-diaminodecane**	1.1009
8	**2-methyl-1,5-diaminopentane**	0.1038

9	**Diethylenetriamine**	0.0415
10	**bis-(2-aminoethyl)-ether**	0.0415
11	**Spermidine**	0.1038
12	**Spermine**	0.3115
13	**Cyclohexane-bismethylenediamine**	0.2081
14	**1,8-diamino-3,6-dioxooctane**	0.001
15	**Tetraethylenepentamine**	0.002
16	**(*S*)-Methylbenzylamine**	0.64

1.0 equivalent of the amine donor was used in each case and pyruvate was used as the amine acceptor.

[00140] The results shown above demonstrate that the transaminase displays the highest activity towards a range of low-cost bulk diamines, in particular long chain diamines, such as diaminooctane and diaminodecane (entries 6 & 7). Particularly good activity towards 1,4-diaminobutane, 1,5-diaminopentane and 1,6-diaminohexane (entries 3, 4 & 5) was also observed. Notable activity is similarly observed with polyamines, including spermidine and spermine (entries 11 & 12). Significantly, this enzyme also displayed good activity towards the simple mono-amine (S)-MBA, suggesting that this new transaminase may display activity towards standard ketones (since TAs catalyze reactions reversibly).Having established that this ATA displays activity towards a diverse range of diamine/polyamine donors in the presence of pyruvate as the amine acceptor, the activity of the AT towards phenylacetone, **S9**, in the presence of 1,5-diaminopentane as the amine donor was investigated (Scheme 5). The enzyme mediated the conversion of **S9** to the corresponding chiral amine in good conversion and excellent *e.e.* (20%, >99% *e.e.*) using only 1.0 equivalent of the amine donor.



Scheme 5

While this conversion appears modest, there is enormous scope to enhance this conversion *via* process optimization (e.g. increased donor concentration, additives designed to trap the cyclic imine by-product) or enzyme engineering (or a combination of the two). Wild-type transaminases typically suffer from substrate/ product inhibition and have modest activities

towards non-native substrates, and evolution is typically required to make these biocatalysts suitable for practical applications. The dual activity displayed by this enzyme towards diamines (as amine donors) and ketones makes it an ideal starting point for directed evolution. Additionally, improved conversions may be obtained with alternative low cost diamine donors (see above table) for which this enzyme displays good activity (e.g. entries 3, 6, 7)

[00141] While specific embodiments of the invention have been described herein for the purpose of reference and illustration, various modifications will be apparent to a person skilled in the art without departing from the scope of the invention as defined by the appended claims.

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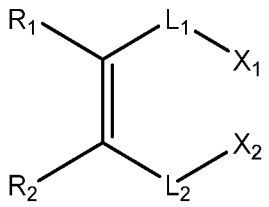
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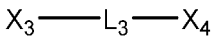
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CLAIMS

1. A process for the preparation of an amine, the process comprising the step of reacting a ketone or aldehyde substrate with an amine donating compound of formula (I) or (II), or a salt thereof, in the presence of a transaminase or aminotransferase



(I)



(II)

wherein

L_1 and L_2 are selected from one of the following options:

L_1 is absent and L_2 is $–[CR_aR_b]_2–$, or
L_1 is $–[CR_aR_b]_2–$ and L_2 is absent, or
L_1 is $–CR_aR_b–$ and L_2 is $–CR_aR_b–$;

L_3 is a straight or branched alkylene, alkenylene or alkynylene linker optionally comprising one or more substituted or unsubstituted heteroatoms disposed within the alkylene, alkenylene or alkynylene linker, wherein L_3 is optionally substituted with one or more R_c;

each group R_a and R_b is independently absent or selected from hydrogen, oxo, halo, trifluoromethyl, trifluoromethoxy, cyano, nitro, hydroxy, mercapto, amino, formyl, carboxy, carbamoyl, ureido, (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl, (1-4C)alkoxy, aryl, (1-4C)alkylthio, (1-4C)alkylsulphinyl, (1-4C)alkylsulphonyl, (1-4C)alkylamino, di-[(1-4C)alkyl]amino, (1-4C)alkoxycarbonyl, *N*-(1-4C)alkylcarbamoyl, *N*,*N*-di-[(1-4C)alkyl]carbamoyl, (2-4C)alkanoyl, (2-4C)alkanoyloxy, (2-4C)alkanoylamino, sulphamoyl, *N*-(1-4C)alkylsulphamoyl and *N*,*N*-di-[(1-4C)alkyl]sulphamoyl;

each group R_c is independently absent or selected from hydrogen, oxo, halo, trifluoromethyl, trifluoromethoxy, cyano, nitro, hydroxy, mercapto, amino, formyl, carbamoyl, ureido, (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl, (1-4C)alkoxy, aryl, (1-4C)alkylthio, (1-4C)alkylsulphinyl, (1-4C)alkylsulphonyl, (1-4C)alkylamino, di-[(1-4C)alkyl]amino, (1-4C)alkoxycarbonyl, *N*-(1-

4C)alkylcarbamoyl, *N*,*N*-di-[(1-4C)alkyl]carbamoyl, (2-4C)alkanoyl, (2-4C)alkanoyloxy, (2-4C)alkanoylamino, sulphamoyl, *N*-(1-4C)alkylsulphamoyl and *N*,*N*-di-[(1-4C)alkyl]sulphamoyl

X_1, X_2, X_3 and X_4 are each selected from NH_2, NHR_d, NR_dR_e, SH and OH, with the proviso that at least one of X_1 and X_2, and at least one of X_3 and X_4, is NH_2;

R_d and R_e are independently selected from (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl and (1-4C)alkoxy; and

R_1 and R_2 are

(iii) independently selected from hydrogen halo, trifluoromethyl, trifluoromethoxy, cyano, nitro, hydroxy, mercapto, amino, formyl, carboxy, carbamoyl, ureido, (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl, (1-4C)alkoxy, (1-4C)alkylthio, (1-4C)alkylsulphinyl, (1-4C)alkylsulphonyl, (1-4C)alkylamino, di-[(1-4C)alkyl]amino, (1-4C)alkoxycarbonyl, *N*-(1-4C)alkylcarbamoyl, *N*,*N*-di-[(1-4C)alkyl]carbamoyl, (2-4C)alkanoyl, (2-4C)alkanoyloxy, (2-4C)alkanoylamino, sulphamoyl, *N*-(1-4C)alkylsulphamoyl and *N*,*N*-di-[(1-4C)alkyl]sulphamoyl, or

(iv) linked, so that when R_1 and R_2 are taken together with the carbon atom to which they are each attached, they collectively form a carbocyclic, heterocyclic, aryl or heteroaryl ring, optionally substituted with one or more substituents selected from halo, trifluoromethyl, trifluoromethoxy, cyano, nitro, hydroxy, mercapto, amino, formyl, carboxy, carbamoyl, ureido, (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl, (1-4C)alkoxy, (1-4C)alkylthio, (1-4C)alkylsulphinyl, (1-4C)alkylsulphonyl, (1-4C)alkylamino, di-[(1-4C)alkyl]amino, (1-4C)alkoxycarbonyl, *N*-(1-4C)alkylcarbamoyl, *N*,*N*-di-[(1-4C)alkyl]carbamoyl, (2-4C)alkanoyl, (2-4C)alkanoyloxy, (2-4C)alkanoylamino, sulphamoyl, *N*-(1-4C)alkylsulphamoyl and *N*,*N*-di-[(1-4C)alkyl]sulphamoyl,

with the proviso that

when X_3 is OH and X_4 is NH_2, the carbon atom to which X_3 is attached is not substituted with oxo, and

when X_4 is OH and X_3 is NH_2, the carbon atom to which X_4 is attached is not substituted with oxo.,

or a salt thereof

2. The process of claim 1, wherein the transaminase or aminotransferase is selected from a group consisting of polyamine aminotransferases (e.g. diamine aminotransferases) and ω-TAs.

3. The process of claim 2, wherein the transaminase or aminotransferase is a ω-TA.

4. The process of claim 1, 2 or 3, wherein the substrate is a ketone and the amine is chiral.

5. The process of any preceding claim, wherein L_3 is a straight or branched (2-12C)alkylene, (2-12C)alkenylene or (2-12C)alkynylene linker optionally comprising one or more substituted or unsubstituted heteroatoms disposed within the alkylene, alkenylene or alkynylene linker, wherein L_3 is optionally substituted with one or more R_c.

6. The process of any preceding claim, wherein L_3 is a straight or branched (2-12C)alkylene, (2-12C)alkenylene or (2-12C)alkynylene linker optionally comprising one or more substituted or unsubstituted heteroatoms selected from N, S and O disposed within the alkylene, alkenylene or alkynylene linker, wherein L_3 is optionally substituted with one or more R_c.

7. The process of any preceding claim, wherein L_3 is a straight or branched (2-12C)alkylene, (2-12C)alkenylene or (2-12C)alkynylene linker optionally comprising one or more heteroatoms selected from N, S and O disposed within the alkylene, alkenylene or alkynylene linker, the one or more N-heteroatoms being optionally substituted with one or more groups selected from (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl, (1-4C)alkoxy or aryl, wherein L_3 is optionally substituted with one or more R_c

8. The process of any preceding claim, wherein R_1 and R_2 are

   i. independently selected from hydrogen halo, trifluoromethyl, trifluoromethoxy, hydroxy, amino, formyl, carboxy, carbamoyl, ureido, (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl, (1-4C)alkoxy, (1-4C)alkylthio, (1-4C)alkylamino, di-[(1-4C)alkyl]amino, (1-4C)alkoxycarbonyl, *N*-(1-4C)alkylcarbamoyl, *N*,*N*-di-[(1-4C)alkyl]carbamoyl, (2-4C)alkanoyl, (2-4C)alkanoyloxy and (2-4C)alkanoylamino; or

ii. linked, so that when R_1 and R_2 are taken together with the carbon atom to which they are each attached, they collectively form a phenyl or naphthalenyl group, or a 5-10 membered heteroaryl ring, optionally substituted with one or more substituents selected from halo, trifluoromethyl, trifluoromethoxy, cyano, nitro, hydroxy, mercapto, amino, formyl, carboxy, carbamoyl, ureido, (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl, (1-4C)alkoxy, (1-4C)alkylthio, (1-4C)alkylsulphinyl, (1-4C)alkylsulphonyl, (1-4C)alkylamino, di-[(1-4C)alkyl]amino, (1-4C)alkoxycarbonyl, *N*-(1-4C)alkylcarbamoyl, *N*,*N*-di-[(1-4C)alkyl]carbamoyl, (2-4C)alkanoyl, (2-4C)alkanoyloxy, (2-4C)alkanoylamino, sulphamoyl, *N*-(1-4C)alkylsulphamoyl and *N*,*N*-di-[(1-4C)alkyl]sulphamoyl.

9. The process of any preceding claim, wherein R_1 and R_2 are

i. independently selected from hydrogen halo, trifluoromethyl, trifluoromethoxy, hydroxy, amino, carboxy, carbamoyl, (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl and (1-4C)alkoxy; or

ii. linked, so that when R_1 and R_2 are taken together with the carbon atom to which they are each attached, they collectively form a phenyl group, or 5-6 membered heteroaryl ring, optionally substituted with one or more substituents selected from halo, trifluoromethyl, trifluoromethoxy, cyano, nitro, hydroxy, mercapto, amino, formyl, carboxy, carbamoyl, ureido, (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl, (1-4C)alkoxy, (1-4C)alkylthio, (1-4C)alkylsulphinyl, (1-4C)alkylsulphonyl, (1-4C)alkylamino, di-[(1-4C)alkyl]amino, (1-4C)alkoxycarbonyl, *N*-(1-4C)alkylcarbamoyl, *N*,*N*-di-[(1-4C)alkyl]carbamoyl, (2-4C)alkanoyl, (2-4C)alkanoyloxy, (2-4C)alkanoylamino, sulphamoyl, *N*-(1-4C)alkylsulphamoyl and *N*,*N*-di-[(1-4C)alkyl]sulphamoyl.

10. The process of any preceding claim, wherein R_1 and R_2 are

i. independently selected from hydrogen halo, trifluoromethyl, trifluoromethoxy, hydroxy, amino, carboxy, carbamoyl, (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl and (1-4C)alkoxy; or

ii. linked, so that when R_1 and R_2 are taken together with the carbon atom to which they are each attached, they collectively form a phenyl group, or 6 membered heteroaryl ring containing 1-3 nitrogen atoms, optionally substituted with one or more substituents selected from halo, trifluoromethyl, trifluoromethoxy, cyano, nitro, hydroxy, mercapto, amino, formyl, carboxy,

carbamoyl, ureido, (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl, (1-4C)alkoxy, (1-4C)alkylthio, (1-4C)alkylsulphinyl, (1-4C)alkylsulphonyl, (1-4C)alkylamino, di-[(1-4C)alkyl]amino, (1-4C)alkoxycarbonyl, *N*-(1-4C)alkylcarbamoyl, *N*,*N*-di-[(1-4C)alkyl]carbamoyl, (2-4C)alkanoyl, (2-4C)alkanoyloxy, (2-4C)alkanoylamino, sulphamoyl, *N*-(1-4C)alkylsulphamoyl and *N*,*N*-di-[(1-4C)alkyl]sulphamoyl.

11. The process of any preceding claim, wherein

L_1 and L_2 are selected from one of the following options:

L_1 is absent and L_2 is $–[CR_aR_b]_2–$, or
L_1 is $–[CR_aR_b]_2–$ and L_2 is absent, or
L_1 is $–CR_aR_b–$ and L_2 is $–CR_aR_b–$;

L_3 is a straight or branched (2-12C)alkylene linker optionally comprising one or more heteroatoms selected from N, S and O disposed within the alkylene linker, the one or more N heteroatoms being optionally substituted with one or more groups selected from (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl, (1-4C)alkoxy or aryl, wherein L_3 is optionally substituted with one or more R_c;

each group R_a and R_b is independently absent or selected from hydrogen, oxo, halo, trifluoromethyl, trifluoromethoxy, hydroxy, amino, carboxy, carbamoyl, (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl, (1-4C)alkoxy and phenyl;
each group R_c is independently absent or selected from hydrogen, oxo, halo, trifluoromethyl, trifluoromethoxy, hydroxy, amino, carbamoyl, (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl, (1-4C)alkoxy and phenyl;

X_1, X_2, X_3 and X_4 are each selected from NH_2, NHR_d and NR_dR_e, with the proviso that at least one of X_1 and X_2, and at least one of X_3 and X_4, is NH_2;

R_d and R_e are independently selected from (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl and (1-4C)alkoxy; and

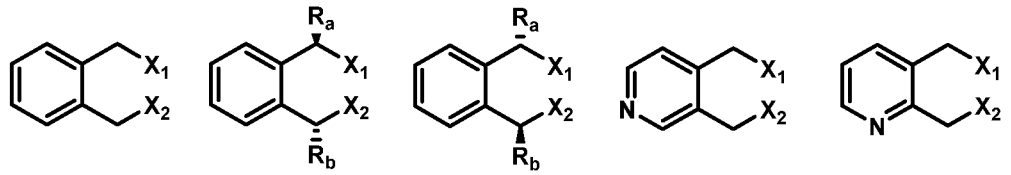
R_1 and R_2 are

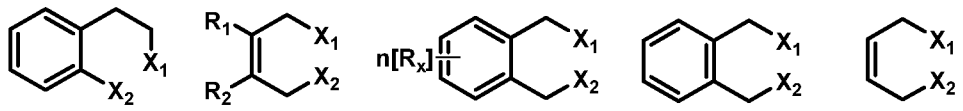
(i) independently selected from hydrogen halo, trifluoromethyl, trifluoromethoxy, hydroxy, amino, carboxy, carbamoyl, (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl and (1-4C)alkoxy, or

(ii) linked, so that when R_1 and R_2 are taken together with the carbon atom to which they are each attached, they collectively form a phenyl group, or 6 membered heteroaryl ring containing 1-3 nitrogen atoms, optionally substituted with one or more substituents selected from halo,

trifluoromethyl, trifluoromethoxy, hydroxy, amino, carboxy, carbamoyl, (1-4C)alkyl, (2-4C)alkenyl, (2-4C)alkynyl, (1-4C)alkoxy, (1-4C)alkylthio, (1-4C)alkylamino and di-[(1-4C)alkyl]amino.

12. The process of any preceding claim, wherein formula (I) is represented by any of formulae (I-a) – (I-j) shown below:





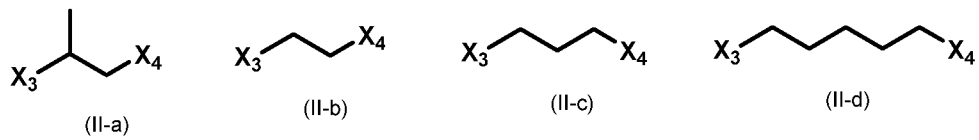
wherein

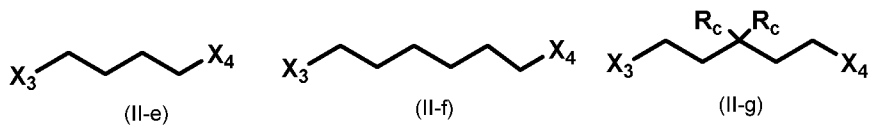
R_a, R_b, X_1 and X_2 are as defined in any preceding claim;

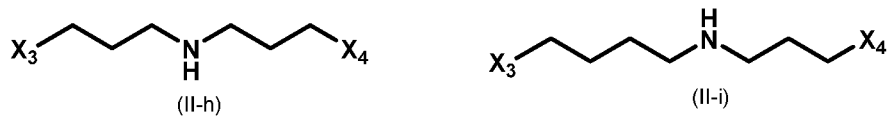
each R_x is independently any of the ring substituents specified in any preceding claim where R_1 and R_2 are linked; and

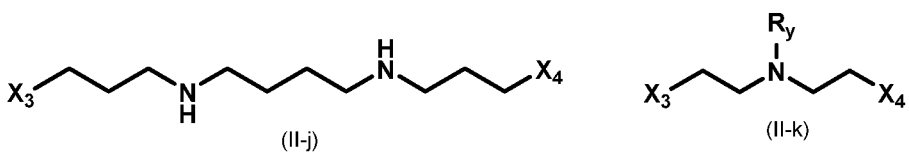
n is a whole integer 1-4.

13. The process of any preceding claim, wherein formula (II) is represented by any of formulae (II-a) – (II-k) shown below:







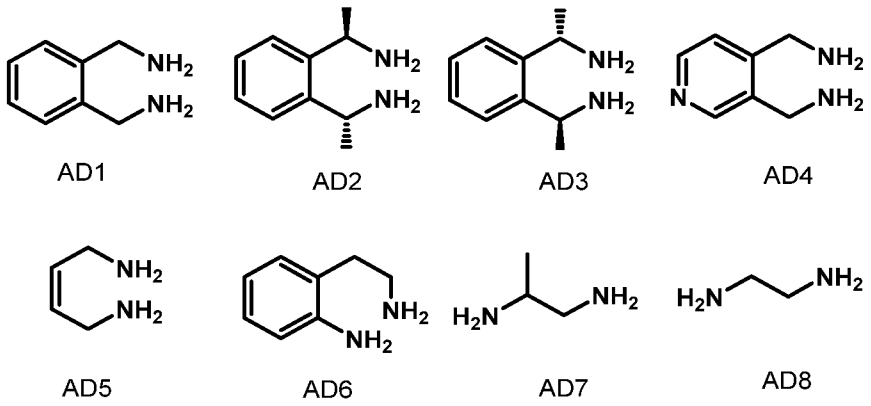


wherein

X_3, X_4 and R_c are as defined in any preceding claim; and

R_y is a heteroatom substituent as defined in any preceding claim.

14. The process of any preceding claim, wherein the amine donating compound is selected from any of the following compounds AD1 – AD8, or a salt thereof:



15. A process for assessing the ability of a transaminase or aminotransferase to catalyse the conversion of a ketone or aldehyde substrate into an amine, the process comprising the steps of:

a) contacting one or more ketone or aldehyde substrates with an amine donating compound of formula (I) or (II) as defined in any preceding claim, in the presence of one or more transaminase or aminotransferase, and

b) analysing the reaction medium for the presence of a by-product,

wherein the presence of a by-product indicates a successful conversion of the ketone or aldehyde substrate into the amine.

16. The process of claim 15, wherein the transaminase or aminotransferase is selected from a group consisting of polyamine aminotransferases (e.g. diamine aminotransferases) and ω-TAs.

17. The process of claim 16, wherein the transaminase or aminotransferase a ω-TA.

18. The process of claim 15, 16 or 17, wherein step a) comprises contacting a plurality of different ketone or aldehyde substrates each with an amine donating compound of formula (I) or (II) as defined in any preceding claim, each in the presence of a single transaminase or aminotransferase.

19. The process of claim 15, 16 or 17, wherein step a) comprises contacting a plurality of identical ketone or aldehyde substrates each with an amine donating compound of

formula (I) or (II) as defined in any preceding claim, each in the presence of a different transaminase or aminotransferase.

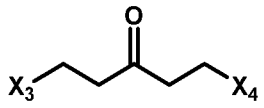
20. The process of claim 15, 16 or 17, wherein step a) comprises contacting a plurality of different ketone or aldehyde substrates each with an amine donating compounds of formula (I) or (II) as defined in any preceding claim, each in the presence of a different transaminase or aminotransferase.

21. The process of any of claims 15 to 20, wherein step b) comprises analysing the reaction medium using one or more of visual analysis using the naked eye, spectrophotometry, chromatography (including HPLC and GPC), IR spectroscopy, mass spectroscopy, colorimetric analysis and NMR spectroscopy.

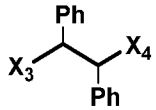
22. The process of any of claims 15 to 21, further comprising, prior to step b), the step of subjecting the reaction medium to oxidative conditions.

23. The process of any of claims 15 to 22, wherein step b) comprises analysing the reaction medium with the naked eye for the presence of a coloured by-product (e.g. a precipitate).

24. The process of any of claims 15 to 23, wherein step a) comprises contacting one or more ketone or aldehyde substrates with an amine donating compound of formula (I) as defined in any preceding claim, or an amine donating compound of formula (II-l) – (II-m) defined below, in the presence of the transaminase or aminotransferase:



(II-l)



(II-m)

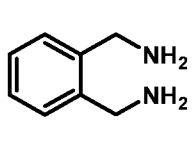
wherein

X_3 and X_4 are each as defined in any of claims 1 to 14.

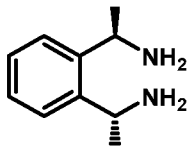
25. The process of any of claims 15 to 24, wherein step a) comprises contacting one or more ketone or aldehyde substrate with an amine donating compound of formula (I-a) – (I-j) as defined in claim 12, or an amine donating compound of formula (II-l) – (II-m) as defined in claim 24, in the presence of one or more transaminase or aminotransferase.

26. The process of any of claims 15 to 25, wherein step a) comprises contacting one or more ketone or aldehyde substrate with an amine donating compound having any of

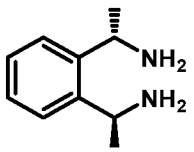
the structures shown below, in the presence of one or more transaminase or aminotransferase:



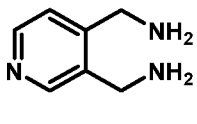
AD1



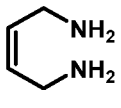
AD2



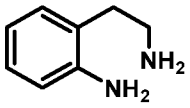
AD3



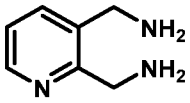
AD4



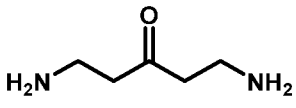
AD5



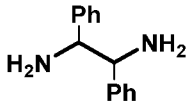
AD6



AD9

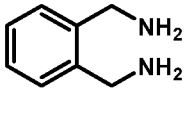


AD10

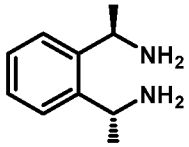


AD11

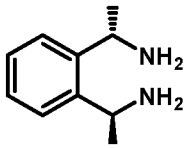
27. The process of any of claims 15 to 25, wherein step a) comprises contacting one or more ketone or aldehyde substrate with an amine donating compound having any of the structures shown below, in the presence of one or more transaminase or aminotransferase:



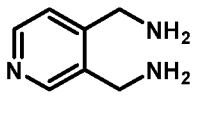
AD1



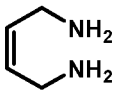
AD2



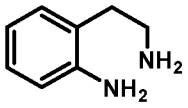
AD3



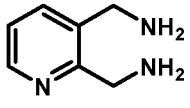
AD4



AD5



AD6



AD9

28. A kit of parts suitable for assessing the ability of a transaminase or aminotransferase to catalyse the conversion of a ketone or aldehyde substrate into an amine in accordance with a process as claimed in any of claims 15 to 27, the kit comprising:

a) one or more amine donating compounds of formula (I) or (II) as defined in any preceding claim, and either or both of:

i. one or more ketone or aldehyde substrates, and

ii. one or more transaminase or aminotransferase.

29. The kit of claim 28, further comprising a set of instructions to instruct an operator to perform a process of any of claims 15 to 27.

30. The kit of claim 28 or 29, further comprising means for analysing for the presence of a by-product.

31. A process for determining whether a sample exhibits transaminase or aminotransferase activity, the process comprising the steps of:

a) providing one or more sample having putative transaminase or aminotransferase activity;

b) contacting the one or more samples of step a) with an amine-donating compound of formula (I) or (II) as defined in any preceding claim; and

c) analysing the reaction medium for the presence of a by-product,

wherein the presence of a by-product indicates that the sample exhibits transaminase or aminotransferase activity.

32. The process of claim 31, wherein the one or more samples are each selected from enzymes, proteins or other biological samples including biological cells (in the form of a tissue sample including cells, cultured cells, or microorganisms), or an extract (such as a cell extract or lysate)

33. The process of claim 31 or 32, wherein step b) comprises contacting the one or more samples of step a) with an amine-donating compound of formula (I) or (II) as defined in any preceding claim, and one or more aldehyde or ketone substrates.

34. The process of any of claims 31, 32 or 33, wherein step c) comprises analysing the reaction medium using one or more of visual analysis using the naked eye, spectrophotometry, chromatography (including HPLC and GPC), IR spectroscopy, mass spectroscopy, colorimetric analysis and NMR spectroscopy.

35. The process of any of claims 31 to 34, wherein step c) comprises analysing the reaction medium with the naked eye for the presence of a coloured by-product (e.g. a precipitate).

36. A kit of parts for determining whether a sample exhibits transaminase or aminotransferase activity in accordance with a process of any of claims 31 to 35, the kit comprising:

a) one or more amine donating compound of formula (I) or (II) as defined in any preceding claim, and

b) instructions for performing a process of any of claims 31 to 35.

37. The kit of claim 36, further comprising one or more aldehyde or ketone substrates.

38. The kit of claim 36 or 37, further comprising means for analysing for the presence of a by-product.

39. A process for preparing a polymer, the process comprising the steps of:
    a) reacting a ketone or aldehyde substrate with an amine donating compound of formula (I) as defined in any preceding claim, in the presence of a transaminase or aminotransferase, such that a reaction by-product is formed, and
    b) subjecting the reaction by-product to conditions suitable for causing polymerisation of the reaction by-product.

40. The process of claim 39, wherein the amine donating compound is AD1 as defined in claim 14, and the polymer is polyisoindole.

41. A process for assessing the ability of an amine oxidase to catalyse the oxidation of an amine donating compound of formula (I) or (II) as defined in any preceding claim, the process comprising the steps of:
    a) contacting one or more amine donating compound of formula (I) or (II) as defined in any preceding claim with an oxidizing agent, in the presence of one or more amine oxidase, and
    b) analysing the reaction medium for the presence of a by-product,
wherein the presence of a by-product indicates a successful oxidation of the amine donating compound.

42. A process for determining whether a sample exhibits amino oxidase activity, the process comprising the steps of:
    a) providing one or more sample having putative amino oxidase activity;
    b) contacting the one or more samples of step a) with one or more amine-donating compound of formula (I) or (II) as defined in any preceding claim and a suitable oxidizing agent; and
    c) analysing the reaction medium for the presence of a by-product,
wherein the presence of a by-product indicates that the sample exhibits amino oxidase activity.

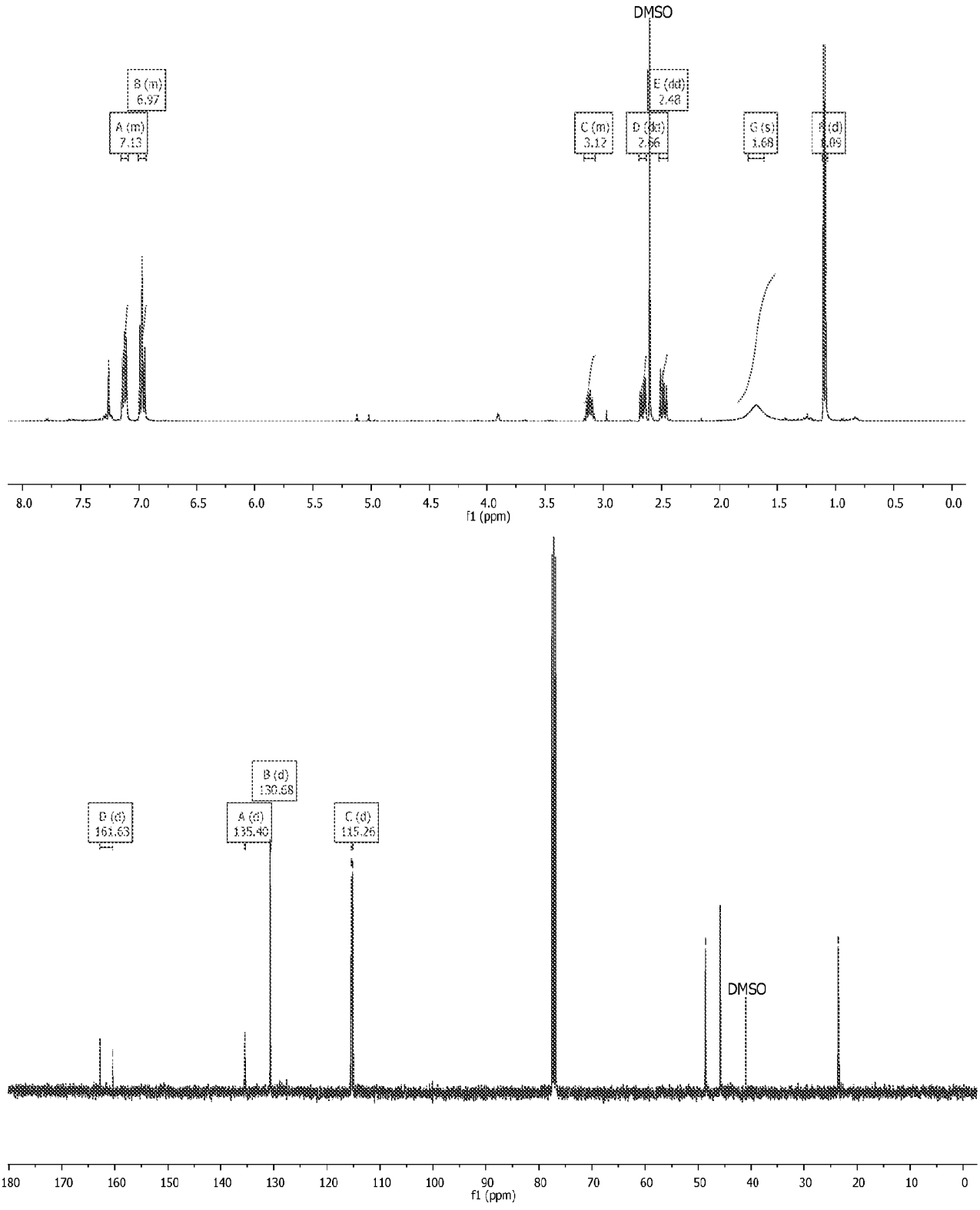

DMSO
B (m)
6.97
A (m)
7.13
E (dd)
2.48
C (m)
3.12
D (dd)
2.66
G (s)
3.68
F (d)
8.09
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)
B (d)
161.63
B (d)
130.68
A (d)
135.40
C (d)
115.26
DMSO
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

FIG. 1

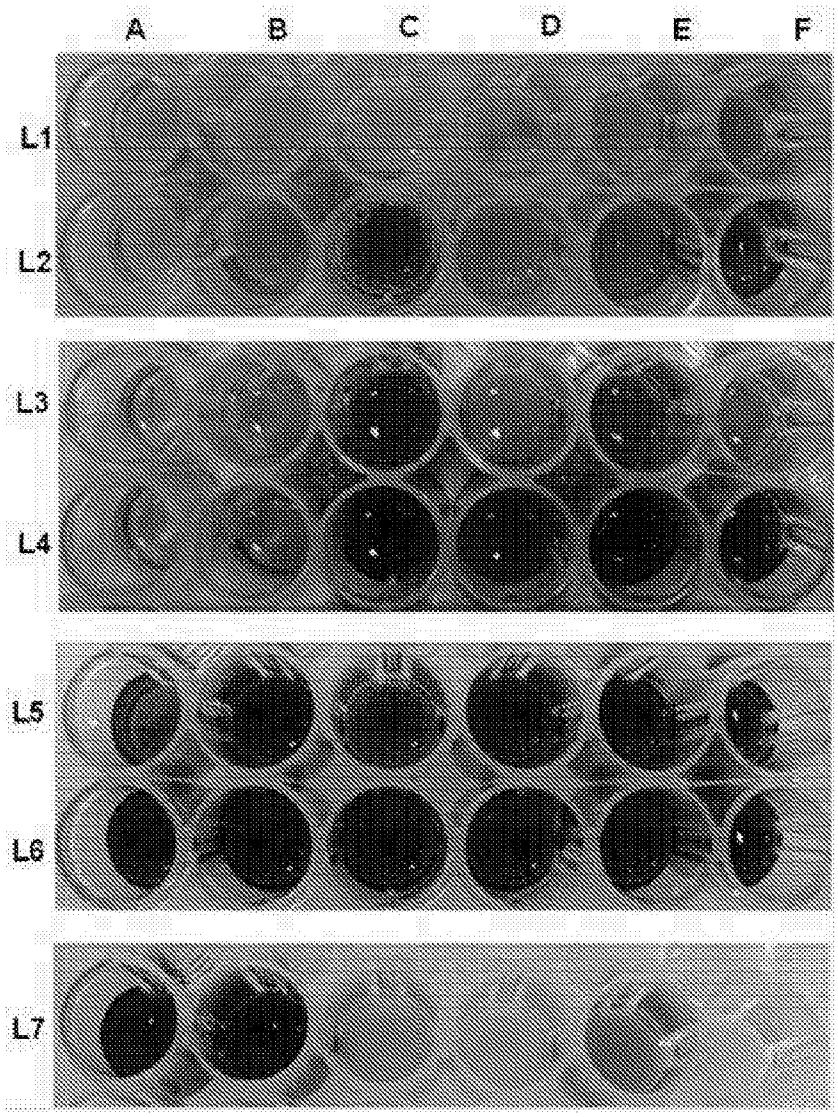


FIG. 2

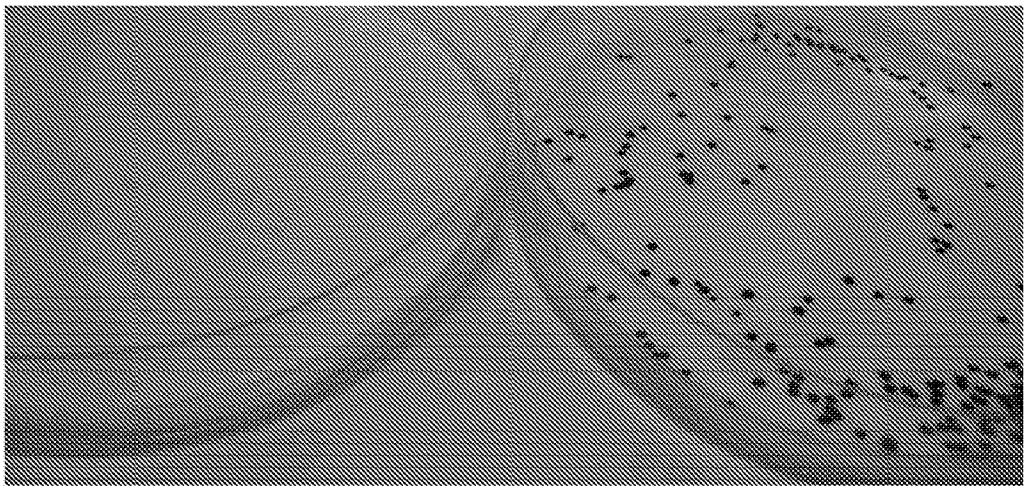


FIG. 3

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2015/052037

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/52 C12P13/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12Q C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 2 354 240 A1 (CARGILL INC [US]) 10 August 2011 (2011-08-10) Abstract; Fig.12. -----	1-4, 8-12, 14-40
A	SAMSONOVA NATALYA N ET AL: "Molecular cloning and characterization of Escherichia coli K12 ygjG gene", BMC MICROBIOLOGY, BIOMED CENTRAL LTD, GB, vol. 3, no. 1, 31 January 2003 (2003-01-31), page 2, XP021014831, ISSN: 1471-2180, DOI: 10.1186/1471-2180-3-2 Abstract; table 2. -----	1-4, 8-12, 14-40

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 September 2015	02/12/2015

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	López García, F

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Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB2015/052037

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

8-10, 12, 27, 39, 40(completely); 1-4, 11, 14-26, 28-38(partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 8-10, 12, 27, 39, 40(completely); 1-4, 11, 14-26, 28-38(partially)

   Methods for the preparation of amine according to claim 1 and methods and kits based on said methods wherein the amine donating compounds is of formula (I).

   ---

2. claims: 5-7, 13(completely); 1-4, 11, 14-26, 28-38(partially)

   Methods for the preparation of amine according to claim 1 and methods and kits based on said methods, wherein the amine donating compound is of formula II.

   ---

3. claims: 41, 42

   Methods for assessing the ability of an amino oxidase to catalyse the oxidation of an amino donating compound of formula I or II according to claim 41 and methods based on said method.

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2013/024453 A1 (EMBIO LTD [IN]) 21 February 2013 (2013-02-21) Abstract; p. 7	1-4, 8-12, 14-40
X	JENNIFER HOPWOOD ET AL: "A fast and sensitive assay for measuring the activity and enantioselectivity of transaminases", CHEMICAL COMMUNICATIONS, vol. 47, no. 2, 1 January 2011 (2011-01-01), pages 773-775, XP055212525, ISSN: 1359-7345, DOI: 10.1039/C0CC02919J Abstract; Table 1.	1-4, 8-12, 14-40
X	SAM MATHEW ET AL: "High throughput screening methods for [omega]-transaminases", BIOTECHNOLOGY AND BIOPROCESS ENGINEERING, vol. 18, no. 1, 1 February 2013 (2013-02-01), pages 1-7, XP055191155, ISSN: 1226-8372, DOI: 10.1007/s12257-012-0544-x Abstract; Scheme 5	1-4, 8-12, 14-40
X	WO 2006/077233 A1 (BASF AG [DE]; ERNST MARTIN [DE]; HAHN THILO [DE]; DAHMEN KIRSTEN [DE];) 27 July 2006 (2006-07-27) p. 1, l. 11	36,38
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X,P	ANTHONY P. GREEN ET AL: "Chiral Amine Synthesis Using [omega]-Transaminases: An Amine Donor that Displaces Equilibria and Enables High-Throughput Screening", ANGEWANDTE CHEMIE INTERNATIONAL EDITION, vol. 53, no. 40, 19 August 2014 (2014-08-19), pages 10714-10717, XP055212461, ISSN: 1433-7851, DOI: 10.1002/anie.201406571 Scheme 1; Table 1.	1-4, 8-12, 14-40

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International application No
PCT/GB2015/052037

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