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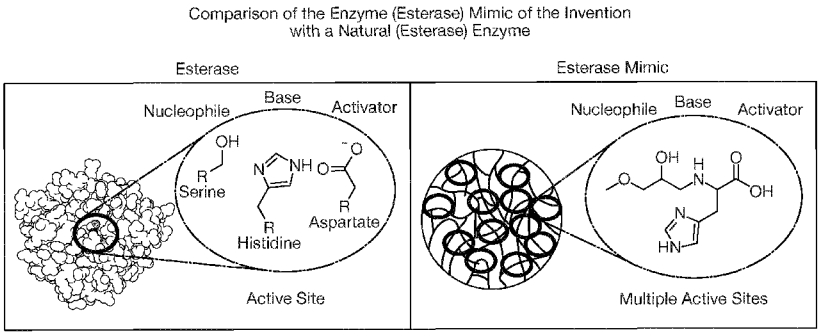
[Continued on next page]

(54) Title: SYNTHETIC ENZYME MIMIC BASED ON A HISTIDINE SCAFFOLD

Fig. 3



(57) Abstract: A synthetic enzyme mimic comprising a support carrying at least one scaffold, the scaffold incorporating multiple functional groups comprising (i) a carboxylate group, (ii) an imidazole group; and (iii) a nucleophilic group such as an amino, hydroxy or thiol group; wherein the functional groups are arranged in close proximity. The invention also provides a process for making a synthetic enzyme, comprising the step of attaching a catalytic unit comprising said scaffold to a support such as a polymer by attachment of a reactive handle such as an azide group to the support and a matching reactive handle to the catalytic unit, in particular a terminal alkynyl group, to form a linker which is preferably an ether linker. Advantageously the attaching is done via click chemistry.

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— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

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SYNTHETIC ENZYME MIMIC BASED ON A HISTIDINE SCAFFOLD

The present application claims priority benefit of EP14198190.2 (filed 16 December 2014) which is incorporated by reference herein in its entirety.

The present invention relates to synthetic enzyme mimics.

Synthetic molecules that mimic enzyme reactivity have been pursued by an active field of researchers for decades with the goals of both developing synthetically useful mimics and understanding fundamental questions with regard enzyme action as well as developing more robust catalysts that would allow them to be employed in more challenging reaction conditions required for certain industrial applications. A long standing challenge in catalysis has been to develop synthetic mimics that can perform similar chemistry inspired by biologically evolved systems. So far, man-made catalysts seem archaic in comparison with nature's elegant design of enzymes in terms of efficiency and specificity.

Publications by Breslow *et al* (Chem. Soc. Rev. 1972, 1, 553; Acc. Chem. Res. 1995, 28, 146; Chem. Rev. 1998, 98, 1997 and J. Biol. Chem. 2009, 284, 1337) disclose enzyme mimicry or catalysis using metal based active sties, and catalytic diads. However, no examples disclose the three functional groups of the catalytic triad, carboyxylate, imidazole and hydroxyl groups, in close proximity, as an artificial catalytic triad.

US4777250 discloses a cyclodextrin based chymotrypsin model having no amino acids but including the three functional groups (a carboxylate group, an imidazole group and a hydroxyl group) of naturally occuring chymotrpsin. However, it has since been proven that cyclodextrin itself catalyses the hydrolysis reaction faster than cyclodextrin attached to the functional groups, so the role of the functional groups in hydrolysis is not disclosed by US4777250 (Tetrahedron Letters, Vol. 30, No.33, pp 4357-4358,1989 (Zimmerman) and *Tetrahedron Lett.* 1989, *30*, 4353 (Breslow, R.; Chung, S.).

The objective of the present invention is to provide an improved synthetic enzyme mimic.

The synthetic enzyme mimic of the invention comprise a carboxylate group, an imidazole group, and a nucleophilic group such as a hydroxyl group. These groups arranged in close proximity. As a result, this "triad" is able to interact with substrates in a manner akin

to an enzyme (in which a triad of amino acid residues is arranged to facilitate catalytic interactions).

Accordingly, in a first aspect the invention provides a synthetic enzyme mimic comprising a support carrying at least one scaffold, the scaffold incorporating multiple functional groups comprising:

(i) a carboxylate group;

(ii) an imidazole group; and

(iii) a nucleophilic group

and wherein the functional groups are arranged in close proximity with each other.

In a second aspect the invention provides a catalytic unit for a synthetic enzyme mimic, comprising a scaffold and attached thereon multiple functional groups comprising:

(i) a carboxylate group

(ii) an imidazole group; and

(iii) a nucleophilic group

wherein the multiple functional groups are in close proximity with each other.

Preferably the nucleophilic group (Nu) is a hydroxyl group (-OH), an amine group, or a thiol group (-SH). More preferably, it is a hydroxyl group or a thiol group.

The invention advantageously provides a synthetic enzyme mimic with at least three catalytic functional groups including, but not limited to, those of the chymotrypsin model thereby facilitating mimicry not only of serine- and cysteine-proteases, esterases, lipases and other enzymes. In other words, the synthetic enzyme mimic of the invention provides a catalytic triad. This are located in close proximity, thereby mimicking the catalytic site of a folded enzyme. The activity is not limited to hydrolytic activity since enzymes may be used to catalyse product synthesis, e.g lipases may be used in chiral synthesis, esterification (transesterification of vegetable oils for bio-diesel) and inter-esterification.

Furthermore the possibility of incorporating multiple 'active sites' via incorporation of multiple scaffolds as described herein allows for improved performance and greater control of the enzyme mimic (see Figure 3) as described hereinbelow.

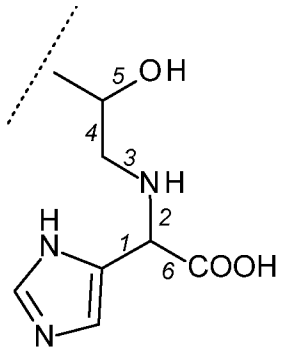
As used herein "polymer" is intended to include both short and long chains and so includes oligomeric forms of scaffold, including alkyl chains. The term also includes co-polymers, homo-polymers etc., linear, cross linked polymers, as well as various architectures such as linear, branched, hyper branched, and dendritic macromolecules.

As used herein "synthetic" is intended to mean non-naturally occurring, and excludes naturally occurring enzymes. For example, it excludes multiple amino acid formations where each amino acid residue provides a single functional group as is found in naturally occurring enzymes.

As used herein the term "scaffold" includes the structure to which the multiple functional groups are attached as specified in the claims but excludes the arrangement of e.g. naturally occurring enzymes whereby each functional group is provided by a different amino acid residue.

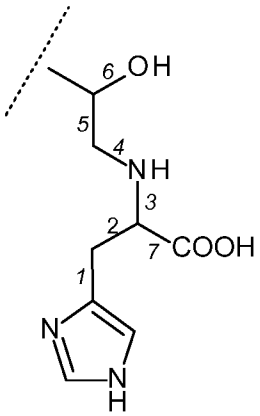
As used herein the term "close proximity with each other" means such that all the functional groups are in functional proximity, e.g. so all can together access so as to act on the relevant atom /bond/s of the substrate molecule. For example, the number of bonds linking each of the three groups (and forming the scaffold) may be 3 to 15. The number of bonds linkings is calculated as a "walk through the molecule" along the longest chain between two of the three groups, plus the number of bonds connecting the third group to that chain. In some cases, the number of bonds is 6 to 12. For example, it may be 6 to 8.

For example, in a scaffold based on 2-amino-2-(1H-imidazol-5-yl)acetic acid and a ring opening epoxide addition:



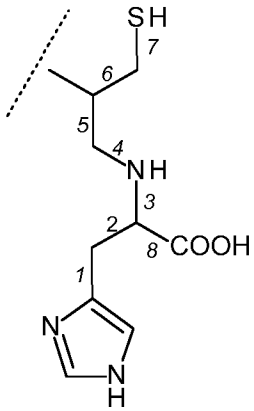
the three functional groups are separated by 6 bonds.

For example, in a scaffold based on 2-amino-3-(1H-imidazol-4-yl)propanoic acid and a ring opening epoxide addition:



the three functional groups are separated by 7 bonds.

For example, in a scaffold based on 2-amino-3-(1H-imidazol-4-yl)propanoic acid and a ring opening epoxide addition to install a thiol nucleophile (the secondary alcohol is used to link with the reactive handle):



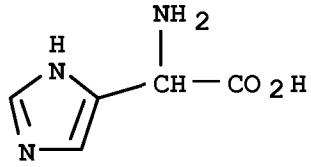
the three functional groups are separated by 8 bonds.

As used herein “histidine” is intended to include histidine derivatives and analogues including all its stereoisomeric (and racemic) forms, and also substituted imidazole derivatives.

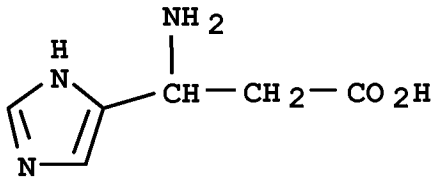
The scaffold preferably comprises an amino group. The inclusion of an amino group is advantageous for linking mechanisms involving functional epoxides as described herein.

The scaffold preferably comprises histidine or is histidine-based, and more preferably based on a single histidine molecule.

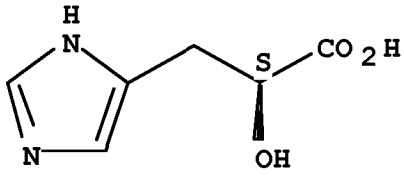
The scaffold preferably comprises any one of the below structures. These structures may be reacted with, for example, a epoxide via a ring opening addition to incorporate a hydroxyl functional group (nucleophile) to provide a scaffold as claimed:



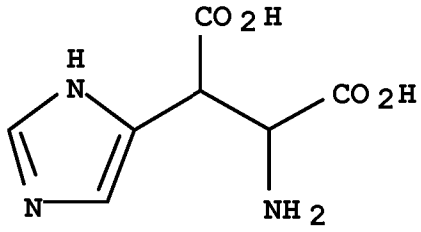
CA Index Name: 1H-Imidazole-5-acetic acid, α-amino-
2-amino-2-(1H-imidazol-5-yl)acetic acid



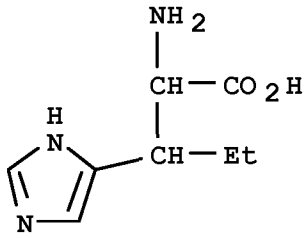
CA Index Name: 1H-Imidazole-5-propanoic acid, β-amino-
3-amino-3-(1H-imidazol-5-yl)propanoic acid



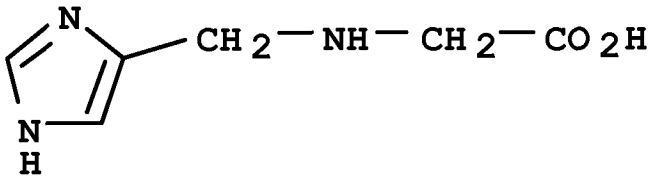
CA Index Name: 1H-Imidazole-5-propanoic acid, α-hydroxy-, (αS)-
(2S)-2-hydroxy-3-(1H-imidazol-5-yl)propanoic acid



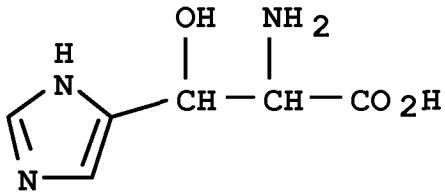
CA Index Name: Imidazole-4-succinic acid, α-amino-, (+)- (8CI)
2-amino-3-(1H-imidazol-5-yl)butanedioic acid



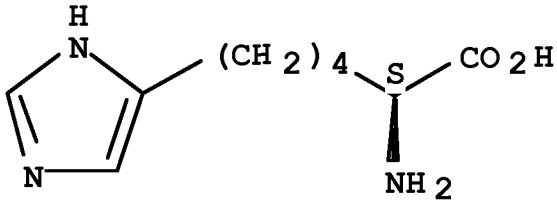
CA Index Name: Histidine, β -ethyl- (9CI)
2-amino-3-(1H-imidazol-5-yl)pentanoic acid



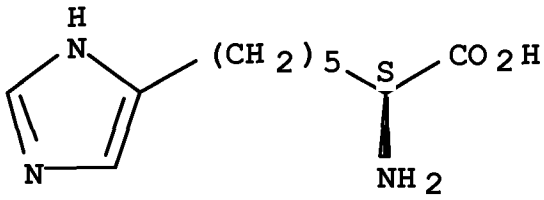
CA Index Name: Glycine, N-(1H-imidazol-5-ylmethyl)-
2-(1H-imidazol-5-ylmethylamino)acetic acid



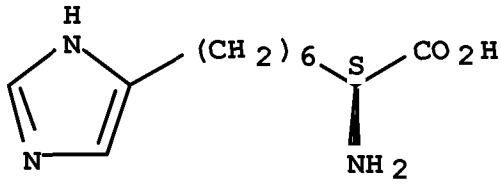
CA Index Name: Histidine, α-hydroxy- (9CI)
2-amino-3-hydroxy-3-(1H-imidazol-5-yl)propanoic acid



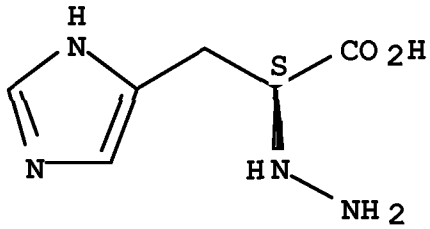
CA Index Name: 1H-Imidazole-5-hexanoic acid, α-amino-, (α S)-
2-amino-6-(1H-imidazol-5-yl)hexanoic acid



CA Index Name: 1H-Imidazole-5-heptanoic acid, α-amino-, (α S)-
2-amino-7-(1H-imidazol-5-ylheptanoic acid

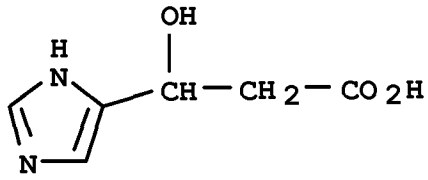


CA Index Name: 1H-Imidazole-5-octanoic acid, α-amino-, (α S)-
2-amino-8-(1H-imidazol-5-yl)octanoic acid

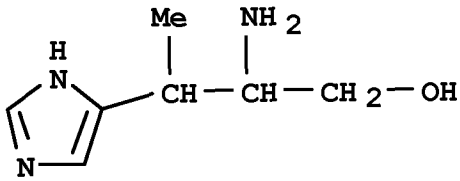


Formula: C6 H10 N4 O2

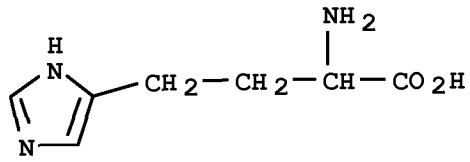
CA Index Name: 1H-Imidazole-4-propanoic acid, α-hydrazino-, (α S)- (9Cl)
2-hydrazino-3-(1H-imidazol-5-yl)propanoic acid



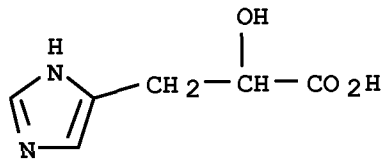
CA Index Name: 1H-Imidazole-5-propanoic acid, β-hydroxy-
3-hydroxy-3-(1H-imidazol-5-yl)propanoic acid



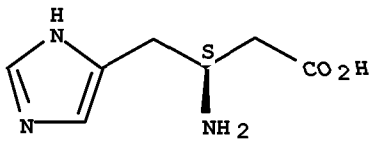
CA Index Name: 1H-Imidazole-5-propanol, β -amino-γ-methyl-
2-amino-3-(1H-imidazol-5-yl)butan-1-ol



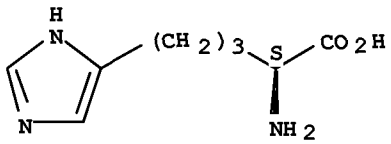
CA Index Name: 1H-Imidazole-5-butanoic acid, α-amino-
2-amino-3-(1H-imidazol-5-yl)propan-1-ol



CA Index Name: 1H-Imidazole-5-propanoic acid, α-hydroxy-
2-hydroxy-3-(1H-imidazol-5-yl)propanoic acid



CA Index Name: 1H-Imidazole-5-butanoic acid, β-amino-, (βS)-
3-amino-4-(1H-imidazol-5-yl)butanoic acid



CA Index Name: 1H-Imidazole-5-pentanoic acid, α-amino-, (αS)-
2-amino-5-(1H-imidazol-5-yl)pentanoic acid

Alternatively or additionally the scaffold may comprise any molecule bearing the functional groups: imidazole and a carboxylic acid group, and further an amino group.

Preferably the support is a solid phase support. For example, the support may be a bead. The support may have a diameter greater than 50 µm, greater than 100 µm,

greater than 200 μm or even greater than 500 μm. Suitable beads are known in the art. This is advantageous because removal of the synthetic enzyme mimic following the reaction (as would be necessary for enzyme-catalysed synthesis of compounds/molecules for cosmetics or drugs) can then be achieved by filtration as opposed to extraction. The support which may be natural or synthetic, preferably comprises a polymer.

Preferably the scaffold is attached to the polymer (support) at a single repeat unit.

Preferably the scaffold is attached to the support via a linker, more preferably an ether-based linker.

Preferably the support comprises a non-reactive polymer, more preferably it is based on polystyrene.

Preferably the support comprises copolymer. The copolymer may comprise a synthesized co-polymer of methyl glycidyl ether and epichlorohydrin. The copolymer may comprise a synthesized co-polymer of styrene and chloromethylstyrene.

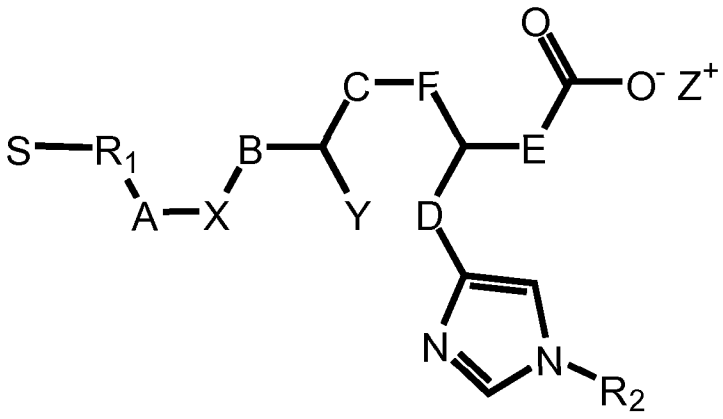
The support may comprise inorganic particles such as silica or zeolite.

The support may comprise a micelle structure.

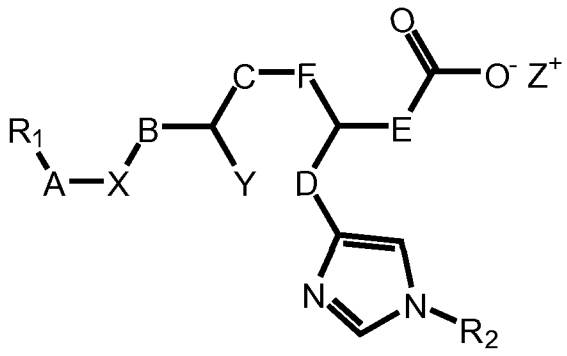
The catalytic unit preferably comprises a reactive handle for attachment to a support as described herein. Preferably the reactive handle comprises, an alkyne, alkene, styrenic, alkyl halide, amine, thiol, carboxylic acid or carboxylic acid anhydride, etc. Most preferably it comprises an alkyne group.

Preferably the reactive alkyne handle comprises terminal alkyne units (an acetylene group).

According to a third aspect of the invention, a synthetic enzyme mimic is provided, having the following structure:



According to a fourth aspect of the invention, a catalytic unit for an enzyme mimic, is provided having the following structure (where R_1 is optional)



wherein both the third and the fourth aspect:
S is the support as described herein.

R_1 is preferably a reactive handle and comprises, an alkyne, alkene, styrenic, alkyl halide, amine, thiol, carboxylic acid or carboxylic acid anhydride, etc. The reactive handle of the catalytic unit will of course be chemically modified by the bonding of the catalytic unit to the support, such that R_1 of the catalytic unit will not be identical to the associated R_1 of the resultant synthetic enzyme mimic incorporating the catalytic unit by attachment using R_1;

For example, R_1 may be a group suitable for undergoing a cycloaddition reaction. For example, it may comprise an alkene or alkyne moiety or an azide. The group may a group primed to under nucleophile substitution (i.e. it may feature a leaving group), for example, it may be an alkyl halide. The group may be a group primed to undergo

nucleophilic addition, for example, it may be a carboxylic acid anhydride. Alternatively, the group may be a nucleophile. For example. it may comprise an amine or thiol group.

Preferably, R_1 is a -C≡CH group.

It will be appreciated that when the catalytic unit and the scaffold are attached, the nature of R_1 changes. In the synthetic enzyme of the invention, for example, R_1 may be a 1,2,3 triazole.

R_2 preferably comprises A substituents. For example, R_2 may be H and/or may be linear or cyclic and may be a short or long alkyl group, e.g. methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, hexadecyl, cyclohexane, etc. aryl, e.g. phenyl, naphthyl, arylalky, e.g. benzyl, oxyethylene, oxypropylene, polyoxyethylene, polyoxypropylene, or any combination thereof, etc;

For example, R_2 may be selected from H, C_{1-20}alkyl, C_{2-20}alkenyl, C_{2-20}alkynyl, C_{3-8}cycloalkyl, $C_{3\ 8}$heterocycyl, C_{6-10}aryl, C_{5-10}heteroaryl, C_{1-20}alkoxy, C_{3-20}alkenoxy, and C_{1-5}alkyl-C_{6-10}aryl. These groups may be unsubstituted or optionally substituted,

For example, R_2 may be selected from H, C_{1-18}alkyl, C_{2-18}alkenyl, C_{3-8}cycloalkyl, phenyl and benzyl. For example, R_2 may be selected from H, C_{1-16}alkyl, C_{2-6}alkenyl, C_{5-8}cycloalkyl, phenyl and benzyl. These groups may be unsubstituted or optionally substituted

Preferably, R_2 is H. In these cases, the imidazole moiety may be considered amphoteric.

X is preferably a divalent atom, for example an O or S atom;

Y preferably comprises a nucleophilic functional group, for example -OH, -SH, $-NH_2$ or -CO_2H functional groups. Preferably, Y comprises –OH, -SH, or $–NH_2$. Preferably, Y is $(CR_3R_4)_n$-Nu, wherein Nu is the nucleophilic functional group and n is an integer (e.g. 0,1,2,3,4). Preferably, n is 0 or 1. Most preferably Y is –OH, –SH, -CH_2OH or -CH_2SH. For example, Y may be –OH or -CH_2SH;

Z is preferably a cationic counterion of the carboxylate functional group. For example, Z may be H, or any metal ion such as Cs, Na, K, ammonium, sulphonium or phosphonium salts, e.g. tetraalkylammonium, tetraalkylsulphonium or tertaalkylphosphonium salts;

Preferably A is a linker group selected from linear and cyclic alkyl, aryl, arylalkyl groups which may or may not incorporate heteroatoms along the backbone, i.e. $-CH_2CH_2O-$;

In some cases, A and X together form an ether based linker. For example, X may be O and A may be CH_2.

In some cases, A and R_1 may together for a terminal alkyne group. For example, R_1 may be a -C≡CH group and A may be a C_{1-4} alkyl. Preferably, A is CH_2 and R_1 and A together form a propargyl group in the catalytic unit.

B, C, and D and E are preferably independently selected connecting groups denoted by $-(CR_3R_4)_n$; where R_3 & R_4 may be H, halogen, a linear and cyclic alkyl, OH, alkoxy, aryl, arylalkyl groups as defined for R_2 above and n is an integer e.g. 0,1,2,3. For B, C, and D, n is preferably 1, 2 or 3. For E, n is preferably, 0, 1, 2, or 3.

For example, R_3 and R_4 may be independently H, OH, halogen, C_{1-4}alkyl or C_{1-4}alkoxy.

Preferably, R_3 and R_4 are both H.

B forms part of a linker attaching the scaffold (the moiety bearing functional groups (i), (ii), and (iii) as described herein) and the support. B may, in some cases, be $(CR_3R_4)_{1-6}$, for example $(CH_3H_4)_{1-6}$, preferably $(CH_3H_4)_{1-3}$. Preferably, B is CH_2.

In the case of C and D, n is preferably selected such that the functional groups are maintained in close proximity to each other;

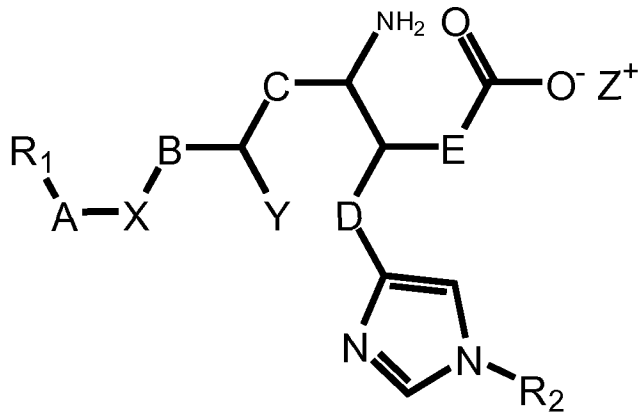
For example, in some cases, both C and D are $(CR_3R_4)_{1-6}$, preferably both $(CH_2)_{1-4}$. Preferably, at least one of C and D is CH_2. In some cases, both C and D are CH_2.

In the case of E, n is preferably selected such that the functional groups are maintained in close proximity to each other;

For example. n may be zero (in other words, E may be a bond) or n may be 1 (in other words, E may be CH_2.

F preferably comprises an amino group (-NH-) or an amino group linked by an alkyl group e.g. F may be CH-NH_2 or (CH)-$(CH_2)_n$–NH_2 where n is an integer 0,1,2,3 etc. limited such that the resultant structure has the functional groups in close proximity to each other. Accordingly, it will be understood that F may comprise a primary amine or a secondary amine. The alkyl group or groups may be modified. In one example F is –NH– providing a histidine-based scaffold.

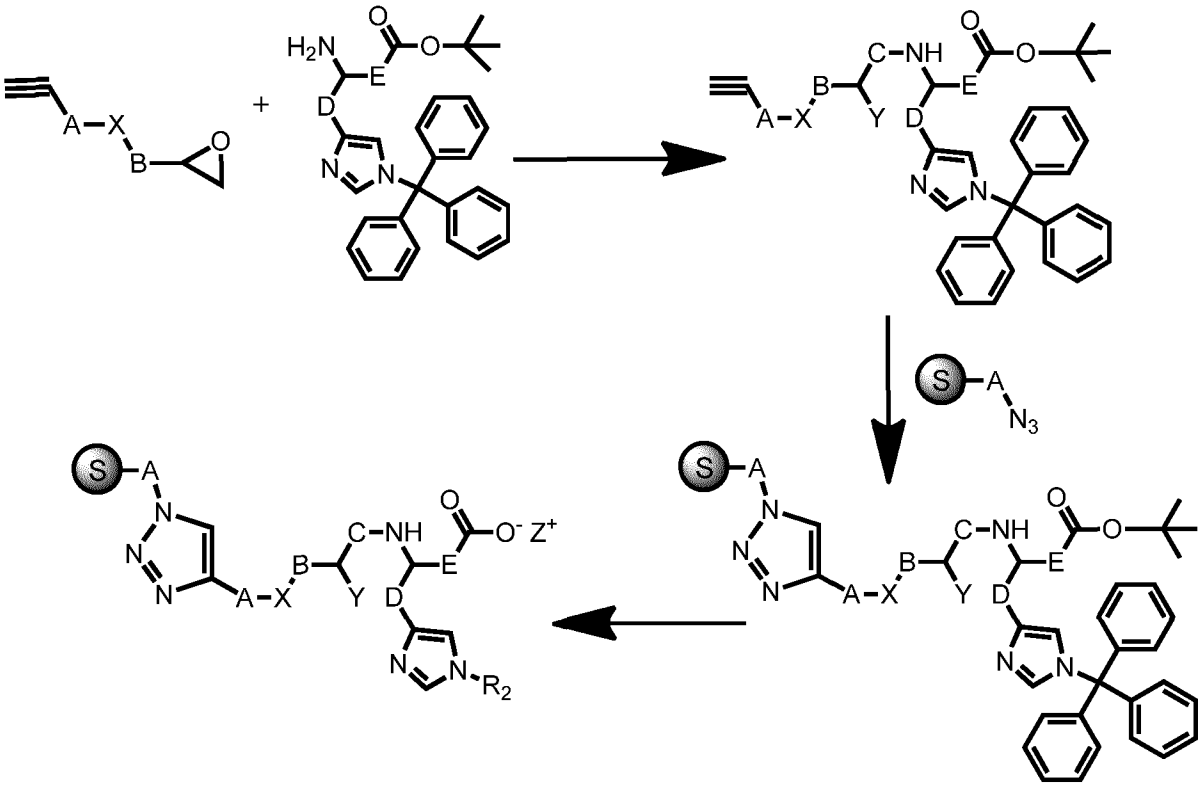
In another example, F is CH-NH_2 and the catalytic unit has the structure



(where R_1, R_2, A –E, Y, and Z are all as described above).

Where appropriate, groups as described herein may be optionally substituted. For example, and not by way of limitation, Suitable substituents may include halogen (F, Cl, Br, I. preferably F or Cl), OH, C_{1-4}alkyl, C_{1-4}alkoxy, or C_{6-10}aryl.

The synthetic enzyme mimic may comprise a triazole (N_3) group. The triazole group may comprise either of the isomers or any derivatives thereof. The triazole may be 1,4-substituted or 1,2-substituted. Preferably, it is 1,4-disubstituted as shown. A reaction scheme showing the construction of the catalytic unit with reactive handle, and attachment to support via a triazole with de-protection step is below:



Advantageously, the catalytic unit of the second and fourth aspects of the invention may be used to make the enzyme mimic of the first and third aspects of the invention.

The support (S) may be azidified, and preferably comprises an azidified polymer. In other words, the support may comprise an azide group, and preferably comprises a plurality of azide groups in repeating polymer units. This advantageously facilitates the use of click chemistry azide-alkyne cycloaddition reactions for linking the scaffold to the support. It will be appreciated that when the support and catalytic unit are attached to each other, the azide group(s) suitably form triazoles, as is conventional in click chemistry methods. The reactive handle is a very versatile moiety that can be used to conjugate the catalytic unit of the invention with a range of supports, utilizing the highly efficient alkyne-azide (optimally copper catalyzed) cycloaddition reaction. The use such reaction chemistry affords quantitative addition/incorporation of the scaffold/s to the support as is described below.

Hydrophobic tuning

Preferably the scaffold further comprises one or more hydrophobic groups for

hydrophobic tuning of the synthetic enzyme mimic. The or each hydrophobic group preferably includes, but is not limited to alkyl chains, said alkyl chain having a carbon chain length Cn, where n is greater than 4, more preferable greater than 8. For example, the hydrophobic group may be a C_{4-30} alkenyl or alkenyl group, preferably a C_{8-30} alkenyl or alkenyl group, for example, a C_{8-20} alkenyl or alkenyl group. Alkyl and alknyl groups may be linear or branched.

Preferably the or each hydrophobic group is added via a quantitative addition reaction to allow for varying the amount of catalytic units loaded onto/incorporated into the support. In this way, the or each hydrophobic group may be functionalised to allow for such quantitative addition reactions. In one example the or each hydrophobic group may comprise n-hexadecyne where n is preferably 1.

Preferably the catalytic unit is incorporated on the scaffold in a weight concentration of preferably in the range 0.01-90 wt % more preferably 0.01-80 wt%, even more preferably 1- 60% and most preferably 20 w%.

Hydrophobic tuning using azidified polymer

With azidified supports/polymers, preferably a proportion of the azides on the polymer are bonded to the scaffold/s and a proportion (preferably the remaining proportion) of the azides are bonded to hydrophobic groups. Preferably the ratio of bonding to the azides is in the range scaffold:hydrophobic groups 80:20 – 20:80 and more preferably is 60:40.

The or each hydrophobic group is preferably functionalized via a reactive handle (which may comprise the same structure as the reactive handle as already described for the catalytic unit, i.e. R1) such as an alkene group. This is preferably at a terminal position. For example the azidified support/polymer may be reacted with n-hexadecyne, where n is an integer >0 but is preferably 1. In other words, the or each hydrophobic group may be straight chain or branched.

In this way the unreacted azides (i.e. those not reacted with the scaffold/s) are utilized to tune the hydrophobicity of the mimic, and hydrophobic pockets are introduced throughout the mimic which improves esterolytic performance.

The selection of a specific loading range is advantageous in increasing the hydrophobicity of the synthetic enzyme mimic.

In one example the catalytic unit may be attached to a support comprising an azidified Merrifield Resin. Merrifield resins are known in the art, and are based on a co-polymer of styrene and chloromethylstyrene.

In a fifth aspect the invention provides a process for making a synthetic enzyme mimic of the first aspect or the third aspect (including all preferred features), the method comprising the steps of attaching a nucleophile e.g. hydroxyl group or thiol group (Y above) to a scaffold and also forming a reactive handle as described herein on the scaffold, preferably both steps being achieved by means of a ring opening reaction using a functional epoxide.

Preferably the ring opening reaction uses an alkyne functionalised epoxide, and more preferably the functional epoxide group comprises acetylene functionalised epoxide. For example, the functionalised epoxide may be 2-(prop-2-ynoxymethyl)oxirane.

The process may have a pre-step wherein the functional groups are first protected in a pre-step by protection of at least one of said functional groups and more preferably at least the carboxylic acid and imidazole functional groups. Preferably the process further comprises the final step of removal of the protection groups.

Preferably the process comprises the step of attaching the catalytic unit to a support, said support as described herein.

Preferably the step of attaching the catalytic unit to a support is by attachment of a reactive handle as described herein to the support to form a linker, preferably an ether linker.

In a further aspect the invention provides a process for constructing catalytic species involving small organic molecules, inorganic materials such as functional silicas, zeolites, as well as soluble and insoluble cross-linked polymer and micelle formation (and combinations thereof) as supports which bear catalytic units: triads, tetrads or higher with multiple functional groups per reactive, catalytic unit.

The process preferably involves two phases comprising:

1) Construction of a catalytic unit comprising one or more functional groups and formation of a reactive handle as described herein and optionally a linker group, thereby optionally further introducing further functional group/s.

2) Attachment of the catalytic unit formed in (1) using said reactive handle and optional linker group A onto a support which can be any small molecule or organic or inorganic polymeric support S as described herein.

For the avoidance of doubt the preferred features of the first and second aspects are also preferred features of the second and third aspects and vice versa.

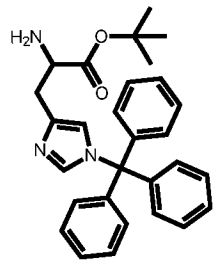
Example of Non Limiting Embodiments of the Invention

Example 1: Synthesis of an exemplary synthetic enzyme on polystyrene support

Protection of histidine functional groups

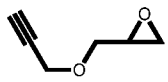
Protective groups on the histidine (e.g. ex L-histidine H6034, H8000 ex Sigma Aldrich) molecule protect the imidazole and carboxylate groups of histidine. Dehalogenating alkylation using trityl chloride (triphenylmethyl chloride: $C_{19}H_{15}Cl$) is used to introduce a trityl protecting group, to protect the reactive imidazole group of histidine.

Tert-butyl ester is used to protect the carboxylate group. The result is a protected histidine:



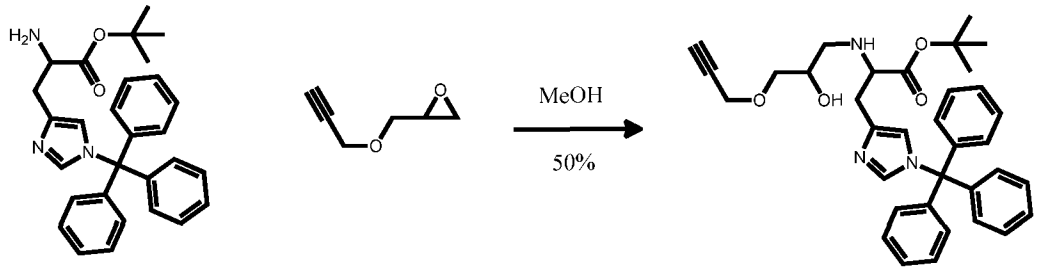
Synthesis of the catalytic unit via ring opening reaction of functional epoxide

The catalytic unit is synthesized by the reaction of protected histidine with a functional epoxide, which in this example it is an acetylene functionalised epoxide:



Starting materials for the following example are the protected histidine: trityl-L-histidine t-butyl ester (BA chemicals, product no. E-3600) and gylcidyl propargyl ether (sigma Aldrich).

Glycidyl propargyl ether (1 mmol, 115 µl) and trityl-L-histidine t-butyl ester (1 mmol, 450mg) is dissolved in 800 µl of methanol and stirred for 48h at 40 °C. Solvent is removed under vacuum and residue purified by column chromatography (eluting with Dichloromethane/methanol, 19:1 by volume) to yield a pale yellow viscous oil (280 mg, 50%).



(1) (2) (3)

The ring opening reaction is highly advantageous as it introduces the acetylene functionality for immobilizing the catalytic unit on to a support and introduces the third functional group (the hydroxyl) in one step (see **Figure 1** for NMR spectrum of isolated compound (3)). This creates the synthetic enzyme precursor molecule (with imidazole and carboxylate groups protected as well as the hydroxyl group).

The incorporation of the acetylene functional group is highly advantageous as it allows for the efficient immobilization of this histidine-based catalytic unit onto a range of synthetic and/or natural supports by means of azide alkyne Huisgen cycloaddition click chemistry. However, this is not limited to an acetylene functionalised molecule as there is a large range of suitable functional epoxides commercially available. For example: 3-(4-chlorobenzoyl)-N-(2-propynyl)-2-oxiranecarboxamid, 3,4-Epoxy-1-butene, Neopentyl glycol diglycidyl ether, Allyl glycidyl ether, Glycidyl acrylate, Glycidyl methacrylate,

Resorcinol diglycidyl ether, (3-Glycidyloxypropyl)trimethoxysilane, 4-Vinyl-1-cyclohexene 1,2-epoxide, mixture of isomers, (+)-Limonene 1,2-epoxide.

Immobilization of catalytic unit onto a polystyrene support

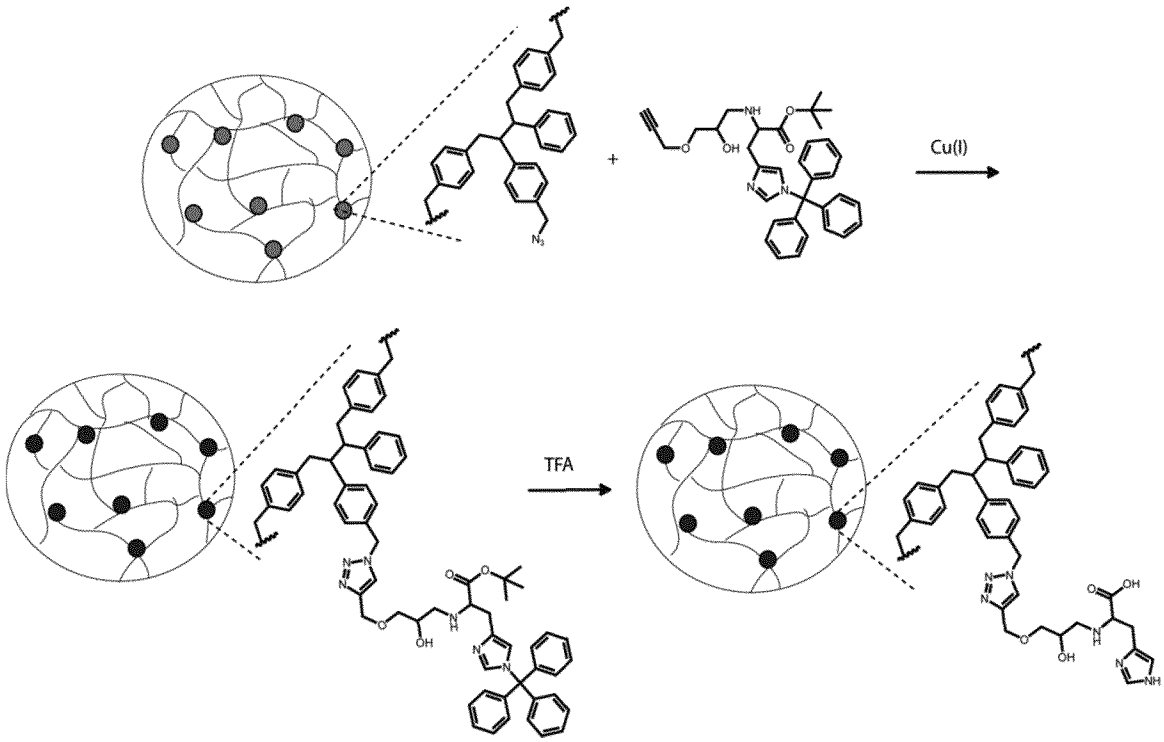
The support in this example is prepared from a modified polystyrene Merrifield resin (" SC-295380" ex Santa Cruz Biotechnology, Inc., (CA)) 3mmol g^{-1} loading and 1% cross linked with divinyl benzene) and provides a hydrophobic, dispersed system exhibiting esterolysis activity.

The Merrifield resin is azidified by reaction with sodium azide. The resultant azide functional resin is then reacted with the synthetic enzyme precursor molecule.

The reaction progress may be monitored by Thin Layer Chromatography (TLC) and the attenuation of the azide peak with Fourier transform infrared spectroscopy (FTIR). Within 2 hours complete disappearance of the acetylene functional molecule by TLC and significantly reduced azide peak via FTIR is observed (**Figure 2**).

Deprotection of the functional groups.

The protecting groups (*t*-butyl and trityl as described above) are then removed by trifluoroacetic acid yielding a synthetic enzyme with *ca.* 3mmol g^{-1} of unprotected enzymatic functionality.



Esterolysis was evaluated using 4-nitrophenyl acetate. Control experiments were performed whereby the esterolysis was measured in pure buffer solutions (no particles) and with particles still containing the protecting groups.

Figure 6 shows the evolution of absorbance at 405 nm with respect to time. Both controls showed a small, linear increase in absorbance, presumably from the inherent hydrolysis of the ester at ph 9.1. Encouragingly, the catalytic triad functional resin shows markedly increased kinetics for the hydrolysis of the nitrophenyl ester, with an increased initial rate and asymptotic curve leveling at higher reaction times.

The triad functional resin was further investigated to cleave the nitrophenyl acetate at varying pH. Figure 7 shows the effect of pH on the catalytic activity of these particles, the particles become less active at lower pH which may help elucidate some of the mechanistic aspects of the catalyst, however further study is required.

Example 2: Immobilisation of catalytic unit on to synthesized co-polymer of methyl glycidyl ether and epichlorohydrin.

Methyl glycidyl ether (MGE) (TCI-America, Inc.) is stirred over calcium hydride, degassed by freeze-pump-thaw (3x), and allowed to stir for 20 hours at room temperature. The

MGE is distilled to a fresh purification flask containing butyl magnesium chloride and is stirred at 0°C for 10 minutes after thawing. The MGE is distilled to a burette and used immediately after purification.

Epichlorohydrin (TCI-America, inc.) is stirred over calcium hydride, degassed by freeze-pump-thaw (3x), and distilled to a burette for storage. Toluene is taking from a dry solvent system.

Preparation of Poly(epichlorohydrin)-co-poly(methyl glycidyl ether):

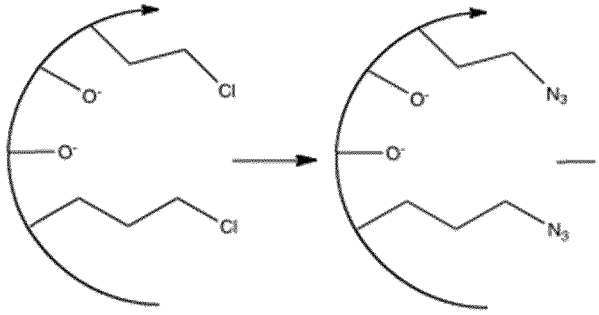
Tetraoctylammonium bromide (Sigma-Aldrich, Inc.) (265 mg) is added to the reactor and dried in vacuo. Monomer burets containing 1.31 g of epichlorohydrin and 7.91 g of MGE were attached to the reactor. Toluene is added and stirred with a magnetic stir bar until the tetraoctylammonium bromide dissolved. The temperature of the reactor assembly is reduced to –30°C. The epichlorohydrin and MGE is then added to the reactor. After the temperature equilibrated, 1mL of 1.0M $Al(iBu)_3$ in hexane is added via gas tight syringe. The polymerization is allowed to warm to room temperature overnight. GPC: *Mn* = 12000 g/mol, PDI = 1.14 relative to polystyrene standards.

Preparation of Poly(glycidyl azide)-co-poly(methyl glycidyl ether):

Poly(epichlorohydrin)-co-poly(methyl glycidyl ether) (5.0g) and Sodium azide (2.0g) is dissolved in DMF and stirred at 50 °C for 5 days. Polymer is precipitated into cold hexanes.

Example 3: Immobilisation of catalytic unit on to porous silica

Functional silica is prepared by grafting with (EtO)3Si(CH2)3Cl and consequent azide exchange with sodium azide:

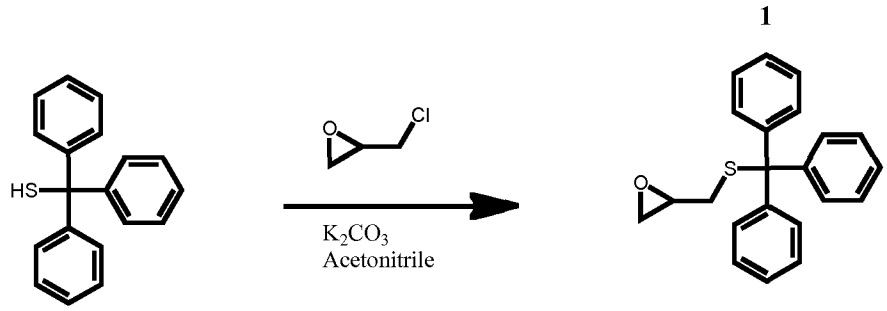


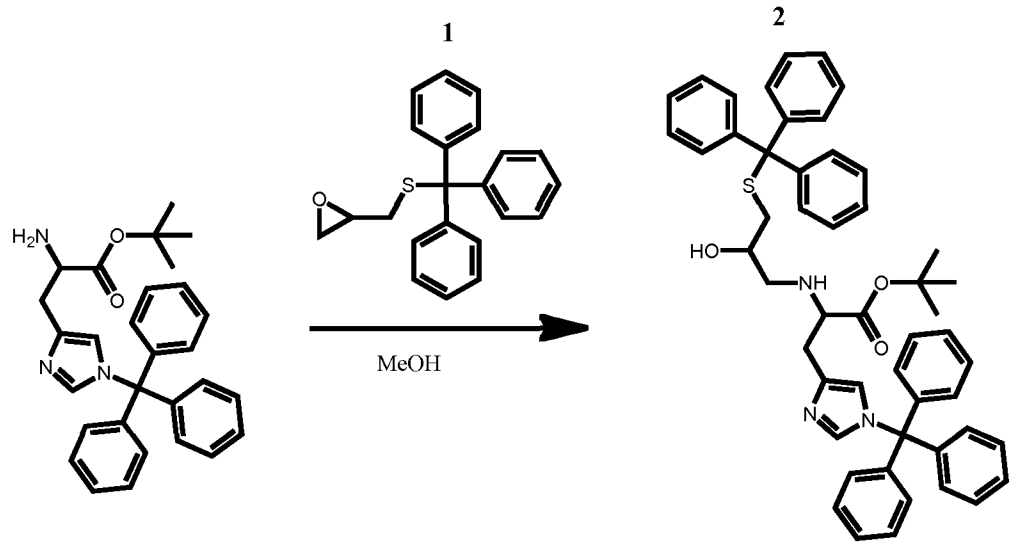
Example 4: A synthetic enzyme with thiol as a nucleophile is prepared as described below.

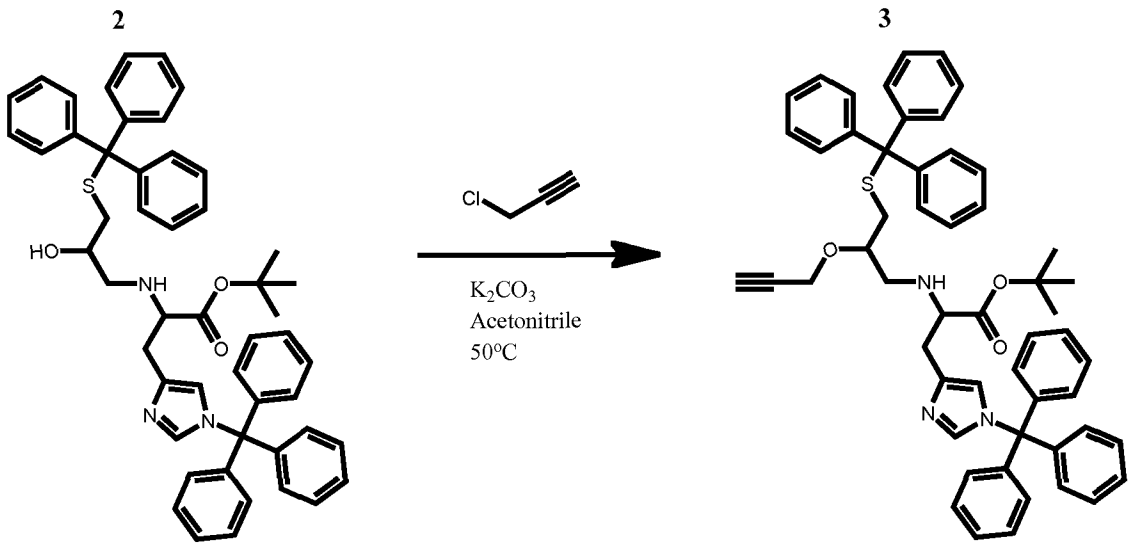
Compound 1. Triphenyl methane thiol (250 mg, 0.90 mmol) is reacted with large excess epichlorohydrin (5 mL) in acetonitrile (1 mL) with K_2CO_3 (256 mg) as base for 48 hr. Dichloromethane (5 mL) is added to precipitate salts. The solution is filtered and dried to yield 1 as a white solid (250 mg, 84%).

Compound 2. Compound 1 (300 mg, 0.90 mmol) and His(Trt)-OtBu (275 mg, 0.60 mmol) are reacted in MeOH at 50°C for 48 hr. The reaction is dried and the product purified via column chromatography (DCM:MeOH gradient) as a colorless wax (75 mg, 16%).

Compound 3. Compound 2 (60 mg, 7.5 mmol) is reacted with excess propargyl chloride (0.75 mL) in acetonitrile (1 mL) at 50°C for 48 hr. Dichloromethane (1 mL) is added to precipitate salts. The solution is filtered and dried to yield 3 as a yellow solid (50 mg, 75%).

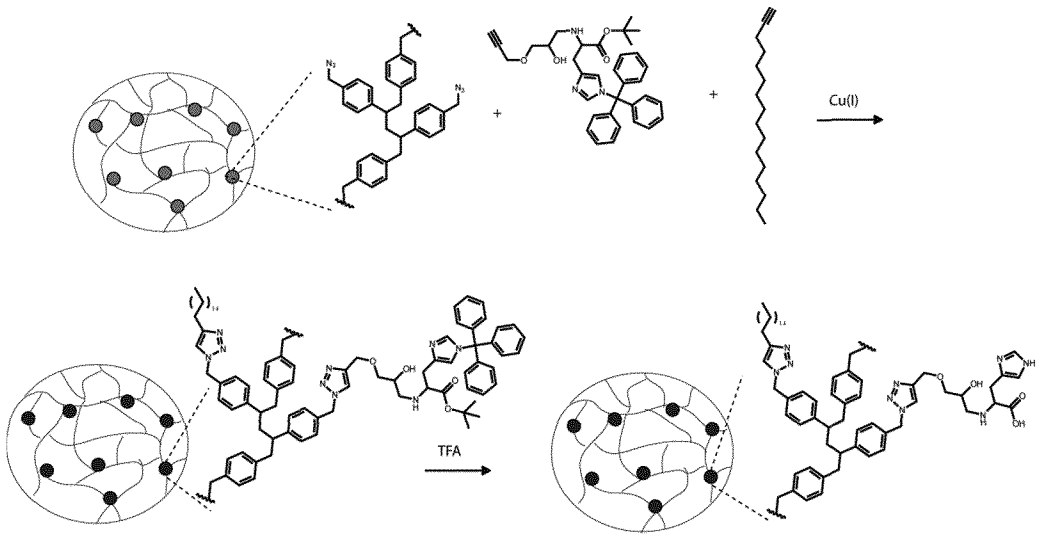

HS
Cl
O
K_2CO_3
Acetonitrile
1
S
O


H_2N
O
N
1
S
MeOH
2
HO
NH
O
N


2
S
HO
NH
O
N
Cl
K_2CO_3
Acetonitrile
50°C
3

Example 5 – Hydrophobic Tuning

The enzyme mimic of Example 1 was tuned for hydrophobicity by introduction of long alkyl chain in the form of hexadecyne-1 (CAS number 629-74-3) in varying amounts.



The initial rates of esterolysis of 4-nitrophenyl acetate by varying amounts of hexadecyne incorporation are shown in **Figure 4**.

The enzyme mimic of Example 5 and an optimized, commercially available Lipase was then evaluated over number different substrates with varying alkyl chain length and the results are shown in **Figure 5**. The enzyme mimic shows superior activity initially for nitrophenyl acetate. The alkyl chain of the substrate indicates esterase activity. Esterases hydrolyse solutions of water-soluble short acyl chain esters and are inactive against water-insoluble long chain esters such as 4-nitrophenyl palmitate which, in turn, are specifically hydrolyzed by lipases.

It is of course to be understood that the invention is not intended to be restricted to the details of the above embodiment which are described by way of example only.

CLAIMS

1. A synthetic enzyme mimic comprising a support carrying at least one scaffold, the scaffold incorporating multiple functional groups comprising:
   (i) a carboxylate group;
   (ii) an imidazole group; and
   (iii) a nucleophilic group
   and wherein the functional groups are arranged in close proximity to each other.

2. A synthetic enzyme mimic according to claim 1 wherein the scaffold is histidine-based.

3. A synthetic enzyme mimic according to claim 1 or 2 wherein the nucleophilic group comprises a hydroxyl group or a thiol group.

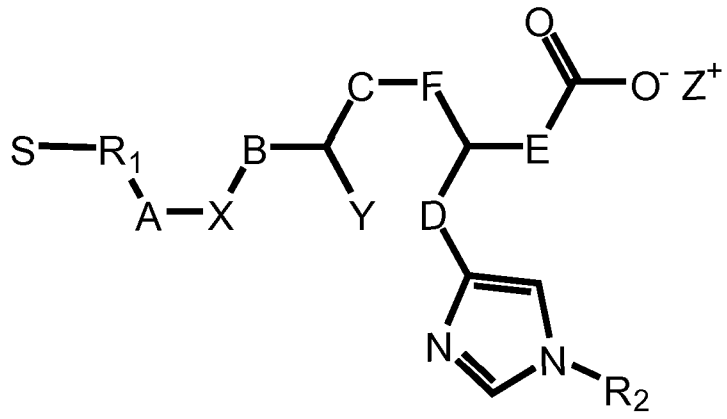
4. A synthetic enzyme mimic according to any preceding claim wherein the support comprises a polymer.

5. A synthetic enzyme mimic according to claim 4 wherein the support comprises a polymer and the scaffold is attached to the polymer at a single repeat unit of said polymer.

6. A synthetic enzyme mimic according to any preceding claim wherein the scaffold is attached to the support via a linker, preferably an ether linker.

7. A synthetic enzyme mimic according to any preceding claim wherein the support further comprises one or more hydrophobic pockets, the or each hydrophobic pocket comprising a hydrophobic group.

8. A synthetic enzyme mimic comprising a support and the following structure:



wherein:

S is a support selected from the group consisting of non reactive polymers, copolymers, inorganic particles such as silica or zeolite, micelle structures;

R_1 is said reactive handle and is selected from the group consisting of alkene, styrenic, halide, amine, thiol, carboxylic acid or carboxylic acid anhydride groups;

R_2 is selected from the group consisting of H and/or alkyl groups methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, hexadecyl, cyclohexane, and/or aryl groups phenyl, naphthyl, and/or arylalky groups benzyl, oxyethylene, oxypropylene, polyoxyethylene, polyoxypropylene, and/ or any combination thereof;

X is a divalent atom selected from the group consisting of O or S;
Y comprises a nucleophilic group selected from -OH, -SH, -NH2 or -CO2H functional groups;

Z is a cationic counter ion selected from the group consisting of H or metal ions which are preferably Cs, Na, K or ammonium, sulphonium or phosphonium salts preferably tetraalkylammonium, tetraalkylsulphonium or tertaalkylphosphonium salts;

A is a linker group selected from the group consisting of linear and cyclic alkyl, aryl, arylalkyl groups;

B, C, D and E are independently selected connecting groups denoted by -(CR3R4)n; where R3 & R4 are independently selected from the group consisting of H, halogen, a linear and cyclic alkyl, OH, alkoxy, aryl, arylalkyl groups as defined for R_2 and wherein n is an integer F comprises an amino group ($-NH_2$) or an amino group linked by an akyl group comprising $CH-NH_2$ or $(CH)-(CH2)_n-NH_2$ where n is an integer 0,1,2,3 etc. wherein the resultant structure has the functional groups in close proximity to each other.

9. A synthetic enzyme mimic according to any preceding claim having esterase activity.

10. A synthetic enzyme mimic according to any preceding claim having lipase activity.

11. A synthetic enzyme mimic according to any preceding claim having protease activity.

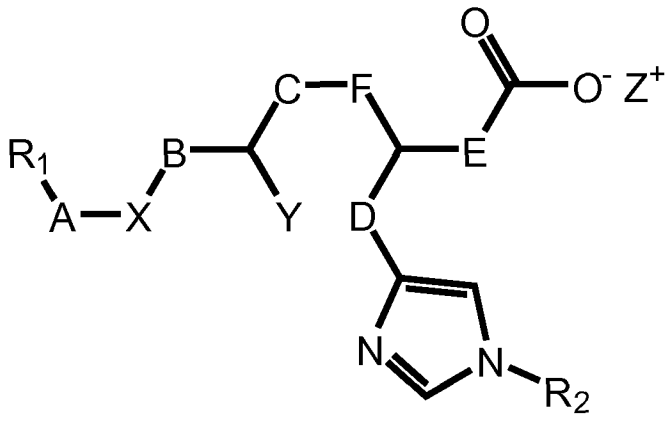
12. A catalytic unit for a synthetic enzyme according to any of claims 1 - 7, comprising a scaffold incorporating multiple functional groups, said multiple functional groups comprising:
(i) a carboxylate group;
(ii) an imidazole group; and
(iii) a nucleophilic group
wherein the multiple functional groups are in close proximity with each other

13. A catalytic unit according to claim 12 wherein the scaffold is histidine-based.

14. A catalytic unit according to claim 12 or claim 13 wherein the nucleophilic group comprises a hydroxyl group or a thiol group.

15. A catalytic unit according to any of claims 12- 14 comprising a reactive handle for attachment to a support.

16. A catalytic unit for a synthetic enzyme mimic comprising the following structure



R_1 is an optional reactive handle and is selected from the group consisting of alkene, styrenic, halide, amine, thiol, carboxylic acid or carboxylic acid anhydride groups;

R_2 is optional and selected from the group consisting of H and/or alkyl groups methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, hexadecyl, cyclohexane, and/or aryl groups phenyl, naphthyl, and/or arylalky groups benzyl, oxyethylene, oxypropylene, polyoxyethylene, polyoxypropylene, and/ or any combination thereof;
X is a divalent atom selected from the group consisting of O or S;
Y is a nucleophilic group selected from the group consisting of -OH, -SH, -NH2 or -CO2H functional groups;

Z is a cationic counterion selected from the group consisting of H or metal ions which are preferably Cs, Na, K or ammonium, sulphonium or phosphonium salts preferably tetraalkylammonium, tetraalkylsulphonium or tertaalkylphosphonium salts;

A is a linker group selected from the group consisting of linear and cyclic alkyl, aryl, arylalkyl groups;

B, C, D and E are independently selected connecting groups denoted by -(CR3R4)n; where R3 & R4 are independently selected from the group consisting of H, a linear and cyclic alkyl, aryl, arylalkyl groups as defined for R_2 and wherein n is an integer;

F comprises an amino group ($-NH_2$) or an amino group linked by an alkyl group comprising $CH-NH_2$ or $(CH)-(CH2)_n-NH_2$ where n is an integer 0,1,2,3 etc. wherein the resultant structure has the functional groups in close proximity to each other

17. A process for making a catalytic unit according to any of claims 12-16, the method comprising the steps of attaching a nucleophilic group to the scaffold and also forming a reactive handle on the scaffold.

18. A process according to claim 17 comprising the step of a ring opening reaction using a functional epoxide.

19. A process for making a synthetic enzyme mimic of any of claims 1-11 comprising the step of attaching the catalytic unit of claims 11-16 to a support.

20. A process according to claim 19 comprising the step of attaching a reactive handle to the support.

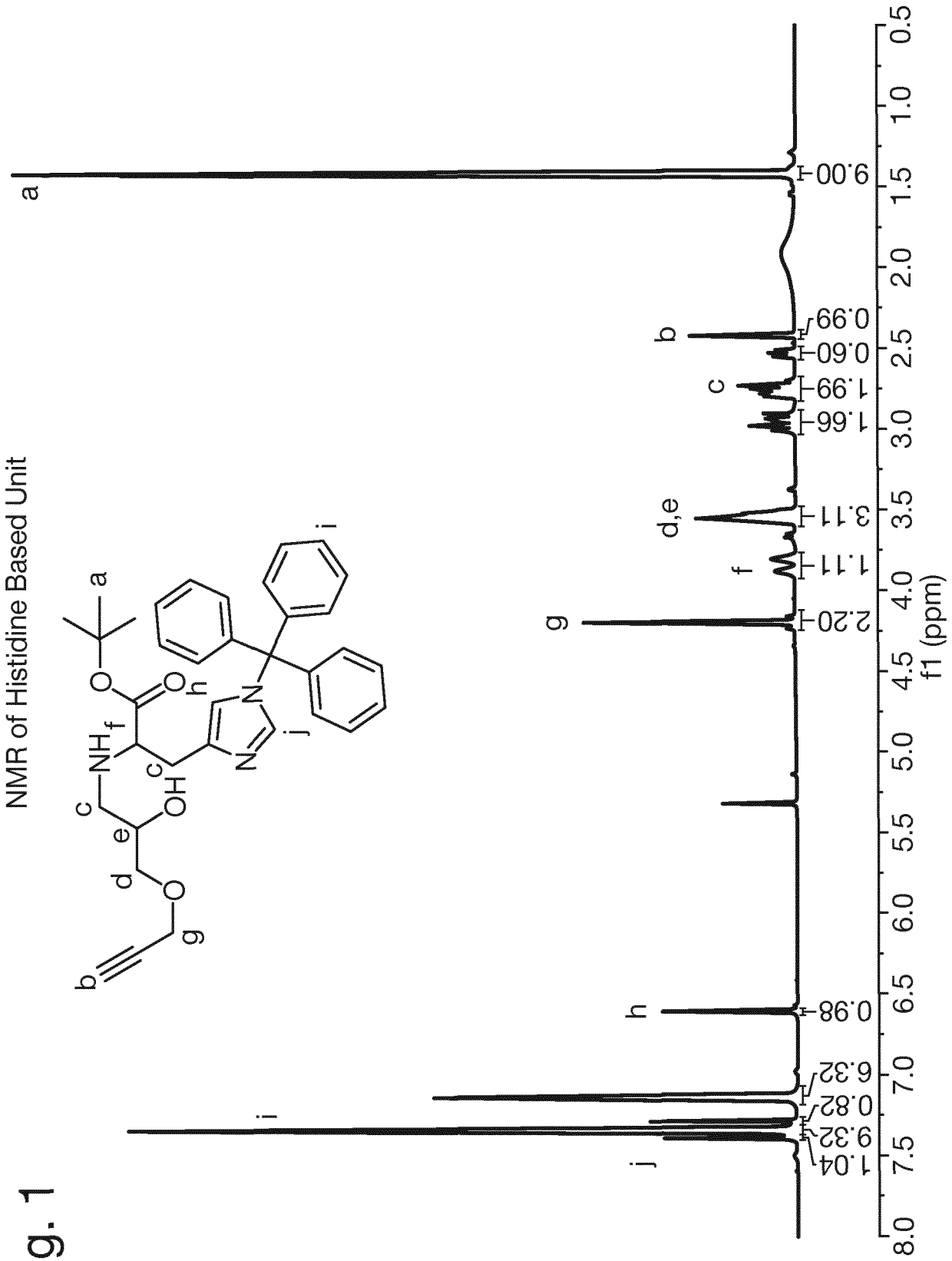

NMR of Histidine Based Unit
a
b
c
d,e
f
g
h
i
j
9.00
0.99
0.60
1.99
1.66
3.11
1.11
2.20
0.98
6.32
0.82
9.32
1.04
f1 (ppm)

Fig. 1

Fig. 2 FTIR Spectrum showing the Disappearance of the Azide Group on the Polystyrene Resin Initial Screening for Catalytic Behavior

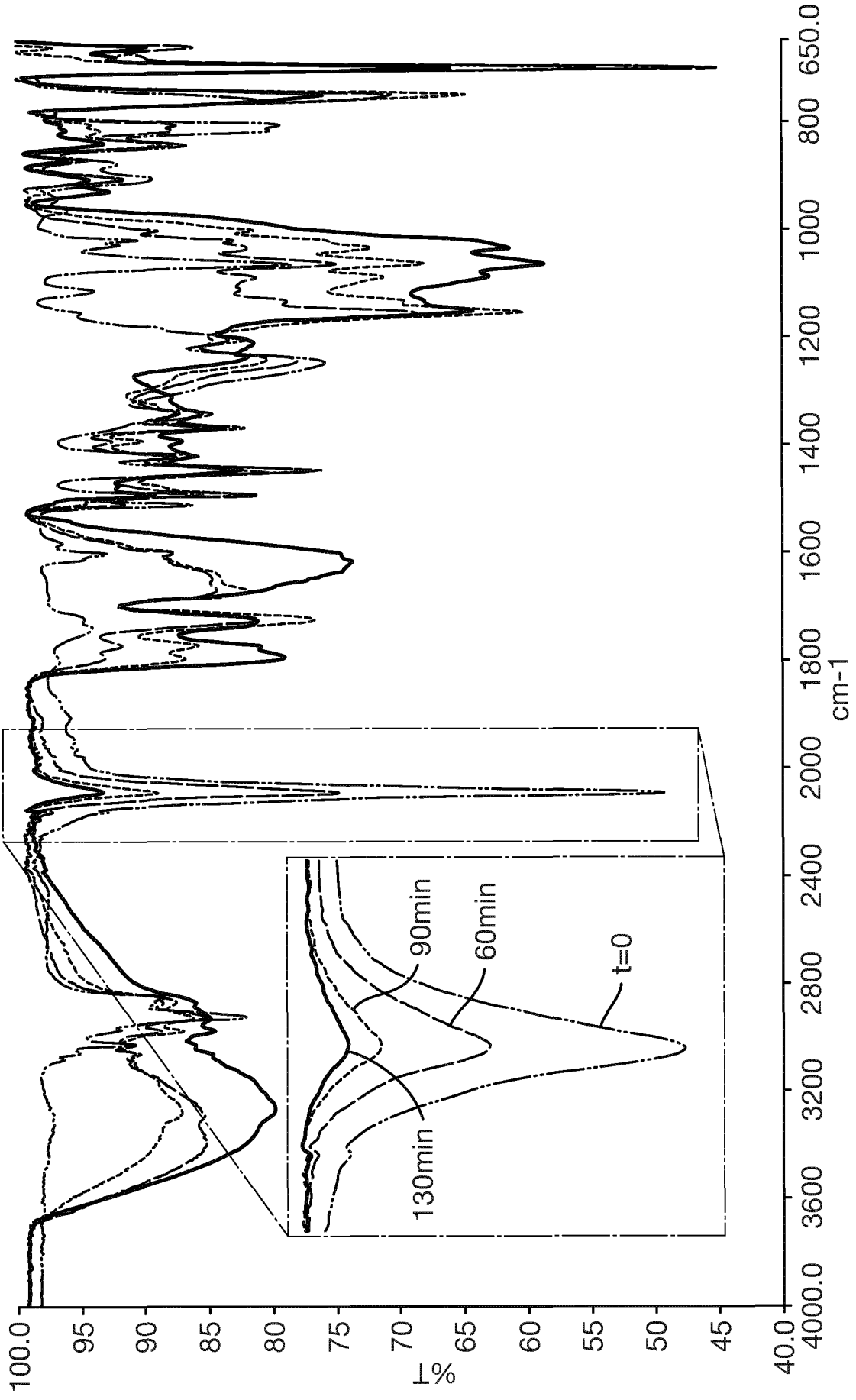


Fig. 3

Comparison of the Enzyme (Esterase) Mimic of the Invention with a Natural (Esterase) Enzyme

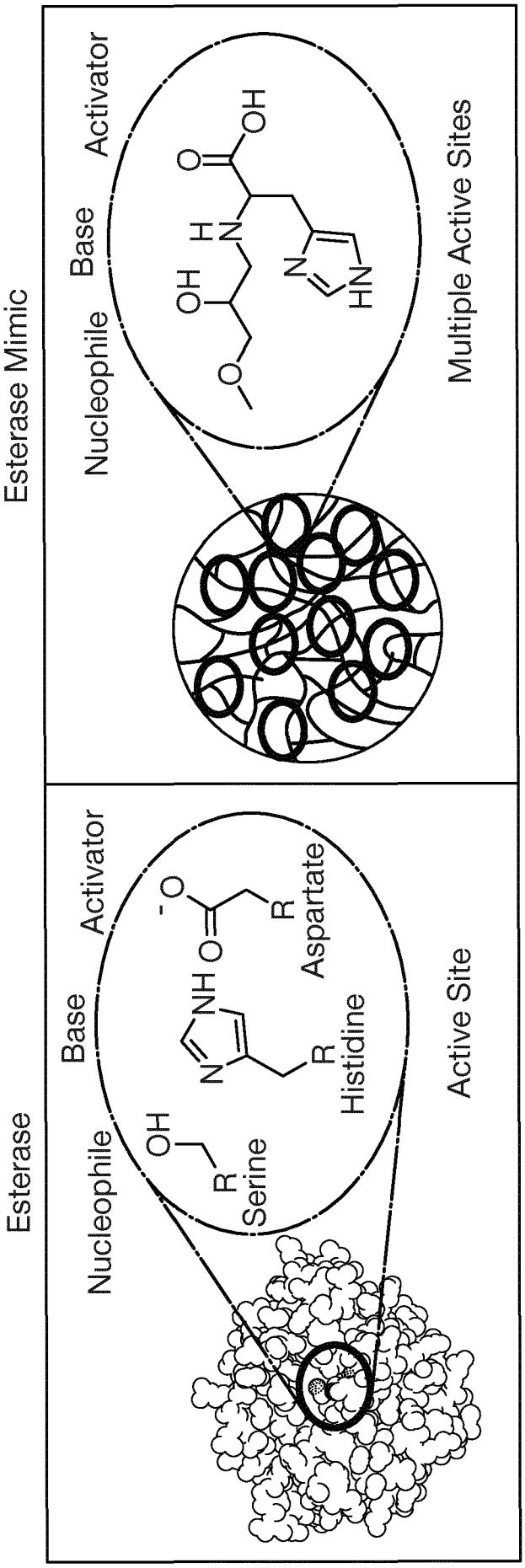


Fig. 4

Initial Rate of Esterolysis of Nitrophenyl Acetate Resin with Different Incorporations of Triad Molecule and Varying Incorporation of Hexadecyne

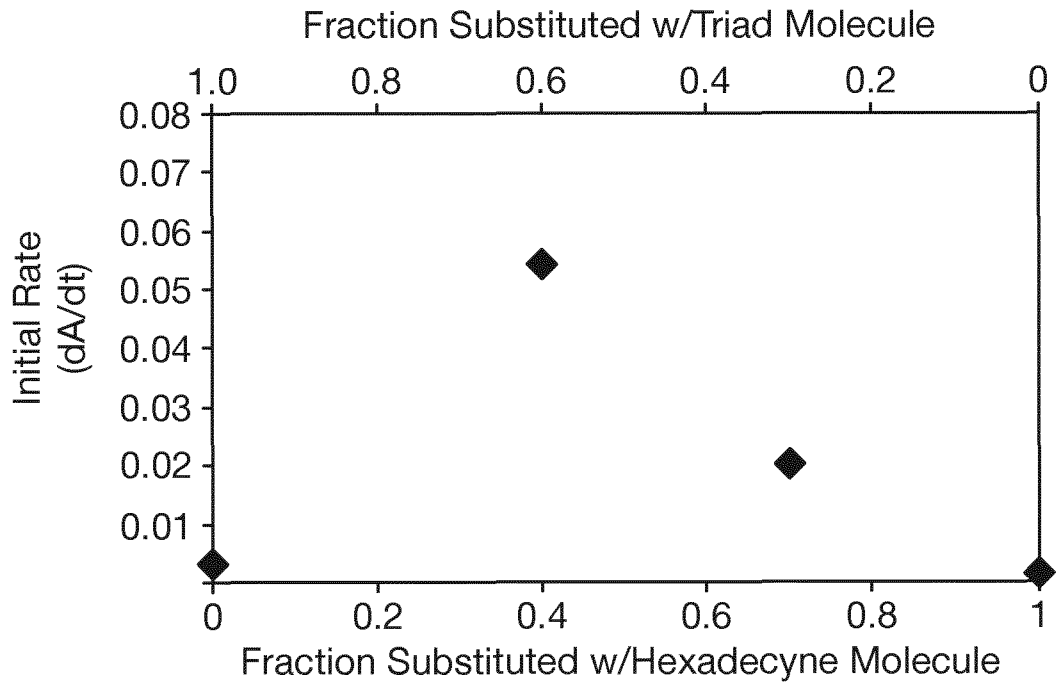


Fig. 5 Esterolysis Catalyzed by Mimic of the Invention using Different Substrates and Background Hydrolysis. All Conditions are Performed at pH9

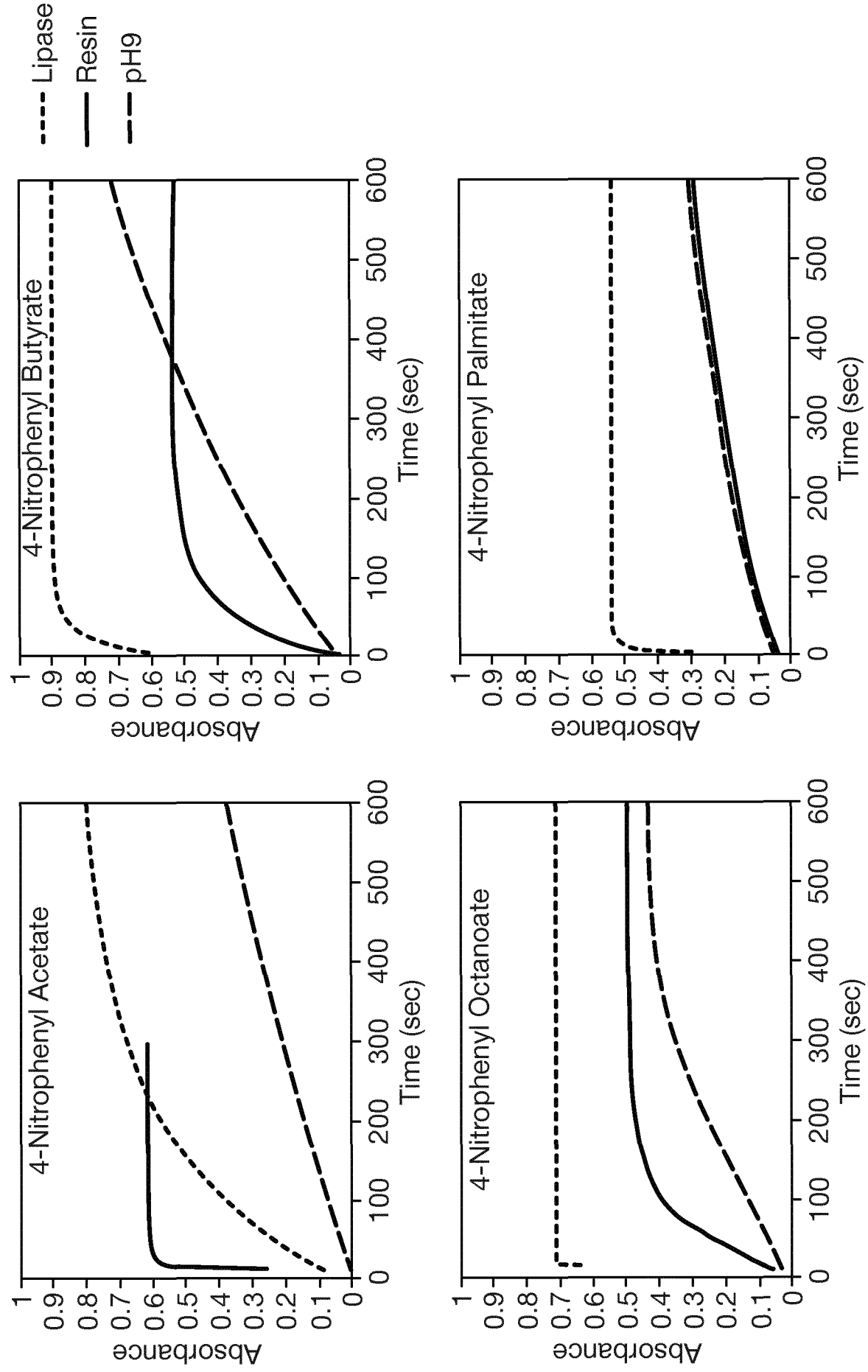


Fig. 6

UV-VIS Analysis of Triad Functional Resin, and Controls Containing Resin with Protected Functionality and Esterolysis in Pure Buffer Conditions

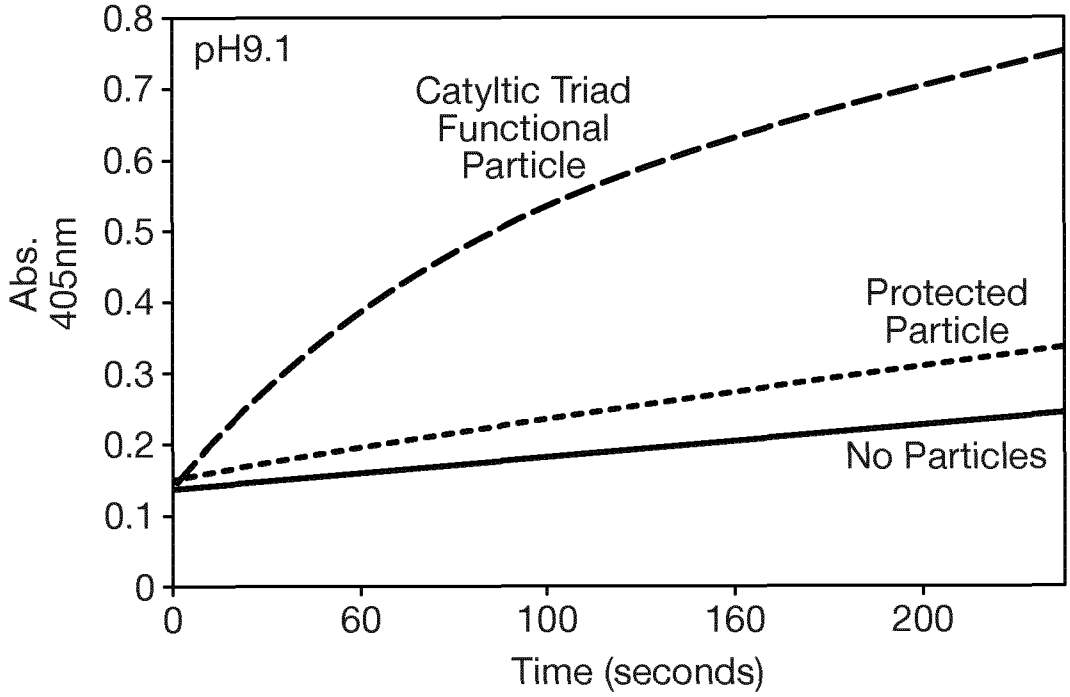
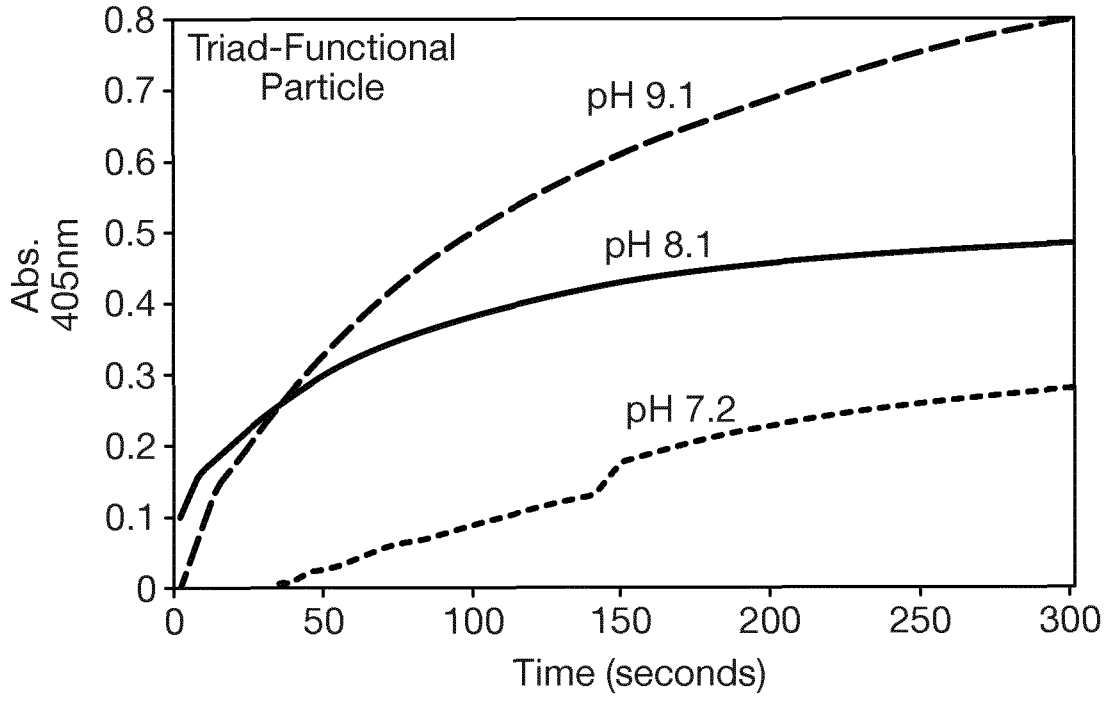


Fig. 7

Esterolysis of Nitrophenyl Acetate Catalyzed by Triad Functional Polystyrene Resin at Different pH



INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2015/079204

A. CLASSIFICATION OF SUBJECT MATTER
INV. B01J31/02 B01J31/06 B01J31/04 C12N9/00
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)
B01J C12N

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, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4 777 250 A (BENDER MYRON L [US] ET AL) 11 October 1988 (1988-10-11) cited in the application column 5, lines 16-62; claim 1; examples abstract ----- -/--	1-7,19, 20

[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
22 February 2016	10/05/2016

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	Goebel, Matthias

1

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

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	RONALD BRESLOW ET AL: "A novel synthesis of substituted imidazoles, and a reexamination of a purported chymotrypsin model", TETRAHEDRON LETTERS, vol. 30, no. 33, 1 January 1989 (1989-01-01), pages 4353-4356, XP055124171, ISSN: 0040-4039, DOI: 10.1016/S0040-4039(00)99358-4 cited in the application page 4353 * Scheme 1 * page 4356, paragraph 1	1-7,19, 20
X	US 2007/184970 A1 (GAO YONG [US]) 9 August 2007 (2007-08-09) paragraphs [0016], [0023] - [0030], [0032], [0074], [0077]; claims 1-10; figures 1D,1E,1G,1H,1J examples 1,2; tables 1,2	1-7,19, 20
X	WO 2010/071462 A1 (UNIV HUMANISTYCZNO PRZYRODNICZ [PL]; POLITECHNIKA LODZKA [PL]; KACA WI) 24 June 2010 (2010-06-24) claim 1; figure 1; examples 3,4,6-8,9; table 1	1-7,19, 20
X	US 6 310 199 B1 (PROMEGA CORP., USA) 30 October 2001 (2001-10-30) column 15, line 29 - column 16, line 21; figures 1,2 examples 3,4,5	1-8
X	EL-KAK, A. ET AL: "Interaction of immunoglobulin G with immobilized histidine: mechanistic and kinetic aspects", JOURNAL OF CHROMATOGRAPHY, vol. 604, no. 1, 1992, pages 29-37, XP026549522, ISSN: 0021-9673, DOI: 10.1016/0021-9673(92)85525-X page 30; figure 1; compounds sorbents A-C	1-8
X	DATABASE CA [Online] CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; INAMI, YOSHIAKI ET AL: "Finely porous polyamide membranes in manufacture of pyrogen-free water", XP002741686, retrieved from STN Database accession no. 1991:49662 abstract	1-8

-/--

1

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	-& JP H21 80626 A (TOYO ROSHI KAISHA, LTD., JAPAN) 13 July 1990 (1990-07-13) abstract Scheme; page 3 -----	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2015/079204

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.: 9-11(completely); 1-8, 19, 20(partially)
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:
see FURTHER INFORMATION sheet PCT/ISA/210

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.:
1-11, 19, 20

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: 1-11, 19, 20

   drawn to a final product comprising a support carrying at least one scaffold, the scaffold incorporating the defined multiple functional groups; a process for producing it from the intermediate product.

   ---

2. claims: 12-18

   drawn to a scaffold incorporating the defined multiple functional groups per se, as intermediate product; a process for producing it.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.2

Claims Nos.: 9-11(completely); 1-8, 19, 20(partially)

1. Cl 1 relates to an extremely large number of possible compounds/supports, since anything from synthetic derivatives of natural amino acids such as Histidine to naturally occurring enzymes is in fact encompassed.

1.1 The attempted delimitation to only "synthetic" enzymes cannot change this finding, since it would be an attempt do delimit a product from the prior art by means of

I. the process for its production, i.e. "non-biosynthetic", which is not compatible with the EPO's interpretation of product-by process claims as, namely, *product* claims (cf. PGL A5.26[1]); and on top of that by

II. an inherent transitional and hence *arbitrary* definition of the generic process and thus the product claimed, since many natural products have already been made synthetically and this trend will continue with scientific advance in the field, a situation similar to that when using likewise transient trademarks arises (PGL 5.39); such a definition further poses the question of sufficiency of disclosure, since if it is not known what is excluded from the total of e.g. enzymes, it is naturally also not known what remains (in analogy to trademarks, cf. PGL 4.25); it further poses the question of where "semi-synthetic" materials belong, i.e. possibly of natural origin but synthetically modified (ambiguity, Art. 6 PCT).

1.2 Furthermore, the terminology "proximity" and "close" [proximity] is relative and thus not clear (Art. 6 PCT). In particular, the precise difference between "in proximity" and "in close proximity" would not be clear.

1.3 Likewise, the delimitation between"scaffold" and "support" is not straight-forward and also of a relative nature, in particular when the support size is within molecular dimensions (cf. D3: 12 nm = 120 Å; D1: cyclodextrin: made up of 6-8 glucose units in one ring) and a coordinative bond (D3), comparable in strength to a covalent bond (D1), is involved.

1.4 Still further, the delimitation between features (iv)/(vi) and (v)/(vi) in said claims is not clear, since both (iv) and (v) are embodiments of (vi), since the most general definition of a nucleophile is a compound with a free (or "lone") pair of electrons (cf. e.g. http://en.wikipedia.org/wiki/Nucleophile). Both carboxylate groups (iv) and imidazole groups (v) have several lone pairs of the respective O and N atoms and are well-known to react as nucleophiles. Hence the definition of subject-matter to be protected is ambiguous , since it is not clear whether a mere second group (iv) or (v) fulfils (vi) or not (Art. 6 PCT).

1.5 Support, a clear definition of subject-matter and disclosure within the meaning of Arts. 5 and 6 PCT are to be found for only a very small proportion of the compounds claimed (see worked exs 1-5 on pages 17-24).

1.6 Non-compliance with the substantive provisions is such that a meaningful search of the whole claimed subject-matter of cl 1 **cannot** be carried out (PGL 9.19).

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

2. The same applies to the production process of these supported compounds defined in cl 19, mutatis mutandis. The generic "scaffold" and "support" is the same, the only difference vs. cl 1 being that they are not yet connected at the outset of the process of cl 19.

Likewise, non-compliance with the substantive provisions is such that a meaningful search of the whole claimed subject-matter of claim 19 **cannot** be carried out (PGL 9.19).

3. With respect to dependent cls 9-11, these appear to attempt to delimit the supported compounds of cl 8 further solely by a desideratum, obscuring the product features necessary to achieve this result (cf. PGL 5.35). The description does not elucidate matters either.

Non-compliance with the substantive provisions is such that a meaningful search of the claimed subject-matter of claims 9-11 cannot be carried out *at all* (Art. 17(2)(b) PCT).

4. Thus, pursuant to PCT Guidelines 9.19 and 9.24, the search of claim(s) 1-8, 19 and 20 was restricted to those claimed compounds/methods which appear to be supported and to a generalisation of their structural formulae.

In view of the many clarity, support and/or disclosure deficiencies in the present cls, these appear to be those of *exs 1-5* and a reasonable generalisation thereof, as expressed by the subject-matter of cls 1/19, with the restrictions imposed by the combination of cls 2 & 3, a "histidine-based " scaffold being interpreted for the present purposes as derived from a compound with carboxylate, imidazole and amino groups, with any degree of further substitution (cf. page 8, lines 24-25), an embodiment thereof being def'd in cl 8.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guidelines C-IV, 7.2), should the problems which led to the Article 17(2) declaration be overcome.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2015/079204

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 4777250 A	11-10-1988	NONE	
US 2007184970 A1	09-08-2007	US 2007184970 A1	09-08-2007
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		US 2001014650 A1	16-08-2001
		WO 0069872 A2	23-11-2000
JP H2180626 A	13-07-1990	----------	