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(54) Abstract Title: **Preparation of solid support particles for chemical reactions**

(57) The invention relates to a process for the preparation of particles suitable for use as supports for solid supported chemical reactions. The use of a metal-containing sacrificial layer to liberate the photolithographically-produced particles results in their having superior utility in multi-step reaction sequences.

GB 2422686 A continuation

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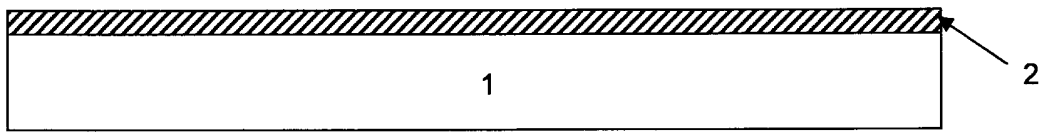


Figure 1

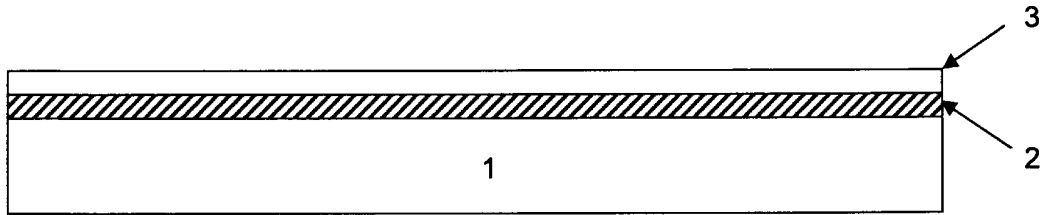


Figure 2

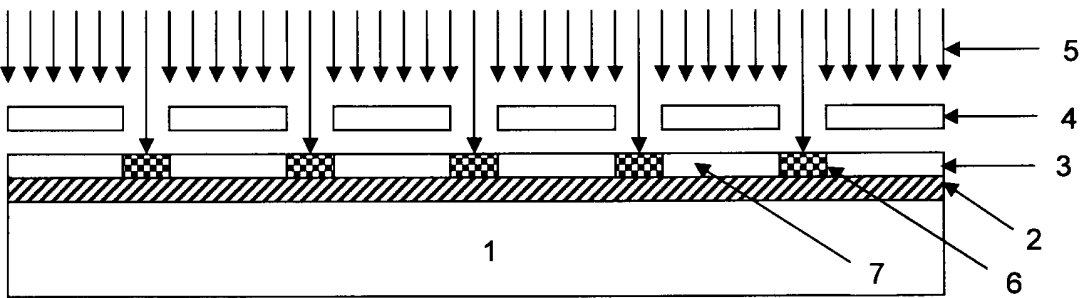


Figure 3

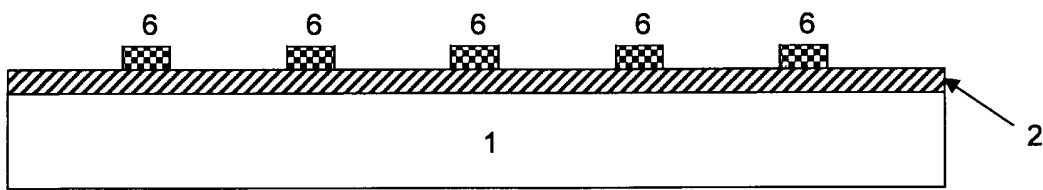


Figure 4

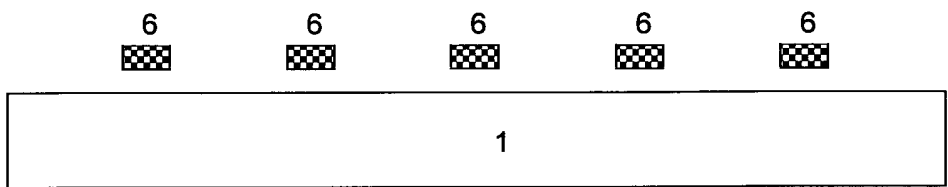


Figure 5

Particles

Field

The present invention relates to a process for the preparation of a particle for use in solid supported chemistry, to particles obtained by such a process, and to a plurality
5 of uses of such particles.

Background

The use of solid supports in synthetic chemistry has been known for a number of years. The basic concept of attaching a starting material to a functionalised polymeric support, and performing transformations of the supported starting material confers a
10 number of advantages over conventional solution-phase synthetic methods, including:

ease of separation of products from reaction mixture;

amenability to iterative synthetic sequences;

ease of purification of final product.

The technique was originally developed for the synthesis of peptides (R.B. Merrifield,
15 J. Am. Chem. Soc. 85, 2149 (1963)). However, it has been substantially expanded and is now routinely used in the preparation of other chemical species such as oligonucleotides, oligosaccharides, heterocycles and others.

More recently, the methods of solid phase synthesis have found utility in the field of combinatorial chemistry. Combinatorial chemistry is a technique for generating large
20 numbers of chemical compounds using relatively few chemical reaction steps. In tandem with high throughput screening, combinatorial chemistry has greatly accelerated the process of generating new compounds for leads as pharmaceuticals, agrochemicals and the like.

In various combinatorial chemistry and biological assay techniques it is known to use
25 solid support particles (e.g. beads) to synthesise chemical entities (such as, for example, polypeptides, carbohydrates, nucleotides and other oligomeric and non-oligomeric compounds) for inclusion as predetermined chemical entities in a chemical library. Additionally, it is also known that such particles can be tagged to make them

identifiable so that they may then be used to test various substances for the presence, or absence, of chemical entities in those substances.

Since identifiable particles are especially useful for high throughput screening, combinatorial chemistry, genomic and proteomic scientific applications, much effort
 5 has gone into the development of tagging techniques used to provide or encode an identifiable particle, or set of identifiable particles, with an identifier. Such identifiers can be read following chemical interactions at the identifiable particles in order that the identifiers can be matched to those indicating the chemical entities in the chemical library or other chemical entities with which the library chemical entities interact.

10 Numerous tagging techniques exist for tagging or coding identifiable particles, such as beads. For example, it is known that particles can be coded using transponders (US5736332, US5981166, US6051377, US6361950, US6376187), magnetic tags, biological tags and various optical techniques. One example uses unique short
 15 sequences of DNA that are attached to different tagged particles which are decoded after chemical processing by using a polymerase chain reaction (PCR). Conventional optical techniques that are currently used to identify particles include the following: fluorescent tagging; infrared (IR) tagging; optical image pattern recognition (GB2306484); Raman tagging (J. Am. Chem. Soc., 25, 10546-10560 (2003)); and quantum dot encoding (Anal. Chem., 76, 2406-2410, (2004)).

20 Prior art

GB2306484 discloses particles suitable for use in combinatorial chemistry techniques. The support particle preferably comprises a first phase comprising a solid support
 suitable for use in combinatorial chemistry techniques, and a second phase containing a machine readable code.

25 GB2334347 discloses a process for the preparation of coded particles comprising steps of

i. coating a face of a wafer of silicon or a similar crystalline material or inert metal or metal alloy with a photo-resist polymer

ii. exposing the coated face of the wafer to ultra-violet radiation through a photolithographic mask, said mask defining the particle size and/or the position of code sites on the particles;

iii. dissolving or otherwise removing either the UV-Exposed or the UV-
5 Unexposed areas of photo-resist polymer;

iv. etching the exposed areas of the wafer, from which the photoresist polymer has been removed, using an appropriate etching agent; and

v. liberating the particles

The processes of this disclosure involves the etching of the silicon wafer (or other
10 suitable solid) itself to form the particles.

US2003/0153092 discloses a process for the preparation of coded microparticles comprising

i. providing a sheet of polymeric material on a substrate;

ii. delineating the sheet into a plurality of particles without destroying the
15 integrity of the substrate;

iii. machine-readably encoding the particles;

iv. removing the particles from the substrate.

Amongst the techniques suitable for removing the particles from the substrate is the
20 removal of a sacrificial layer. A preferred sacrificial layer is a 3 μm layer of photoresist. Release is effected using a diluted developer.

A problem that remains is to provide particles suitable as supports for multi-step synthetic sequences.

A further problem that remains is to provide improved particles suitable for use in solid supported chemical reaction sequences.

25 The present invention addresses the problems of the prior art.

Summary of the invention

According to a first aspect, there is provided a process for the preparation of a particle for use as a solid support in chemistry comprising

- 5 i. coating a laminar support material comprising at least a sacrificial layer with a photopolymerisable material;
- ii. exposing the coated face of the support to radiation through a photolithographic mask, said mask defining the particle size and shape;
- 10 iii. selectively removing the exposed or unexposed areas of photopolymerisable material;
- iv. reducing the integrity of the sacrificial layer to liberate said particle;

characterised in that the sacrificial layer comprises a metal or metals.

15 According to a second aspect, there is provided a particle obtainable by a process of the invention.

According to a third aspect, there is provided the use of a particle of the invention as a support for solid supported chemistry.

According to a fourth aspect, there is provided the use of a particle of the invention in the preparation of an oligopeptide.

20 According to a fifth aspect, there is provided the use of a particle of the invention in the preparation of an oligonucleotide.

According to a sixth aspect, there is provided the use of a particle of the invention in the preparation of a chemical library.

25 According to a seventh aspect, there is provided a process for the preparation of a library of compounds comprising steps of

- i. providing a plurality of encoded particles of the invention;

- ii. dividing the particles into a plurality of portions;
- iii. subjecting each portion to a specific chemical reaction;
- iv. reading the code of a particle;
- v. recombining the portions; and
- 5 vi. repeating steps i to v n times, wherein n is an integer.

Brief description of the figures

Figure 1 – shows a laminar support material comprising substrate material (1) and sacrificial layer (2).

10 Figure 2 – shows a laminar support material bearing a layer of photopolymerisable material (3).

Figure 3 – shows laminar support material bearing a layer of photopolymerisable material (3) being exposed to radiation (5) through photolithographic mask (4) to give exposed (6) and unexposed (7) regions of photopolymerisable material (3).

15 Figure 4 – shows laminar support material after development of the exposed photopolymerisable material, with particles (6) attached to sacrificial layer.

Figure 5 – shows particles (6) liberated from substrate material (1) after removal of sacrificial layer (2).

Detailed description

Laminar support material

20 As used herein, the term “laminar support material” refers to a material comprising one or more layers and having a substantially uniplanar face.

Preferably, the laminar material comprises at least a substrate material. More preferably, the substrate material comprises silicon or a glass material. Most preferably, the substrate material consists of silicon or a glass material.

If the laminar support material comprises more than one layer, the layers may be held together using any suitable means. For example, an intermediate adhesive layer may be necessary or expedient.

5 In some embodiments, the laminar support material may consist of only a sacrificial layer.

Sacrificial layer

As used herein, the term "sacrificial layer" refers to the layer of the laminar support material which can be reduced in integrity in order to liberate the particle of the invention.

10 The sacrificial layer of the present invention comprises a metal or metals. The metal may exist as a pure metal (i.e. a layer of elemental metal), as an alloy, or as a dispersion of metal particles within a matrix material. Preferably, the sacrificial layer consists of a metal or an alloy of metals.

15 Preferably, the sacrificial layer comprises at least aluminium. More preferably, the sacrificial layer is an alloy comprising aluminium. More preferably, the sacrificial layer consists essentially of aluminium.

Surprisingly, the use of sacrificial layers comprising a metal or metals results in a particle having markedly superior properties as a support for use in solid-supported chemistry, especially in multi-step reaction sequences.

20 Preferably, the thickness of the sacrificial layer is less than 1000 nm. More preferably, the thickness of the sacrificial layer is less than 500 nm. More preferably, the thickness of the sacrificial layer is less than 200 nm. More preferably, the thickness of the sacrificial layer is less than 100 nm. Most preferably, the thickness of the sacrificial layer is less than 50 nm.

25 Preferably, the thickness of the sacrificial layer is more than 1 nm. More preferably, the thickness of the sacrificial layer is more than 2 nm. More preferably, the thickness of the sacrificial layer is more than 5 nm. Most preferably, the thickness of the sacrificial layer is at least 10 nm.

Preferably, the thickness of the sacrificial layer is between 1 and 1000 nm. More preferably, the thickness of the sacrificial layer is between 5 and 100 nm. Most preferably, the thickness of the sacrificial layer is between 10 and 50 nm.

Preferably, the sacrificial layer is applied to the adjoining layer of the laminar support material by a sputtering process. The term sputtering refers to the process of dislodging atoms from a target material to coat a thin film onto a substrate.

Photopolymerisable material

As used herein, the term "photopolymerisable material" refers to a composition comprising at least one monomer which is capable of undergoing polymerisation when exposed to radiation.

Preferably, the photopolymerisable material is a u.v. polymerisable material. That is, the composition is capable of undergoing polymerisation when exposed to ultraviolet light.

Preferably, the photopolymerisable material is present as a solution for the purposes of coating on to the laminar support material. Suitable solvents include water and organic solvents.

A preferred photopolymerisable material is SU-8. SU-8 comprises three components; an EPON epoxy resin, an organic solvent, and a photoinitiator. The EPON resin of SU-8 is a multifunctional glycidyl ether derivative of bisphenol-A novolac used to provide high-resolution patterning for semiconductor devices. The second component is gamma-butyrolactone (GBL), an organic solvent. The quantity of the solvent determines the viscosity of the solution, which determines final thickness of the spin-coated film. Along with GBL, cyclopentanone is also used as a solvent for SU-8. The third component is a triarylium-sulfonium salt, a photoinitiator which is approximately 10 wt % of EPON SU-8. SU-8 resin can be cationically polymerised by utilizing a photoinitiator which generates strong acid upon exposure to ultraviolet light (365 to 436 nm) and the acid facilitates polymeric cross-linking during post-exposure bake.

To enable the use of the particles formed from polymerised photopolymerisable material as supports in combinatorial chemistry, it is necessary that the polymerised material bears reactive functional groups. The term "reactive functional group" as

used herein refers to a chemical group present in the polymerised photopolymerisable material capable of reacting with a small molecule (i.e. one present in solution) to form a covalent bond.

5 Examples of reactive functional groups are halogen (especially fluoro, chloro, bromo and iodo), hydroxy, carboxy (CO_2H), carbonyl, amino, epoxy and thiol.

The reactive functional group may be introduced subsequent to polymerisation of the photopolymerisable material. For example, if the polymerised material comprises unfunctionalised aromatic rings, it may be chloromethylated in a manner analagous to the preparation of Merrifield resins (R.B. Merrifield, J. Am. Chem. Soc. 85, 2149
10 (1963)).

However, it is preferred that the polymerised photopolymerisable material possesses reactive functional groups without the necessity of introducing these subsequent to polymerisation.

For example, when the photopolymerisable material comprises an epoxy resin (as in
15 the case of SU-8), the polymerised material will comprise at least an amount of epoxy groups.

The photopolymerisable material may be coated on the laminar support material using any appropriate technique. It may be applied as a sheet that is simply adhered to the laminar support material. If the polymerisable material is a liquid, it may be applied as
20 a spray or aerosol. Alternatively, it may be painted on using a brush or roller. A preferred technique for coating the laminar support material with photopolymerisable material is spin coating.

Spin-coating consists of dispensing photopolymerisable material solution over the wafer surface and rapidly spinning the wafer until it becomes dry. Typical spin-
25 coating processes are conducted at final spin speeds of 3000-7000 rpm for a duration of 20-30 seconds.

The thickness of the coat of photopolymerisable material is preferably between 0.1 and 100 μm , more preferably between 0.5 and 50 μm , still more preferably between 1 and 25 μm .

Optionally, it may be necessary to pre-treat the laminar support material with a layer of adhesive or primer in order to achieve satisfactory binding of the support material to the photopolymerisable material. Preferably, such a primer comprises hexamethyldisilazane (such as Microposit primer).

- 5 Optionally, it may be necessary to heat treat the laminar support material (for example at about 200 °C for about 30 minutes) in order to effect dehydration. This also assists in achieving good adhesion of the photopolymerisable material to the laminar support material.

- 10 After the laminar support material has been coated with photopolymerisable material, it may be necessary or convenient to subject the coated material to one or more subsequent treatment steps prior to irradiation.

- 15 When the photopolymerisable material is applied as a solution, a soft baking step is preferably employed. A soft bake is done to: i) drive away the solvent from the applied photopolymerisable material; ii) improve the adhesion of the photopolymerisable material to the laminar support material; and iii) anneal the shear stresses introduced during the coating. Soft baking may be performed using one of several types of ovens (e.g., convection, infrared, hot plate). Soft-bake ovens must provide well-controlled and uniformly distributed temperatures and a bake environment that possesses a high degree of cleanliness. The recommended temperature range for soft baking is between 90-100 °C, while the exposure time needs to be established based on the heating method used and the resulting properties of the soft-baked resist. These factors will be readily determined by one skilled in the art.

Exposure to radiation

- 25 After the laminar support material has been coated with photopolymerisable material, and optionally subjected to one or more intervening treatments (preferably soft baking), the coated face is exposed to radiation (figure 3).

- 30 The radiation is of a wavelength and intensity to cause regions of photopolymerisable material exposed to it to polymerise. These factors will be dependent on the nature of the photopolymerisable material, and the skilled person will be able to determine them

For example, when the photopolymerisable material is SU-8, polymerisation is suitably achieved by exposure to ultraviolet (u.v.) radiation for between 1 and 30, preferably about 18 seconds, at an intensity of between 1 and 50 mw cm^{-2} , preferably about 13.6 mw cm^{-2} .

5 Post exposure treatment

Preferably, subsequent to exposure to radiation, the polymerised photopolymerisable material is subjected to one or more post exposure treatment steps in order to make the polymerised material more physically and chemically durable during later stages.

- 10 Preferably, the post exposure treatment steps comprise at least a post exposure bake. Such a post exposure bake serves to further cross-link the polymerised material and increase its strength and durability.

Preferably, the post exposure bake is conducted at about 65 °C for about 1 minute, followed by about 95 °C for about 5 minutes.

Photolithographic mask

- 15 The coated face of the laminar support material is exposed to radiation through a photolithographic mask (figure 3). The principles of photolithography are well known from the field of semiconductor device fabrication. The photolithographic mask consists in essence of portions of material transparent to radiation, and portions of material opaque to radiation arranged in the desired pattern, such that when radiation
20 is applied to the coated face of the laminar support material through the mask, the pattern is transposed onto the coated face.

The photolithographic mask defines the size and shape of the particles by means of the above-mentioned transparent and opaque portions.

Selective removal

- 25 Subsequent to exposure to radiation and optional post-exposure treatment, either the polymerised regions (i.e. those exposed to radiation) are removed to leave the unpolymerised regions, or the unpolymerised (i.e. unexposed) regions are removed to leave the polymerised regions (figure 4). This is referred to as "development". Preferably, the unpolymerised regions are removed.

The person skilled in the art will be able to determine suitable conditions for the selective removal of either the exposed or unexposed regions.

Preferably, the unexposed photopolymerisable material is selectively removed from the coated laminar substrate by treatment with organic solvent. Preferably, the
 5 organic solvent is polypropylene glycol methyl ether acetate (PGMEA). It is particularly convenient to immerse the laminar substrate in a bath of such a solvent. Again, the skilled person will be able to determine the optimum duration of such a treatment step to ensure substantially complete removal of unexposed regions of photopolymerisable material while having little or no effect on the exposed regions.

10 After such a development step, the coated laminar substrate may be washed to remove excess solvent.

Optionally, after such development and optional washing steps, the developed coated laminar substrate may be subjected to a post-development bake.

Very preferably, the development step discloses or reveals portions of sacrificial
 15 material between the residual polymerisable material (i.e. the adhering particles; (6) in figure 4).

Liberation of particles

After the abovementioned development step, the developed coated laminar substrate comprises particles of the invention adhering to the laminar substrate. In order to
 20 liberate the particles, the integrity of the sacrificial layer is reduced (figure 5).

Reduction of the integrity of the sacrificial layer may be achieved by any one of a number of means. In a broad sense, any degree of reduction of integrity is acceptable provided that it serves to liberate the particles from the laminar support material.

25 Preferably, the integrity of the sacrificial layer is reduced by at least partially dissolving the metal component.

The metal component may be at least partially dissolved by treating the developed coated laminar substrate with an etch. Suitable etches for a variety of metals are known, and the skilled person will be able to select an appropriate one.

When the sacrificial layer is or comprises aluminium, it is preferred that the etch comprises hydroxide ions. Highly preferably, the etch comprises tetraalkylammonium hydroxide. Very highly preferably, the etch comprises tetramethylammonium hydroxide.

- 5 A highly preferred etch is Microposit MF 319 developer, available from Rohm and Haas. This is an aqueous solution comprising 97-98 wt. % water, 0.1 – 1 wt. % surfactant and balance tetramethylammonium hydroxide.

- Preferably, the particles are liberated by immersing the developed coated laminar substrate in the etch. The liberated particles are then removed by filtration and
 10 washed. Preferably, the developed coated laminar substrate is immersed in the etch for a period of less than 1 minute. More preferably, the developed coated laminar substrate is immersed in the etch for a period of less than 30 seconds. More preferably, the developed coated laminar substrate is immersed in the etch for a period of less than 20 seconds. More preferably, the developed coated laminar
 15 substrate is immersed in the etch for a period of less than 10 seconds. It has been found that a reduction in the time the particles are exposed to the etch results in improved performance of the particles as substrates for solid supported chemistry.

Size and shape of particles

- As will be appreciated, the thickness of the particles will be chiefly determined by the
 20 thickness of the coat of photopolymerisable material originally applied to the laminar substrate. The photolithographic mask determines the ultimate shape of the particles in the remaining two dimensions once liberated from the laminar substrate. The particles may be of any desired size and shape that is convenient for the intended end use.

- 25 Preferably, the particles are of square or rectangular cross section. More preferably, the particles are rectangles of from 1 to 500 μm by 1 to 500 μm .

Particle encoding

Optionally, and highly preferably, the particles may comprise an encoded identifier, or tag, which enables the individual particles to be distinguished from one another.

In one embodiment, the particles of the invention are encoded by means of their shape. Such a scheme is described in US2003/0153092, which is incorporated herein by reference in its entirety.

5 Alternatively, each particle may be encoded by pits, grooves, notches, bumps, ridges or by fluorescent, coloured or monochrome markings (for example, bar codes). Any conventional technique may be used to apply such encoding motifs, for example by printing. Alternatively, magnetic or radiofrequency identifiers may be incorporated or attached.

10 The particles may also be encoded using Raman barcoding, comprising spectroscopic bar codes that incorporate infrared and Raman-active groups that are identifiable using standard infrared or Raman spectrometers. Such a scheme is described in J. Am. Chem. Soc., 25, 10546, 2003.

15 Preferably, the particles are encoded with a diffraction pattern identifier such that the particles give an identifiable diffraction pattern when exposed to a particular wavelength of radiation.

Encoding may be performed at any stage of the preparation of the particles. In a preferred embodiment, the photolithographic mask comprises encoding means, so that encoding occurs during exposure of the photopolymerisable material to radiation.

20 In an alternative preferred embodiment, the particles are encoded by embossing features into the photopolymerisable layer by means of a rigid stamp. This technique is referred to as nanoimprinting.

Alternatively, the encoding of the particle can be achieved by embossing, imprinting, injection moulding, laser ablation or direct write (e-beam) techniques.

25 In an alternative preferred embodiment, encoding occurs in a secondary coating / exposure / development sequence subsequent to development of the primary coating (i.e. after step iii).

30 The exposed and developed layer of photopolymerisable material is coated with a secondary coat of photopolymerisable material. The coated face is exposed to radiation through a second photolithographic mask, and developed in a similar manner to that described above.

Initial functionalisation of particles

In order to conduct subsequent solid supported chemical reactions, the particles may bear reactive functional groups or may have these introduced as described above, for example by chloromethylation.

- 5 Preferably, the particles are further functionalised by reaction with a functionalising agent.

As used herein, the term “functionalising agent” refers to a reagent or mixture of reagents which are capable of reacting with the reactive functional groups to render the particles more suitable for use as a solid support in subsequent reaction sequences.

10

Examples of functionalising agents are those comprising thiols, alcohols, and amines.

Preferably, the functionalising agent comprises one or more amines. Preferably, the amine is selected from ammonia, alkylamines and arylamines (anilines). Preferably, the amine comprises one, two, three or more than three amine groups.

- 15 Preferably, the amine is a polyoxyalkyleneamine or polyether amine. A more preferred amine is a Jeffamine. A still more preferred amine is Jeffamine₈₀₀.

Preferably, the reactive functional group is an epoxide, and the functionalising agent is an amine. In this case, the functionalised particles will bear amine groups that are capable of undergoing further reaction.

20 Attachment of linker group

Subsequent to liberation of the particles and optional functionalisation, a linker group may optionally be attached.

As used herein, the term “linker group” refers to a chemical moiety capable of forming a chemical bond with both the particles (optionally functionalised) and partaking in further chemical reactions. Preferably, the linker group is cleavable at the end of the reaction sequence to furnish the desired product.

25

Preferably, the linker group is introduced by reaction of the optionally functionalised particles with a compound containing at least two functional groups.

Suitable linker groups are those derived from amino acids (for example α amino acids such as the natural amino acids, β amino acids, γ amino acids, δ amino acids, 1,5 amino acids and 1,6 amino acids), dicarboxylic acids (such as oxalic, maleic, fumaric and succinic acids), and derivatives and protected versions thereof. The person
 5 skilled in the art will be able to determine which conditions to use to attach a particular linker group to the particles of the invention

In a preferred embodiment, the linker is an amino acid. A preferred linker group is 6-aminohexanoic acid.

In the embodiment wherein the particles bear an amine reactive functional group, a
 10 linker group bearing a carboxylic acid moiety is conveniently introduced using standard amide coupling conditions. Suitable techniques are described in "The Chemical Synthesis of Peptides" by J Jones, Oxford University Press, 1994.

It will be apparent to those skilled in the art that sensitive functional groups may need to be protected and deprotected during synthesis of a compound of the invention. This
 15 may be achieved by conventional techniques, for example as described in "Protective Groups in Organic Synthesis" by T W Greene and P G M Wuts, John Wiley and Sons Inc. (1991), and by P.J.Kocienski, in "Protecting Groups", Georg Thieme Verlag (1994).

Preferably, the linker group is attached by reaction of the particles with N-protected 6-aminohexanoic acid, or a reactive derivative thereof, followed by subsequent
 20 deprotection. Preferably, the protecting group is fluorene-9-yl methoxycarbonyl (Fmoc). Suitable reactive derivatives are those formed by the reaction of the N-protected 6-aminohexanoic acid with a diisocyanate (particularly hexamethyldiisocyanate (DIC)), or hydroxybenzotriazole (HOBt).

In an alternative preferred embodiment, the linker group is derived from a
 25 hydroxycarboxylic acid (a molecule possessing both a hydroxyl and carboxylic acid group). A highly preferred hydroxycarboxylic acid group is 4-hydroxymethyl-phenoxy acetic acid (HMPA). HMPA may be coupled to particles bearing reactive amine groups using standard amide bond formation conditions as described above.

Solid supported synthesis

The particles of the invention may be used as supports in an identical manner to conventional solid supports. The particles of the invention are amenable to multi-step synthetic sequences, and provide products in good purity and yield.

Peptide synthesis

- 5 A typical solid phase peptide synthesis scheme involves attaching a first amino acid or peptide group to the particles of the invention via the carboxyl moiety of the peptide or amino acid. This leaves the amine group of the resin bound material available to couple with additional amino acids or peptide material. Thus, the carboxyl moiety of the additional amino acid or peptide desirably reacts with the free amine group of the
- 10 particle bound material. To avoid side reactions involving the amine group of the additional amino acid or peptide, such amine group is masked with a protecting group during the coupling reaction. Two well-known amine protecting groups are the BOC group and the Fmoc group. Many others also have been described in the literature. After coupling, the protecting group on the N-terminus of the resin bound peptide may
- 15 be removed, allowing additional amino acids or peptide material to be added to the growing chain in a similar fashion. In the meantime, reactive side chain groups of the amino acid and peptide reactants, including the particle bound peptide material as well as the additional material to be added to the growing chain, typically remain masked with side chain protecting groups throughout the synthesis.
- 20 After the desired peptide has been assembled in this fashion, it may be liberated from the particles. Suitable conditions for this liberation step depend on the nature of the particle and in particular the linker group as defined above (where present). The skilled person will be able to determine conditions for the liberation of the peptide.

- A preferred class of peptides of the present invention are those that incorporate from
- 25 about 2 to about 100, preferably from about 4 to about 50, residues of one or more amino acids. Residues of one or more other monomeric, oligomeric, and/or polymeric constituents optionally may also be incorporated into a peptide. Non-peptide bonds also may be present. For instance, the peptides of the invention may be synthesized to incorporate one or more non-peptide bonds. These non-peptide bonds may be
- 30 between amino acid residues, between an amino acid and a non-amino acid residue, or between two non-amino acid residues. These alternative non-peptide bonds may

be formed by utilizing reactions well known to those in the art, and may include, but are not limited to imino, ester, hydrazide, semicarbazide, and azo bonds.

The principles of the present invention may be advantageously used to synthesise the following peptide material, fragment intermediates thereof, and/or analogs from a particle after solid phase synthesis: enkephalins, oxytocin, vasopressin, felypressin, 5 pitressin, lypressin, desmopressin, terlipressin, atosiban, adrenocorticotrophic hormone, insulin, secretin, calcitonins, luteinizing hormone-releasing hormone (LH-RH) and analogues, leuprolide, deslorelin, triptorelin, goserelin, buserelin, nafarelin, cetorelix, ganirelix, parathyroid hormone, human corticotropin-releasing factor, ovine 10 corticotropin-releasing factor, growth hormone releasing factor, somatostatin, lanreotide, octreotide, thyrotropin releasing hormone (TRH), thymosin-1, thymopentin (TP-5), cyclosporine and integrilin. A particularly preferred peptide is Leu-enkephalin.

The nature and use of protecting groups for peptide synthesis is well known in the art. Generally, a suitable protecting group is any sort of group that that can help prevent 15 the atom or moiety to which it is attached, e. g. , oxygen or nitrogen, from participating in undesired reactions during processing and synthesis. Protecting groups include side chain protecting groups and amino-or N-terminal protecting groups. Protecting groups can also prevent reaction or bonding of carboxylic acids, thiols and the like.

A side chain protecting group refers to a chemical moiety coupled to the side chain (i. 20 e., R group in the general amino acid formula $H_2N-C(R)(H)-COOH$) of an amino acid that helps to prevent a portion of the side chain from reacting with chemicals used in steps of peptide synthesis, processing, etc. The choice of a side chain-protecting group can depend on various factors, for example, type of synthesis performed, processing to which the peptide will be subjected, and the desired intermediate 25 product or final product. The nature of the side chain protecting group also depends on the nature of the amino acid itself. Generally, a side chain protecting group is chosen that is not removed during deprotection of the amino groups during the solid phase synthesis ("orthogonal protection"). Therefore the amino protecting group and the side chain protecting group are preferably not the same.

30 In some cases, and depending on the type of reagents used in solid phase synthesis and other peptide processing, an amino acid may not require the presence of a side-

chain protecting group. Such amino acids typically do not include a reactive oxygen, nitrogen, or other reactive moiety in the side chain.

Examples of side chain protecting groups include acetyl (Ac), benzoyl (Bz), tert-butyl, triphenylmethyl (trityl), tetrahydropyranyl, benzyl ether (Bzl) and 2,6-dichlorobenzyl (DCB), t-butoxycarbonyl(BOC), nitro, p-toluenesulfonyl (Tos), adamantyloxycarbonyl, xanthyl (Xan), benzyl, 2,6-dichlorobenzyl, methyl, ethyl and t-butyl ester, benzyloxycarbonyl (Z), 2-chlorobenzyloxycarbonyl (2-Cl-Z), t-amyloxycarbonyl (Aoc), and aromatic or aliphatic urethane type protecting groups, and photolabile groups such as nitroveritryl oxycarbonyl (NVOC); and fluoride labile groups such as trimethylsilyl oxycarbonyl (TEOC).

Preferred side chain protecting groups include t-Bu group for Tyr (Y), Thr(T) ; Ser (S) and Asp (D) amino acid residues; the Trt group for His (H), Gln (Q) and Asn (N) amino acid residues; and the Boc group for Lys (K) and Trp (W) amino acid residues.

An amino-terminal protecting group includes a chemical moiety coupled to the alpha amino group of an amino acid. Typically, the amino-terminal protecting group is removed in a deprotection reaction prior to the addition of the next amino acid to be added to the growing peptide chain, but can be maintained when the peptide is cleaved from the support. The choice of an amino terminal protecting group can depend on various factors, for example, type of synthesis performed and the desired intermediate product or final product.

Examples of amino-terminal protecting groups include acyl-type protecting groups, such as formyl, acrylyl (Acr), benzoyl (Bz) and acetyl(Ac); aromatic urethane-type protecting groups, such as benzyloxycarbonyl (Z) and substituted Z, such as p-chlorobenzyloxycarbonyl, p-nitrobenzyloxycarbonyl, p-bromobenzyloxycarbonyl, p-methoxybenzyloxycarbonyl; aliphatic urethane protecting groups, such as t-butyloxycarbonyl (BOC), diisopropylmethoxycarbonyl, isopropyloxycarbonyl, ethoxycarbonyl, allyloxycarbonyl; cycloalkyl urethan-type protecting groups, such as 9-fluorenyl-methyloxycarbonyl (Fmoc), cyclopentyloxycarbonyl, adamantyloxycarbonyl, and cyclohexyloxycarbonyl; and thiourethane-type protecting groups, such as phenylthiocarbonyl. Preferred protecting groups include 9-fluorenyl-methyloxycarbonyl (Fmoc), 2-(4-biphenyl)-propyl (2) oxycarbonyl (Bpoc), 2-phenylpropyl (2)-oxycarbonyl (Poc) and t-butyloxycarbonyl (Boc).

Optionally, the peptide may incorporate one or more labels such as a chromophore, fluorophore, biotin or a magnetic or electron-dense entity. Preferred fluorophores include fluorescein, eosin, Alexa Fluor, Oregon Green, Rhodamine Green, tetramethylrhodamine, Rhodamine Red, Texas Red, coumarin and NBD.

5 **Oligonucleotide synthesis**

Solid phase chemical synthesis of DNA fragments is conveniently performed using protected nucleoside phosphoramidites. In this approach, the 3'-hydroxyl group of an initial 5'-protected nucleoside is first covalently attached to the particle of the invention, optionally via a linker (preferably a succinic acid derivative). Synthesis of
 10 the oligonucleotide then proceeds by deprotection of the 5'-hydroxyl group of the attached nucleoside, followed by coupling of an incoming nucleoside-3'-phosphoramidite to the deprotected hydroxyl group. Alternatively, synthesis may be conducted in the 5'-3' direction. Suitable methodologies are for example disclosed in "Oligonucleotide synthesis: A practical approach" M.J. Gait, IRL Press, 1992.

15 Residues of one or more other monomeric, oligomeric, and/or polymeric constituents optionally may also be incorporated into an oligonucleotide. Non-nucleotide bonds also may be present. For instance, the oligonucleotides of the invention may be synthesized to incorporate one or more non-nucleotide bonds. These non-nucleotide bonds may be between nucleotide residues, between a nucleotide and a non-
 20 nucleotide residue, or between two non-nucleotide residues. Non-nucleotide linkages include phosphorothioates, phosphonates, amides and others. Non-DNA base phosphoramidates may also be incorporated, and may function as linkers, labels, or functional groups (such as biotin).

Coupling is achieved with any one of a number of coupling reagents known to those
 25 skilled in the art. Preferred coupling reagents are uronium salts, particularly TBTU, and diisocyanates, particularly DIC, and ETT (5-ethylthio-1*H*-tetrazole).

The resulting phosphite triester is finally oxidized to a phosphotriester to complete the internucleotide bond. The steps of deprotection, coupling and oxidation are repeated until an oligonucleotide of the desired length and sequence is obtained. The
 30 completed oligonucleotide may optionally be cleaved from the particle. Again, the skilled person will be able to determine suitable conditions for achieving this result.

The chemical group conventionally used for the protection of nucleoside 5'-hydroxyls is preferably dimethoxytrityl ("DMT"), which is removable with acid.

Optionally, the oligonucleotide may incorporate one or more labels such as a chromophore, fluorophore, biotin or a magnetic or electron-dense entity. Preferred
5 fluorophores include fluorescein, eosin, Alexa Fluor, Oregon Green, Rhodamine Green, tetramethylrhodamine, Rhodamine Red, Texas Red, coumarin and NBD fluorophores.

Small molecule synthesis

In addition to peptides and oligonucleotides, the particles of the invention may be
10 used as supports in the solid phase synthesis of many other classes of compound which have been demonstrated to be amenable to solid phase synthesis using conventional supports. Heterocycles are a preferred class of compounds.

Library synthesis

The particles of the invention have special utility in the preparation of libraries of
15 chemical compounds.

In a typical procedure, a suspension of particles is separated into three portions, each of which is then exposed to a specific reagent, A1, A2, or A3, which becomes chemically bound to the particles. The three suspensions of particles are then recombined within a single vessel and thoroughly mixed before being divided once
20 again into three separate parts, each part containing a proportion of all three compound types. The large number of particles involved in the process ensures that statistically the numbers of each type of compound in each vessel are approximately equal. The three suspensions are then further exposed to the same or different reagents B1, B2, or B3, and this results, for example in compounds A1B1, A2B1, and
25 A3B1 in the first vessel; A1B2, A2B2, and A3B2 in the second vessel; and A1B3, A2B3 and A3B3 in the third. The suspensions are then remixed in a single vessel and the process repeated. In this way, a substantial number of different compounds may be produced in a relatively small number of reaction steps. At the end of the processing the identity of the compound(s) showing the desired properties may
30 subsequently be deduced.

The use of the particles of the invention, when tagged or encoded as described above, in such a sequence confers the advantage that the process steps to which each individual particle has been subjected, and consequently the nature of the chemical species appended thereto, can be determined by reading the tag or code.

- 5 Such libraries of compounds are advantageously employed in binding, inhibition, or other chemical or biochemical activities.

In a preferred embodiment, a compound library affixed to a plurality of particles of the invention is brought into association with a target species. Particles bearing chemical compounds which bind to the target species are identified, and the nature of the
10 chemical compound is established. Highly preferably, the nature of the chemical compound is identified by means of the tag or encoding element present on the particle.

The target species may be a protein, an enzyme, receptor, micro organism, or a DNA or RNA fragment. The target species may bear a means for detection such as a
15 radiolabel, fluorescence label, or visual label.

Examples

The present invention will now be described only by way of example. However, it is to be understood that the examples also present preferred embodiments of the present invention, as well as preferred routes for making same and useful intermediates in the
20 preparation of same.

Example 1. Fabrication of particles

Microfabrication techniques were developed to construct bars based on SU-8 photolithographic process and lift off of the patterned SU-8 bars from the substrate
25 (Si).

Processing of Al as a sacrificial layer

The silicon substrates were prepared by cleaning them in fuming nitric acid for 20 minutes. 50 nm Al was coated by evaporation of pure aluminium by Al E-Gun Evaporator. An Aluminium sacrificial layer is preferred to S1813 which was also tested as particles made with the Al sacrificial layer performed better in later chemistry.

SU-8 photolithography process

The Al coated silicon wafers were baked at 200° C for 1 hour. To increase the adhesion of SU-8 with Al, Microposit primer was spin coated. SU-8 was then spin coated onto the silicon substrate coated with Al. Following the spin coating, a soft bake step was performed. The polymer was cross-linked by exposure to UV light for 18 seconds (13.6 mw cm⁻²). A post exposure bake of 65° C for 1 minute and 95° C for 5 minutes was performed to further cross-link the SU-8 to be resistant to solvent during processing and also to be resistant to the solvents used for later chemistry. Finally, the wafers were developed in polypropylene-glycol-methyl-ether-acetate (PGMEA) for 2 minutes.

Releasing of SU-8 bars

Rom and Haas MF 319 developer was used to dissolve the Al in order to release the bars from the substrates. After immersing the wafers into the solution and leaving them for few seconds, the bars were filtered and washed off with water followed by acetone.

Chemistry on SU-8 particles

Example 2. Initial functionalisation of particles.

SU8 (100 mg) was treated with the Jeffamine₈₀₀ (500 mg) and acetonitrile (500 µl) and heated to 65 °C in an oven overnight. The support was washed with acetonitrile (7 x 800 µl) followed by THF (7 x 800 µl) and dried under vacuum at room temperature for 4 h. Jeffamine₈₀₀ is preferred to simply alkylamines.

Example 3. Attachment of N-Fmoc-6-aminohexanoic acid.

N-Fmoc-6-aminohexanoic acid (5.0 mg, 14 µmol) was dissolved in DMF (100 µl) and DIC (2 µl, 13 µmol) was added. The mixture was shaken for 8 min at room

temperature. HOBt (2 mg, 15 μmol) was added and the mixture was shaken for 5 min at r.t. The mixture was added to Jeffamine functionalised SU8 particles (1.50 μmol based on free $-\text{NH}_2$ groups) suspended in DMF (300 μl) and the mixture was heated to 60 $^\circ\text{C}$ for 1h. The support was washed with DMF (7 x 800 μl) followed by THF (7 x 800 μl) and dried under vacuum at room temperature for 4 h.

Example 4. Attachment of HMPA linker.

HMPA (17 mg, 90 μmol) was dissolved in DMF (100 μl) and DIC (14 μl , 90 μmol) was added. The mixture was shaken for 8 min at room temperature. HOBt (12 mg, 90 μmol) was added and the mixture was shaken for 5 min at room temperature. The mixture was added to amine functionalised SU8 (30 μmol based on free $-\text{NH}_2$ groups) suspended in DMF (300 μl) and the mixture was shaken for 1h at room temperature. The support was washed with DMF (7 x 800 μl) and the procedure was repeated. The support was washed with DMF (7 x 800 μl) followed by THF (7 x 800 μl) and dried under vacuum at room temperature for 4 h.

Example 5. Attachment of first amino acid.

N-Fmoc-Leucine (36 mg, 100 μmol) was dissolved in DMF (50 μl) and DIC (16 μl , 100 μmol) was added. The mixture was shaken for 8 min at room temperature. DMAP (1.5 mg, 10 μmol) was added and the mixture was added to HMPA SU8 (30 μmol based on $-\text{OH}$ groups) suspended in DMF (300 μl) and the mixture was shaken for 1h at room temperature. The support was washed with DMF (7 x 800 μl) and the procedure was repeated twice. The support was washed with DMF (7 x 800 μl) followed by THF (7 x 800 μl) and dried under vacuum at room temperature for 4 h.

Example 5. Attachment of subsequent amino acids.

N-Fmoc-amino acid (25 μmol) was dissolved in DMF (50 μl) and TBTU (8 mg, 25 μmol), HOBt (0.5 mg, 4 μmol) and DIPEA (4 μl , 25 μmol) were added. The mixture was shaken for 2 min and the mixture was added to the deprotected SU8 (8 μmol based on $-\text{NH}_2$ groups) suspended in DMF (300 μl) and the mixture was shaken for 1h at r.t. The support was washed with DMF (3 x 800 μl) followed by THF (2 x 800 μl)

and Et₂O (3 x 800 µl). The completeness of the reaction was monitored by ninhydrin test. After a negative ninhydrin test the N-terminus Fmoc group was removed.

Example 6. Cleavage of peptides.

The support was treated with TFA/phenol (98/2 % v/w, 25 ml/g resin) for 90 min at room temperature. The support was filtered and washed with TFA (3 x 1 ml). The combined filtrates were evaporated under vacuum and the remainder oil was titrated with Et₂O. The solid that precipitated was washed with Et₂O and dried under vacuum at room temperature for 4 h.

Example 6. Attachment of succinylated nucleoside

2'-O-succinyl-5'-O-DMT-cytosine was dissolved in DMF and TBTU (8 mg, 25 µmol), HOBt (0.5 mg, 4 µmol) and DIPEA (4 µl, 25 µmol) were added. The mixture was shaken for 2 min and the mixture was added to the SU-8 particles functionalised with Jeffamine and aminohexanoic acid linkers suspended in DMF (300 µl) and the mixture was shaken for 1h at room temperature. The support was washed with DMF (3 x 800 µl) followed by THF (2 x 800 µl) and Et₂O (3 x 800 µl). The completeness of the reaction was monitored by ninhydrin test. The succinylated nucleoside can also be coupled using DIC and HOBt conditions.

Example 7. Automated DNA synthesis

SU-8 particles derivatised with succinylated base were subjected to multiple cycles of standard automated DNA synthesis using 5'-O-DMT-3'-O-phosphoramidite nucleosides. Coupling was performed with ETT as activator. After synthesis the DNA was deprotected and cleaved from the solid support by standard methods. The resulting solution was freeze dried and purified by HPLC.

Example 8. Coupling amino-modified oligonucleotides to SU-8 microparticles.

Amine modified SU-8 particles were treated with succinic anhydride to generate a carboxy modified surface. 5'-amino modified oligonucleotides (with or without fluorescent labels) were coupled to the surface with EDC.

Example 9. Synthesis of Leu-Enkephalin

Following the procedures above amine functionalised SU-8 particles were prepared with Jeffamine and aminohexanoic acid linker and an HMPA linker. The initial amino acid Leucine was coupled on to this support followed by phenylalanine, 2 x glycine, and tyrosine. The peptide was cleaved from the resin as described. The dry solid
 5 was analysed by HPLC-MS and found to be identical to a synthetic standard.

Example 10. Synthesis of 5'-T₁₅C

SU-8 particles derivatised with Jeffamine and aminohexanoic acid linker, and with succinyl-DMT-cytosine, were subjected to 15 cycles of DNA synthesis in which 15 x thymidine phosphoramidites were coupled. Following deprotection, cleavage and
 10 HPLC purification the oligonucleotide product was analysed by capillary electrophoresis which showed the presence of an oligonucleotide that co-migrated with a synthetic standard.

Example 11. DNA hybridisation on oligonucleotide modified SU-8 particles

DNA-modified particles were suspended in an aqueous buffer containing fluorescently
 15 labelled oligonucleotide complementary to that on the particles. The increase in fluorescence on hybridisation was monitored using a Fluorescence Activated Cell Sorter. Incubation of the particles with a non-complementary fluorescently labelled oligonucleotide gave significantly lower fluorescence measurements by FACS.

Example 12. Synthesis and enzymatic cleavage of HIV protease substrate

20 A peptide substrate of the HIV protease with a terminal fluorophore was synthesised on SU-8 particles. Upon treatment with the protease the particle fluorescence due to the fluorophore was lost due to peptide cleavage. The loss of fluorescence was monitored using a Fluorescence Activated Cell Sorter.

Example 13. Hybridisation of DNA to *in situ* synthesised oligonucleotides

25 DNA oligonucleotides were synthesised on SU-8 particles using a non-cleavable linker and deprotected *in situ*. The DNA-modified particles were then suspended in an aqueous buffer containing fluorescently labelled oligonucleotide complementary to that on the particles. The increase in fluorescence on hybridisation was monitored using a Fluorescence Activated Cell Sorter. Incubation of the particles with a non-

complementary fluorescently labelled oligonucleotide gave significantly lower fluorescence measurements by FACS.

All publications mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described methods and system
5 of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention
10 which are obvious to those skilled in chemistry or related fields are intended to be within the scope of the following claims.

CLAIMS

1. A process for the preparation of a particle for use as a solid support in chemistry comprising
 - i. coating a laminar support material comprising at least a sacrificial layer with a photopolymerisable material;
 - ii. exposing the coated face of the support to radiation through a photolithographic mask, said mask defining the particle size and shape;
 - iii. selectively removing the exposed or unexposed areas of photopolymerisable material;
 - iv. reducing the integrity of the sacrificial layer to liberate said particle;

characterised in that the sacrificial layer comprises a metal or metals.
2. The process according to claim 1 wherein the sacrificial layer consists essentially of a metal or metals.
3. The process according to claim 1 or 2 wherein one of the metals is aluminium.
4. The process according to claim 3 wherein the sacrificial layer consists essentially of aluminium.
5. The process according to any preceding claim wherein the sacrificial layer is less than 100 nm thick.
6. The process according to any preceding claim wherein the laminar support material further comprises a substrate layer.
7. The process according to claim 6 wherein the substrate layer comprises silicon.
8. The process according to any preceding claim wherein the photopolymerisable material comprises at least a reactive functional group when polymerised.

9. The process according to claim 8 wherein the photopolymerisable material comprises at least an epoxide group when polymerised.
10. The process according to any preceding claim comprising a further step of reacting the particle with a functionalising agent.
- 5 11. The process according to claim 10 wherein the functionalising agent comprises a polyoxyalkyleneamine or polyether amine.
12. The process according to any preceding claim comprising the further step of reacting the particle with a linker group.
13. The process according to claim 12 wherein the linker group is an amino acid.
- 10 14. The process according to claim 13 wherein the linker group is 6-aminohexanoic acid.
15. The process according to claim 14 wherein the linker group is a hydroxycarboxylic acid.
- 15 16. The process according to claim 15 wherein the hydroxycarboxylic acid is 4-hydroxymethyl-phenoxy acetic acid
17. The process according to any preceding claim comprising an additional step of incorporating an identifier.
18. The process according to claim 17 wherein the identifier comprises a pattern of refractive index variations that give rise to a predetermined diffraction pattern.
- 20 19. A process for the preparation of a polymer particle for use as a solid support in chemistry comprising
 - i. coating a laminar support material comprising at least a sacrificial layer with a composition comprising monomer;
 - 25 ii. selectively polymerising said monomer to define the particle size and shape;

- iii. selectively removing unpolymerised material;
 - iv. reducing the integrity of the sacrificial layer to liberate said particle;
- characterised in that the sacrificial layer comprises a metal or metals.
20. A particle obtainable by a process as claimed in any one of claims 1 to 19.
- 5 21. A particle obtained by a process as claimed in any one of claims 1 to 19.
22. Use of a particle as claimed in claim 20 or 21 as a support for solid supported chemistry.
23. Use of a particle as claimed in claim 20 or 21 in the preparation of an oligopeptide.
- 10 24. Use of a particle as claimed in claim 20 or 21 in the preparation of an oligonucleotide.
25. Use of a particle as claimed in claim 20 or 21 in the preparation of a chemical library.
26. A process for the preparation of a library of compounds comprising steps of
- 15 i. providing a plurality of encoded particles as claimed in claim 20 or 21;
- ii. dividing the particles into a plurality of portions;
- iii. subjecting each portion to a specific chemical reaction;
- iv. reading the code of a particle;
- v. recombining the portions; and
- 20 vi. repeating steps i to v n times, wherein n is an integer.
27. A process substantially as hereinbefore described with reference to the examples or figures.
28. A particle substantially as hereinbefore described with reference to the examples or figures.

29. A use substantially as hereinbefore described with reference to the examples or figures.

Application No: GB0518478.3

Examiner: Tony Rudge

Claims searched: 1-29

Date of search: 28 May 2006

Patents Act 1977: Search Report under Section 17

Documents considered to be relevant:

Category	Relevant to claims	Identity of document and passage or figure of particular relevance
A	1, 19 at least	GB2306484 A (UNIVERSITY OF HERTFORDSHIRE), page 13, lines 9 et seq
A	1, 26 at least	US2002/0137059 A1 (WU ET AL)
X	"	US2003/0129654 A1 (RAVKIN ET AL)

Categories:

X Document indicating lack of novelty or inventive step	A Document indicating technological background and/or state of the art
Y Document indicating lack of inventive step if combined with one or more other documents of same category.	P Document published on or after the declared priority date but before the filing date of this invention
& Member of the same patent family	E Patent document published on or after, but with priority date earlier than, the filing date of this application

Field of Search:

Search of GB, EP, WO & US patent documents classified in the following areas of the UKC^X :

G2X

Worldwide search of patent documents classified in the following areas of the IPC

B01J; G01N; G03F

The following online and other databases have been used in the preparation of this search report

EPODOC, WPI