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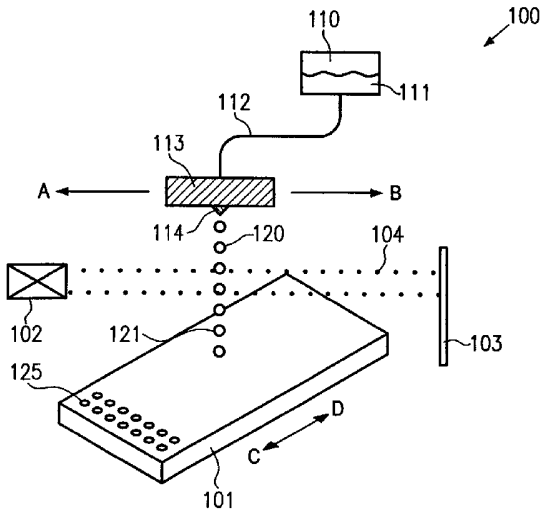
(54) Title: METHOD AND DEVICE FOR THE INTERMOLECULAR TRANSPORT OF BOND CLEAVAGE ACTIVATION ENERGY THROUGH SPACE



(57) Abstract: The present invention relates to a method for cleaving chemical bonds whereby the activation energy for bond cleavage is delivered through space by a carrier molecule, which can be switched from its ground state to an excited state upon irradiation. Furthermore, the present invention relates to a device for the intermolecular transfer of bond cleavage activation energy from carrier molecules to target molecules of the bond to be cleaved comprising a storage tank, comprising said carrier molecules, means (113) for delivering said carrier molecules through an activation area (104), activation means (102) and an exchange area (101) comprising the target molecule. Furthermore, the present invention addresses the use of the method according to the invention for effecting the light-addressed 5' to 3' or 3' to 5' in-situ synthesis of nucleic acid derivatives on solid supports for the production of biochips or genechips.

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METHOD AND DEVICE FOR THE INTERMOLECULAR TRANSPORT OF BOND CLEAVAGE ACTIVATION ENERGY THROUGH SPACE

The present invention relates to a method for cleaving chemical bonds and to a device for the intermolecular transport of bond cleavage activation energy and in particular to a method of effecting 5' to 3' or 3' to 5' nucleic acid synthesis. The method can inter alia be used to prepare arrays of oligomers bound to a support via their 5' or 3' termini.

Methods for cleaving chemical bonds are widely known and used in chemistry, biochemistry and related technological fields. For example, bond cleavage, also termed as "homolytic dissociation" in molecules is achieved by:

- heating (thermal dissociation). The energy for bond cleavage is supplied by an external source of thermal energy.
- irradiating (photochemical or photolytic dissociation). The energy for bond cleavage is supplied in form of photons.
- applying an electric current in an appropriate medium (electrolytic dissociation).

The necessary bond cleavage energy (the so-called activation energy, comprising the "true" bonding energy or bonding enthalpy Δ H) is different for each chemical bond and depends further even for identical bonds on the specific chemical environment of each bonding partner.

Photochemical cleavage of chemical bonds or photochemically induced reactions in general requires a highly sophisticated set up, especially when carried out on a large scale. Furthermore, all of the interfering reaction partners have to be present in the same reactor or reactor system to obtain a reaction by irradiation with the appropriate wavelengths. The irradiation process may also often lead to undesired side reactions and side products which lower the overall yield and require additional purification steps of the products.

This is especially onerous in the field of photo-addressed in-situ-synthesis of nucleic acids on solid supports, especially regarding the manufacture of so called biochips or genechips (nucleic acid chips). Usually, two main techniques for the in-situ photolytic synthesis of nucleic acid derivatives (oligonucleotides) on solid supports are used. The first method termed "photolithographic method" employs photolithographic masks, as

for example disclosed in US Patent No. 5,510,270. Accordingly, a plurality of small regions termed "spots" form an array on one surface of a solid support. Every spot contains a plurality of identical nucleosides or nucleotides. These nucleosides/nucleotides also carry photolabile protecting groups at their 3' or 5' termini. Irradiation of all or a selected number of the spots leads to photolytic cleavage of the chemical bond between the photolabile protecting groups and the nucleosides/nucleotides. After deprotection, a reaction with new protected nucleosides/nucleotides is carried out. The reaction is continued until the nucleotide chains have their final lengths. To prevent that dispersed light will also touch neighbouring spots, causing the deprotection of non-selected nucleosides/nucleotides, masks with holes having diameters corresponding to the dimension of the spots have to be used. The use of masks is onerous and cost-expensive because more than 100 of these masks have to be used for the production of a biochip with nucleotides comprising more than 25 nucleosides in length. Thus, costs for the above-mentioned synthesis are high.

An alternative method for the photo-addressed in-situ-synthesis of oligonucleotides using photosensitive protecting groups was proposed by Bogoslawski, J. (2001), Drug Discovery and Development, 3, page 15 – 16. The traditional masks with holes are replaced by virtual masks comprising a plurality of addressable micro-mirrors. This method is termed "maskless array synthesis" (MAS). This method allows to irradiate defined arrays on the chip surface with for example UV-light by using up to 780 000 micro mirrors.

US Patent 5,472,672 describes a polymer synthesis apparatus for the synthesis of oligonucleotides by drop for drop sequential addition of polymer units (nucleosides/nucleotides) in a solution onto a solid support, preferably into a microtiter well of an usual microtiter plate. The reagent solution is supplied via a head assembly having a plurality of nozzles. The head assembly may for example comprise a piezoelectric pump as described in US Patent 5,474,796. The polymer units are brought to reaction by classical chemistry as described in said US Patent in each well to form the oligonucleotide chain. A major disadvantage of this method is the addition of strong acids and bases which attack the material of the head assembly and have to be washed away from the well after completion of the reaction.

It was therefore an object of the present invention to provide a new and effective method for cleaving chemical bonds, in particular in cleaving chemical bonds in

molecules during the in-situ-synthesis of oligonucleotides in the manufacture of biochips. The problem underlying the invention has been solved by a method for cleaving chemical bonds in a target molecule, whereby the activation energy for bond cleavage is transported through space by a carrier molecule from a first place to a second place where the activation energy is transferred to said target molecule and whereby the first place and the second place are located at spatially different places.

The "spatial decoupling" of the activation of a carrier molecule, i.e. providing the carrier molecule with activation energy, and the subsequent reaction of a target molecule (i.e. bond cleavage by intermolecular transport) results in a large decrease of undesired side products. The cleavage reaction can take place immediately after the transfer of the activation energy from the carrier molecule to the target molecule or after a certain time period depending on he specific target molecule and the bond to be cleaved.

It is preferred that the cleavage reaction takes place where the activation energy transfer took place. But in another embodiment of the present invention, the cleavage reaction takes place elsewhere.

Further the spatial decoupling results in the elimination of side reactions which are always present when irradiating a reaction mixture containing all reaction partners. In the method according to the invention, only the carrier molecule carries the activation energy for bond cleavage. The carrier molecule is activated in a first (remote) place and carries the necessary activation energy through space to a second place where the activation energy transfer to a target molecule and if desired a photochemical reaction will take place, i.e. the cleavage of selected chemical bonds in a target molecule. Excess activation energy carried by the molecule to the place of cleavage is dissipated very fast by transfer to vibrational energy thus avoiding any damage to the target molecule which is in contrast to direct photolysis where excess irradiation provides direct damage to the target molecule. The method according to the invention is also far superior to prior art methods where chemical cleavage is achieved by a deprotection agent (i.e. acids or bases), as these chemicals remain active (i.e. further undesired reactions, rearrangements etc) until they are either removed or neutralized.

The term "carrier molecule" as used herein comprises molecules with electrons in energy levels in the ground state able to be activated by radiation energy whereby the activated electrons will be in an energy level in the excited (or activated) state with

higher energy than the energy level of the ground state. It is understood that when a plurality of carrier molecules is present, they can be the same or different.

The term "target molecule" denotes every molecule, capable of performing a chemical reaction, either an intramolecular bond cleavage or a reaction with a reaction partner when the activation energy necessary for performing said chemical reaction is delivered by a carrier molecule.

The term "excited state" as used herein means any electronically excited state of a molecule. An "excited state" may for example be the triplet state if the ground state is the singlet state, or the singlet state if the ground state is the triplet state. But any other ground and excited states of molecules are comprised within the invention.

It is preferred, that the carrier molecule is in its ground state and is irradiated in a first place prior to contacting a target molecule with a bond to be cleaved in a second place. From the ground state, a carrier molecule can reach upon irradiation other energy states able to store energy over a period of time and thereby to transfer this energy through space to a different location. It is understood that the scope of the invention comprises molecules which have either a singlet or a triplet ground state.

In a preferred embodiment of the invention, the carrier molecule is present in an excited state upon irradiation. Preferably, the carrier molecule has a stable excited state and an excited state lifetime which is long enough to allow the transport of the activated carrier molecule through space to another location. It is evident from the foregoing that carrier molecules can be present in a singlet or a triplet excited state. It is also clear from the foregoing that the term "an excited state" denotes any excited state of a carrier molecule, i.e. comprising its first, second, etc. excited state.

It is especially preferred, that the absolute energy level of the excited state of the carrier molecule is at least equal or higher than the activated energy level, necessary for bond cleavage in a target molecule, thus delivering sufficient energy suitable for bond cleavage.

In a further especially preferred embodiment, the method according to the invention comprises the following steps:

a) activating said carrier molecule in an activation area by irradiating said carrier molecule,

b) transporting said activated carrier molecule from step a) through space to an exchange area comprising a target molecule, whereby said activation area and said exchange area are located at spatially different places,

c) transferring in said exchange area the activation energy from said carrier molecule to the target molecule.

The method according to the invention therefore allows the selective delivery or transport of the necessary activation energy for a bond cleavage reaction from a first place (activation area) to a second place (exchange area). This process is also termed as intermolecular energy transport. At the exchange area, selected target molecules with bonds to be cleaved are present and are activated by the transfer of the energy from the carrier molecules to the target molecules. As a result, no activation energy is lost and the presence of undesired side products is considerably decreased.

The term "exchange area" denotes a defined region close to a surface of a solid support. Typical examples are – but are not limited to – wells of a microtiter plate, single spots of an array of spots on a surface or in a well, a reaction vessel, etc.

The term "transferring" in step c) comprises every method by which energy transfer from one molecule to another can take place. Typically, the direct physical contact from one molecule to another molecule is sufficient. Other types of transfer are "through space mechanisms", for example in solution, dispersion, solid state etc. via vibrational transfer when the molecules are spatially close enough. Typical mechanisms for this type of energy transfer are for example disclosed in T. Förster, Fluoreszenz organischer Verbindungen, ch. 13, Vandenhoeck & Rupprecht, Göttingen 1951. It is understood that the mechanisms by which the transfer takes place in the present invention are not limited thereto.

It is preferred that the amount of carrier molecules to be activated can be freely selected. This also allows the specific activation of only a few selected target molecules or a large amount of target molecules to be reacted.

It is further preferred, that the transport of said carrier molecule to said exchange area can be spatially addressed. This provides for the possibility of activating only a few molecules in the exchange area without addressing molecules in adjacent spots or wells.

The method according to the invention is for example used in the manufacture of nucleic acid, protein, peptide or carbohydrate chips.

The problem of the present invention is further solved by a method of effecting the light-addressed 5' to 3' or 3' to 5' synthesis of nucleic acid derivative comprising the reaction of selected nucleosides and/or nucleotides comprising suitable photolabile protecting groups at their 3' or 5'O-position under suitable reaction conditions, whereby a selected carrier molecule is passed through an activation area comprising an electromagnetic field before being delivered to an area comprising a suitable nucleoside and/or nucleotide with a photolabile functional group.

The method according to the invention allows the selected deprotection of nucleosides and/or nucleotides by transporting only a selected amount of carrier molecules to said selected nucleosides and/or nucleotides in order to effect chain extension reactions only at selected areas.

Advantageously, the electromagnetic field, which may be a temporary or a stationary field is generated by a laser source. Laser sources are well known and widely available, may supply high doses of energy within a chosen and spatially confined space, are easy to operate and the set-up according to the invention does not require high performance optical set-ups and thus can be reasonable cheap and miniaturizable. Laser light provides a source of coherent non-dispersed monochromatic light compared to for example light generated by widely used polychromatic Hg-lamps as in prior art.

It is preferred that the carrier molecule switches from its ground to an excited state when passing through the electromagnetic field and that its excited state lifetime is in the range of 50 to 100 ms in order to carry the activation energy without any energy loss from the activation area to the exchange area. The switch may be e.g. from a singlet ground state to an excited triplet state or from a triplet ground state to an excited singlet state.

The underlying problem of the present invention is further solved by a device for the intermolecular transfer of bond cleavage activation energy from a carrier molecule to a target molecule with a bond to be cleaved, comprising a storage tank containing said carrier molecule, means for transporting said carrier molecule to an activation area, activation means and an exchange area comprising the target molecule.

The device according to the invention allows a non-sophisticated set-up and provides the selective transport of carrier molecules containing stored activation energy to

selected spatially resolved places (wells, spots) to an activation area without the risk of activating other spatially resolved places of the exchange area.

Advantageously, the exchange area comprises a solid support. In another preferred embodiment said exchange area is a reaction vessel. The solid support or reaction vessel is either transparent or non-transparent for UV/VIS radiation or may be also translucent. As a non-limiting example and for illustrative purposes only, an exchange area may comprise an 96-well microtiter plate, a glass slide and the like. Inside the well is an array of spots where target molecules which are the same or different are located.

The method according to the invention therefore allows addressing only target molecules in selected wells and/or spots.

In another preferred embodiment the solid support is non-transparent for UV/VIS radiation (light). This allows an increase of the choice of materials for the manufacture of the solid support. Non-transparent substrates heat up considerably when irradiated directly as used in prior art. The heating up causes severe cleavage of surface bound molecules as an undesired side reaction.

The devices and processes known from prior art can therefore not be used with temperature sensitive target molecules, which degrade when the support is irradiated directly. Advantageously, the present invention avoids the heating-up of the substrates and, therefore, temperature sensitive molecules can be used on a non-transparent support without the danger of decomposition.

In a further preferred embodiment, said support comprises an array of spatially resolved areas (spots) comprising said target molecules, therefore allowing to build up large molecular libraries at a time.

It is especially preferred that the solid support is movable in one direction, thus allowing to select specific single spots containing target molecules along that a line on the solid support where carrier molecules have to be delivered.

It is advantageous that the activation area is located above the exchange area, thus allowing to use gravitational force for the transport of said activated carrier molecules to the exchange area. But it is also within the scope of the invention to arrange the activation area below the exchange area whereby the advantages of this "below" arrangement are discussed below.

In an especially preferred embodiment, said activation area comprises an electromagnetic field, which is usually easy to generate and to maintain in order to activate the carrier molecules.

In a further preferred embodiment said device comprises delivery means for delivering said carrier molecules to said activation area which are arranged above said activation area, also using gravitational force for the transport of said carrier molecules without the necessity of a sophisticated setup.

Advantageously said delivery means are moveable in one direction, preferentially perpendicular to the moving direction of the solid support, thus adding two further degrees of freedom when selecting a single spot on the solid support. Since the moving directions of said exchange area and said delivery means are perpendicular to one another, each desired individual spot on said solid support can be selected and delivered individually with specific and selected amounts and/or volumina of said carrier molecules. The delivery means further comprises outlet means for controlling a spatially addressed transfer of said carrier molecules to said spatially resolved area on said support.

A person skilled in the art is fully aware, that the scope of the invention does not only comprise the above mentioned features alone but also combinations thereof and that the scope of the invention comprises also every single feature in relation to the invention as described herein.

For illustrative purposes only, the invention is further described in detail in the following description of preferred embodiments with respect to figure 1, whereby the description is not meant to limit the scope of the invention.

Figure 1 shows schematically a set-up for a device according to the invention.

Figure 1 shows a device 100 comprising a solid support 101 which is preferably in the form of a so-called "chip" essentially as known by a person skilled in the art. The material thereof may be freely selected among those which are known for the intended purpose e.g. coated glass supports, plastic supports (nylon, polypropylene), etc.

Chip 101 represents the exchange area according to the invention and has a two- or three-dimensional array of spots 125 comprising target molecules. The target molecules may be in their pure form or comprised within a solution or dispersion etc. They may also be generated via sol/gel synthesis in situ or ex situ.

Typical target molecules comprise but are not limited to protected or non-protected nucleotides or nucleotides comprising 2 to 20 nucleosides, amino acids and their derivatives, oligo- (consisting of 2-9 amino acids), poly- (consisting of 10-100 amino acids), and macropeptides (consisting of more than 100 amino acids), carbohydrates and their derivatives, proteins, etc.

The term "array" as used herein denotes a set of compounds maintained in a specified spatial distribution, e.g. in the wells of a multi-well plate, in pins held in a rack or at the tip of optical fibres arranged in a bunch.

More specifically, the array can have any suitable geometrical arrangement, usually perpendicularly arranged lines consisting of small spots or two- or three-dimensional micro-objects. The spots may also comprise porous structures like zeolithes, nanotubes, silicones, siloxanes, etc. It is understood that any other suitable geometrical arrangement is also within the scope of the invention.

Chip 101 is mounted on a high resolution stepper device not shown in figure 1. Any stepper device able to move chip 101 in the desired direction can be used and such stepper devices are essentially known by a person skilled in the art. Chip 101 can move in one direction, as shown in figure 1 by the arrow and denoted by the letters C and D. Chip 101 is arranged between a laser source 102, e.g. an UV laser source like a dye-laser, an LED-laser, a YVO4 or a YAG-laser. The laser is arranged in a range of between 1 – 200 mm, preferably 1 – 50 mm and most preferably 1 - 20 mm above chip 101. The laser generates an electromagnetic radiation field 104, which represents the activation area according to the invention. Opposite laser 102, reflecting means 103, which are for example a mirror or a plurality of mirrors, are arranged to reflect the laser light and to create a large activation area 104.

A printer head 113 is arranged above said activation area 104. Printer head 113 is for example a modified or unmodified ink-jet or laser printer head. But in another embodiment of the invention, printer head 113 is arranged below the activation area 104. This "below" arrangement is particularly advantageous if a solution/solvent with low surface tension, for example toluene, DMSO, isopropyl alcohol and the like is used which is not kept in a printer head 113 arranged above said activation area 104 and would therefore uncontrollably splash onto the substrate.

Printer head 113 is movable in the directions as represented by the arrows and the letters A and B. The direction A to B is perpendicular to the direction C to D of chip 101 on the stepper device. This set up allows to focus and to deliver the activated carrier

molecules 111 injected from printer head 113 to one or more selected spots 125 on the surface of chip 101. Printer head 113 has an outlet 114, preferably a nozzle, which is adjustable in order to choose the volume of the droplets to be injected onto the surface of chip 101.

Printer head 113 may comprise one or a plurality of nozzles. Two important factors concerning the liquid delivery of the carrier molecules through outlets 114, especially through nozzles are: a) how to eject a droplet cleanly so that a drop is not left hanging on the end of the nozzle b) how to keep the contents of a spot 125 comprising the target molecules from splashing when droplets 120 comprising the carrier molecules are delivered onto said spot 125. Hence, a person skilled in the art will choose the way of delivering said carrier molecules depending on the nature of the solution, suspension etc. to dispense droplets 120 comprising the carrier molecules in a continuous stream, a series of pulses or in droplet form.

The use of a plurality of printer heads 113 is also within the scope of the invention. Another embodiment of a printer head 113 according to the invention comprises a piezoelectric pump in an arrangement as for example disclosed in US Patent 5,474,796.

Printer head 113 is connected with a storage tank 110 containing said carrier molecules 111. It is self-evident from the foregoing that in the case of a plurality of printer heads 113, each single printer head 113 is connected with a storage tank, thus allowing for example to have reservoirs of different carrier molecules in each storage tank 110. It is also within the scope of the invention that a plurality of printer heads 113 are connected to a single storage tank 110. A further preferred embodiment comprises building blocks for the selective synthesis of oligonucleotide chains in one or more storage tanks 110, so that reactions and chain extension may be carried out on different or on identical spots simultaneously by a proper choice of the order of injection of carrier molecules and building blocks.

The connection 112 is for example any tube or hollow article suited for this purpose and essentially known by a person skilled in the art. Preferably the connection 112 is flexible and semi-resilient, for example a TEFLON® tube.

The carrier molecules may be either in solution, or in their pure state or in the form of a suspension or a dispersion inside the storage tank 110. The concentration of the carrier molecules when present in solution, suspension or dispersion should not exceed 15 wt.-% in order to prevent degradation upon irradiation. Preferred are 1 to 10 wt.-%, most preferred are 1 to 5 wt.-%.

Representative non-limiting examples of carrier molecules according to the invention comprise acridone, xanthone, thioxanthone, benzene, naphthalenes and their substituted or unsubstituted derivatives, e.g. N-methylacridone, 2-ethylthioxanthone, 2-anilino-naphthalene, naphtho-[1, 2-c] [1, 2, 5]-thiadiazole, benzo-[*b*]-fluorene, 5,7-dimethoxy-3-thionyl-coumarine, 1,2-cycloheptandione, 3-acetyl-6-bromo-coumarine, 2-bromo-9-acridinone, 4,4'-dibenzylbiphenyl, 2, 6-dithiocoffeine, 1,4-dibromonaphthalene, dibenzo-[*fg,op*]-naphtalene, 10-phenyl-9-acridinone, 2-methyl-5-nitro-imidazol-1-ethanol, 1-(2-naphthoyl)-aziridine, 9-(2-naphthoyl)-carbazole, 4,6'-diamino-2-phenyl-benzooxazole, p-thiophenyl, 3-acetyl-phenanthrene, dinaphtho-[1, 2-*b*:2',1'-]-thiophene, (E)-piperylene, β-methyl-(E)-styrene, 2-phenyl-benzothiazole, chinoxaline, 9,9'-biphenantryl, naphtho-[1, 2-*c*] [1,2,5]-oxabiazole, phenothiazine, 2-ethoxy-naphthalene, 9-phenyl-9-stibafluorene, 9,10-antrachinone, 4,4'-dichlorodiphenyl. Other suitable compounds are disclosed in S. L. Murov ,I. Carmichael and G. L. Hug, Handbook of Photochemistry, Marcel Dekker, Inc., New York 1993.

In an especially preferred embodiment, thioxanthone and its 9-alkyl derivatives, Michlers ketone and acridone and its derivatives are used. Preferred solvents are for example DMSO (dimethylsulfoxide), isopropanol, acetonitrile, DMEU N,N'-dimethylethylene urea, DMPU (N,N'-dimethylpropylene urea).

From outlet means 114 of printer head 113 small droplets 120 of carrier molecules are injected towards selected spot(s) 125 of chip 101. Upon passing the activation zone, i.e. electromagnetic radiation field 104, the molecules in said droplets 120 switch to an excited state, for example from the singlet state to the excited triplet state because they are pumped with sufficient energy to allow a change of the energy level. The excitation can for example be carried out with a pulsed Nd-YVO4 frequency tripled UV laser (diode current max. 21A, frequency max. 100 KHz, impulse width between 35μs and 170μs, voltage between 0.5V and 1.5V).

As a representative example, droplets of a solution of 5 wt% thioxanthone as carrier molecule in DMEU were passed through a field generated by a pulsed Nd-YVO4 frequency tripled UV laser (diameter 1mm) with an impulse width of 118μs and a voltage of 0.972V. 3 impulses (4ms length) with a delay of 100μs were used. The speed of the droplets was measured as 2,89 m/s. Therefore, the time the droplets spent in the laser field was 346 μs which proved to be sufficient for excitation of the carrier molecules. Other time periods were 150 μs, 200 μs, 250 μs, or 300 μs, which were all sufficient to achieve the required excitation (energy storage).

It is also within the scope of the invention that any other sources of electromagnetic radiation, which may induce energy level changes in molecules, can be used for the purpose of the present invention, for example sources generating microwave, infrared or Raman radiation.

The energy "stored" in the molecule in its excited state, for example in its triplet or singlet state is at least equal or larger than the necessary activation energy for bond cleavage in the target molecules contained in said spots 125 on the surface of chip 101. Specific target molecules are for example but not limited to typical nucleic acid derivatives with NPPOC-protecting groups or similar protecting groups essentially known by a person skilled in the art.

When passing the electromagnetic radiation field 104 said droplets of carrier molecules 120 now contain the molecules in their excited state and are represented in figure 1 as "excited droplets" 121. These droplets 121 are now coming in contact with defined spots 125 on the surface of chip 101 comprising for example protected nucleic acid derivatives (target molecules). In the above-mentioned example 3'-thymidin with a NPPOC photolabile protecting group was synthesized by using the MAS technology.

The carrier molecules (thioxanthone in the example above) now transfer their stored energy on to the target molecules which undergo bond cleavage reaction (deprotection) between thymidine and NPPOC. A fluorescent protecting group (Cy3, 568 nm) was coupled to the deprotected sites by MAS technology and read-out by known fluorescence detection techniques. The results showed that deprotection of NPPOC protected 3-thymidine by activated thioxanthone was successfully achieved on every selected spot.

The deprotection time, i.e. the time for cleavage of the bond between the protecting group and the residue of the nucleic acid derivative depends on the nature of the carrier molecule and of the target molecule, the solvents used, the travel distance of the carrier molecule in its excited state and the reaction atmosphere (quenching of the excited carrier molecules is quite frequently observed when using an oxygen atmosphere, depending on the nature of the carrier molecule, solvent and the oxygen level).

In another embodiment of the invention the target molecules contain acid labile protective groups and/or second functional groups. When brought in contact with the carrier molecules the second functional groups of the target molecules generate an acid in situ which enables in-situ cleavage of the acid labile protecting groups.

The method according to the present invention in combination with the device according to the invention allows the growing of selected oligonucleotide chains only on selected spots 125 on the surface of the chip 101, because in a preferred embodiment of the invention, each outlet means 114 can be addressed individually. Usually, the target molecules are in solution. Excess solvent may therefore be pumped away by putting the chip 101 into a vacuum chamber after reaction, also allowing the removal of oxygen, which could influence the energy transfer. In a further preferred embodiment, the spots 125 are arranged in an array of wells in a microtiter plate. The device is preferably configured to use a 96-well microtiter plate, aligned in 12 equally spaced apart rows. The microtiter plate is preferably fabricated from a chemically inert material such as polypropylene or the like. It is of course obvious from the foregoing that any number or arrangement of rows and columns can be employed within the scope of the present invention.

Compared to a controlled supply of light in usual photochemical set ups, no optical set ups and arrangements are necessary thus no energy loss occurs due to the optics. Further, this set up does not require any photolithographic masks and is suited perfectly for mass production of chips of a flexible and individual design.

The design is also suited for multiplexing by beam splitting and using multi-channel print head devices. Furthermore, the method according to the invention in combination with said device offers the advantage that there is no cross-talk between individual micro-locations, i.e. spots 125, because the energy storage can be selected in such a way that the lifetime of the excited state of the carrier molecule is too short to travel to distant locations, thus preventing undesired remote activation or reactions in a non controlled manner.

* * * * *

PATENT CLAIMS

1. Method for cleaving chemical bonds in a target molecule, characterized in that the activation energy for bond cleavage is transported through space by a carrier molecule from a first place to a second place where the activation energy is transferred to said target molecule and whereby the first place and the second place are located at spatially different places.

2. Method according to claim 1, characterized in that the carrier molecule in its ground state is provided in the first place with the activation energy.

3. Method according to claim 2, characterized in that the activation energy is provided by irradiation.

4. Method according to claim 3, characterized in that the carrier molecule is present in an excited state upon irradiation.

5. Method according to any of the preceding claims, characterized in that the absolute energy level of the excited state of the carrier molecule is at least equal or higher than the activated energy level for bond cleavage in a target molecule.

6. Method according to claim 5, comprising the following steps:

   a) Activating said carrier molecule in an activation area by irradiating said carrier molecule,

b) transporting said activated carrier molecule from step a) through space to an exchange area comprising a target molecule, whereby said activation area and said exchange area are located at spatially different places,

c) transferring the activation energy from said carrier molecule to said target molecule in said exchange area.

7. Method according to claim 6, characterized in that the amount of carrier molecules to be activated can be freely selected.

8. Method according to claim 7, characterized in that the transport of said carrier molecules to said exchange area can be spatially addressed.

9. Method of effecting the light-addressed 5' to 3' or 3' to 5' synthesis of a nucleic acid derivative, comprising the reaction of selected nucleosides and/or nucleotides comprising suitable photolabile protecting groups at their 3' or 5' O-position under suitable reaction conditions, characterized in that a selected carrier molecule is passed through an activation area comprising an electromagnetic field where the carrier molecule is provided with energy sufficient for a bond cleavage before being delivered to an area comprising a suitable nucleoside and/or nucleotide with a photolabile protecting group.

10. Method according to claim 9, characterized in that the electromagnetic field is a stationary field.

11. Method according to claim 9, characterized in that the electromagnetic field is a temporary field.

12. Method according to claim 10 or 11, characterized in that the electromagnetic field is generated by a laser source.

13. Method according to claim 12, characterized in that the carrier molecule switches from its ground to an excited state when passing through the electromagnetic field.

14. Method according to claim 13, characterized in that the carrier molecule has an excited state lifetime of more than 50 ms.

15. Device (100) for the intermolecular transfer of bond cleavage activation energy from a carrier molecule to a target molecule with a bond to be cleaved, comprising a storage tank (110), containing said carrier molecule, means (113) for delivering said carrier molecule to an activation area (104), activation means (102) and an exchange area (101) comprising the target molecule.

16. Device according to claim 15, characterized in that the exchange area (101) comprises a solid support.

17. Device according to claim 16, characterized in that the solid support is non-transparent for UV/VIS radiation.

18. Device according to claim 17, characterized in that said support comprises an array of spatially resolved areas (125), comprising said target molecules.

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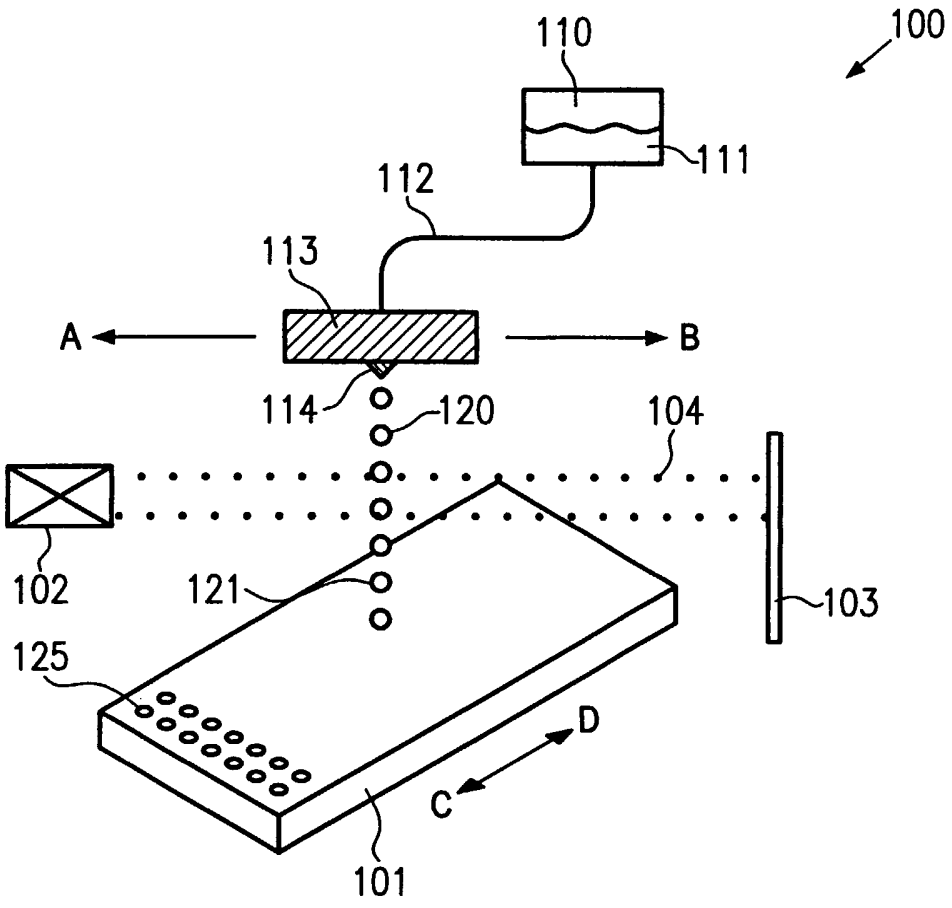

FIG.1