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(54) Benævnelse: **DIARYLSULPHID SKELET INDEHOLDENDE fotolabile beskyttelsesgrupper**

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EP-A2- 0 295 233
WO-A1-00/21936
WO-A2-2004/074300
WO-A2-2007/092854
US-A- 4 863 507
US-A1- 2010 048 610

DESCRIPTION

Background of the Invention

[0001] The present invention relates to photoactivable protecting groups containing a diarylsulphid chromophore, a method for the synthesis thereof and their use as photoactivable protecting groups using maskless photolithography based array synthesis.

[0002] Photolabile protecting groups (PLPG) play an important role in blocking functional groups present in nucleosides, nucleotides, sugars and amino acids, which are used for the synthesis of biomolecules, e.g. nucleic acids and their derivatives, proteins, peptides and carbohydrates. Additionally, PLPG have the advantage that deprotection of the protected functional group can be performed simply via light exposure. Therefore, PLPG provide the basis for the photolithography based spatially resolved synthesis of oligonucleotides or peptides on solid supports. The major advantage of this technique is that high resolution microarrays can be produced. Such high resolution microarrays are of great significance for the analysis of biomolecules in medicine and pharmaceutical research, as they provide the possibility to perform high throughput and cost-effective analysis of multiple samples on a single array.

[0003] The use of PLPG for the synthesis of microarrays is well known in the art. Commonly used PLPG for photolithography based oligonucleotide synthesis are for example α-methyl-6-nitropiperonyl-oxycarbonyl (MeNPOC) (Pease, et al., Proc. Natl. Acad. Sci. USA 91 (1994) 5022-5026), 2-(2-nitrophenyl)-propoxycarbonyl (NPPOC) (Hasan, et al., Tetrahedron 53 (1997) 4247-4264). Commonly used PLPG for photolithography based peptide synthesis are for example nitroveratryloxycarbonyl (NVOC) (Fodor, et al., Science 251 (1991) 767-773) and 2-nitrobenzyloxycarbonyl (NBOC) (Patchornik, et al., J. Am. Chem. Soc. 92 (1970) 6333-6335).

[0004] The major drawback of the prior art PLPG is that light at a wavelength of approximately 365 nm or shorter has to be used for the deprotection of the protected functional groups. Light sources, which are suitable to generate such wavelength, are e.g. mercury arc lamps, excimer lasers, UV-LEDs and frequency multiplied solid-state lasers. Such light sources are characterized by high purchase costs, provide limited luminous power and have a short life-time leading to high overall costs of operation. Since some of the above mentioned light sources contain hazardous substances, e.g. mercury, appropriate actions to secure occupational safety and proper disposal are necessary further increasing the costs.

[0005] Optical devices used for the photolithography based synthesis of oligonucleotides or peptides, such as micro mirror devices (WO 03/065038), are primarily designed for the visible wavelength range of approximately 380 to 780 nm, i.e. such devices carry an antireflective or protective antiscratch coating optimized for transparency for the respective visible wavelength range. Thus, the near UV wavelength of 365 nm, necessary for the deprotection of the functional groups protected with the PLPG known in the state of the art, require optical devices which are optimized for near UV wavelengths. Since most of the optical devices are optimized for the use with visible light, such optimization often comprise removing the coating intended for the use with visible light from the optical devices and/or coating the optical device with materials intended for use with near UV or UV light.

[0006] Furthermore, some of the above mentioned light sources produce a broad spectrum of wavelengths, e.g. mercury arc lamps emit light from the UV- to the IR-range, both of which have disadvantageous effects concerning the synthesis of biomolecules. UV-light for example can be absorbed by the synthesized DNA leading to random breaks within the strand by phosphate backbone radical cleavage, Guanine base oxidation and subsequent strand break or photodimerization, especially of thymine bases. Furthermore, UV-light can also lead to the destruction of certain amino acids, such as tryptophan by radical oxidation or cysteine and methionine by sulfur oxidation. As a result DNA or peptide microarrays might be of low quality due to undefined lengths of the synthesized DNA strands and peptides, respectively.

[0007] In contrast, IR-light leads to warming of the optical devices which results in deformation of the optical device. In case of the use of a micro mirror device, for example, an increase of the temperature of 1°C of the device leads to a drift of the reflected light of approximately 10 µm and thus to a loss of focus on the respective feature on the microarray. In view of the needed accuracy in photolithography based synthesis of oligonucleotides or peptides on solid supports, such aberration would lead to reduced quality of the arrays. Consequently, additional effort and costs are required to remove undesired UV- and IR-wavelengths (e.g. by filters) produced by the light sources necessary for a deprotection at 365 nm in order to ensure the quality of oligonucleotide or peptide arrays.

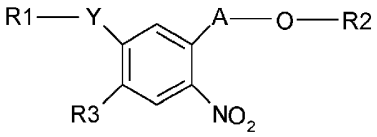
[0008] EP1589024A1 and WO2004074300A2 also disclose photocleavable protecting groups.

[0009] The object of the present invention is therefore the provision of PLPG, which do not show the above mentioned drawbacks of the prior art. Thus, PLPG are presented herein, which are suitable for the deprotection of the functional groups using visible light. Consequently, harmless and cost-effective light sources as well as regular optical elements can be used for the photolithography based oligonucleotide and peptide synthesis.

Brief Description of the Invention

[0010] In a first aspect, the invention is directed to photolabile protecting groups containing a diarylsulphid chromophore having the general formula

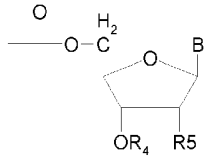
[Formula Ib]



wherein Y is S and

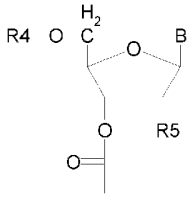
A is selected from the group consisting of -CH_2-, -CH_2-CH_2-, -$CH(CH_3)$-, - $CH(CH_3)$-CH_2-, and R1 is an unsubstituted or substituted aryl- or heteroaryl-group, and R3 is H, a methyl group or an ethyl group, and wherein R2 is H, forms a phosphoramidite, H-phosphonate or phosphate triester, or wherein R2 is

[Formula II]



or wherein R2 is

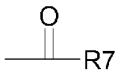
[Formula III]



wherein R4 is H, or OR4 forms a phosphoramidite, H-phosphonate or phosphate triester and wherein R5 is H, OH, a halogen or XR6, wherein X is O or S and R6 is H, an alkyl-group, aryl-group, or OR6 forms a phosphoramidite, phosphodiester, phosphotriester or H-phosphonate or an acetal or a silicone moiety, and wherein B is selected from the group consisting of adenine, cytosine, guanine, thymine, uracil, 2,6-diaminopurine-9-yl, hypoxanthin-9-yl, 5-methylcytosinyl-1-yl, 5-amino-4-imidazolecarboxylic acid-1-yl or 5-amino-4-imidazolecarboxylic acid amide-3-yl, wherein when B is adenine, cytosine or guanine the primary amino group optionally has a protecting group or when B is thymine or uracil at the O^4 position is optionally a protecting group,

or wherein R2 is

[Formula IV]



wherein R7 is a natural amino acid, a non-natural amino acid or an amino acid derivative forming an urethan bond to formula Ib or wherein formula IV represents the carboxy function of a natural amino acid, a non-natural amino acid or an amino acid derivative, forming an ester bond to formula Ib.

[0011] R1 preferably is a phenyl-group, a tert-butyl-phenyl group, a 1- or 2-naphthyl-group, a 2-pyridyl-group an aminophenyl-group, an N-alkylaminophenyl-group, an N-Acylaminophenyl-group, a carboxyphenyl-group, a phenylcarboxylic ester or an amide, and/or A preferably is -$CH(CH_3)$-CH_2- and/or R2 is a phosphoramidite or - $P(OCH_2CH_2CN)(N\text{-}iPr_2)$ and/or R4 preferably is H

and/or R5 preferably is H and/or R7 preferably is a natural amino acid. B is selected from the group consisting of adenine, cytosine, guanine, thymine or uracil, more preferably when B is adenine, cytosine or guanine the protecting group is phenoxyacetyl-, 4-tert-butyl-phenoxyacetyl-, 4-isopropyl-phenoxyacetyl- or dimethylformamidino-residues, when B is adenine the protecting group is benzoyl- or p-nitro-phenyl-ethoxy-carbonyl- (p-NPPOC)-residues, when B is guanine the protecting group is isobutyroyl-, p-nitrophenylethyl (p-NPE) or p-NPEOC-residues and when B is cytosine the protecting group is benzoyl-, isobutyryl- or p-NPEOC-residues.

[0012] The compounds of the present invention may be used for a variety of different applications. In one aspect, the invention is directed to the use of the compounds as photoactivable protecting groups using maskless photolithography. In one embodiment the compounds are used for the maskless photolithography based DNA array synthesis as intermediate or permanent OH-protecting group in nucleoside derivatives at the 3'-OH end or the 5'-OH end. Further, the compounds are used for the maskless photolithography based peptide array synthesis as NH-protecting group in amino acids. In another embodiment the compounds are used for the maskless photolithography based peptide array synthesis as COOH-protecting group in amino acids and/or for the maskless photolithography based synthesis of carbohydrates as OH-protecting group and/or for orthogonal protecting group strategy as SH-protecting group. In another embodiment the compounds are used for the maskless photolithography having a wavelength of 374 to 405 nm, preferably of 390 nm.

[0013] In another aspect, the invention is directed to a method for the synthesis of a diarylsulphid backbone containing photolabile protecting group as described above comprising the steps of

1. a) Provision of p-diethylbenzene as a starting material
2. b) Bromination of the phenylring
3. c) Nitration of the obtained compound in Nitric- and Sulfuric Acid in the position para- to the Bromine
4. d) Purification and crystallization
5. e) Hydroxymethylation of the compound at the benzylic position
6. f) Conversion of the aromatic bromine group to the arylsulfide using thiophenol
7. g) Purification
8. h) Conversion of the alcohol to chlorocarbonate
9. i) Reaction of the chlorocarbonate with a nucleoside and reaction of the nucleoside with a phosphitylating agent, or

[0014] Reaction of the chlorocarbonate with an amino acid derivative.

[0015] In one embodiment, R1 is a phenyl-group, a tert-butyl-phenyl group, a 1- or 2-naphthyl-group, a 2-pyridyl-group, A is -$CH(CH_3)$-CH_2- and R3 is H or an ethyl group.

Figures

[0016]

Figure 1:
Half-lives of the PLPG according to the invention are shown in dependence of the solvent used at a wavelength of 390 nm. Light exposure was performed for 2, 4, 6 s or 2, 4, 6, 8 s or 2, 4, 6, 8, 12 s, respectively.

Figure 2:
Half-lives of the PLPG according to the invention are shown in dependence of the solvent used at a wavelength of 404 nm. Light exposure was performed for 1, 2, 3, 4 min or 1, 2, 3, 5 min, respectively.

Figure 3:
UV absorption characteristics of PLPG.

Figure 4:
Synthesis pathways of disulfide-PLPG-amino acids.

Figure 5:
Synthesis pathways of disulfide-PLPG-nucleotides.

Figure 6:
Synthesis pathways of further PLPG according to the invention.

Figure 7:
Alternative synthesis pathway of PLPG without further alkyl substituents.

Figure 8:

Microarray scan of a peptide array containing the target sequence of an anti-V5 antibody synthesized according to the invention using Disulfide-PLPG-amino acids.

Detailed Description of the Invention

[0017] The following definitions are set forth to illustrate and define the meaning and scope of various terms used to describe the invention herein.

[0018] The term "unsubstituted" is used herein as known to the expert skilled in the art and refers to a hydrocarbon chain which fully consists of carbon and hydrogen.

[0019] The term "substituted" is used herein as known to the expert skilled in the art and refers to a replacement of a chemical group or substituent (typically H or OH) with a functional group, and particularly contemplated functional groups include electrophilic groups (e. g., C(O)-OR, C(X)-OH, etc.), nucleophilic (e. g., -NH2, - OH, -SH, -NC, etc.), ionic groups (e. g., -NH3-), polar groups (e. g.,-OH), non-polar groups (e. g., aryl, alkyl, alkenyl, alkynyl, etc.), and halogens (e. g., - F,-Cl), and combinations thereof.

[0020] The term "protecting group" is used herein as known to the expert skilled in the art and refers to a substituent, functional group, ligand, or the like, which is bonded (e.g., via covalent bond, ionic bond, or complex) to a potentially reactive functional group and prevents the potentially reactive functional group from reacting under certain reaction conditions. Potentially reactive functional groups include, for example, amines, carboxylic acids, alcohols, double bonds, and the like. Protecting groups according to the invention are photo labile protecting groups, which include, but are not limited to, 2-Nitrobenzyloxycarbonyl-(NBOC), 2-nitrophenyl-ethyloxycarbonyl (NPEOC), 2-(3,4-methylenedioxy-2-nitrophenyl)-propyloxy-carbonyl (MeNPPOC), 2-(3,4-methylenedioxy-2-nitrophenyl)-oxycarbonyl (MeNPOC), 2-(2-nitrophenyl)-propyloxycarbonyl (NPPOC), dimethoxy-benzo-inylyl-oxycarbonyl (DMBOC), 2-(2-nitrophenyl)-ethylsulfonyl (NPES), (2-nitrophenyl)-propylsulfonyl (NPPS), and the like.

[0021] The term "aryl" is used herein as known to the expert skilled in the art and refers to an aromatic residue consisting solely of hydrogen and carbon atoms, such as a phenyl (C_6H_5-), naphthyl ($C_{10}H_7$-) pyrenyl- or anthracenyl ($C_{14}H_9$-) residue. The aryl can be substituted or unsubstituted with e.g. alkyl groups, such as methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl, or tert-butyl, or alkoxy- such as methoxy- ethoxy- or isopropoxy- or halogen atoms, such as bromide, chloride, or fluoride.

[0022] The term "heteroaryl" is used herein as known to the expert skilled in the art and refers to to a cyclic aromatic group having five or six ring atoms wherein at least one ring atom is selected from the group consisting of oxygen, sulfur, and nitrogen, and the remaining ring atoms are carbon. The heteroaromatic ring may form a fused heteroaromatic system together with other aryl- or heteroaryl- rings such as benzothiophene, benzimidazole, pteridine or alloxazine.

[0023] The term "alkyl" is used herein as known to the expert skilled in the art and refers to a univalent residue consisting only of carbon and hydrogen atoms. The alkyls form homologous series with the general formula C_nH_{2n+1}. The alkyl can be a straight or branched alkyl, for example the alkyl can be a secondary alkyl which is branched with the central carbon atom linked to two carbon residues or a tertiary alkyl which is branched with the central carbon atom linked to three carbon residues.

[0024] The letter A in the group -A-O- represents a "fragmentation linker" comprising from 1 to 2 linearly, covalently connected atoms such as methylene- or ethylene-. The term "fragmentation linker" is used herein as known to the expert skilled in the art and relates to a moiety which is used as a moiety in photochemistry that effects the light-induced fission of the PLPG by transforming the primary photoprocess into a chemical cleavage reaction. Accordingly, in a first aspect the divalent group -A- refers to a linking group which connects the functional group R2 with the nitrophenyl-chromophore. In one embodiment the 1 to 2 atom chain of the linking group A can be fully comprised of hydrogen and carbon atoms in form of a substituted or unsubstituted, branched or linear, saturated or unsaturated hydrocarbon chain.

[0025] The hydrocarbon chain can also be branched having one or more alkyl groups, wherein the alkyl group can be methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl, or tert-butyl.

[0026] Such a hydrocarbon chain can also be substituted by e.g. halogen atoms. Accordingly, from 1 hydrogen atom to 3

hydrogen atoms of the respective hydrocarbon chain can be substituted through e.g. halogen.

[0027] The term "branched" in context with the definition of the term linking group is used herein as known to the expert skilled in the art and refers to the presence of a side-chain at the main chain of the molecule or moiety. Accordingly, a branched linking group can be a hydrocarbon chain as defined above having one or more alkyl groups as side chain, wherein the alkyl group is methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl, or tert-butyl, preferably a methyl or ethyl group. In the branched hydrocarbon chain represented by A from one to all carbon atoms can have one or more alkyl groups as defined above.

[0028] The term "saturated" in context with the definition of the term linking group is used herein as known to the expert skilled in the art and relates to a linking group in which all members of the group are connected to the respective adjacent atom(s) through single bonds. Accordingly, a saturated hydrocarbon chain is represented by the formula $-(CH_2)_n-$ with n being an integer ranging from 1 to 2.

[0029] The term functional group" is used herein as known to the expert skilled in the art and refers to any of numerous combinations of atoms that form parts of chemical molecules, that undergo characteristic reactions themselves, and that in many cases influence the reactivity of the remainder of the molecule. Typical functional groups are hydroxyl, carboxyl, aldehyde, carbonyl, amino, azide, alkynyl, thiol and nitril.

[0030] The term "solid support" is used herein as known to the expert skilled in the art and refers to any insoluble and rigid or semi-rigid inorganic or organic material, preferably having a large surface area to which surface organic molecules can be attached through bond formation or absorbed through electronic or static interactions such as through bond formation through a functional group.

[0031] The term "biomolecule" is used herein as known to the expert skilled in the art and refers to any organic molecule that is produced by a living organism or to any artificially produced derivatives of such compounds, including large polymeric molecules such as proteins, polysaccharides, carbohydrates, lipids, nucleic acids and oligonucleotides as well as small molecules such as primary metabolites, secondary metabolites, and natural products.

[0032] The term "nucleic acid" is used herein as known to the expert skilled in the art and refers to a macromolecule composed of chains of monomeric nucleotides, wherein each nucleotide consists of three components: a nitrogenous heterocyclic base, which is either a purine or pyrimidine; a pentose sugar; and a phosphate group.

[0033] The term "natural amino acid" is used herein as known to the expert skilled in the art and refers to one of the 20 canonical amino acids used for protein biosynthesis as well as all amino acids which can be incorporated into proteins during translation (including pyrrolysine and selenocysteine). The 20 canonical amino acids include histidine, alanine, valine, glycine, leucine, isoleucine, aspartic acid, glutamic acid, serine, glutamine, asparagine, threonine, arginine, proline, phenylalanine, tyrosine, tryptophan, cysteine, methionine and lysine.

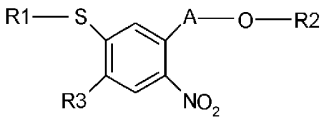
[0034] The term "non-natural amino acid" is used herein as known to the expert skilled in the art and refers to organic compounds that are not among those encoded by the standard genetic code or incorporated into proteins during translation. Furthermore, the term "non-natural amino acid" refers to organic compounds that do not occur naturally. Therefore, non-natural amino acids include amino acids or analogs of amino acids, but are not limited to, the D-isostereomers of amino acids, citrulline, homocitrulline, homoarginine, hydroxyproline, homoproline, ornithine, 4-amino-phenylalanine, cyclohexylalanine, α-aminoisobutyric acid, N-methyl-alanine, N-methyl-glycine, norleucine, N-methyl-glutamic acid, tert-butylglycine, α-aminobutyric acid, tert-butylalanine, 2-aminoisobutyric acid, α-aminoisobutyric acid, 2-aminoindane-2-carboxylic acid, selenomethionine, dehydroalanine, lanthionine, γ-amino butyric acid, and derivatives thereof wherein the amine nitrogen has been mono- or di-alkylated.

[0035] The term "peptide" is used herein as known to the expert skilled in the art and refers to organic compounds made of amino acids arranged in a linear chain and joined together by peptide bonds between the carboxyl and amino groups of adjacent amino acid residues.

[0036] The term "amino group" is used herein as known to the expert skilled in the art and refers to primary ($-NH_2$), secondary ($-NHR_1$), or tertiary ($-N\,R_1\,R_2$), and in cationic form, may be quaternary ($-N\,R_1\,R_2\,R_3$). Examples of amino groups include, but are not limited to, $-NH_2$, $-NHCH_3$, $-NHC(CH_3)_2$, $-N(CH_3)_2$ and $-N(CH_2CH_3)_2$. Examples of cyclic amino groups include, but are not limited to, aziridino, azetidino, pyrrolidino, piperidino, piperazino, morpholino, and thiomorpholino.

[0037] The term "maskless photolithography" is used herein as known to the expert skilled in the art and refers to a technique for the synthesis of DNA- or peptide-microarrays without the use of photographic masks. The maskless photolithography uses an array of optical switching elements that are individually addressable and operable under software control. Examples for such optical switching elements are micro mirror devices. A preferred micro mirror device is the Digital Light Processor (DLP) from Texas Instruments, Inc.

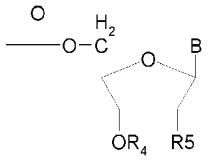
[0038] The present invention relates to diarylsulphid chromophore containing PLPG which can be used for the photolithography based oligonucleotide and peptide synthesis having the structure
[Formula Ia]



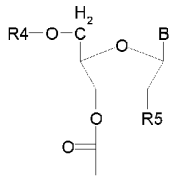
wherein A is selected from the group consisting of -CH2-, -CH_2-CH_2-, - $CH(CH_3)$-, -$CH(CH_3)$-CH_2-, -CH2-CH(Alky,Aryl)- and -CH(CH3)-CH(Alkyl, Aryl)-

R1 is an unsubstituted or substituted aryl- or heteroaryl-group or a condensed aryl-or heteroaryl- group, and R3 is H, a methyl group or an ethyl group, and

wherein R2 is
[Formula II]



or wherein R2 is
[Formula III]

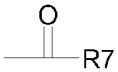


wherein R4 is H, an alkyl-group, aryl-group, or OR4 forms a phosphoramidite, H-phosphonate or phosphate triester and

wherein R5 is H, OH, a halogen or XR6, wherein X is O or S and R6 is an alkyl-group, aryl-group, or OR6 forms a phosphitamide-group, phosphodiester, phosphotriester or H-phosphonate or an acetal or a silicone moiety and

wherein B is selected from the group consisting of adenine, cytosine, guanine, thymine, uracil, 2,6-diaminopurine-9-yl, hypoxanthin-9-yl, 5-methylcytosinyl-1-yl, 5-amino-4-imidazolecarboxylic acid-1-yl or 5-amino-4-imidazolecarboxylic acid amide-3-yl, wherein when B is adenine, cytosine or guanine the primary amino group optionally has a protecting group or when B is thymine or uracil at the O^4 position is optionally a protecting group,

or wherein R2 is
[Formula IV]



wherein R7 is a natural amino acid, a non-natural amino acid or an amino acid derivative, including but not limited to α- or β-amino acids, forming an urethan bond to formula Ia,

or wherein formula IV represents the carboxy function of a natural amino acid, a non-natural amino acid or an amino acid derivative, forming an ester bond to formula Ia, including but not limited to α- or β-amino acids.

[0039] In another embodiment compounds according to formula Ia are used, characterized in that R1 is a phenyl-group, a tert-

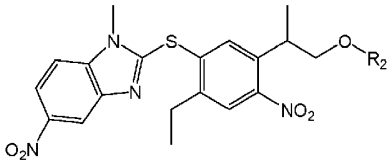
butyl-phenyl group, a 1- or 2-naphthyl-group or a 2- or 4-pyridyl-group, A is -CH(CH_3)-CH_2-, R4 is H and R5 is H, R4 is H and R5 is OH or OSi(Alkyl3).

[0040] In another embodiment compounds according to formula Ia are used, characterized in that B is selected from the group consisting of adenine, cytosine, guanine, thymine, 5-methylcytosineor uracil.

[0041] In another embodiment compounds according to formula Ia are used, characterized in that, when B is adenine, cytosine or guanine the protecting group is phenoxyacetyl-, 4-tert-butyl-phenoxyacetyl-, 4-isopropyl-phenoxyacetyl- or dimethylformamidino-residues, when B is adenine the protecting group is a benzoyl-residue, when B is guanine the protecting group is a isobutyroyl-residue and when B is cytosine the protecting group is benzoyl-- or isobutyroyl -residues. In another embodiment compounds according to formula Ia are used, characterized in that R7 is a natural amino acid.

[0042] The diarylsulphid chromophore containing PLPG which can be used for the photolithography based oligonucleotide and peptide synthesis preferably have the structures:

[Formula V]



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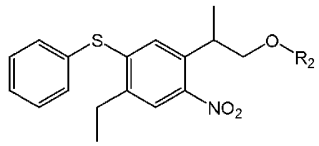
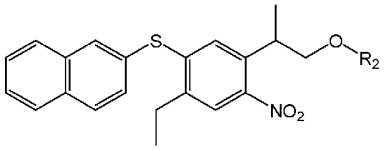
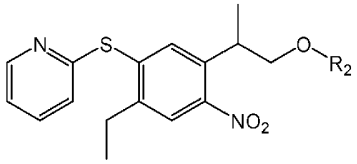


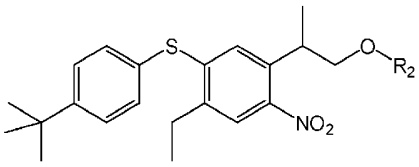
[Formula VII]



[Formula VIII]



[Formula IX]



[0043] The present invention further relates to the use of the compounds according to formula Ia as photoactivable protecting group using maskless photolithography. In one embodiment of the invention micro mirror devices are used to perform a spatial selective exposure of the oligonucleotide and peptide microarrays to visible light in order to deprotect nucleotides and amino acids, respectively, in the exposed areas during the synthesis process. Deprotection of nucleotides and amino acids, respectively, lead to the release of the next linkage site for the respective next nucleotide or amino acid. The next nucleotide or amino acid which should be coupled to the released linkage site within the specific areas is simply added by its provision within a solvent plus an activating reagent which is poured onto the array. This strategy is repeated until oligonucleotides and oligopeptides, respectively, of the desired lengths and design are obtained. Using this strategy it is possible to produce highly dense

microarrays of at least 10 000 and preferably 100 000 to 500 000 features per cm^2.

[0044] The PLPG according to the invention can be removed by using visible light in a range from 375 nm to 420 nm, preferably in the range from 390 to 405 nm. More preferred for deprotection are the wavelengths of 390 nm and 404 nm, respectively. Both wavelengths can be generated using light sources which are much less expensive as compared to light sources necessary to perform deprotection in the near UV range at approximately 365 nm. Preferably, solid state lasers within the range from 375 nm to 420 nm, preferably 390 nm and 404 nm, are used as light sources to remove the PLPG according to the invention. More preferably, LEDs (light emitting diodes) with sufficient emission within the range from 375 nm to 420 nm, preferably 390 nm and 404 nm, are used as light sources to remove the PLPG according to the invention. Especially LEDs are low cost products as they are produced in high quantities, e.g. for the use in Blu-ray Players.

[0045] In a further embodiment micro mirror devices are used, which are optimized for the use of visible light in the range of 375 nm to 420 nm, preferably in the range of 390 to 410 nm, more preferably at 390 nm and 404 nm, respectively. In a further embodiment the coating of the micro mirror devices remain on the devices in order to be used with visible light. Devices that are used for UV- or near UV-light have to be optimized for that purpose, i.e. the coating on the micro mirror elements has to be removed by polishing.

[0046] In another embodiment, LCD displays or a beam splitter can be used as virtual masks between the light source and the synthesis area.

[0047] Photolithographic synthesis of the oligonucleotides and peptides, respectively, can be performed on a support, preferably a solid support. The support can be made of any material known by the skilled person used for such a purpose, preferably the support is made of plastic, silicon, diamond carbon or glass. More preferably, plastic or glass is used as a support, much more preferred as material is optical grade polyolefin or optical grade microscope glass slides. The support can be provided in any form, such as beads, gels, plates, membranes, slides or preferably chips. The support can be transparent or non-transparent, preferably the support exhibits at least 30 %, preferably at least 60 %, most preferably at least 90 % light transmission at a wavelengths of between 375 nm to 410 nm.

[0048] The PLPG according to the invention can be used in any process for oligonucleotide synthesis known by the skilled person where protected nucleosides or nucleotides are necessary. Preferably, the PLPG-nucleotides as described herein can be used for the synthesis of oligonucleotides in solution, more preferably the PLPG-nucleotides as described herein can be used for the synthesis of oligonucleotides on a solid support. The synthesis can be performed by any standard method known in the state of the art. More preferably the synthesis can be performed by using photolithographic techniques, such as maskless techniques wherein a micro mirror device is used to expose light to spatial selected features on a microarray as explained above.

[0049] Solvents known by the skilled person can be used during oligonucleotide synthesis, such as acetonitrile.

[0050] The PLPG associated to nucleosides or nucleotides for oligonucleotide synthesis can be used in a concentration within the solvents of 1 mmol/L to 100 mmol/L. Preferably in a concentration of 10 mmol/L to 40 mmol/L. More preferably, the PLPG-nucleotides can be used in a concentration of 25 mmol/L.

[0051] The PLPG associated to nucleosides or nucleotides can be used in connection with sensitizing agents known by the skilled person, which increase the effectiveness of the deprotection reaction. As sensitizing agents can be particularly used benzophenone, xanthone and thioxanthone derivates, like e.g. thioxanthen-9-one, alkylthioxanthen-9-ones, as for example isopropylthioxanthen-9-one, 2-ethylthioxanthen-9-one, 2-chloro-thioxanthen-9-one, 1,4-dimethoxythioxanthen-9-one.

[0052] Oligonucleotide microarrays can be used for a variety of purposes, including but not limited to sequence capturing, comparative genomic hybridization (CGH), CHIP-chip analysis, DNA-methylation analysis, gene expression analysis and comparative genome sequencing.

[0053] In another embodiment compounds according to formula Ia are used in the maskless photolithography based DNA array synthesis as intermediate or permanent OH-protecting group in nucleoside derivatives at the 3'-OH end or the 5'-OH end as carbon ester, wherein the synthesis can be performed in 3'-5'-direction or in 5'-3'-direction.

[0054] If the PLPG is located at the 5'-end, the nucleotide carries a phosphoramidite group on its 3'-end, which can be reacted with a free -OH group on the solid support to form a stable elongated oligonucleotide. After all oligonucleotides are synthesized, all PLPG are removed and the oligonucleotide still bound to the solid support has a free 5'-OH.

[0055] If however the PLPG is located at the 3'-end, the nucleotide carries a phosphoramidite group on its 5'-end, which can be reacted with any free -OH group on the solid support to form a stable elongated oligonucleotide. After all oligonucleotides are synthesized, all PLPG are removed and the oligonucleotide still bound to the solid support has a free 3'-OH.

[0056] While both types of immobilization allow for hybridization based assays, only the oligonucleotides that exhibit a free 3'-OH may be used for enzymatic reactions for detection, labeling, capping or elongation by ligation or enzymatic polymerization. The PLPG according to the invention can further be used in any process for peptide synthesis known by the skilled person where protected amino acids are necessary. The used amino acids can be non-natural amino acids, amino acid derivatives and preferably natural amino acids. Preferably, the PLPG as described herein can be used for the synthesis of oligopeptides in solution, more preferably the PLPG as described herein can be used for the synthesis of oligopeptides on a solid support. The synthesis can be performed by any standard method known in the state of the art. More preferably the synthesis can be performed by using photolithographic techniques, such as techniques where a micro mirror device is used to expose visible light to spatial selected features on a microarray as explained above.

[0057] It has been shown that the deprotection reaction is dependent on the solvent used during the peptide synthesis process. Solvents known by the skilled person can be used during peptide synthesis. Preferably, polar solvents like dimethylsulfoxide (DMSO), n-methylpyrrolidone (NMP), acetonitrile (MeCN) or isopropanol can be used. Said solvents can contain certain additives, preferably imidazole, hydroxylamine and WATER. Imidazole can be added at concentrations of 0.1 % to 3 % (v/v), preferably of 0.5 % to 1.5 % (v/v), more preferably imidazole can be added at a concentration of 1 % (v/v). Hydroxylamine can be added at concentrations of 0.1 % to 3 % (v/v), preferably of 0.2 % to 1 % (v/v), more preferably hydroxylamine can be added at a concentration of 1 % (v/v). Water can be added at concentrations of 0.1 % to 20 % (v/v), preferably of 1 % to 17 % (v/v), more preferably water can be added at a concentration of 1 % (v/v). Most preferred as solvents are DMSO, DMSO + 1 % imidazole, NMP + 0.5 % hydroxylamine, MeCN + 1 % H_2O, MeCN + 1 % H_2O + 1 % imidazole, isopropanol + 1 % imidazole, isopropanol + 12 % H_2O + 1 % imidazole.

[0058] The PLPG associated to amino acids for peptide synthesis can be used in a concentration within the solvents of 0.1 mmol/L to 0.5 mmol/L. Preferably in a concentration of 0.2 mmol/L to 0.4 mmol/L. More preferably, the PLPG can be used in a concentration of 0.3 mmol/L.

[0059] The PLPG associated to amino acids can be used in connection with sensitizing agents known by the skilled person, which increase the effectiveness of the deprotection reaction.

[0060] Oligopeptide microarrays can be used for a variety of purposes, including but not limited to screening of antibody libraries, quantitative or qualitative analysis of biological samples, biomarker discovery, enrichment of scarce proteins, depletion of high abundant proteins, analysis of protein-protein-interactions, analysis of DNA-protein-interactions or RNA-protein-interactions.

[0061] In another embodiment compounds according to formula Ia are used for the maskless photolithography based peptide array synthesis as NH-protecting group in amino acids as urethan. The PLPG is used as NH-blocked free acid, activated ester, acid halogenide, anhydride, intermolecular or intramolecular as N-carboxy-anhydride (NCA).

[0062] In another embodiment the compounds according to formula Ia are used for the maskless photolithography based peptide array synthesis as COOH-protecting group in amino acids as ester for inverse direction of synthesis.

[0063] The PLPG according to the invention can further be used in any process known by the skilled person where protected sugars are necessary. The sugars used can be compounds, such as aldohexoses and aldopentoses. Preferably, the PLPG as described herein can be used for the synthesis of carbohydrates, glycoproteins and proteoglycans in solution, more preferably the PLPG as described herein can be used for the synthesis of carbohydrates, glycoproteins and proteoglycans on a solid support. The synthesis can be performed by any standard method known in the state of the art. More preferably the synthesis can be performed by using photolithographic techniques, such as techniques where a micro mirror device is used to expose visible light to spatial selected features on a microarray as explained above.

[0064] Carbohydrate microarrays can be used for a variety of purposes, including but not limited to analysis of saccharide-protein-interactions, high-throughput analysis of proteins and cells, analysis of glycans and their molecular interactions,

[0065] In another embodiment the compounds according to formula Ia are used for the maskless photolithography based

synthesis of carbohydrates, glycoproteins, proteoglycans, and the like, as OH-protecting group as ether.

[0066] In another embodiment the compounds according to formula Ia are used as SH-protecting group for orthogonal strategies as ether, ester or thiocarbonate.

[0067] In another embodiment the compounds according to formula Ia are used as photoactivable protecting groups for releasing an biologically active structure for the initiation of a polymerase reaction or a ATP-dependent biochemical conversion.

[0068] The present invention further relates to the use of the compound according to formula Ia, characterized in that light is used for the maskless photolithography having a wavelength of 375 to 405 nm, preferably of 390 nm.

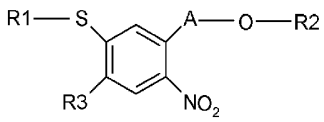
[0069] The present invention further relates to a method for producing the diarylsulphid backbone containing PLPG which can be used for the photolithography based oligonucleotide and peptide synthesis, wherein the method comprises the following steps:

1. a) Providing as a starting material p-Diethylbenzene.
2. b) Bromination of the phenyl ring in one position by the action of molecular bromine and purification by distillation.
3. c) Nitration of the obtained compound in Nitric- and Sulfuric Acid in the position para- to the Bromine and isolation and purification by column chromatography on silica gel and crystallization.
4. d) Hydroxymethylation of the compound by the action of para-Formaldehyde in DMSO and Triton B at the benzylic position.
5. e) Conversion of the aromatic bromine group to the aryl sulfide by action of the appropriate thiophenol, thionaphthol etc in DMF potassium carbonate and catalytic amounts of copper(II) salt and purification by column chromatography on silica gel.
6. f) Conversion of the previous alcohol to the chlorocarbonate by action of triphosgen in THF and triethylamine.
7. g) Reaction of the chlorocarbonate with the appropriate nucleoside and further reacting the nucleoside with a phosphitylating agent to the appropriate phospshoramidite, or
   reaction of the chlorocarbonate with the appropriate amino acid derivative.

[0070] The following examples are provided to aid the understanding of the present invention, the true scope of which is set forth in the appended claims. It is understood that modifications can be made in the procedures set forth without departing from the spirit of the invention.

[0071] Further embodiments are included by the following items:

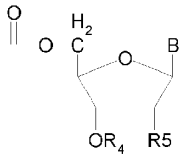
1. 1. A compound of the formula
   [Formula Ia]



wherein A is selected from the group consisting of -CH_2-, -CH_2-CH_2-, - $CH(CH_3)$-, -$CH(CH_3)$-CH_2-, and

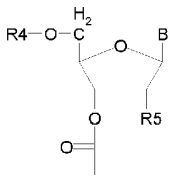
R1 is an unsubstituted or substituted aryl- or heteroaryl-group, and R3 is H, a methyl group or an ethyl group, and

wherein R2 is
[Formula II]



or wherein R2 is

[Formula III]



wherein R4 is H, forms a phosphoramidite, H-phosphonate or phosphate triester, and

wherein R5 is H, OH, a halogen or XR6, wherein X is O or S and R6 is H, an alkyl-group, aryl-group, or OR6 forms a phosphoramidite, phosphodiester, phosphotriester, H-phosphonate or an acetal or silicone moiety, and

wherein B is selected from the group consisting of adenine, cytosine, guanine, thymine, uracil, 2,6-diaminopurine-9-yl, hypoxanthin-9-yl, 5-methylcytosinyl-1-yl, 5-amino-4-imidazolecarboxylic acid-1-yl or 5-amino-4-imidazolecarboxylic acid amide-3-yl, wherein when B is adenine, cytosine or guanine the primary amino group optionally has a protecting group or when B is thymine or uracil at the O^4 position is optionally a protecting group, or

wherein R_2 is
[Formula IV]

wherein R7 is a natural amino acid, a non-natural amino acid or an amino acid derivative forming an urethan bond to formula Ia, or

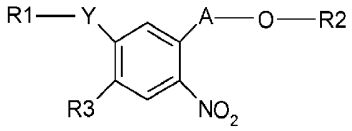
wherein formula IV represents the carboxy function of a natural amino acid, a non-natural amino acid or an amino acid derivative, forming an ester bond to formula Ia.

2. 2. The compound according to item 1, characterized in that R1 is a phenyl-group, a tert-butyl-phenyl group, a 1- or 2-naphthyl-group or a 2-pyridyl-group.
3. 3. The compound according to item 1 or 2, characterized in that A is $-CH(CH_3)-CH_2-$.
4. 4. The compound according to items 1 to 3, characterized in that R3 is H or an ethyl group.
5. 5. The compound according to items 1 to 4, characterized in that R4 is H and R5 is H.
6. 6. The compound according to items 1 to 5, characterized in that B is selected from the group consisting of adenine, cytosine, guanine, thymine or uracil.
7. 7. The compound according to items 1 to 6, characterized in that, when B is adenine, cytosine or guanine the protecting group is phenoxyacetyl-, 4-tert-butyl-phenoxyacetyl-, 4-isopropyl-phenoxyacetyl- or dimethylformamidino-residues, when B is adenine the protecting group is benzoyl- or p-nitro-phenyl-ethoxy-carbonyl- (p-NPPOC)-residues, when B is guanine the protecting group is isobutyroyl-, p-nitrophenylethyl (p-NPE) or p-NPEOC-residues and when B is cytosine the protecting group is benzoyl-, isobutyryl- or p-NPEOC-residues.
8. 8. The compound according to items 1 to 4, characterized in that R7 is a natural amino acid.
9. 9. Use of the compound according to items 1 to 8 as photoactivable protecting group using maskless photolithography.
10. 10. Use of the compound according to item 7 for the maskless photolithography based DNA array synthesis as intermediate or permanent OH-protecting group in nucleoside derivatives at the 3'-OH end or the 5'-OH end.
11. 11. Use of the compound according to item 8 for the maskless photolithography based peptide array synthesis as NH-protecting group in amino acids.
12. 12. Use of the compound according to item 8 for the maskless photolithography based peptide array synthesis as COOH-protecting group in amino acids.
13. 13. Use of the compound according to item 8 for the maskless photolithography based synthesis of carbohydrates as OH-protecting group.
14. 14. Use of the compound according to item 8 for orthogonal protecting group strategy as SH-protecting group.
15. 15. Use of the compound according to items 8 to 13, characterized in that light is used for the maskless photolithography having a wavelength of 374 to 405 nm, preferably of 390 nm.
16. 16. A method for preparing a diarylsulphid backbone containing photolabile protecting group according to one of the items 1 to 8 comprising the steps of
    1. a) Provision of p-diethylbenzene as a starting material
    2. b) Bromination of the phenylring
    3. c) Nitration of the obtained compound in Nitric- and Sulfuric Acid in the position para- to the Bromine
    4. d) Purification and crystallization

5. e) Hydroxymethylation of the compound at the benzylic position
6. f) Conversion of the aromatic bromine group to the arylsulfide using thiophenol
7. g) Purification
8. h) Conversion of the alcohol to chlorocarbonate
9. i) Reaction of the chlorocarbonate with a nucleoside and reaction of the nucleoside with a phosphitylating agent, or Reaction of the chlorocarbonate with an amino acid derivative.

17. 17. The method according to item 16, characterized in that R1 is a phenyl-group, a tert-butyl-phenyl group, a 1- or 2-naphthyl-group or a 2-pyridyl-group.
18. 18. The method according to item 16 or 17, characterized in that A is -CH(CH_3)-CH_2-.
19. 19. The method according to item 16 to 18, characterized in that R3 is H or an ethyl group.

[0072] The present disclosure further relates to diarylsulphid chromophore containing PLPG which can be used for the photolithography based oligonucleotide and peptide synthesis having the structure
[Formula Ib]

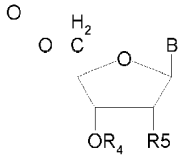


Wherein Y is S and

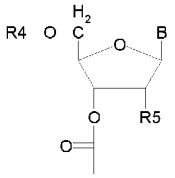
A is selected from the group consisting of -CH2-, -CH_2-CH_2-, -CH(CH_3)-, - CH(CH_3)-CH_2-, -CH2-CH(Alky,Aryl)- and -CH(CH3)-CH(Alkyl, Aryl)-

R1 is an unsubstituted or substituted aryl- or heteroaryl-group or a condensed aryl-or heteroaryl- group, and R3 is H, a methyl group or an ethyl group, and wherein R2 is H, forms a phosphoramidite, H-phosphonate or phosphate triester, or

wherein R2 is
[Formula II]



or wherein R2 is
[Formula III]

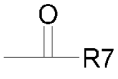


wherein R4 is H, an alkyl-group, aryl-group, or OR4 forms a phosphoramidite, H-phosphonate or phosphate triester and

wherein R5 is H, OH, a halogen or XR6, wherein X is O or S and R6 is an alkyl-group, aryl-group, or OR6 forms a phosphitamide-group, phosphodiester, phosphotriester or H-phosphonate or an acetal or a silicone moiety and

wherein B is selected from the group consisting of adenine, cytosine, guanine, thymine, uracil, 2,6-diaminopurine-9-yl, hypoxanthin-9-yl, 5-methylcytosinyl-1-yl, 5-amino-4-imidazolecarboxylic acid-1-yl or 5-amino-4-imidazolecarboxylic acid amide-3-yl, wherein when B is adenine, cytosine or guanine the primary amino group optionally has a protecting group or when B is thymine or uracil at the O^4 position is optionally a protecting group,

or wherein R2 is
[Formula IV]



wherein R7 is a natural amino acid, a non-natural amino acid or an amino acid derivative, including but not limited to α- or β-amino acids, forming an urethan bond to formula Ib,

or wherein formula IV represents the carboxy function of a natural amino acid, a non-natural amino acid or an amino acid derivative, forming an ester bond to formula Ib, including but not limited to α- or β-amino acids.

[0073] In another embodiment compounds according to formula Ib are used, characterized in that R1 is a phenyl-group, a tert-butyl-phenyl group, a 1- or 2-naphthyl-group, an aminophenyl-group, an N-alkylaminophenyl-group, an N-Acylaminophenyl-group, a carboxyphenyl-group, a phenylcarboxylic ester, an amide or a 2- or 4-pyridyl-group, A is $-CH(CH_3)-CH_2-$, R2 is a phosphoramidite or $-P(OCH_2CH_2CN)(N\text{-}iPr_2)$, R3 is H or an ethyl group, R4 is H and R5 is H, R4 is H and R5 is OH or OSi(Alkyl3).

[0074] In another embodiment compounds according to formula Ib are used, characterized in that B is selected from the group consisting of adenine, cytosine, guanine, thymine, 5-methylcytosineor uracil.

[0075] In another embodiment compounds according to formula Ib are used, characterized in that, when B is adenine, cytosine or guanine the protecting group is phenoxyacetyl-, 4-tert-butyl-phenoxyacetyl-, 4-isopropyl-phenoxyacetyl- or dimethylformamidino-residues, when B is adenine the protecting group is a benzoyl-residue, when B is guanine the protecting group is a isobutyroyl-residue and when B is cytosine the protecting group is benzoyl-- or isobutyroyl -residues.

[0076] In another embodiment compounds according to formula Ib are used, characterized in that R7 is a natural amino acid.

[0077] The present disclosure further relates to the use of the compounds according to formula Ib as photoactivable protecting groups using maskless photolithography. In one embodiment of the disclosure micro mirror devices are used to perform a spatial selective exposure of the oligonucleotide and peptide microarrays to visible light in order to deprotect nucleotides and amino acids, respectively, in the exposed areas during the synthesis process. Deprotection of nucleotides and amino acids, respectively, lead to the release of the next linkage site for the respective next nucleotide or amino acid. The next nucleotide or amino acid which should be coupled to the released linkage site within the specific areas is simply added by its provision within a solvent plus an activating reagent which is poured onto the array. This strategy is repeated until oligonucleotides and oligopeptides, respectively, of the desired lengths and design are obtained. Using this strategy it is possible to produce highly dense microarrays of at least 10 000 and preferably 100 000 to 500 000 features per cm^2.

[0078] The PLPG according to the disclosure can be removed by using visible light in a range from 375 nm to 420 nm, preferably in the range from 390 to 405 nm. More preferred for deprotection are the wavelengths of 390 nm and 404 nm, respectively. Both wavelengths can be generated using light sources which are much less expensive as compared to light sources necessary to perform deprotection in the near UV range at approximately 365 nm. Preferably, solid state lasers within the range from 375 nm to 420 nm, preferably 390 nm and 404 nm, are used as light sources to remove the PLPG according to the disclosure. More preferably, LEDs (light emitting diodes) with sufficient emission within the range from 375 nm to 420 nm, preferably 390 nm and 404 nm, are used as light sources to remove the PLPG according to the disclosure. Especially LEDs are low cost products as they are produced in high quantities, e.g. for the use in Blu-ray Players.

[0079] In a further embodiment micro mirror devices are used, which are optimized for the use of visible light in the range of 375 nm to 420 nm, preferably in the range of 390 to 410 nm, more preferably at 390 nm and 404 nm, respectively. In a further embodiment the coating of the micro mirror devices remain on the devices in order to be used with visible light. Devices that are used for UV- or near UV-light have to be optimized for that purpose, i.e. the coating on the micro mirror elements has to be removed by polishing.

[0080] In another embodiment, LCD displays or a beam splitter can be used as virtual masks between the light source and the synthesis area.

[0081] Photolithographic synthesis of the oligonucleotides and peptides, respectively, can be performed on a support, preferably a solid support. The support can be made of any material known by the skilled person used for such a purpose, preferably the support is made of plastic, silicon, diamond carbon or glass. More preferably, plastic or glass is used as a support, much more preferred as material is optical grade polyolefin or optical grade microscope glass slides. The support can be provided in any form, such as beads, gels, plates, membranes, slides or preferably chips. The support can be transparent or non-transparent, preferably the support exhibits at least 30 %, preferably at least 60 %, most preferably at least 90 % light transmission at a wavelengths of between 375 nm to 410 nm.

[0082] The PLPG according to the disclosure can be used in any process for oligonucleotide synthesis known by the skilled person where protected nucleosides or nucleotides are necessary. Preferably, the PLPG-nucleotides as described herein can be used for the synthesis of oligonucleotides in solution, more preferably the PLPG-nucleotides as described herein can be used for the synthesis of oligonucleotides on a solid support. The synthesis can be performed by any standard method known in the state of the art. More preferably the synthesis can be performed by using photolithographic techniques, such as maskless techniques wherein a micro mirror device is used to expose light to spatial selected features on a microarray as explained above.

[0083] Solvents known by the skilled person can be used during oligonucleotide synthesis, such as acetonitrile.

[0084] The PLPG associated to nucleosides or nucleotides for oligonucleotide synthesis can be used in a concentration within the solvents of 1 mmol/L to 100 mmol/L. Preferably in a concentration of 10 mmol/L to 40 mmol/L. More preferably, the PLPG-nucleotides can be used in a concentration of 25 mmol/L.

[0085] The PLPG associated to nucleosides or nucleotides can be used in connection with sensitizing agents known by the skilled person, which increase the effectiveness of the deprotection reaction. As sensitizing agents can be particularly used benzophenone, xanthone and thioxanthone derivates, like e.g. thioxanthen-9-one, alkylthioxanthen-9-ones, as for example isopropylthioxanthen-9-one, 2-ethylthioxanthen-9-one, 2-chloro-thioxanthen-9-one, 1,4-dimethoxythioxanthen-9-one.

[0086] Oligonucleotide microarrays can be used for a variety of purposes, including but not limited to sequence capturing, comparative genomic hybridization (CGH), CHIP-chip analysis, DNA-methylation analysis, gene expression analysis and comparative genome sequencing.

[0087] In another embodiment compounds according to formula Ib are used in the maskless photolithography based DNA array synthesis as intermediate or permanent OH-protecting group in nucleoside derivatives at the 3'-OH end or the 5'-OH end as carbon ester, wherein the synthesis can be performed in 3'-5'-direction or in 5'-3'-direction.

[0088] If the PLPG is located at the 5'-end, the nucleotide carries a phosphoramidite group on its 3'-end, which can be reacted with a free -OH group on the solid support to form a stable elongated oligonucleotide. After all oligonucleotides are synthesized, all PLPG are removed and the oligonucleotide still bound to the solid support has a free 5'-OH.

[0089] If however the PLPG is located at the 3'-end, the nucleotide carries a phosphoramidite group on its 5'-end, which can be reacted with any free -OH group on the solid support to form a stable elongated oligonucleotide. After all oligonucleotides are synthesized, all PLPG are removed and the oligonucleotide still bound to the solid support has a free 3'-OH.

[0090] While both types of immobilization allow for hybridization based assays, only the oligonucleotides that exhibit a free 3'-OH may be used for enzymatic reactions for detection, labeling, capping or elongation by ligation or enzymatic polymerization.

[0091] The PLPG according to the disclosure can further be used in any process for peptide synthesis known by the skilled person where protected amino acids are necessary. The used amino acids can be non-natural amino acids, amino acid derivatives and preferably natural amino acids. Preferably, the PLPG as described herein can be used for the synthesis of oligopeptides in solution, more preferably the PLPG as described herein can be used for the synthesis of oligopeptides on a solid support. The synthesis can be performed by any standard method known in the state of the art. More preferably the synthesis can be performed by using photolithographic techniques, such as techniques where a micro mirror device is used to expose visible light to spatial selected features on a microarray as explained above.

[0092] It has been shown that the deprotection reaction is dependent on the solvent used during the peptide synthesis process. Solvents known by the skilled person can be used during peptide synthesis. Preferably, polar solvents like dimethylsulfoxide (DMSO), n-methylpyrrolidone (NMP), acetonitrile (MeCN) or isopropanol can be used. Said solvents can contain certain additives, preferably imidazole, hydroxylamine and water. Imidazole can be added at concentrations of 0.1 % to 3 % (v/v), preferably of 0.5 % to 1.5 % (v/v), more preferably imidazole can be added at a concentration of 1 % (v/v). Hydroxylamine can be added at concentrations of 0.1 % to 3 % (v/v), preferably of 0.2 % to 1 % (v/v), more preferably hydroxylamine can be added at a concentration of 1 % (v/v). Water can be added at concentrations of 0.1 % to 20 % (v/v), preferably of 1 % to 17 % (v/v), more preferably water can be added at a concentration of 1 % (v/v). Most preferred as solvents are DMSO, DMSO + 1 % imidazole, NMP + 0.5 % hydroxylamine, MeCN + 1 % H_2O, MeCN + 1 % H_2O + 1 % imidazole, isopropanol + 1 % imidazole, isopropanol + 12 % H_2O + 1 % imidazole.

[0093] The PLPG associated to amino acids for peptide synthesis can be used in a concentration within the solvents of 0.1 mmol/L to 0.5 mmol/L. Preferably in a concentration of 0.2 mmol/L to 0.4 mmol/L. More preferably, the PLPG can be used in a concentration of 0.3 mmol/L.

[0094] The PLPG associated to amino acids can be used in connection with sensitizing agents known by the skilled person, which increase the effectiveness of the deprotection reaction.

[0095] Oligopeptide microarrays can be used for a variety of purposes, including but not limited to screening of antibody libraries, quantitative or qualitative analysis of biological samples, biomarker discovery, enrichment of scarce proteins, depletion of high abundant proteins, analysis of protein-protein-interactions, analysis of DNA-protein-interactions or RNA-protein-interactions.

[0096] In another embodiment compounds according to formula Ib are used for the maskless photolithography based peptide array synthesis as NH-protecting group in amino acids as urethan. The PLPG is used as NH-blocked free acid, activated ester, acid halogenide, anhydride, intermolecular or intramolecular as N-carboxy-anhydride (NCA).

[0097] In another embodiment the compounds according to formula Ib are used for the maskless photolithography based peptide array synthesis as COOH-protecting group in amino acids as ester for inverse direction of synthesis.

[0098] The PLPG according to the disclosure can further be used in any process known by the skilled person where protected sugars are necessary. The sugars used can be compounds, such as aldohexoses and aldopentoses. Preferably, the PLPG as described herein can be used for the synthesis of carbohydrates, glycoproteins and proteoglycans in solution, more preferably the PLPG as described herein can be used for the synthesis of carbohydrates, glycoproteins and proteoglycans on a solid support. The synthesis can be performed by any standard method known in the state of the art. More preferably the synthesis can be performed by using photolithographic techniques, such as techniques where a micro mirror device is used to expose visible light to spatial selected features on a microarray as explained above.

[0099] Carbohydrate microarrays can be used for a variety of purposes, including but not limited to analysis of saccharide-protein-interactions, high-throughput analysis of proteins and cells, analysis of glycans and their molecular interactions,

[0100] In another embodiment the compounds according to formula Ib are used for the maskless photolithography based synthesis of carbohydrates, glycoproteins, proteoglycans, and the like, as OH-protecting group as ether.

[0101] In another embodiment the compounds according to formula Ib are used as SH-protecting group for orthogonal strategies as ether, ester or thiocarbonate.

[0102] In another embodiment the compounds according to formula Ib are used as photoactivable protecting groups for releasing an biologically active structure for the initiation of a polymerase reaction or a ATP-dependent biochemical conversion.

[0103] The present disclosure further relates to the use of the compound according to formula Ib, characterized in that light is used for the maskless photolithography having a wavelength of 375 to 405 nm, preferably of 390 nm.

[0104] The present disclosure further relates to a method for producing the diarylsulphid backbone containing PLPG which can be used for the photolithography based oligonucleotide and peptide synthesis, wherein the method comprises the following steps:

1. a) Providing as a starting material p-Diethylbenzene.
2. b) Bromination of the phenyl ring in one position by the action of molecular bromine and purification by distillation.
3. c) Nitration of the obtained compound in Nitric- and Sulfuric Acid in the position para- to the Bromine and isolation and purification by column chromatography on silica gel and crystallization.
4. d) Hydroxymethylation of the compound by the action of para-Formaldehyde in DMSO and Triton B at the benzylic position.
5. e) Conversion of the aromatic bromine group to the aryl sulfide by action of the appropriate thiophenol, thionaphthol etc in DMF potassium carbonate and catalytic amounts of copper(II) salt and purification by column chromatography on silica gel.
6. f) Conversion of the previous alcohol to the chlorocarbonate by action of triphosgen in THF and triethylamine.
7. g) Reaction of the chlorocarbonate with the appropriate nucleoside and further reacting the nucleoside with a phosphitylating agent to the appropriate phospshoramidite, or
   reaction of the chlorocarbonate with the appropriate amino acid derivative.

Example 1:

Evaluation of the Half-life of 5PyS4EtNPPOC-Thymidine in Dependence of the Solvent at a Wavelengths of 390 nm and 404 nm

[0105] To evaluate the half-life of the PLPG, 5PyS4EtNPPOC-Thymidine was dissolved in a concentration of c = 0.3 mmol/L in the solvents given in Figs. 1 and 2. As solvents dimethylsulfoxide (DMSO), n-methylpyrrolidone (NMP), acetonitrile (MeCN) and isopropanol were used. Imidazole, hydroxylamine or water were added to the solvents as depicted in the table. In case of an irradiation wavelength of 390 nm (Fig. 1), light exposure was performed for 2, 4, 6 s or 2, 4, 6, 8 s or 2, 4, 6, 8, 12 s, respectively, to induce deprotection of threonine. In case of an irradiation wavelength of 404 nm (Fig. 2), light exposure was performed for 1, 2, 3, 4 min or 1, 2, 3, 5 min, respectively, to induce deprotection of thymidine. Subsequently, the solution was analyzed by HPLC in order to evaluate the time period necessary to deprotect 50 % of the initial amount of the protected thymidine. The half-lives were then extrapolated from the durations resulting from the exposure times. As can be taken from Figs. 1 and 2, in case of a wavelengths of 390 nm, fastest deprotection was achieved with DMSO (2.1 s), NMP + 0.5 % hydroxylamine (1.8 s), MeCN + 1 % H_2O(2. s) and isopropanol + 12 % H_2O + 1 % imidazole (2.2 s). In case of a wavelengths of 404 nm, fastest deprotection was achieved with DMSO + 1 % imidazole (2.5 min), NMP + 0.5 % hydroxylamine (1.8 min), MeCN + 1 % H_2O + 1 % imidazole (2.6 min) and isopropanol + 12 % H_2O + 1 % imidazole (3.8 min). Concerning the significant time differences between 390 nm (seconds) and 404 nm (minutes), it has to be taken into consideration that in case of the former the power output of the lamp was 15 W, whereas the in case of the latter the power output of the lamp was 0.08 W.

Example 2:

UV Absorption Characteristics of PLPG

[0106] UV absorption for different PLPG at the wavelengths commonly used is depicted in Fig. 3. The appropriate derivatives of phenylalanine with the PLPG according to the invention were dissolved at a concentration of 1 mg/mL in UV grade methanol. UV spectra were recorded in a scanning photometer and absorption values were taken at the given wavelengths. Molar extinction coefficients were calculated from the molecular weight using Lambert Beers law. Deprotection speed of any PLPG is approximately the product of triplett quantum yield times molar extinction coefficient. It may thus be estimated, that PhS-phenylalanine deprotects 15 times more efficient as BTA-phenylalanine and about 25 times more efficient as NPPOC-phenylalanine at an irradiation wavelength 390 nm.

Example 3:

Synthesis of a Peptide Array Containing the Target Sequence of an Anti-V5 Antibody Using Disulfide-PLPG-Amino Acids

[0107] Target-epitope: (H)G KPIPNPLLGLDS T-(OH)

[0108] Peptide features on the array were synthesized in a pattern of varying density on a Roche Nimblegen Maskless Array Synthesizer according to the synthesis scheme in Fig 8, at a light dose of:

Area 1:	2.5 s irradiation at 190 mW/sq.cm [365 nm]
Area 2:	3.5 s irradiation at 190 mW/sq.cm [365 nm]
Area 3:	4.5 s irradiation at 190 mW/sq.cm [365 nm]

[0109] Exposure was in NMP/hydroxylamine (1 %). The standard irradiation time for NPPOC amino acids at this lamp intensity would have been 50 s. All features of the array contained the same V-5 antigen sequence. Coupling was under standard

conditions, 30 mM amino acid, 30 mM activator (HBTU and HOBT) and 60 mM Hünig base in peptide grade DMF sequentially coupled a Greiner 3D-Amino-functionalized microscope slide. Washing between cycles and between coupling and irradiation was with NMP. Final deprotection of the array was achieved by soaking in trifluoro acetic acid, water, triisopropylsilane 97,5:2:0,5 for 1 h. After thorough washing with water, the array was incubated with anti-V5-antibody (labeled with Cy-3 fluorescent dye), obtained from Sigma in the manufacturers recommended buffer system at 1:10 000 dilution (0.1 µg / mL) overnight at room temperature. After washing with buffer and drying the array was scanned at the appropriate filter setting in a Roche Nimblegen MS 200 fluorescent scanner at 2 µm resolution. Images were analyzed in Nimblescan and Genepix (Molecular Dynamics) software packages.

[0110] The results show excellent signal intensity over the three doses as shown in Fig. 8b, indicating complete photodeprotection at less than 500 mW*s, whereas NPPOC-amino acids would require about 10.000 mW*s to achieve the same result.

[0111] In addition, the same experiment was conducted on a Roche Nimblegen Maskless Array Synthesizer according to the synthesis scheme in Fig. 8a, at a Light Dose of:

Area 1-30: 1-30 s irradiation at 90 mW/sq.cm [390 nm]

Incubation, staining and washing was done as mentioned above.

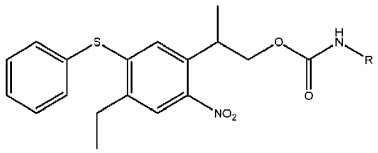
[0112] The results show excellent signal intensity with a maximum at about 21 s irradiation at 390 nm as shown in Fig. 8c, indicating complete photodeprotection at less than 2.000 mW*s, whereas NPPOC-amino acids are insufficiently deprotected at 390 nm and do not give signals attributable to the peptides made.

Example 4:

Synthesis of Disulfide-PLPG-Amino Acids

[0113] Synthesis pathways are depicted in the respective figures as indicated below.

[0114] General formula of phenyl-thio-NPPOC-amino acids



a) 2-(2-Nitro-4-ethyl-5-thiophenyl-phenyl)propanol ("PhSNPPOH")

[0115]

1,4-Diethylbezene	1902.6 g	14.18 mol	1Eq
Bromine	2288 g	14.32 mol	1.01Eq
Iron (powder)	26 g		

[0116] The corresponding synthesis pathway is depicted in Fig. 4a. A few drops of bromine are added to a mixture of 1902.6 g 1,4-diethylbenzene and 26 g of iron powder. The mixture is stirred at ambient temperature until HBr evolution starts. Then the mixture is cooled in an ice bath and further 2288 g of bromine are added under vigorous stirring over a period of approximately 5 h. Then the ice bath is removed and the mixture is stirred over night at ambient temperature. The reaction mixture is washed with water, saturated $NaHCO_3$ solution and again with water. The crude product is diluted with toluene, concentrated and distilled in vacuum (approximately 5 mbar / 82-84°C).

[0117] 2740 g 2,5-diethyl-bromobenzene, a colorless liquid, are obtained (yield: 90 % of theory).

[0118] ^{1}H-NMR (300MHz, DMSO):

[0119] 7.37 ppm (d, 1H, Ar-H); 7.20 ppm (d, 1H, Ar-H); 7.12 ppm (dd, 1H, Ar-H); 2.65 ppm (q, 2H, Ar-C H_2-CH_3); 2.56 ppm (q, 2H, Ar-CH_2-CH_3), 1.20-1.15 ppm (m, 6H, 2x CH_3).

b) 2,5-Diethyl-4-nitro-bromobenzene

[0120]

2,5-Diethyl-bromobenzene	426 g	2 mol
HNO_3 (65%)	202 ml	
H_2SO_4 conc.	241 ml	

[0121] The corresponding synthesis pathway is depicted in Fig. 4b. Under ice cooling 241 mL of H_2SO_4 conc. are added slowly to 202 mL of HNO_3 (65%). Under ice cooling and vigorous stirring this mixture is dropped slowly into 426 g of 2,5-diethyl-bromobenzene (dosing time 2 h). The reaction mixture is stirred over night at ambient temperature. Then the mixture is poured on ice, diluted with dichloromethane, washed twice with water and finally with saturated $NaHCO_3$-solution. The organic phase is diluted with toluene, concentrated in vacuum and purified by fast filtration (silica gel, mobile phase iso-hexane).

[0122] 217 g of a slightly yellow oil are obtained (yield: 42 % of theory).

[0123] ^{1}H-NMR (300MHz, DMSO):

7.74 ppm (s, 2H, Ar-H); 4.72 ppm (t, 1H, OH); 3.54-3.48 ppm (m, 2H, HO-CH_2), 3.26-3.14 ppm (m, 1H, HO-CH_2-CH); 2.74 ppm (q, 2H, Ar-CH_2-CH_3); 1.25-1.15 ppm (m, 6H, 2x CH_3).

c) 2-(2-Nitro-4-ethyl-5-bromophenl)propan-1-ol ("BrEtNPPOH")

[0124]

2,5-Diethyl-4-nitro-bromobenzene	1000	g 3.87 mol	1 Eq
Paraformaldehyde	418.8 g	4.65 mol	1.2 Eq
Triton B (40% in methanol)	1090 ml		
DMSO	5.21		
Acetic Acid	400 ml		

[0125] The corresponding synthesis pathway is depicted in Fig. 4c. A mixture of 1000 g 2,5-diethyl-4-nitro-bromobenzene, 418.8 g paraformaldehyde, 1090 mL triton B (40% in methanol) and 5.2 L DMSO is heated for 2 h at 80 to 90°C. The heating is switched off and the mixture is stirred for further 4 h. 400 mL of acetic acid are added. The mixture is diluted with water to a volume of approximately 15 L and extracted twice with 2 L of toluene. The toluene extract is washed twice with 1 L of water and then concentrated in vacuum. The crude product is purified by chromatography (silica gel, gradient: iso-hexane to iso-hexane/EtOAc 30%).

[0126] Yield: 521 g 2-(2-Nitro-4-ethyl-5-bromophenyl)propan-1-ol as a brown oil (46 % of theory) plus 77 g of lesser purity.

[0127] ^{1}H-NMR (300MHz, DMSO):

7.74 ppm (s, 2H, Ar-H); 4.72 ppm (t, 1H, OH); 3.54-3.48 ppm (m, 2H, HO-CH_2), 3.26-3.14 ppm (m, 1H, HO-CH_2-CH); 2.74ppm (q, 2H, Ar-CH_2-CH_3); 1.25-1.15 ppm (m, 6H, 2x CH_3).

d) 2-(2-Nitro-4-ethyl-5-thiophenylphenyl)propanol (PhSNPPOH)

[0128]

BrEtNPPOH	450 g	1.56 mol	1 Eq
Thiophenol	172 g	156 mol	1 Eq
K_2CO_3	324 g	2.34 mol	1.5 Eq
DMF	2 L		

[0129] The corresponding synthesis pathway is depicted in Fig. 4d. A mixture oft the reactants and DMF was stirred at 140-160°C for 5 h. After cooling to 110°C, the solvent is removed by distillation under vacuum. The residue was treated with approximately 2.5 L water and extracted with approximately 1 L dichlormethane. The organic phase was washed with dilute NaOH and water, then evaporated to dryness *in vacuo*, further distilled with an azeotropic toluene/ethanol-mixture and purified by column chromatography on silica gel in 5 to 30 % ethylacetate in hexanes.

[0130] Yield: 352 g clear, yellow oil (71 %)

e) 2-(2-Nitro-4-ethyl-5-thiophenylphenyl)propanol chlorocarbonate ("PHSNPPOC-Cl"

[0131]

PhSNPPOH	352 g	1.11 mol	1 Eq
Triethylamin	112.2 g	1.11 mol	1 Eq
Triphosgen	219.4 g	2.22 mol Phosgen	2 Eq
THF	ca. 1.7 L		

[0132] The corresponding synthesis pathway is depicted in Fig. 4e. 219.4 g triphosgene was dissolved in 1 L dry THF under stirring for 30 min. Under ice-cooling, a solution of 352 g PhSNPPOH and 112.2g NEt_3 in 700 mL dry THF was added slowly over a period of 3 h. After standing overnight, the icebath was replaced by a water bath at 40°C and excess phosgen and about 1 L THF removed *in vacuo*. The suspension was filtered, the residue washed with little THF and filtrates evaporated to dryness *in vacuo*.

[0133] Yield: 410.3 g yellow crystals (97 %)

[0134] The material is pure for further use without purification.

f) PhSNPPOC-Glycine-OH

[0135]

Glycine	5.81 g	0.0774 mol	1 Eq
PhSNPPOC-Cl	29.4 g	0.0774 mol	1 Eq
Na_2CO_3	18.1 g	0.1703 mol	2.2 Eq
Water	190 mL		
THF	150 mL		

[0136] The corresponding synthesis pathway is depicted in Fig. 4f. 5.81 g glycine und 18.1 g Na_2CO_3 are dissolved in 190 mL water and 60 mL THF. The solution is stirred in an ice-bath and is dropwise treated with a solution of 29.4 g PhSNPPOC-Cl in 90 mL THF. Stirring is continued for 20 min. THF was evaporated and the solution adjusted to pH 11. The solution is extracted twice with approximately 500 mL Hexane/Ethylacetate 1:1, the pH is adjusted to 2,5 with dilute HCl and extracted with approximately 500 mL ethylacetate. The organic phase is washed with approximately 500 mL water and evaporated to dryness. The product is purified by column chromatography on silica gel with methanol in dichlormethane (0 to 3 %).

[0137] Yield: 21 g pale yellow amorphous foam (65%)

g) PhSNPPOC-Proline-OH

[0138]

Proline	8.6 g	0.075 mol	1 Eq
PhSNPPOC-Cl	28.5 g	0.075 mol	1 Eq
Na_2CO_3	17.5 g	0.165 mol	2.2 Eq
Water	1000 ml		
THF	1200 ml		

[0139] The corresponding synthesis pathway is depicted in Fig. 4g. 8.6 g proline and 17.5 g Na_2CO_3 are dissolved in 1000 mL water and 1000 mL THF. The solution is stirred in an ice-bath and is dropwise treated with a solution of 28.5 g PhSNPPOC-Cl in 200 mL THF. Stirring is continued for 20 min. THF was evaporated and the solution adjusted to pH 11. The solution is extracted twice with approximately 500 mL ethylacetate, the pH is adjusted to 2.5 with dilute HCl and extracted with approximately 500 mL ethylacetate. The organic phase is washed with approximately 500 mL water and evaporated to dryness. The product is purified by column chromatography on silica gel with methanol in dichlormethane (0 to 2 %).

[0140] Yield: 21,7g pale yellow amorphous foam (63%)

h) PhSNPPOC-Isoleucine-OH

[0141]

Isoleucine	9.97 g	0.076 mol	1 Eq
PhSNPPOC-Cl	28.9 g	0.076 mol	1 Eq
Na_2CO_3	26.8 g	0.25 mol	3.3 Eq
Water	300 ml		
THF	300 ml		

[0142] The corresponding synthesis pathway is depicted in Fig. 4h. 9.97 g isoleucin and 26.8 g Na_2CO_3 are dissolved in 300 mL water and 200 mL THF. The solution is stirred in an ice-bath and is dropwise treated with a solution of 28.9 g PhSNPPOC-Cl in 90 mL THF. Stirring is continued for 20 min. THF was evaporated and the pH of the solution adjusted to 9.5.

[0143] The solution is extracted twice with approximately 500 mL hexane/ethylacetate 1:1, the pH is adjusted to 3.2 with dilute HCl and extracted with approximately 500 mL ethylacetate. The organic phase is washed with approximately 500 mL water and evaporated to dryness. The product is purified by column chromatography on silica gel with methanol in dichlormethane (0 to 2 %).

[0144] Yield: 15 g pale yellow oil (42 %)

i) PhSNPPOC-AsparticAcid-OH

[0145]

Aspartate	10.5 g	0.0789 mol	1 Eq
PhSNPPOC-Cl	30.0 g	0.0789 mol	1 Eq
Na_2CO_3	23.0 g	0.22 mol	2.8 Eq
Water	1000 ml		
THF	1200 ml		

[0146] The corresponding synthesis pathway is depicted in Fig. 4i. 10,5g aspartate and 23 g Na_2CO_3 are dissolved in 1000 mL water and 1000 mL THF. The solution is stirred in an ice-bath and is dropwise treated with a solution of 30 g PhSNPPOC-Cl in 200 mL THF. Stirring is continued for 20 min. THF was evaporated. The solution is extracted twice with approximately 500 mL hexane/ethylacetate 1:1, the pH is adjusted to 2 with dilute HCl and extracted with approximately 500 mL ethylacetate. The organic phase is washed with approximately 500 mL Water and evaporated to dryness. The product is purified by column chromatography on silica gel with methanol in dichlormethane (0 to 2 %).

[0147] Yield: 28 g pale yellow amorphous foam (74 %)

i) PhSNPPOC-Asparagine-OH

[0148]

Asparagine	12.7 g	0.0848 mol	1 Eq
PhSNPPOC-Cl	32.2 g	0.0848 mol	1 Eq
Na_2CO_3	19.8 g	0.1866 mol	2.2 Eq
Wasser	1000 ml		
THF	1200 ml		

[0149] The corresponding synthesis pathway is depicted in Fig. 4j. 12.7 g asparagine and 19.8 g Na_2CO_3 are dissolved in 1000 mL water and 1000 mL THF. The solution is stirred in an ice-bath and is dropwise treated with a solution of 32.2 g PhSNPPOC-Cl in 200 mL THF. Stirring is continued for 20 min. THF was evaporated. The solution is extracted twice with approximately 500 mL ether, the pH is adjusted to 2 with dilute HCl and extracted with approximately 500 mL ethylacetate. The organic phase is washed with approximately 500 mL water and evaporated to dryness. The product is purified by crystallization from ethylacetate.

[0150] Yield: 28 g pale yellow crystals (73 %)

k) PhSNPPOC-Leucine-OH

[0151]

Leucine	12.1 g	0.092 mol	1 Eq
PhSNPPOC-Cl	35.0 g	0.092 mol	1 Eq
Na_2CO_3	21.5 g	0.202 mol	2.2 Eq
Water	250 ml		
THF	250 ml		

[0152] The corresponding synthesis pathway is depicted in Fig. 4k. 12.1 g leucine and 21.5 g Na_2CO_3 are dissolved in 250 mL

water and 200 mL THF. The solution is stirred in an ice-bath and is dropwise treated with a solution of 35 g PhSNPPOC-Cl in 50 mL THF. Stirring is continued for 20 min. THF was evaporated. The solution is extracted twice with approximately 300 mL hexane/ethylacetate 1:1, the pH is adjusted to 3 with dilute HCl and extracted with approximately 500 mL ethylacetate. The organic phase is washed with approximately 500 mL water and evaporated to dryness. The product is purified by column chromatography on silica gel with methanol in dichlormethane (0 to 3 %).

[0153] Yield: 40 g yellow oil (91 %)

1) PhSNPPOC-C_6-Spacer

[0154]

6-Amino-hexanoic Acid	3.45 g	0.0263 mol	1 Eq
PhSNPPOC-Cl	10.0 g	0.0263 mol	1 Eq
Na_2CO_3	6.1 g	0.0579 mol	2.2 Eq
Water	300 ml		
THF	200 ml		

[0155] The corresponding synthesis pathway is depicted in Fig. 4l. 3.45 g 6-Amino-hexanoic Acid and 6.1 g Na_2CO_3 are dissolved in 300 mL water and 120 mL THF. The solution is stirred in an ice-bath and is dropwise treated with a solution of 10 g PhSNPPOC-Cl in 80 mL THF. Stirring is continued for 20 min. THF was evaporated. The pH was adjusted to 10.5. The solution is extracted twice with approximately 300 mL ether, the pH is adjusted to 2.3 with dilute HCl and extracted with approximately 500 mL ethylacetate. The organic phase is washed with approximately 500 mL water and evaporated to dryness. The product is purified by column chromatography on silica gel with methanol in dichlormethane (0 to 5 % and acetic acid 0.5 %).

[0156] Yield: 10.4g pale yellow oil (83 %)

m) PhSNPPOC-Lysine(Boc)-OH

[0157]

Fmoc-Lysine(Boc)-OH	37.0 g	0.079 mol	1 Eq
Piperidine	33.6 g	0.395 mol	5 Eq
THF	1400 ml		
Na_2CO_3	18.4 g	0.174 mol	2.2 Eq
PhSNPPOC-Cl	30.0 g	0.079 mol	1 Eq

[0158] 37.0g Fmoc-lysine(Boc)-OH are dissolved in 400ml THF and treted with 33.6g piperidine for 3 h under stirring with a mechanical stirrer blade. TLC indicated complete FMOC removal after that period. Water (approximately 2 L) was added and stirred for another 30 min. The precipitate was filtered with suction. The clear filtrate was charged with 18.4 g Na_2CO_3 and evaporated to dryness. Evaporation was continued until all piperidine was removed, by repeatedly addition of water and distillation. The residue was dissolved in approximately 1 L water and treated with 800 mL THF. The solution is stirred in an ice-bath and is dropwise treated with a solution of 30 g PhSNPPOC-Cl in 200 mL THF. Stirring is continued for 20 min. THF was evaporated. The pH is adjusted to 2 with dilute HCl and extracted with approximately 500 mL ethylacetate. The organic phase is washed with approximately 500 mL Water and evaporated to dryness. The product is purified by column chromatography on silica gel with methanol in dichlormethane (0 to 1 %).

[0159] Yield: 28.3 g pale orange amorphous foam (60 %)

n) PhSNPPOC-Serine(t-Bu)-OH

[0160]

Fmoc-Serine(t-Bu)-OH	30.3 g	0.079 mol	1 Eq
Piperidine	33.6 g	0.395 mol	5 Eq
THF	1600 ml		
Na_2CO_3	18.4 g	0.174 mol	2.2 Eq
PhSNPPOC-Cl	30.0 g	0.079 mol	1 Eq

[0161] 30.3 g Fmoc-Serine(Boc)-OH are dissolved in 600 mL THF and treted with 33.6 g piperidine for 3 h under stirring with a mechanical stirrer blade. TLC indicated complete FMOC removal after that period. Water (approximately 2 L) was added and stirred for another 60 min. The precipitate was filtered with suction. The clear filtrate was charged with 18.4 g Na_2CO_3 and evaporated to dryness. Evaporation was continued until all piperidine was removed, by repeatedly addition of water and distillation. The residue was dissolved in approximately 600 mL water, filtered and treated with 800 mL THF. The solution is stirred in an ice-bath and is dropwise treated with a solution of 30 g PhSNPPOC-Cl in 200ml THF. Stirring is continued for 20 min. THF was evaporated. The pH is adjusted to 2 with dilute HCl and extracted with approximately 500 mL ethylacetate. The organic phase is washed with approximately 500 mL water and evaporated to dryness. The product is purified by column chromatography on silica gel with 1 % methanol in dichlormethane.

[0162] Yield: 30.9 g pale yellow amorphous foam (77 %)

o) PhSNPPOC-Threonine(t-Bu)-OH

[0163]

Fmoc-Thr(t-Bu)-OH	31.4 g	0.079 mol	1 Eq
Piperidin	33.6 g	0.395 mol	5 Eq
THF	1300 ml		
Na_2CO_3	18.4 g	0.174 mol	2.2 Eq
PhSNPPOC-Cl	30.0 g	0.079 mol	1 Eq

[0164] 31.4 g Fmoc-Thr(t-Bu)-OH are dissolved in 600 mL THF and treted with 33.6 g piperidine for 4 h under stirring with a mechanical stirrer blade. TLC indicated complete FMOC removal after that period. Water (approximately 3 L) was added and stirred for another 30 min. The precipitate was filtered with suction. The clear filtrate was charged with 18.4 g Na_2CO_3 and evaporated to dryness. Evaporation was continued until all piperidine was removed, by repeatedly addition of water and distillation. The residue was dissolved in approximately 600 mL Water, filtered and treated with 600 mL THF. The solution is stirred in an ice-bath and is dropwise treated with a solution of 30 g PhSNPPOC-Cl in 100 mL THF. Stirring is continued for 20 min. THF was evaporated. The pH is adjusted to 2 with dilute HCl and extracted with approximately 500 mL ethylacetate. The organic phase is washed with approximately 500ml water and evaporated to dryness. The product is purified by column chromatography on silica gel with 1 % methanol in dichlormethane.

[0165] Yield: 30.9 g pale yellow amorphous foam (69 %)

p) PhSNPPOC-Histidine(Trt)-OH

Step 1:

[0166]

Fmoc-His(Trt)-OH	100 g	0.161 mol	1 Eq

Piperidine	140 g	1.614 mol	10 Eq
THF	2000 ml		

[0167] 100g Fmoc-His(Trt)-OH are dissolved in 2000 mL THF and treated with 140 g piperidine for 2 h under stirring with a mechanical stirrer blade. TLC indicated complete FMOC removal after that period.

[0168] Water (approximately 4 L) was added and stirred for another 30 minutes. The precipitate was filtered with suction. The clear filtrate was concentrated to remove all THF. The pH was adjusted to 2.5 with dilute HCl and the mixture stirred overnight.

[0169] Filtration yielded 53 g colorless crystals (83 %), which were dried in the air overnight.

Step 2:

[0170]

H-His(Trt)-OH	20.9 g	0.0526 mol	1 Eq
PhSNPPOC-Cl	20.0 g	0.0526 mol	1 Eq
Na_2CO_3	12.3 g	0.116 mol	2.2 Eq
THF	800 ml		

[0171] The crystals from above, 20 g, and 12.3 g Na_2CO_3 were dissolved in approximately 800 mL water and 700 mL THF. The solution is stirred in an ice-bath and is dropwise treated with a solution of 20 g PhSNPPOC-Cl in 100 mL THF. Stirring is continued for 20 min. THF was evaporated. The pH is adjusted to 1.5 with dilute HCl and extracted with approximately 500 mL ethylacetate. The organic phase is washed with approximately 500 mL water and evaporated to dryness. The product is purified by column chromatography on silica gel with 0-1 % methanol in dichlormethane and acetic acid (0.01 %).

[0172] Yield: 10 g pale amorphous foam (26 %)

Example 5:

Synthesis of Disulfide-PLPG-Nucleotides

5-PhSNPPOC-dB-3'-PA's

a) 5'-PhSNPPOC-dT

[0173]

Thymidine	19.1 g	0.0789 mol	1 Eq
PhSNPPOC-Cl	30.0 g	0.0789 mol	1 Eq
Pyridine	300 ml		
Dichloromethane	50 ml		

[0174] A solution of 19.1 g thymidine in 300 mL dry pyridine is stirred in an ice-bath. Dropwise, a solution of 30.0 g PhSNPPOC-Cl in 50 mL dichloromethane is added.

[0175] After 10 min continued stirring, the solution is washed twice with 800 mL water and evaporated to dryness. The residue is co-evaporated with a mixture of toluene/ethanol. Purification was accomplished by column chromatography on silica gel in methanol (0 to 2.5 %) in dichloromethane.

[0176] Yield: 28 g pale yellowish amorphous foam (60 %)

b) 5'-PhSNPPOC-dT-3'-PA

[0177]

5'-PhSNPPOC-dT	27.1 g	0.0463 mol	1 Eq
DCI	2.7 g	0.0232 mol	0.5 Eq
P-Reagent	13.5 g	0.0449 mol	0.97 Eq
Dichloromethane	300 ml		

[0178] A mixture of the reactants, vigorously dried and under exclusion of moisture, was stirred overnight at room temperatutre. Hexane was added until a slight turbidity remains. After 10 min stirring, the precipitate is filtered by suction and the crude product purified by column chromatography on silica gel with a gradient from 65 % to 80 % ethylacetate in hexane.

[0179] Yield: 29.7 g pale yellowish amorphous foam (81 %)

[0180] P-NMR: 144,4 (m) ppm , 94 % pure

c) 5'-PhSNPPOC-dC^{Ac}

[0181]

DC^{AC}	28.3 g	0.105 mol	1 Eq
PhSNPPOC-Cl	40 g	0.105 mol	1 Eq
Pyridin	650 ml		
Dichlormethan	100 ml		

[0182] A solution of 28.3 g N-(acetyl)-2'-deoxy-cytidine was co-evaporated twice with 200 mL pyridine, dissolved in in 250 mL dry pyridine and stirred in an ice-bath. Dropwise, a solution of 40.0 g PhSNPPOC-Cl in 100 mL dichloromethane is added. After 10 min continued stirring, the solution is washed twice with 800 mL water and evaporated to dryness. The residue is co-evaporated with a mixture of toluene/ethanol. Purification was accomplished by column chromatography on silica gel in methanol (0 to 2.5 %) in dichloromethane.

[0183] Yield: 31 g pale yellowish amorphous foam (48 %)

d) 5'-PhSNPPOC-dC^{Ac}-3'-PA

[0184]

5'-PhSNPPOC-dC^{Ac}	29.0 g	0.0473 mol	1 Eq
DCI	2.8 g	0.0237 mol	0.5 Eq
P-Reagent	13.8 g	0.0459 mol	0.97 Eq
Dichloromethane	300 ml		

[0185] A mixture of the reactants, vigorously dried and under exclusion of moisture, was stirred overnight at room temperature. Hexane was added until a slight turbidity remains. The crude product is purified by column chromatography on silica gel with a gradient from 65 % to 80 % ethylacetate in hexane.

[0186] Yield: 21.5 g pale yellowish amorphous foam (56 %)

[0187] P-NMR: 144,6 (m) ppm , 99 % pure

e) 5'-PhSNPPOC-dA^{tac}

[0188]

dA^{tac}	46.3 g	0.105 mol	1 Eq
PhSNPPOC-Cl	40 g	0.105 mol	1 Eq
Pyridine	650 ml		
Dichloromethane	100 ml		

[0189] A solution of 46.3 g N-(tert-butyl-phenoxyacetyl)-2'-deoxy-adenosine was co-evaporated twice with 200 mL pyridine, dissolved in in 250 mL dry pyridine and stirred in an ice-bath. Dropwise, a solution of 40.0 g PhSNPPOC-Cl in 100 mL dichloromethane is added. After 10 min continued stirring, the solution is washed twice with 800 mL sodium bicarbonate solution and water and evaporated to dryness. The residue is co-evaporated with a mixture of toluene/ethanol. Purification was accomplished by column chromatography on silica gel in methanol (0 to 1.5 %) in dichloromethane.

[0190] Yield: 35 g pale yellowish amorphous foam (42 %)

f) 5'-PhSNPPOC-dA^{tac}-3'-PA

[0191]

5'-PhSNPPOC-dA^{tac}	32.3 g	0.0412 mol	1 Eq
DCI	2.4 g	0.0206 mol	0.5 Eq
P-Reagent	12.0 g	0.0399 mol	0.97 Eq
Dichloromethane	300 ml		

[0192] A mixture of the reactants, vigorously dried and under exclusion of moisture, was stirred overnight at room temperature. Hexane was added until a slight turbidity remains. After 10 min stirring, the precipitate is filtered by suction and the crude product purified by column chromatography on silica gel with a gradient from 50 % to 65 % ethylacetate in hexane.

[0193] Yield: 32 g pale yellowish amorphous foam (79 %)

[0194] P-NMR: 144,3 (m) ppm , 99 % pure

g) 5'-PhSNPPOC-dG^{tac}

[0195]

dG^{tac}	48.2 g	0.105 mol	1 Eq
PhSNPPOC-Cl	40 g	0.105 mol	1 Eq
Pyridin	800 ml		
Dichlormethan	60 ml		

[0196] A solution of 48.2 g N-(tert-butyl-phenoxyacetyl)-2'-deoxy-guanosine was co-evaporated twice with 200 mL pyridine, dissolved in in 400 mL dry pyridine and stirred in an ice-bath. Dropwise, a solution of 40.0 g PhSNPPOC-Cl in 600 mL dichloromethane is added. After 10 min continued stirring, the solution is washed twice with 800 mL water and evaporated to dryness. The residue is co-evaporated with a mixture of toluene/ethanol. Purification was accomplished by column chromatography on silica gel in methanol (0 to 5 %) in dichloromethane.

[0197] Yield: 35 g pale yellowish amorphous foam (42 %)

h) 5'-PhSNPPOC-dG^{tac}-3'-PA

[0198]

5'-PhSNPPOC-dG^{tac}	34.0 g	0.0425 mol	1 Eq
DCI	2.5 g	0.0212 mol	0.5 Eq
P-Reagent	12.4 g	0.0412 mol	0.97 Eq
Dichloromethane	500 mL		

[0199] A mixture of the reactants, vigorously dried and under exclusion of moisture, was stirred overnight at room temperature. Hexane was added until a slight turbidity remains. The crude product is purified by column chromatography on silica gel with a gradient from 50 % to 65 % ethylacetate in hexane.

[0200] Yield: 24.5 g pale yellowish amorphous foam (58 %)

[0201] P-NMR: 144,5 (m) ppm , 98 % pure

Example 6:

Synthesis of Further Diarylsulfide PLPG According to the Invention

a) 5-(t-Butyphenyl-thio)-4-ethyl-2-nitrophenyl-2'-propan-1'-ol (t-Butylthio-NPPOH)

[0202]

4-t-Bu-Thiophenol	25.0 g	0.150 mol	1.1 Eq
BrEt-NPPOH	39.0 g	0.135 mol	1 Eq
K_2CO_3	31.1 g	0.225 mol	1.7 Eq
DMF	200 mL		

[0203] A mixture of the reactants was stirred at 100°C for 3 h. DMF was distilled off *in vacuo*. The residue was dissolved in dichloromethane, washed twice with water and evaporated to dryness *in vacuo*. The obtained residue was suspended in hexanes, stirred overnight and filtered off by suction. The crystals were dried.

[0204] Yield: 43 g pale yellow powder (85%)

[0205] ^{1}H-NMR (300MHz, DMSO):

[0206] 7.72ppm (s, 1H, Nitro-Ar-H); 7.50ppm (m, 2H, Ar-H t-Bu-Ph); 7.38ppm (m, 2H, Ar-H t-Bu-Ph); 6.88ppm (s, 1H, Nitro-Ar-H); 4.67ppm (s, 1H, OH); 3.25- 3.21ppm (m, 3H, Ar-CH(Me)-CH_2-OH); 2.73ppm (q, 2H, Ar-CH_2-CH_3); 1.29ppm (s, 9H, CH_3 t-Bu); 1.20ppm (t, 3H, Ar-CH_2-CH_3; 0.95ppm (d, 3H, Ar-CH(CH_3)-CH_2-OH)

b) Naphthyl-thio-NPPOH

[0207]

BrEt-NPPOH	20.6 g	0.0715 mol	1 Eq
2-Thionaphtol	11.5 g	0.0715 mol	1 Eq
Kaliumcarbonat	14.8 g	0.107 mol	1.5 Eq
DMF	100 mL		

[0208] A mixture of the reactants was refluxed for 1.5 h and further stirred overnight at room temperature. The residue was diluted with 1.5 L water and extracted with dichloromethane. The organic extract was washed twice with water, evaporated to dryness *in vacuo* and purified by column chromatography on silica gel with ethylacetate (0 - 30 %) in hexane.

[0209] Yield: 9.0 g yellow oil (34%)

[0210] ^{1}H-NMR (300MHz, DMSO):

8.07ppm (m, 1H, Naphthyl-H), 8.00 - 7.90ppm (m, 3H, Naphthyl-H); 7.77ppm (s, 1H, Nitroaromatic-H); 7.61-7.54ppm (m, 2H, Naphthyl-H); 7.47-7.41ppm (m, 1H, Naphthyl-H); 7.11ppm (s, 1H, Nitroaromatic-H); 4.65ppm (t, 1H, OH); 3.30-3.15ppm (m, 3H, Ar-CH(CH_3)-CH_2-OH); 2.77ppm (q, 2H, Ar-CH_2-CH_3); 1.21ppm (t, 3H, Ar-CH_2-CH_3); 0.92ppm (d, Ar-CH(CH_3)-CH_2-OH)

c) Nitrobenzimidazol-S-NPPOH

[0211]

BrEt-NPPOH	6.0 g	0.0208 mol	1 Eq
2-Merkapto-5-nitro-benzimidazol	4.06 g	0.0208 mol	1 Eq
Kaliumcarbonat	4.3 g	0.0312 mol	1.5 Eq
DMF	50 mL		

[0212] A mixture of the reactants was refluxed for 3 h and further stirred overnight at room temperature. The residue was diluted with 0.5 L water and extracted with dichloromethane. The organic extract was washed twice with water, evaporated to dryness *in vacuo* and purified by column chromatography on silica gel with ethylacetate (0 - 30 %) in hexane.

[0213] Yield: 5.8 g yellow oil (69 %)

[0214] ^{1}H-NMR (300MHz, DMSO):

13.35ppm (s, 1H, NH); 8.35ppm (dd, 1H, Nitrobenzimidazole-H); 7.95ppm (s, 1H, Nitroaromatic-H); 7,75ppm (s, 1H, Nitroaromatic-H); 7.63ppm (1H, d, Nitrobenzimidazole-H); 4.71ppm (s, 1H, OH); 3.47ppm (d, 2H, HO-CH_2); 3.19ppm (m, 1H, Ar-CH(CH_3)-CH_2-OH); 2.79ppm (q, 2H, Ar-CH_2-CH_3), 1.21-1.15ppm (m, 6H, 2 x CH_3)

d) Pyridyl-S-NPPOH

[0215]

BrEt-NPPOH	133.5 g	0.463 mol	1 Eq
2-Merkaptopyridin	51.5 g	0.463 mol	1 Eq
Kaliumcarbonat	96.0 g	0.695 mol	1.5 Eq
DMF	600 mL		

[0216] A mixture of the reactants was stirred at 140°C for 4 h. DMF was distilled off *in vacuo*. The residue was dissolved in dichloromethane, washed twice with water and evaporated to an oil *in vacuo*. The residue was purified by column chromatography on silica gel with ethylacetate (0 - 30 %) in hexane.

[0217] Yield: 87.9 g clear yellow oil (60 %)

[0218] ^{1}H-NMR (300MHz, DMSO):

8.43ppm (m, 1H, Py-H); 7.82ppm (s, 1H, Nitroaromatic-); 7.70ppm (m, 1H, Py-H); 7.65ppm (s, 1H, Py-H), 7.21ppm (m, 1H, Py-H); 4.75ppm (t, 1H, O<u>H</u>); 3.47ppm (t, 2H, HO-C<u>H$_2$</u>); 3.20ppm (m, 1H, HO-CH$_2$-C<u>H</u>CH$_3$); 2.72ppm (q, 1H, C<u>H$_2$</u>-Benzylic); 1.15 (m, 6H, 2 x CH$_3$)

<u>e) 2,5-Diethyl-4-phenoxy-nitrobenzene</u>

[0219]

Phenole	8.0 g	0.085 mol	1.1 Eq
NaH (60% in Parafine)	3.4 g	0.085 mol	1.1 Eq
4-Bromo-2,5-diethyl-nitrobenze	20.0 g	0.078 mol	1 Eq
DMF	60 ml		

[0220] Under vigorous stirring 3.4 g of NaH (60% in parafine) was carefully added to a solution of 8.0 g phenole in 60 ml of DMF. When the gas evolution was finished, 20.0 g of 4-bromo-2,5-diethyl-nitrobenzene were added to the mixture. The reaction mixture was stirred for 1.5 h at 170°C. Then the reaction mixture was cooled to ambient temperature and poured into 600 ml of water. The resulting emulsion was extracted with hexane. The hexane was distilled of and the distillation residue was dried over night in vacuum. The distillation residue was dissolved in hexane and purified by column chromatography (Silica / hexane).

[0221] Yield: 2.2g slightly coloured oil

[0222] ^{1}H-NMR (DMSO):

7.96ppm (s, 1H, Ar-H); 7.48 - 7.40ppm (m, 2H, Ph-H); 7.25-7.18ppm (m, 1H, Ph-H); 7.09-7.03ppm (m, 2H, Ph-H); 6.78ppm (s, 1H, Ar-H); 2.72ppm (q, 2H, ArC<u>H$_2$</u>-CH$_3$); 2.68ppm (q, 2H, Ar-C<u>H$_2$</u>-CH$_3$); 1.18ppm (t, 3H, Ar-CH$_2$-C<u>H$_3$</u>); 1.07ppm (t, 3H, Ar-CH$_2$-C<u>H$_3$</u>).

<u>Example 7:</u>

Alternative Synthesis of the Diarylsulfide-PLPG with R3 = H [Formula I]

<u>a) 3-Acetamido-ethylbenzol</u>

[0223]

3-Ethyl-anilin	550 g	4.54 mol
Acetanhydride	1100 mL	

[0224] Within approximately 4 h, 550 g of 3-Ethyl-anilin were added to acetanhydride. The mixture was stirred overnight at room temperature (DC-control hexan/EtOAc 1:1).The reaction mixture was evaporated to dryness in *vacuo*. The distillation residue was distilled in high vacuum (Temp.: 210°C , Head-Temp.: 145°C).

[0225] Yield: 710g yellow oil (96%).

b) 3-Acetamido-6-nitro-ethylbenzol

[0226]

3-Acetamido-ethylbenzol	237.0 g	1.452 mol
H_2SO_4 conc.	622 mL	
HNO_3 conc.	91.0 g	

[0227] 237.0 g 3-Acetamido-ethylbenzol were added dropwise to 622 mL H_2SO_4 conc. in that the temperature of the mixture did not exceed 20°C. The mixture was cooled to -30°C. Subsequently, 91.0 g HNO_3 conc. were added dropwise, in that the inner temperature of the mixture did not exceed -20°C. The mixture was thawed to -10°C and poured into 1800 g of ice. The aqueous phase was separated and extracted with 2x 200 mL of ether. The precipitate of 3-acetamido-6-nitro-ethylbenzol was combined to with the ether extracts and dissolved therein. The ether solution was washed with 100 mL of water and evaporated.

c) 3-Ethyl-4-nitro-aniliniumbromide

[0228]

3-Acetamido-6-nitro-ethylbenzol (raw product)	approximately 1.45 mol
Hydrobromic acid (48%)	400 mL

[0229] The raw product was suspended in 400 mL hydrobromic acid (48%) and heated for 0.5 h to boiling (3-ethyl-4-nitro-aniliniumbromide starts to crystallize, which is associated with a significant increase of the reaction volume). The mixture was cooled to room temperature under stirring and subsequently cooled to 5°C on ice. The suspension was removed by suction, resuspended in 200 mL cold hydrobromic acid (48%) and filtered again, followed by washing on the nutsch filter with approximately 50 mL cold hydrobromic acid (48%).

[0230] Yield: 450 g humid product

d) 3-Brom-6-nitro-ethylbenzol

3-Ethyl-4-nitro-aniliniumbromide ca. 450 g ca. 1.45 mol

(raw product, humid)

[0231]

Hydrobromic acid (48%)	250 mL
Water	400 mL
$NaNO_2$	107.6 g
Water	550 mL

[0232] The humid product of the previous approach was suspended in a solution of 250 mL hydrobromic acid (48%) in 400 mL of water. A solution of 107.6 g $NaNO_2$ was added dropwise to 550 mL water on ice, in that the temperature of the mixture did not exceed 12°C. The mixture was stirred for 30 min at 0°C and filtered.

Sandmayer-Conversion:

[0233]

Diazoniumsalt-solution	ca. 1.45 mol
Copper powder	84.9 g
$CuSO_4 \times 5H_2O$	212.3 g
Hydrobromic acid (48%)	670 mL

[0234] The diazoniumsalt-solution was added dropwise to a mixture of 84.9 g copper powder, 212.3 g $CuSO_4 \times 5H_2O$ and 670 mL hydrobromic acid (48%) on ice, in that the temperature of the mixture did not exceed 15°C. The mixture was stirred over night at room temperature, filtered and the organic phase was separated. The aqueous phase was extracted with dichloromethane. The combined organic phases were filtered under usage of a thin layer of silica gel and then evaporated to dryness in *vacuo*. 167.8 g of raw product was yielded. The distillation residue was distilled in high vacuum (Temp.: 155°C , Head-Temp.: 85°C). Yield: 144.3 g yellow oil (43% via 4 steps).

e) 2-(2-Nitro-5-brom-phenyl)propanol

[0235]

3-Bromo-6-nitro-ethylbenzol	309.6	g 1.346 mol	1 Eq
Paraformaldehyd	42.4 g	1.413 mol	1.05 Eq
Kalium-tert-Butylat	37.8 g	0.337 mol	0.25 Eq
DMSO	900 mL		

[0236] To a solution of 309.6 g 3-bromo-6-nitro-ethylbenzol and 42.4 g paraformaldehyde in 300 mL DMSO, 37.8 g potassium-tert-butylat was added in small portions, in that the temperature increased to 40-50°C. The mixture was stirred over night at room temperature. 900 mL toluol was added to the mixture and washed with 3x 450 mL aqueous NaOH (10%) and subsequently with 450 mL water. The organic phase was evaporated to dryness *in vacuo*. 319 g of raw product was yielded, which was then purified using chromatography:
Column: 700 g silica gel, diameter 8.5 cm, equilibrated with n-hexan. The raw product was dissolved in 100 mL toluol and loaded onto the column. Elution was performed using the following gradient: 2.5 L n-hexan,

Ethylacetat/n-hexan	1:100	1 L
	1:50	1.5 L
	1:20	1 L
	1:6	2 L
	1:5	1.5 L

Yield: 236.06 g 2-(2-Nitro-5-bromo-phenyl)propanol (67%).

f) PhSNPPOH without ethyl-group

[0237]

Br-NPPOH	5.0 g	0.0192 mol	1 Eq
Ph-SH	2.3 g	0.0211 mol	1.1 Eq
K_2CO_3	4.5 g	0.0326 mol	1.7 Eq
DMF	50 mL		

[0238] A mixture of the above listed components were mixed 3 h at 120°C and then over night at 70°C. The reaction mixture was evaporated to dryness *in vacuo*. Dichloromethane was added to the distillation residue and washed with water, with diluted sodium hydroxide and again with water. The organic phase was evaporated and purified using chromatography (Stationary phase: Silica gel equilibrated with iso-hexane; Gradient: iso-hexane/5% ethyl acetate to iso-hexane/20% ethyl acetate).

[0239] Yield: 2.7 g red-brown oil (48%)

REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

- WO03065038A [0005]
- EP1589024A1 [0008]
- WO2004074300A2 [0008]

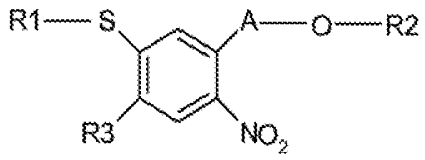
Non-patent literature cited in the description

- **PEASE et al.** Proc. Natl. Acad. Sci. USA, 1994, vol. 91, 5022-5026 [0003]
- **HASAN et al.** Tetrahedron, 1997, vol. 53, 4247-4264 [0003]
- **FODOR et al.** Science, 1991, vol. 251, 767-773 [0003]
- **PATCHORNIK et al.** J. Am. Chem. Soc., 1970, vol. 92, 6333-6335 [0003]

Patentkrav

1. En forbindelse med formlen

Formel Ia

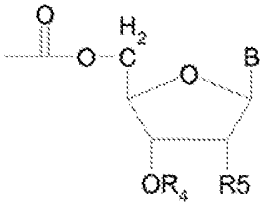


hvor A er valgt fra gruppen bestående af $-CH_2-$, $-CH_2CH_2-$, $-CH(CH_3)-$, $-CH(CH_3)-CH_2-$ og

R1 er en usubstitueret eller substitueret aryl- eller heteroaryl-gruppe, og R3 er H, en methylgruppe eller en ethylgruppe, og

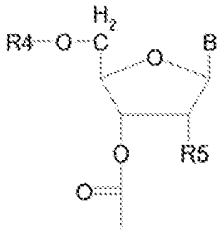
hvor R2 er

Formel II



eller hvori R2 er

Formel III



hvor R4 er H, danner en phosphoramidit, H-phosphonat eller phosphat triester, og

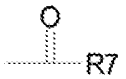
hvor R5 er H, OH, halogen eller XR6, hvor X er O eller S, og R6 er H, en alkyl-gruppe, aryl-gruppe eller OR6 danner en phosphoramidit, phosphodiester, phosphotriester, H-phosphonat eller en acetal eller silikone del og

hvor B er valgt fra gruppen bestående af adenin, cytosin, guanin, thymin, uracil, 2,6-diaminopurin-9-yl, hypoxanthin-9-yl, 5-methylcytosinyl-1-yl, 5-amino-4-imidazolecarboxylsyre-1-yl eller 5-amino-4-imidazolecarboxylsyreamid-3-yl, hvor, når B er adenin, cytosin eller guanin den primære aminogruppe eventuelt har en

beskyttelsesgruppe, eller når B er thymin eller uracil ved O^4position eventuelt er en beskyttelsesgruppe, eller

hvor R2 er

Formel IV



hvor R7 er en naturlig aminosyre, en ikke-naturlig aminosyre eller et aminosyrederivat, der danner en urethan binding til formlen Ia eller

hvor formel IV betegner carboxyfunktionen af en naturlig aminosyre, en ikke-naturlig aminosyre eller et aminosyrederivat, der danner en esterbinding med formlen Ia.

2. Forbindelsen ifølge krav 1, **kendetegnet ved, at** R1 er en phenyl-gruppe, en tert-butyl-phenylgruppe, en 1- eller 2-naphthyl-gruppe eller en 2-pyridyl-gruppe.

3. Forbindelsen ifølge krav 1 eller 2, **kendetegnet ved, at** A er -CH (CH_3) -CH_2-.

4. Forbindelsen ifølge krav 1 til 3, **kendetegnet ved, at** R3 er H eller en ethylgruppe.

5. Forbindelsen ifølge krav 1 til 4, **kendetegnet ved, at** R4 er H, og R5 er H.

6. Forbindelsen ifølge krav 1 til 5, **kendetegnet ved, at** B er valgt fra gruppen bestående af adenin, cytosin, guanin, thymin eller uracil.

7. Forbindelsen ifølge krav 1 til 6, **kendetegnet ved, at**, når B er adenin, cytosin eller guanin er beskyttelsesgruppen phenoxyacetyl-, 4-tert-butyl-phenoxyacetyl-, 4-isopropyl-phenoxyacetyl- eller dimethylformamidino-rester, når B er adenin er beskyttelsesgruppen benzoyl- eller p-nitro phenyl-ethoxy-carbonyl- (p-NPPOC) -rester, når B er guanin er beskyttelsesgruppen isobutyroyl-, p-nitrophenylethyl (p-NPE) eller p-NPEOC-rester, og når B er cytosin er beskyttelsesgruppen benzoyl -, isobutyryl- eller p-NPEOC-rester.

8. Forbindelsen ifølge krav 1 til 4, **kendetegnet ved, at** R7 er en naturlig aminosyre.

9. Anvendelse af forbindelsen ifølge kravene 1 til 8 som fotoaktiverbar beskyttelsesgruppe ved hjælp maskeløs fotolitografi.

10. Anvendelse af forbindelsen ifølge krav 7 til det maskeløse fotolitografi baserede DNA-array syntese som mellemprodukt eller permanent OH-beskyttelsesgruppe i nukleosid-derivater ved 3'-OH enden eller 5'-OH enden.

11. Anvendelse af forbindelsen ifølge krav 8 til maskeløs fotolitografi baseret peptid-array syntese som NH-beskyttelsesgruppe på aminosyrer.

12. Anvendelse af forbindelsen ifølge krav 8 til maskeløs fotolitografi baseret peptid-array syntese som COOH-beskyttelsesgruppe på aminosyrer.

13. Anvendelse af forbindelsen ifølge krav 8 til maskeløs fotolitografi baseret syntese af kulhydrater som OH-beskyttelsesgruppe.

14. Anvendelse af forbindelsen ifølge krav 8 til ortogonal beskyttelsesgruppes-strategi som SH-beskyttelsesgruppe.

15. Anvendelse af forbindelsen ifølge krav 8 til 13, **kendetegnet ved, at** lys anvendes til maskeløs fotolitografi med en bølgelængde på 374 til 405 nm, fortrinsvis 390 nm.

16. Fremgangsmåde til fremstilling af en diarylsulphid skelet indeholdende en fotolabil beskyttelsesgruppe ifølge et af kravene 1 til 8 omfattende trinnene

a) tilvejebringelse af p-diethylbenzen som udgangsmateriale

b) bromering af phenylringen

c) nitrering af den opnåede forbindelse i salpeter- og svovlsyre i position para- til brom

d) oprensning og krystallisation

e) hydroxymethylering af forbindelsen ved benzyl-positionen

f) omdannelse af den aromatiske bromgruppe til arylsulfidet under anvendelse af thiophenol

g) oprensning

h) omdannelse af alkoholen til chlorcarbonat

i) omsætning af chlorcarbonat med et nukleosid og omsætning af nukleosidet med et phosphityleringsmiddel eller
omsætning af chlorcarbonat med et aminosyrederivat.

17. Fremgangsmåden ifølge krav 16, **kendetegnet ved, at** R1 er en phenyl-gruppe, en tert-butyl-phenylgruppe, en 1- eller 2-naphthyl-gruppe eller en 2-pyridyl-gruppe.

18. Fremgangsmåden ifølge krav 16 eller 17, **kendetegnet ved, at** A er -CH (CH_3) -CH_2- .

19. Fremgangsmåden ifølge kravene 16 til 18, **kendetegnet ved, at** R3 er H eller en ethylgruppe.

DRAWINGS

Fig. 1

Solvent	Half-life	Irradiation-wavelength	Experiment No.	Series	Exposure time
DMSO	2.1 s	390 nm	SB10-132	1324	2,4,6,8,12 s
DMSO	2.4 s	390 nm	SB10-132A	1341	2,4,6 s
DMSO + 1 % Imidazol	3.7 s	390 nm	SB10-132	1325	2,4,6,8,12 s
NMP	2.6 s	390 nm	SB10-132	1326	2,4,6,8,12 s
NMP + 0.5 % Hydroxylamin	1.8 s	390 nm	SB10-132	1327	2,4,6,8,12 s
MeCN	2.2 s	390 nm	SB10-132	1328	2,4,6,8,12 s
MeCN + 1 % H_2O	2.0 s	390 nm	SB10-132	1329	2,4,6,8,12 s
MeCN + 1 % H_2O + 1 % Imidazol	2.1 s	390 nm	SB10-132	1330	2,4,6,8,12 s
MeCN + 16.3 % H_2O	2.9 s	390 nm	SB10-132A	1335	2,4,6,8 s
MeCN + 16.3 % H_2O + 1 % Imidazol	2.3 s	390 nm	SB10-132A	1336	2,4,6,8 s
Isopropanol	4.1 s	390 nm	SB10-132A	1337	2,4,6,8 s
Isopropanol + 1 % Imidazol	3.3 s	390 nm	SB10-132A	1338	2,4,6,8 s
Isopropanol + 12 % H_2O	4.3 s	390 nm	SB10-132A	1339	2,4,6,8 s
Isopropanol + 12 % H_2O + 1 % Imidazol	2.2 s	390 nm	SB10-132A	1340	2,4,6,8 s

Fig. 2

Solvent	Half-life	Irradiation-wavelength	Experiment No.	Series	Exposure time
DMSO	3.3 min	404 nm	SB10-128	1272	1,2,3,4 min
DMSO + 1 % Imidazol	2.5 min	404 nm	SB10-128	1273	1,2,3,4 min
DMSO	3.8 min	404 nm	SB10-130	1284	1,2,3,5 min
DMSO + 1 % Imidazol	2.9 min	404 nm	SB10-130	1285	1,2,3,5 min
NMP	2.1 min	404 nm	SB10-130	1286	1,2,3,5 min
NMP + 0.5 % Hydroxylamin	1.8 min	404 nm	SB10-130	1287	1,2,3,5 min
MeCN	3.0 min	404 nm	SB10-130	1288	1,2,3,5 min
MeCN + 1 % H_2O	3.1 min	404 nm	SB10-130	1289	1,2,3,5 min
MeCN + 1 % H_2O + 1 % Imidazol	2.6 min	404 nm	SB10-130	1290	1,2,3,5 min
Isopropanol	5.6 min	404 nm	SB10-139	1304	1,2,3,5 min
Isopropanol + 1 % Imidazol	3.8 min	404 nm	SB10-139	1305	1,2,3,5 min

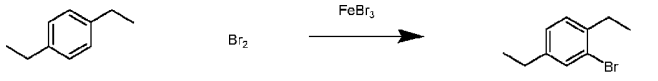
Fig. 3

Compound	MW	M1 365 nm	M2 365 nm	M1 390 nm	M2 390 nm	M1 404 nm	M2 404 nm	ε(365 nm)	ε(390 nm)	ε(404 nm)
NPPOC-Phe	449,49	0,066	0,069	0,014	0,016	0,003	0,004	302,4	67,0	16,3
BTA-Phe	553,53	0,095	0,094	0,019	0,019	0,005	0,005	522,4	104,4	26,7
NaphS-Phe	635,68	0,869	0,858	0,334	0,330	0,133	0,131	5489,3	2111,1	839,5
PhS-Phe	585,63	0,771	0,765	0,263	0,262	0,094	0,094	4497,8	1536,5	551,3
PyS-Phe	586,61	0,346	0,340	0,090	0,088	0,026	0,026	2014,0	522,6	151,8

Fig. 4

a) 2-(2-Nitro-4-ethyl-5-thiophenyl-phenyl)propanol ("PhSNPPOH")

2,5-Diethyl-bromobenzene

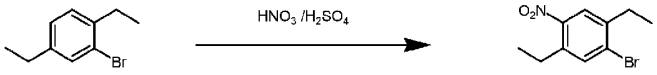


$C_{10}H_{14}$
Mol. Wt.: 134,22

Br_2
Mol. Wt.: 159,81

$C_{10}H_{13}Br$
Mol. Wt.: 213,11

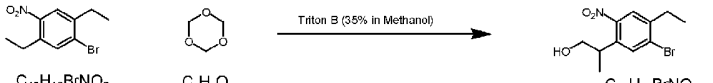
b) 2,5-Diethyl-4-nitro-bromobenzene



$C_{10}H_{13}Br$
Mol. Wt.: 213,11

$C_{10}H_{12}BrNO_2$
Mol. Wt.: 258,11

c) 2-(2-Nitro-4-ethyl-5-bromophenyl)propan-1-ol ("BrEtNPPOH")



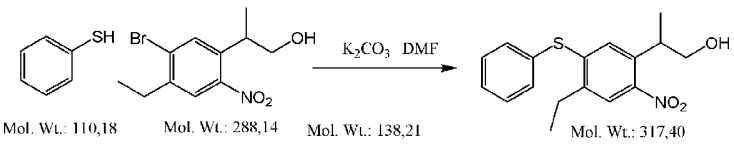
$C_{10}H_{12}BrNO_2$
Mol. Wt.: 258,11

$C_3H_6O_3$
Mol. Wt.: 90,08

$C_{11}H_{14}BrNO_3$
Mol. Wt.: 288,14

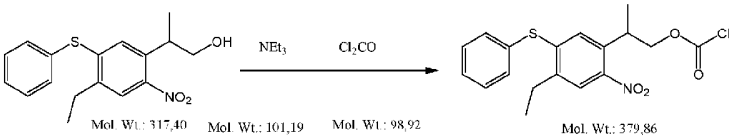
Fig. 4 cont.

d) 2-(2-Nitro-4-ethyl-5-thiophenylphenyl)propanol (PhSNPPOH)



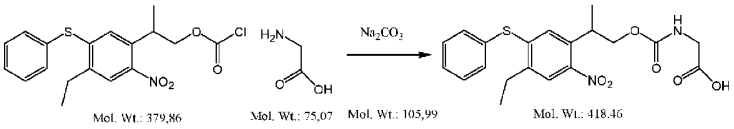
Mol. Wt.: 110,18 Mol. Wt.: 288,14 Mol. Wt.: 138,21 Mol. Wt.: 317,40

e) 2-(2-Nitro-4-ethyl-5-thiophenylphenyl)propanol Chlorocarbonate („PhSNPPOC-Cl")



Mol. Wt.: 317,40 Mol. Wt.: 101,19 Mol. Wt.: 98,92 Mol. Wt.: 379,86

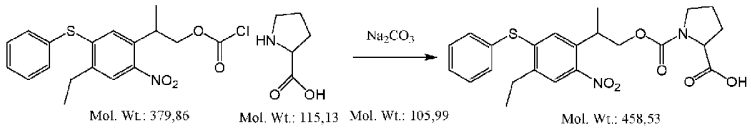
f) PhSNPPOC-Glycine-OH



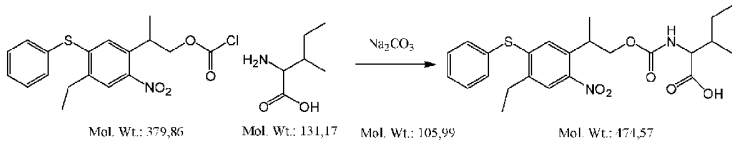
Mol. Wt.: 379,86 Mol. Wt.: 75,07 Mol. Wt.: 105,99 Mol. Wt.: 418.46

Fig. 4 cont.

g) PhSNPPOC-Proline-OH



h) PhSNPPOC-Isoleucine-OH



i) PhSNPPOC-AsparticAcid-OH

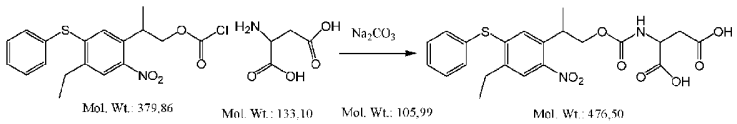
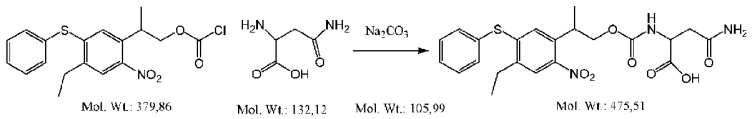
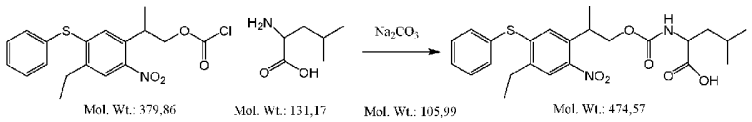


Fig. 4 cont.

j) PhSNPPOC-Asparagine-OH



k) PhSNPPOC-Leucine-OH



l) PhSNPPOC-C_6-Spacer

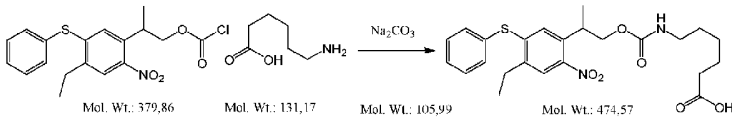
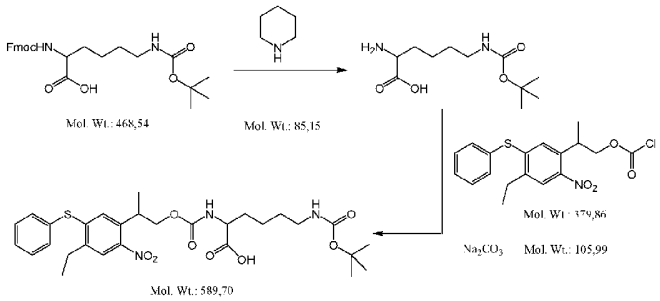


Fig. 4 cont.

m) PhSNPPOC-Lysine(Boc)-OH



n) PhSNPPOC-Serine(t-Bu)-OH

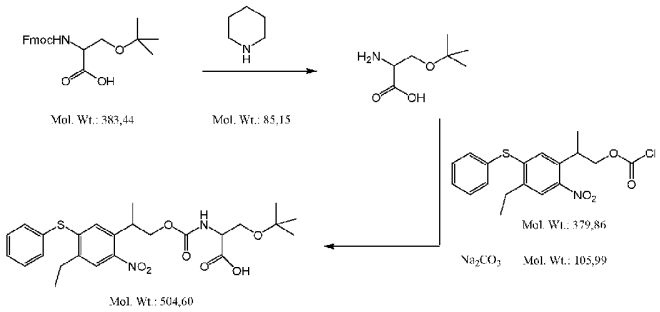
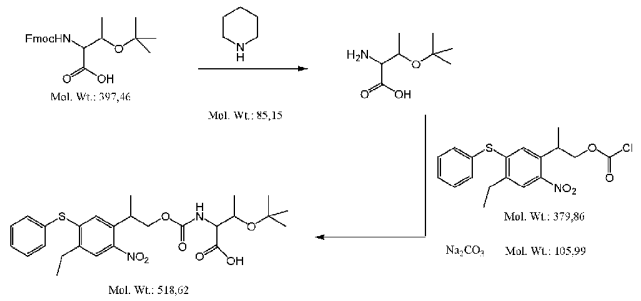


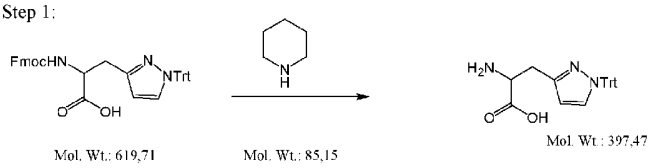
Fig. 4 cont.

o) PhSNPPOC-Threonine(t-Bu)-OH



p) PhSNPPOC-Histidine(Trt)-OH

Step 1:



Step 2:

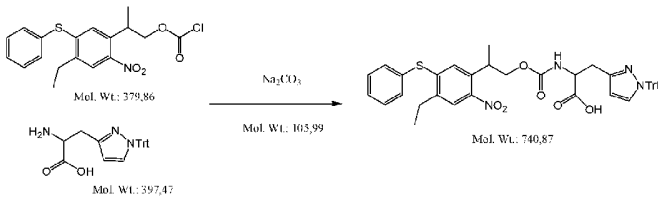
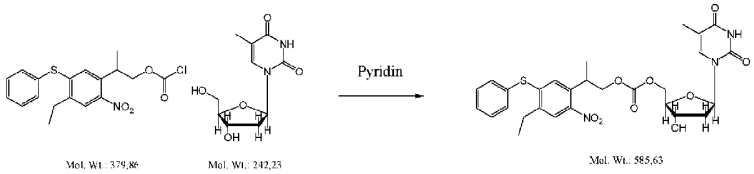
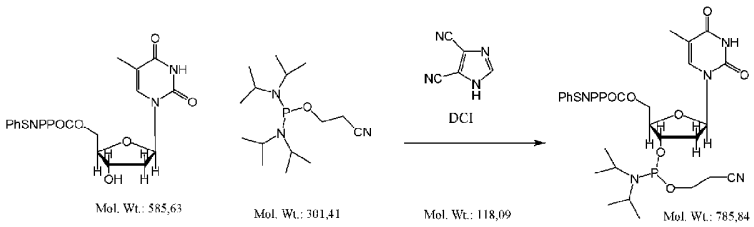


Fig. 5

a) 5´-PhSNPPOC-dT



b) 5´-PhSNPPOC-dT-3´-PA



c) 5´-PhSNPPOC-dC^{Ac}

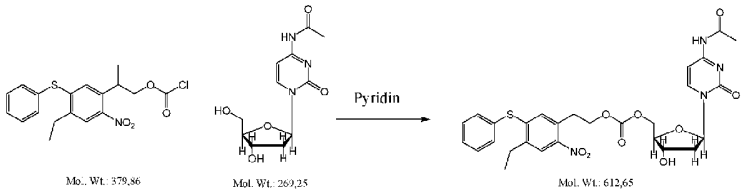
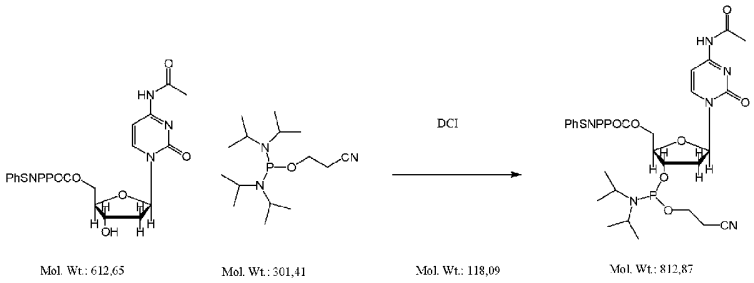
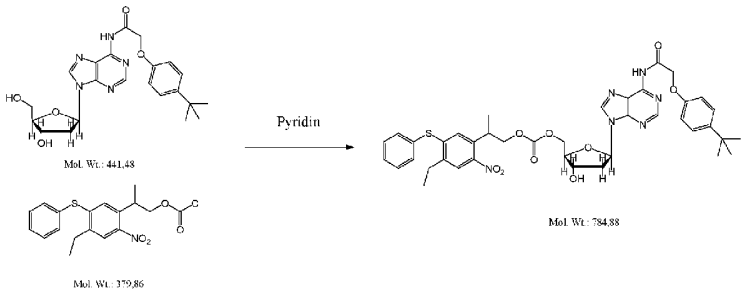


Fig. 5 cont.

d) 5´-PhSNPPOC-dC^{Ac}-3´-PA



e) 5´-PhSNPPOC-dA^{tac}



f) 5´-PhSNPPOC-dA^{tac}-3´-PA

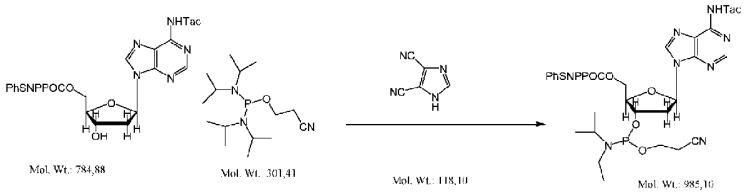


Fig. 5 cont.

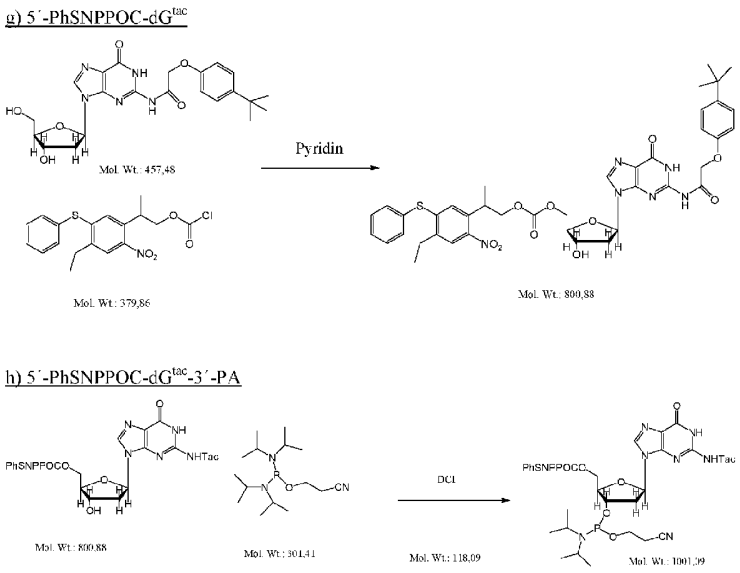
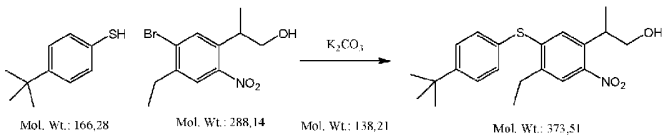

g) 5´-PhSNPPOC-dG^tac
Pyridin
Mol. Wt.: 457,48
Mol. Wt.: 379,86
Mol. Wt.: 809,88
h) 5´-PhSNPPOC-dG^tac-3´-PA
PhSNPPOCO
NHTac
Mol. Wt.: 809,88
Mol. Wt.: 304,41
DCI
Mol. Wt.: 118,09
Mol. Wt.: 1001,09

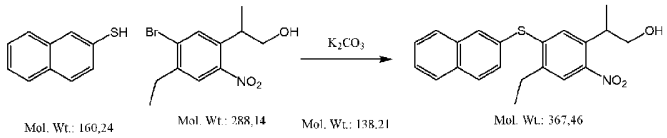
Fig. 6

a) 5-(t-Butylphenyl-thio)-4-ethyl-2-nitrophenyl-2′-propan-1′-ol (t-Butylthio-NPPOH)



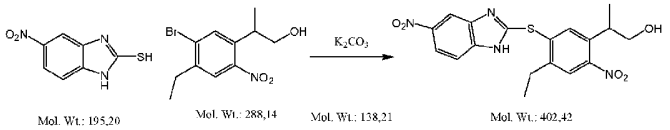
Mol. Wt.: 166,28 Mol. Wt.: 288,14 Mol. Wt.: 138,21 Mol. Wt.: 373,51

b) Naphthyl-thio-NPPOH



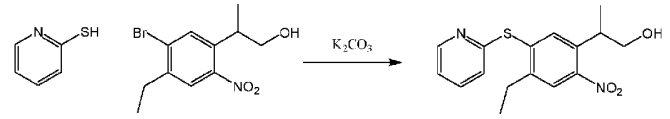
Mol. Wt.: 160,24 Mol. Wt.: 288,14 Mol. Wt.: 138,21 Mol. Wt.: 367,46

c) Nitrobenzimidazol-S-NPPOH



Mol. Wt.: 195,20 Mol. Wt.: 288,14 Mol. Wt.: 138,21 Mol. Wt.: 402,42

d) Pyridyl-S-NPPOH



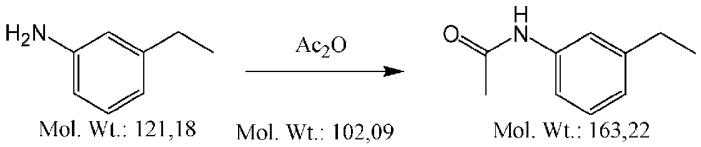
Mol. Wt.: 111,16 Mol. Wt.: 288,14 Mol. Wt.: 138,21 Mol. Wt.: 318,39

Fig. 6 cont.

e) 2,5-Diethyl-4-phenoxy-nitrobenzene

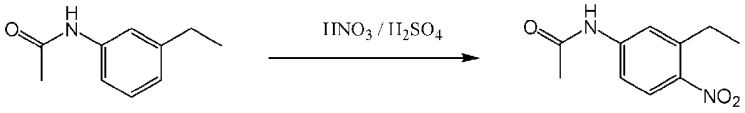
Fig. 7

a) 3-Acetamido-ethylbenzol



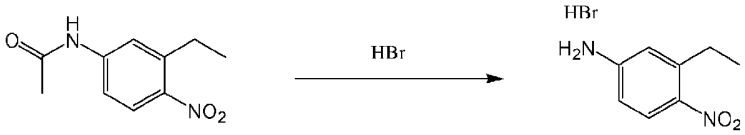
Mol. Wt.: 121,18 Mol. Wt.: 102,09 Mol. Wt.: 163,22

b) 3-Acetamido-6-nitro-ethylbenzol



Mol. Wt.: 163,22 Mol. Wt.: 208,21

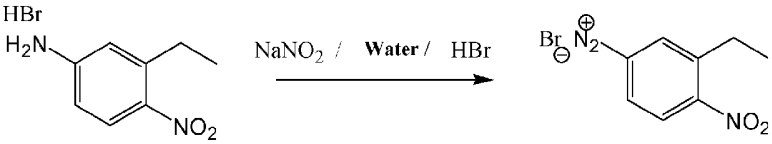
c) 3-Ethyl-4-nitro-aniliniumbromide



Mol. Wt.: 208,21 Mol. Wt.: 247,09

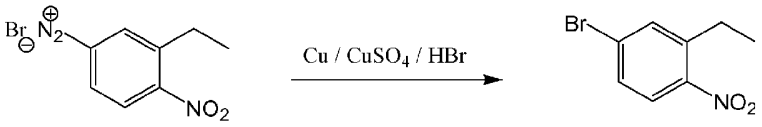
Fig. 7 cont.

d) 3-Brom-6-nitro-ethylbenzol



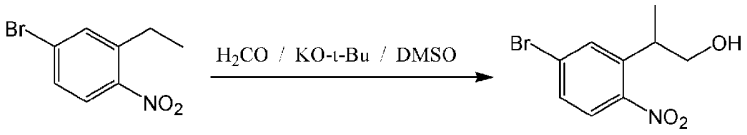
Mol. Wt.: 247,09 Mol. Wt.: 258,07

Sandmayer-Reaction



Mol. Wt.: 258,07 Mol. Wt.: 230,06

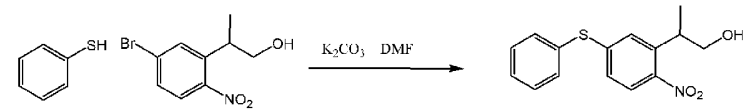
e) 2-(2-Nitro-5-brom-phenyl)propanol



Mol. Wt.: 230,06 Mol. Wt.: 260,08

Fig. 7 cont.

f) PhS-NPPOH



Mol. Wt.: 110,18 Mol. Wt.: 260,08 Mol. Wt.: 138,21 Mol. Wt.: 289,35

Fig. 8

a)

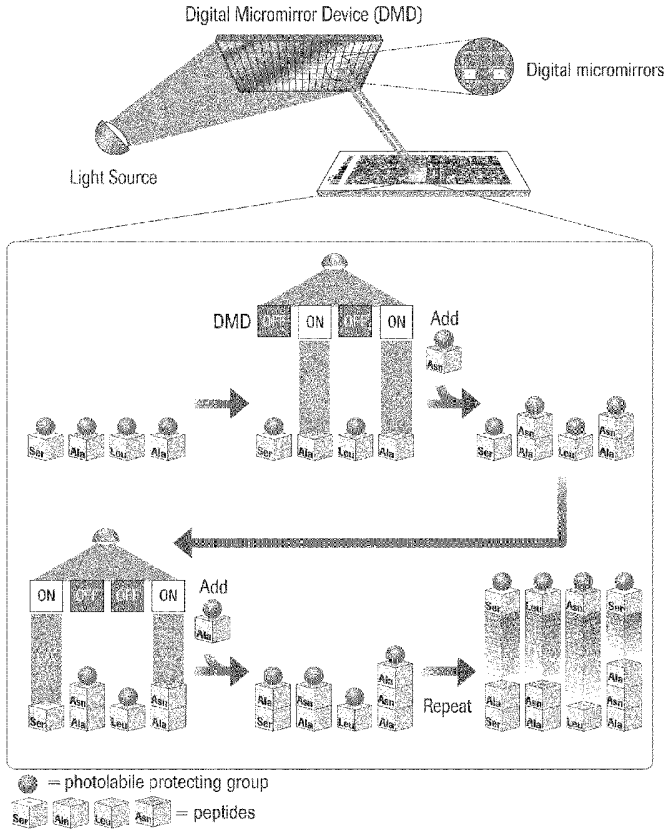

Digital Micromirror Device (DMD)
Digital micromirrors
Light Source
DMD
ON
Add
Repeat
= photolabile protecting group
= peptides

Fig. 8 cont.

b)

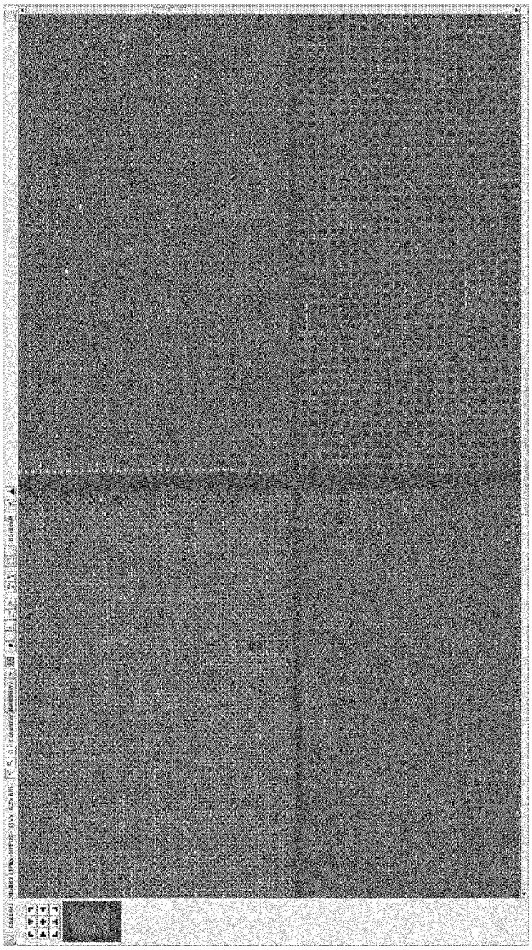


Fig. 8 cont.

c)