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(54) **Titre** : GROUPE AMINO CONTENANT DES MATRICES SUPPORT, LEUR UTILISATION ET LEUR OBTENTION

(54) **Title**: AMINO GROUP CONTAINING SUPPORT MATRICES, THEIR USE AND MANUFACTURE

(57) **Abrégé/Abstract**:

A support matrix comprising a polyvinyl backbone $[(-CH_2CH_2)_n]$ to which a nucleoside/nucleotide, a deoxynucleoside/deoxynucleotide or an oligonucleotide is attached via an available 3'-position while having the available 5'-position protected or unprotected and, if present, having remaining hydroxy or amino groups protected, wherein said support matrix is porous and the pore size diameters are within the interval of 10-2000 Å for more than 10 % of the pore volume.

ABSTRACT

A support matrix comprising a polyvinyl backbone [(-CH_2CH_2)$_n$] to which a nucleoside/nucleotide, a deoxynucleoside/deoxynucleotide or an oligonucleotide is attached via an available 3'-position while having the available 5'-position protected or unprotected and, if present, having remaining hydroxy or amino groups protected, wherein said support matrix is porous and the pore size diameters are within the interval of 10-2000 Å for more than 10 % of the pore volume.

AMINO GROUP CONTAINING SUPPORT MATRICES, THEIR USE AND MANUFACTURE.

Technical field

This invention concerns support matrices comprising a polymeric hydrocarbon backbone $[(-CH_2CH_2)_n]$ to which amino groups and/or derivatized amino groups are attached via link structures. This backbone will further on be called "polyvinyl backbone" or simply "backbone".

The expressions "a derivatized amino group" and "a group derived from an amino group" means that the amino group has been chemically transformed to a group in which the nitrogen of the original amino group remains bound to a link structure.

In earlier known support matrices cross-linking structures and groups containing pure amino groups and derivatized amino groups have replaced hydrogens in the polyvinyl backbone. Hydrogens have also been replaced by other groups, such as alkyls (methyl, ethyl etc), various forms of aryls (such as phenyl, vinyl phenyl, ethyl phenyl etc), acyloxy, aryloxy, alkoxy etc. These groups have sometimes been further derivatized.

This type of support matrices can be used as a solid phase in adsorption and partition processes, such as chromatography, and in solid phase organic synthesis, and as support in cell culturing and for catalysts, such as enzymes.

This type of support matrices has previously been prepared by copolymerising mono-vinyl compounds with di-, tri and polyvinyl compounds, with at least one of the monomers containing a functionality that can be transformed to an amino group after the polymerisation. One alternative has been to copolymerise chloromethyl styrene with divinyl benzene followed by treatment with ammonia. Another alternative has been conversion of residual vinyl groups to groups containing an amine function.

It has also been suggested that similar support matrices can be produced by ammonia gas plasma treatment of various polymers containing a polyvinyl backbone. See for instance US 5,369,012. Gas phase plasma treatment gives a more or less random introduction of functional groups on carbon atoms in the backbone and/or in pending groups.

Another possible method of making similar support matrices is nitration of cross-linked polystyrenes followed by reduction of the nitro groups to amino groups. Nitrations are generally carried out under very harsh conditions leading to a number of side reactions like oxidations. The subsequent reduction step is characterized by relatively low yields resulting in unreacted nitro groups on the polystyrene. In highly cross-linked materials these reactions are very difficult to use successfully. Unwanted nitro groups attached directly on the polyvinyl backbone are obtained in side reactions.

Previously known support matrices thus have been relatively cumbersome to manufacture because of the extra steps necessary for introducing the amine functions and of the side reactions occurring.

For porous matrices carrying amino groups, we have found that the availability of the amino groups in a given matrix often vary. This has not been considered optimal for certain applications, for instance solid phase synthesis of oligonucleotides and adsorption processes.

Commercially available support matrices comprising polyvinyl backbones for use in solid phase synthesis of oligonucleotides are sold with the first monomer attached to the matrix. The loading has been about 90 μmole of the first nucleotide per gram matrix (Primer Support High Load 30; Amersham Pharmacia Biotech AB, Uppsala, Sweden).

If not otherwise specified, the terms nucleotide, deoxynucleotide, amino acid residue, DNA, RNA, oligo-/polypeptide includes their respective synthetic analogue, such as PNA (WO 9220703) and LNA for DNA.

Investigations of polymerizations involving aminostyrenes with other compounds comprising polymerisable unsaturation have been described in: Imoto et al, Bull. Chem. Soc. Jap. 49(5) (1976) 1342-1345; Donya et al Russ. J. Appl. Chem. 71(1) (1998) 132-137 (translated from Zhurnal Prikladnoi Khimii 71(1) (1998) 127-32); Russ. J. Appl. Chem. 67(3:2) (1994) 400-404 (translated from Zhurnal Prikladnoi Khimii 67(3) (1994) 450-454); Donya et al., Vysokomol Soedin Ser A Ser B 36(12) (1994) 2068-2073; Donya et al., Vysokomol Soedin Ser A Ser B 37(5) (1995) 752-757; Donya et al., Vysokomol Soedin Ser B 34(8) (1992) 3-8; and Donya et al., Vysokomol Soedin Ser A 34(12) (1992) 2068-2073. In none of these articles there is described the manufacture of crosslinked polymers that are supposed to support specific functionalities (support matrices).

It has been reported that homopolymerisations of aminostyrenes may be self-inhibiting. See Donya et al., Ukr. Khim. Zh. (Russ. Ed.) 56(9) (1990) 984-990.

Masuda (Makromol. Chem. 190 (1989) 1007-1014) has reported about solvent effects in radical polymerisation of styrene with aminostyrene.

US 4,275,184 (Bargain et al, Rhone-Poulenc Industries) describes vinyl monomers in which the vinyl group may be linked to an aromatic moiety via a silicon group. The aromatic moiety may be functionalized with an amino group. These monomers are not vinyl aromatics.

Objects of the invention

- A first object is to provide a simplified method for manufacturing support matrices carrying amino groups and/or derivatized amino groups, both of which are bound via a link structure to a polyvinyl backbone.
- A second object is to provide support matrices that have an improved availability of amino groups and of derivatized forms thereof.
- A third object is to provide improved support matrices for solid phase synthesis of oligonucleotides and oligopeptides, wherein said matrices are improved in accordance with the first and second objects and leading to improved yields of a desired oligonucleotide/oligopeptide.
- A fourth objective is to provide improved support matrices of the above-mentioned type for use in solid phase synthesis of organic compounds, the improvement being a loading (covalent attachment) of ≥ 100 μmole, such as ≥ 150 μmole or even ≥ 200 μmole of an organic reagent per gram dry matrix. These figures in particular refer to support matrices in which the organic reagent is a nucleotide, deoxynucleotide, or an analogue thereof.
- A fifth object is to provide improved support matrices for the solid phase synthesis of organic compounds, with particular emphasis of combinatorial libraries of small organic molecules

The invention

It has now been recognized that these objects can be met in case one or more amino-(C_{0-10})hydrocarbon vinyl aromatic monomers, optionally having the amino group acylated, are copolymerised with other vinyl monomers. In other words essentially all amino groups in the matrix obtained after polymerisation will be of the same type, i.e. either a pure amino group or an acylated form thereof.

The first aspect of the invention thus encompasses a method for the manufacture of a support matrix to which amino groups and/or acylated forms thereof are attached. The method comprises the step of copolymerising one or more monovinyl monomer (monomer I) with one or more di- tri- or polyvinyl monomers (monomer II). The method is characterized in that a part of the vinyl monomers carries an amino-(C_{0-10})hydrocarbon group or corresponding amido group (acylated amino-(C_{0-10})hydrocarbon) attached to an aromatic ring (monomer III). After the polymerisation the polymer material formed may be collected and further processed. The amino groups and/or acylated forms thereof may subsequently be transformed to a desired functionality. Acylated amino, for instance, may be transformed by hydrolysis to the corresponding amino group. The amino groups may be primary, secondary or tertiary.

The amino-(C_{0-10})hydrocarbon group is preferably a pure amino group, such as in amino monovinyl benzenes (for instance amino styrene) and amino vinyl naphthalenes, i.e. with the hydrocarbon group being non-existent. The amino group may be derivatized, such as in amides. The vinyl group is directly attached to the aromatic ring.

The hydrocarbon group, if present in the amino-(C_{0-10})hydrocarbon group may be straight, branched or cyclic. It may be aromatic or non-aromatic, such as containing a phenylene or consisting of a pure alkylene chain, respectively, i.e. the carbon chain linking the amino group to the aromatic ring preferably contains no heteroatom.

Monomer III is part of monomer I and/or monomer II.

Monomer III may be isoforms of amino-(C_{0-10})hydrocarbon vinyl aromatics in which a vinyl group and an amino-(C_{0-10})hydrocarbon group are ortho, meta or para to each other. Monomer III may also comprise amino-(C_{0-10})hydrocarbon vinyl naphthalenes in which either of the vinyl group or the amino-(C_{0-10})hydrocarbon group is in the 1- or 2-position.

The amino group, which may be optionally acylated, in monomer III complies with the formula

$$-NR_1R_2$$

in which R_1 and R_2 are selected among

a) hydrogen,

b) straight, branched and cyclic hydrocarbon groups or corresponding acyl groups.

Each of the hydrocarbon groups may have a hydrocarbon chain of 1-20 carbon atoms, optionally substituted with one or more hydroxy, alkoxy or amino groups and containing a nitrogen (amino nitrogen), an oxygen (ether oxygen) or a sulphur (thioether sulphur) atom replacing a carbon atom at one or more positions in the chain.

More specifically R_1 and R_2 encompass alkyls, aralkyls arylalkyls and aryls, for instance substituted or unsubstituted phenyl containing none, one or more heteroatoms, such as nitrogen or oxygen. Alkoxy is preferably C_{1-7} alkoxy. The nitrogen of an amino group that may be present in an R_1 or R_2 may carry one or two hydrocarbon groups, such as C_{1-7} alkyl.

One or both of R_1 and R_2 may be according to (b) above.

Compounds to be used as monomer I are alkenes, acrylates/ methacrylates, acrylamides/methacrylamides, acrylnitriles/ methacrylnitriles, vinyl aromatics like monovinyl benzenes such as unsubstituted or substituted variants thereof (meta, orto and para isomers), etc. Monomer I may also be an alkyl vinyl benzene, for instance with a C_{1-10} alkyl substituent, such as ethyl. Vinyl ethers, styryl ethers (with the ether oxygen attached to a carbon) etc are other examples. Monovinyl forms of Monomer III with one vinyl group are part of monomer I.

Compounds to be used as monomer II are alkadienes, bisforms of acrylates/methacrylates and acrylamides/methacrylamides, divinyl aromatics like various forms of divinyl benzenes (for instance meta, orto and para). Corresponding forms having more than two vinyl groups may in principle also be used. Monomer II acts as a cross-linker. Monomer II may also be alkylated forms and ether forms of the divinyl monomers just mentioned. Forms of monomer III with two or more vinyl groups are part of monomer II.

In a typical polymerisation mixture the %-amounts are:

- monomer I: ≥ 0.5 % such as ≥ 20 % and ≤ 99.5 %, such as ≤ 95 %
- monomer II: ≥ 0.5 % such as ≥ 5 % and ≤ 99.5 %, such as ≤ 80 %
- monomer III: ≥ 0.5 % and ≤ 80 %

Increasing the %-amount of monomer II will increase the rigidity of the final polymer and reduce the ability to swell in organic solvents.

The exact selection of %-amounts will depend on the use contemplated of the support matrix.

For uses in which the matrix does not need to withstand pressure, for instance from a liquid flow, typical %-amounts are:

- monomer I: ≥ 95 % such as ≥ 98 % and ≤ 99.5 %
- monomer II: ≥ 0.5 % and ≤ 5 % such as ≤ 2 %
- monomer III: ≥ 5 % such as ≥ 15 % and ≤ 80 % such as ≤ 50 %

The matrices obtained are of particular importance for most kinds of step-wise solid phase synthesis of organic compounds, except for nucleic acid synthesis.

For uses in which the matrix need to withstand pressure, typical %-amounts are:

- monomer I: ≥ 10 % such as 50 % and ≤ 90 % such as ≤ 80 %
- monomer II: ≥ 5 % such as ≥ 10 % and ≤ 80 % such as ≤ 50 %
- monomer III: ≥ 0.5 % such as ≥ 2 % and ≤ 40 % such as ≤ 35 % and even ≤ 20 %

The matrices obtained are of particular importance for applications in which a matrix is placed in a column/vessel and a liquid flow containing reagents is allowed to pass through. Typical uses are chromatographic applications and solid phase synthesis of nucleic acids.

The percentages given above are w/w and calculated on the total amount of polymerisable vinyl monomers in the polymerisation mixture.

The polymerisation conditions will depend on the vinyl monomers used and on demands on the final polymer. Depending on the vinyl polymers used, the polymerisation may be performed as an anionic, a cationic or a free radical polymerisation.

It is preferred to select monomers (monomer I, II and III) that have relative reactivities in free radical polymerisation as close as possible to each other.

One of the most preferred kind of polymerisations for the invention utilizes free radicals and an initiating system. Initiating systems are electron irradiation, γ-radiation, radical initiators etc. Typical initiators are chemical, thermal and irradiation initiators. Thermal initiators are often preferred. They have their best efficiency in the range of 30-90°C. The presence of a free amino group in monomer III makes it advantageous not to select initiators that significantly react with the free amino group under the polymerisation conditions selected.

Thermal/chemical initiators are azo compounds (for instance 2,2'-azobis(2,4-dimethylvaleronitrile), azoisonitriles, peroxides (for instance benzoylperoxide), persulphates.

Redox systems may also be used, for instance Fenton's reagent (hydrogen peroxide + Fe^{2+}).

Irradiation initiators typically work in the UV region and often have a benzophenone structure or are benzoyl derivatives. If needed the selected initiating system is combined with an appropriate accelerator.

It is well-known that an efficient free radical polymerisation involving aminostyrene and/or other amino-(C_{0-10})hydrocarbon vinyl aromatic compounds (in particular amino vinyl aromatic compounds) may be counteracted by the fact that these monomers may act as chain transfer agents and/or inhibitors during radical polymerization.

One route for minimizing this problem is by removing oxygen from the polymerization system as is well known in the field.

A second route is to include in the polymerisation mixture a polymerisation effector substance that overcome the drawbacks of amino-containing vinyl aromatic compounds as defined above, in particular an amino vinyl aromatic compound. Suitable effector substances may act as chain transfer agents and/or agents neutralizing the possibly self-inhibiting property of the amino-containing vinyl aromatics used. The efficiency of this kind of effectors will vary between different effector substances and also between different radical polymerisation systems. The amount and kind of effector should be selected such that the polymerisation reaction is able to proceed so as to give an amino group-containing support matrix as defined in the context of the present invention. The effector may be a selected solvent component, a separate chain transfer agent etc. It is also possible to envisage that certain kind of initiators may have a dual or triple function with respect to acting as an initiator and/or a chain transfer agent and/or an agent neutralizing the self-inhibiting properties of amino substituted vinyl aromatics. For the definition and illustration of chain transfer agents and initiators, see Odian, George G., Principles of Polymerization, 3rd ed.,"A Wiley-Interscience publication", (1991), pp 211-267. By selecting the proper polymerisation effector substance it is possible to promote the normal polymerization behavior of vinyl compounds (e.g. vinyl aromatic compounds) in polymerisation mixtures. The mixtures concerned contain both type I and type II monomers as defined above including also polymerizable amino-containing vinyl aromatic compounds (e.g. amino vinyl aromatic compounds).

A third variant is to use an amino group-containing monomer in which the amino group is protected, for instance in acylated form. The drawbacks of the free amino group will then be minimized with no or very low disturbance of the polymerisation reaction. After polymerisation the free amino group is formed by deprotection, for instance by hydrolysis when being used in acylated form during the polymerisation.

The appropriate conditions applied, including polymerisation efficient amounts will have to be optimized in relation to initiator, kind of monomers, chain transfer agents etc from case to case as outlined in the experimental part (Part A) for aminostyrene, divinyl benzene and other styrenes. See also the back-ground technology, for instance as defined in the patent documents and publications discussed above.

Slow reacting vinyl groups typically require specific precautions. This can be complied with by appropriately selecting initiator-accelerator systems or by utilizing electron irradiation as is known in the field. Allyl groups and other alkene groups which have only hydrogens and/or sp^3-hybridised carbons bound to the C-C-double bonds are examples of slow reacting vinyl groups.

Polymerisation may take place in conventional o/w-emulsions/ suspensions and dispersions to give more or less spherical particles provided the appropriate conditions are applied as known in the field. Similarly also bulk polymerisation may be utilized, possibly with subsequently disintegrating the block into particles, if so found appropriate.

Emulsifiers and stabilizers typical for polymerisations in emulsions, suspensions and dispersions can also be used in the present invention. Well known emulsifiers and stabilizers are sodium dodecyl sulphate, alkylated oligo ethylene glycols, cellulose derivatives etc.

One can also envisage that polymerisations are carried out in so called inverse emulsions (w/o-emulsions) in which the conditions have been selected such that the water droplets break up to a macroporous network having open spherical cavities communicating with each other (water act as a porogen). See for instance EP 60,138 and US 5,200,433. This can be extended to so called w/o/w emulsions in which the inner w/o-emulsion is in form of drops forming HIPEs. See WO 9531485. This kind of emulsions is often called high internal phase emulsions (HIPE).

By including an appropriate porogen into a polymerisation mixture, porous materials in form of particles/beads and monolithic plug material can be accomplished. Porogens typically are compounds that separate out and form channels/pores when the polymer is formed. Porogens may be in form of liquids, solids or gases and shall be possible to remove after polymerisation. Porogens in form of liquids are typically capable of completely dissolving the monomers used but not the polymer chain created. This means that the polymer chains will separate out and form a polymer material containing a pore system filled with the porogen. The characteristics of the pore system will depend on the amount and type of porogen. In general porogens with solubility parameter values near the solubility parameter value of the polymer result in elastic gel-like porous material with relatively high proportion of smaller pores. Aromatic solvents, such as toluene, xylene, mesitylene etc, have the potential to give this effect in case vinyl aromatics are polymerised.

The use of porogens with larger differences between the solubility parameter value of the porogen and of the polymer often results in more rigid porous material with a lower proportion of smaller pores. This effect will, for vinyl aromatics be obtained with, for instance, alcanols and aliphatic hydrocarbons as porogens. Often used porogens of this type have been alkanes, such as heptane, alkanols, such as decanol. Also mixtures of liquid compounds have been used as porogens.

With respect to vinyl aromatic monomers and oligonucleotide solid phase synthesis, porogens containing liquid aromatic solvents are preferred as porogens, possibly in combination with other liquids that are miscible with them.

Polymers may also be used as porogens

The general principles outlined above for selection of porogen also apply to polymers based on vinyl monomers that are non-aromatic.

The porous materials obtained may have pore sizes, pore volumes and pore surface areas as known in art for vinyl polymers. The pore size diameters thus may be within the interval of 10 Å to 1000 µm. The optimal selection of pore diameters typically depends on the use contemplated and can, for instance, be selected according to rules known in the field.

For certain applications it may be important that a certain fraction of the pores is above or below a certain limit or within a certain interval. It has now been found that for the synthesis on porous solid phases, in particular of oligonucleotides and their analogues, optimal pore size diameters of the support matrix should be in the interval of 10-2000 Å, such as 100-1000 Å, for more than 10 %, such as more than 30 %, of the pore volume. The best porogens found so far are toluene, xylene and mesitylene for obtaining these pore characteristics in matrices based on vinyl aromatic monomers. Most likely also other solvents having the comparable properties with respect to dissolving vinyl aromatic monomers and to become adsorbed by polymers obtained therefrom may be used.

The pores often are irregular meaning that it often is difficult to estimate their sizes. The figures given above and in the experimental part for pore sizes and pore size distributions refer to values obtained by size exclusion chromatography (SEC) of polystyrenes in tetrahydrofuran and linking the molecular weight of the polystyrenes to pore sizes according to the formula:

$$\phi(\text{Å}) = 0.62\ (M_w)^{0.59}$$

where $\phi(\text{Å})$ is pore diameter in Å, and M_w is mean molecular weight of the polystyrene. The method approximate irregular pores to cylindrical pores and has been described by Halasz et al (Angew. Chem. Int. Engl. 17 (1978) 901-908) and Halasz et al (Angew. Chem. Int. Engl. 19 (1980) 24-28).

In case the material is in particle form, its mean particle size typically is in the range of 1-1000 µm, preferably 3-1000 µm or 3-500 µm, i.e. particle sizes that normally are not obtained by emulsion polymerisation. The particles may have an irregular shape, such as obtained by disintegrating blocks obtained from bulk polymerisations, or spherical (beaded) as obtained from polymerisation in suspensions. The particles may be monodisperse or polydisperse, with monodisperse particle populations having more than 95% of the particles with sizes within their mean diameter ± 5%.

A particular important method for manufacturing the particles according to the invention is by utilizing so called seed particles, preferably including a first step comprising swelling the seed particles and then a second step comprising uptake of monomers before polymerisation. See for instance US 4,336,173. As
described in US 4,336,173 this method means

i) preparing an aqueous emulsion or dispersion comprising the steps of
   (A) providing a dispersion or emulsion containing seed particles, preferably comprising a polymer and preferably having a diameter < 1 µm, and a Substance I which comprises one or more materials having a molecular weight < 5000 and a water solubility < 10^{-2} g/l and being substantially absorbed by said seed particles,
   (B) adding to said dispersion or emulsion of step (A)
      (a) a Substance II which comprises one or more partly water-soluble materials having a water-solubility at least 10 times higher that that of substance I, whereby Substance II diffuses into the seed particles containing Substance I at a rate substantially in excess of the rate of departure of Substance I from said seed particles, the amount of said Substance II diffusing into said particles containing Substance I being at least 20 times that of the original seed particles used in step (A) based on volume, and

(b) necessary emulsifiers and initiators for polymerisation;

said Substance I and Substance II comprising materials containing (a) one or more of the monomers polymerisable with included initiators and (b) liquids not being polymerisable under the same conditions as the monomers;

ii) polymerisation by activating the initiator.

Subsequent to the polymerisation according to the present inventive method, the group $-NR_1R_2$ may be transformed by methods known in the field to

$$-N^{(+)}R_1'R_2'R_3'.$$

R_3' is a group that may or may not be present. (+) means that the nitrogen is positively charged when R_3' is present (ammonium form) and uncharged when R_3' is absent.

When R_3' is absent, R_1' and R_2' in addition of being selected among the same groups as R_1 and R_2 may be selected from groups having the formula -A-X in which

(a) A is an organic bridge structure and

(b) X is a structure containing

  (i) one or more reactive groups capable of reacting with a substance carrying a nucleophilic or an electrophilic group or with a free radical to covalently attach said substance or a part thereof to said support matrix, or

  (ii) one or more groups that can be transformed to such a reactive group, or

  (iii) a member of an affinity pair mediating affinity binding of the other member of the pair.

When R_3' is present, the group $-N^{(+)}R_1'R_2'R_3'$ is an ammonium group and carries a positive charge. R_1', R_2' and R_3' are groups providing an amine/ammonium functionality to the nitrogen. They are thus selected among those of R_1' and R_2'

that provide an sp^3-hybridised carbon or an aromatic carbon directly to the nitrogen of $-N^{(+)}R_1'R_2'R_3'$.

Typically A comprises a stable organic chain in which there are one or more organic structural elements selected from (a) straight, branched or cyclic hydrocarbon chains comprising 1-20 carbon atoms, (b) ether, (c) thioether, (d) amide structures, (e) ester, (f) azo, (g) secondary or tertiary amine structures, etc. The preferred structures typically have a hydrolytic stability that is comparable to or higher than acetamide, for instance. In some applications it may be advantageous to include structures having a lowered stability in order to enable selective cleavage at a certain location. See for instance the discussion below about solid phase synthesis of polymers.

By the term "stable" above is contemplated that the organic chain does not unintentionally deteriorate or react with any of the groups present in the inventive support matrix or under the conditions applied during its use.

The structure A may be common for at least two of R_1', R_2' and R_3'. Structure A may thus have a terminal group that together with the nitrogen in $-N^{(+)}R_1'R_2'R_3'$ forms -N=N-, -N=C<, $-N^+\equiv N$ etc. In $-N^+\equiv N$, -A-X is $\equiv N$ which means that A and X coincides.

Reactive groups as defined above and present in structure X are well known in the field. Hydroxy, amino, thiol, carboxy etc and their activated forms are typical examples.

Structure X may be a nucleotide or deoxynucleotide or a nucleic acid (DNA, RNA, oligonucleotide, and analogues such as PNA and LNA), which at its 3'-position (the terminal one for oligonucleotides) is linked via A to the support matrix and at its 5'-position (the terminal one for oligonucleotides) is protected or unprotected. If present, amino groups, phosphate groups and hydroxy groups preferably are in protected forms.

The 2'-position is preferably protected in case X is a nucleotide.

For the 5'-position a familiar protecting group is dimethoxytrityl (DMtr).

For amino groups, which are present in the base moiety, the optimal protecting group depends on the nucleoside/ nucleotide, deoxynucleotide/deoxynucleoside or nucleic acid concerned. Typical amino protecting groups for deoxy forms are: adenosine - benzoyl/phenoxyacetyl, cytosine - benzoyl/isobutyryl/acetyl, guanine - isobuturyl/isopropyl phenoxyacetyl, tymidine - none, and for nucleosides/nucleotides: adenosine - phenoxyacetyl, cytosine - acetyl, guanosine - isopropylphenoxy acetyl, uracil - none.

A typical phosphate protecting group is β-cyanoethyl. In certain variants a phosphate oxygen may be replaced with sulphur, i.e. in an oligonucleotide chain, the phosphate group may be $-P(-S^-)(=O)-$ $[-P(=S)(-O^-)-]$.

For the 2'-position of the sugar moiety, t-butyldimethylsilyl is a typical protecting group.

The organic structure A is selected so that the reaction cycles in oligonucleotide solid phase synthesis can be performed without releasing the growing oligonucleotide chain, i.e. the structure A is stable under the various steps up to the final release step.

For oligonucleotide synthesis and the like, structure A typically provides an ester function to the 3'-position of the first nucleotide of the growing nucleotide chain while the other structural elements of A are more stable. Other structural elements in A thus may be amide, pure hydrocarbon chain, ether, thioether etc. In an alternative, the ester group may be replaced with a single or repetitive silyl ether structure. See for instance WO 9209615 and WO 9808857. It is however obvious to one who is skilled in the art that many other variants of structure A also fall within the scope of this invention.

X may also be an amino acid residue or an oligopeptide linked at its carboxy end to a support matrix as defined above. The amino group, e.g. an α-amino group, may be protected or unprotected as known in the field. If present, other carboxy groups and other amino groups and also thiol group may be protected in a manner known in the field. The stability requirements for the bridge structure A are in this case different from those valid for solid phase oligonucleotide synthesis, but well known to people in the field.

X may also be a member of a so called affinity pair and used to affinity bind ("affinity adsorb") the other member of the pair to the support matrix. Well-known affinity pairs are (a) positively and negatively entities (ion exchange; with groups selected among primary, secondary, tertiary and quaternary ammonium, sulphonate, sulphate, phosphonate, phosphate and carboxy bound covalently to a support matrix), (b) antibodies and antigens/haptens, (c) lectins and carbohydrate structures, (d) IgG binding proteins and IgG etc. This kinds of Xs are often used in aqueous solutions and the selectivity and specificity in binding may often be disturbed in case the contact surface between the aqueous medium and the solid phase is hydrophobic. Thus, in this mode of the invention it is often important to hydrophilize the surface, for instance by having hydroxyl group containing entities attached as A which in turn may exhibit X, i.e. the member of the affinity pair. Hydrophilization procedures and procedures for covalently linking affinity binders to solid phases are well known in the field.

The second aspect of the invention is a support matrix comprising

(a) a polymeric hydrocarbon backbone [polyvinyl backbone, $(-CH_2CH_2)_n$] and

(b) one or more amino groups or groups derived from amino groups in which the nitrogen of the amino group is retained. These groups are attached to said backbone via a link structure containing an arylene group, which structure binds to the backbone by replacing a hydrogen.

Other hydrogens of the backbone may be replaced as discussed in the introductory part (page 1). The link structure is selected from the same kind of carbon chains as defined for R_1 and R_2 above.

The matrix of the second aspect of the invention is characterized in that each of said amino groups or groups derived from an amino group is directly attached via their nitrogen to the arylene group.

In the preferred variants the amino groups and groups derived therefrom comply with the structure:

$$-N^{(+)}R_1''R_2''R_3''$$

R_1'', R_2'' and R_3'' are selected from the same groups as R_1', R_2' and R_3'. (+) has the same meaning as above.

The inventive support matrices typically have a content of $-N^{(+)}R_1'R_2'R_3'$ groups in the interval 0.01-6 mmole/g of the polymer, with preference for 0.1-4 mmole/g. These figures in particular apply to the support matrices as they are obtained from the polymerization, i.e. the amino groups are $-NR_1R_2$.

The preferred variants of the second aspect require the backbone to be cross-linked. Cross-linking may have been accomplished as known in the field, either by including monomers having two or more vinyl groups in the polymerisation process or by a separate cross-linking after the polymerisation by the use of bifunctional electrophilic/nucleophilic cross-linking agents.

Typically, there are essentially no nitrogens directly attached to the backbone according to the second aspect, i.e. an essential part of the $-N^{(+)}R_1''R_2''R_3''$ groups is attached to the backbone via a link structure, preferably comprising an arylene group. In the preferred variants there are essentially no nitro groups or groups derived from side reactions created as a consequence of a nitration and subsequent reduction of nitro groups to amino groups.

Variants in which $-N^{(+)}R_1''R_2''R_3''$ derives from a nitrogen-containing group introduced by gas plasma treatment (ammonia or a gaseous analogues thereof) of a support matrix comprising the above-mentioned backbone are excluded from the preferred variants of this aspect of the invention.

Except for the location of the group $-N^{(+)}R_1''R_2''R_3''$ directly on an aryl moiety, the matrix of the second aspect of the invention may have any of the features discussed above for the matrices manufactured according to the first aspect of the invention.

A third aspect of the invention is a method for using the support prepared by the method of the invention in the various fields indicated in the introductory part above. In principle the various steps for each respective use are per se known, without exclusion of future developments.

One use is the step-wise solid phase synthesis of an organic compound. Typically the synthesis is also cyclic. The organic compound may be a polymer composed of one or more different analogous difunctional monomers. By the term difunctional is contemplated that one function in a monomer can participate in a nucleophilic/electrophilic displacement reaction with the second function. When running this type of reaction in a cyclic mode such that the reaction product of one cycle is used as the starting compound in the next cycle, a polymer chain composed of various monomers in sequence will be formed. For each cycle one monomer unit is added to the growing polymer chain that is anchored at one end to the support matrix. A typical protocol comprises:

1. Providing a support matrix as defined above carrying an X which comprises one of the monomers linked to the support matrix via one of its functional groups while the second functional group is protected,
2. If applicable deprotecting the second functional group,
3. Reacting said second functional group with a monomer that is activated at its first functional group, and protected at its second functional group.
4. Repeating steps 2-3 a predetermined number of times with the same or different monomer,
5. Optionally deprotecting and releasing the formed polymer from the support.

In case the monomers used contain further functional groups that can participate in the reactions, they are appropriately protected. Typically, washing steps are included between steps 1-5.

This mode of the invention is useful, among others, for the solid phase synthesis of regular and modified oligonucleotides (DNA, RNA, PNA (WO 9220703), LNA etc) and oligopeptides.

There should be no free nucleophilic groups in A or in the support matrix capable of giving side reactions with the reagents used. Typical prohibited groups are OH- or amino groups,

The synthesis of nucleic acids (for instance DNA or RNA oligonucleotides and analogues) on support matrices are known in the field. Thus starting from a support matrix carrying an X that is equal to a protected nucleoside/deoxynucleoside, a typical protocol comprises:

1. Deprotecting the 5'-protected deoxynucleoside/ deoxynucleotide linked to the support matrix,

2. Reacting the 5'-deprotected deoxynucleoside with an activated amidite of a desired 5'-protected deoxynucleoside,
3. Oxidating the phosphite group linking the deoxynucleoside deprotected in step 1 to the deoxynucleoside introduced in step 2 to a phosphate group or a thiophosphate group,
4. Capping of unreacted 5'-deprotected deoxynucleoside,
5. Repeating steps 1-4 a predetermined number of times with the same or a different deoxynucleoside in each cycle.
6. Deprotecting and releasing the produced DNA (oligonucleotide) from the matrix.

Typically there are also washing steps between steps 1-6. Except for steps 3 and 6 all steps are run under anhydrous conditions, typically in acetonitrile (steps 1, 2, and 4). The procedure for RNA synthesis is analogous, with nucleosides replacing deoxynucleosides. Provided that the conditions are kept fully anhydrous under step 2, the amount of amidite added may be down to 1.5 equivalents of the first monomer attached to the matrix.

The protocols for oligonucleotide synthesis are mostly run in columns/vessels in which a support matrix in form of particles packed to a bed has been placed. The reagent and washing solutions are then allowed to pass through the column, preferably in plug flow. The particles are preferably porous and may be monodisperse or polydisperse as defined above. For suitable pore size diameters see above.

For a review on oligonucleotide synthesis on solid phase, see Sanghvi Y S et al.; "Chemical Synthesis and Purification of Phosphothioate Antisense Oligonucleotides", In: "Manual of Antisense Methodology" G Hartman and S Endrés (Eds) Kluwer Academic Publishers (1998) pages 1-19. See OligoPilot User Manual, Amersham Pharmacia Biotech AB, in particular chapter 4.

The protocols for oligopeptide synthesis are analogous to the protocols for oligonucleotide synthesis. Thus starting from a support matrix carrying an X that is equal to an amino acid residue protected at its amino group, for instance an α-amino group, and running, a typical protocol comprises:

1. Deprotecting the amino group, for instance an α-amino group, of the lastly introduced amino acid residue;
2. Reacting the amino group deprotected in step 1 with a properly protected and activated amino acid derivative;
3. Repeating steps 1-2 a predetermined number of times with the same or different amino acid;
4. Deprotecting and releasing the oligopeptide formed from the support matrix.

See further Peptidsyntese: Miklos Bodanszky Peptide Chemistry Springer Verlag 1988.

In case the support matrices as defined above are used for affinity adsorption, X is a member of an affinity pair as defined above. This use comprises bringing the support matrix and a liquid, typically an aqueous liquid, containing the other member of the affinity pair in contact with each other. The conditions are selected to promote affinity binding and are in principle regarded as per se known in the field. Subsequently the support matrix is separated from the liquid and if so desired the affinity adsorbed member can be released and further processed.

A fourth aspect of the invention comprises a nucleoside/nucleotide, a deoxynucleoside/deoxynucleotide, oligonucleotide, or an analogue of these compounds, attached at its 3'-position (terminal for oligonucleotide) via a linker structure B to a porous support matrix composed of a polyvinyl backbone. The attached entity is preferably protected at 2´-, amino- and phosphate positions and protected or unprotected at the 5'-position (terminal for oligonucleotide). In this aspect of the present invention, the support matrix is characterized in that the pore size diameters are within the interval of 10-

2000 Å, such as 100-1000 Å, for more than 10 %, such as more than 30 %, of the pore volume. See above. In a preferred variant the particles may be monodisperse as defined above having a mean diameter in the interval 5-100 µm with preference for 10-50 µm. The linker structure B provides a group binding to the 3'-position in the same way as described above for the second aspect of the invention. Structure B plus the nucleoside/nucleotide, the deoxynucleoside/deoxynucleotide or the oligonucleotide may or may not be part of the group $-N^{(+)}R_1''R_2''R_3''$ which is linked via an arylene to the polyvinyl backbone.

EXPERIMENTAL PART

POLYMERISATIONS

Monomers: Divinylbenzene (technical grade) containing 80 % (w/w) meta and para divinyl benzene and 20 % (w/w) mainly meta and para ethyl vinyl benzene. Styrene (technical grade). Amino vinyl benzene (aminostyrene, vinylaniline) was from Oakwood (USA). Monomer I: meta and para ethyl benzene + amino vinylbenzene (monomer III) + optionally also styrene. Monomer II: meta and para divinyl benzene.

A. POROUS PLUGS (MONOLITHS) AND POPULATIONS OF POLYDISPERSE (POLYSIZED) PARTICLES.

Polymerisation 1: Radical polymerization of styrene, divinylbenzene, and aminostyrene in the absence of oxygen.

In a 50 mL 3-necked round bottomed flask, the initiator 2,2'-azobis(2,4-dimethylvaleronitrile) (V-65, Wako) (0.526 g, 2.12 mmol) was dissolved in toluene (23.34 g) (porogen). After that the monomers, divinylbenzene (3.001 g, 23.1 mmol), styrene (6.013 g, 57.6 mmol), and aminostyrene (1.000 g, 8.39 mmol) were added. The mixture was stirred with a glass rod to make a homogeneous solution. The reaction flask was then fitted with a water condenser and put in an oil bath at room temperature. Nitrogen gas was flushed through the mixture via a tube fitted with a Pasteur pipette. A low flow of nitrogen gas was maintained under the surface of the reaction mixture for 30 minutes. During this time the nitrogen gas flow was let out via the cold water condenser. After that the Pasteur Pipette was lifted above the surface of the reaction mixture, and left there with a slow nitrogen flow for the remainder of the experiment. Next, the oil bath was heated to 70°C and the reaction was left like that over night. After 21 hours, the reaction flask was taken out of the oil bath and slowly cooled to room temperature. By now the reaction mixture had turned into a wet, soft rubber like polymer plug. The plug was divided into small fragments using a spatula and put into a glass filter funnel. The fragments were then washed with toluene (10 x 50 mL). The washing procedure was very slow since toluene swelled the polymer plug very good. Halfway through the washing procedure the fragments were put into a crystallization bowl and cut into smaller pieces using a scalpel to get a better effect of the washing. Remaining toluene in the polymer fragments after the washing procedure was evaporated in a vacuum oven (25°C, ~1 mbar, 24 h). During the evaporation the polymer fragments hardened enough to be crushed into even smaller particles. Finally the particles were further ground into even smaller particles.

The presence of aminostyrene monomeric units in the product was shown by FTIR, Elemental Analysis, Pyrolysis GC-MS, and Ninhydrin Colour Test.

Polymerisation 2: Radical polymerization of styrene, divinylbenzene, and aminostyrene in the presence of a chain transfer agent (CBr_4)

In four different test tubes, the initiator 2,2′-Azobis(2,4-dimethylvaleronitrile) (V-65) and the chain transfer agent carbontetrabromide (CBr_4) were dissolved in toluene (also porogen). Then divinylbenzene, styrene and aminostyrene were added. Amounts according to Tables 1-3 were used. The test tubes were then thoroughly shaken for a few seconds to make a homogeneous mixture. Every test tube was then flushed with nitrogen gas below the surface of the reaction mixture for 5-10 minutes to remove any oxygen and then fitted with a septum. Furthermore, for every test tube, a balloon fitted with a syringe was filled with nitrogen. The syringe needles were then pierced through the septa to ensure that no oxygen would enter the test tubes. The polymerization reactions were started by placing all the test tubes in an oil bath at a temperature of 60°C, where they were left over night. The following day a spatula was used to fragmentize the produced polymer plugs. Finally the polymer fragments were washed with methanol (4 x 10 mL) and toluene (10 x 10 mL) and dried in a vacuum oven (25°C, ~1 mbar, 24 h).

The first set of reactions (Table 1) showed nearly no signs of any polymerization. Only the sample with the smallest amount of CBr_4 in combination with the highest amount of the initiator, had turned slightly more viscous.

***Table 1** - Amounts in polymerisation 2:*

	A1	B1	C1	D1
Aminostyrene (g)	0.105	0.102	0.107	0.107
Styrene (g)	0.601	0.599	0.599	0.612
Divinylbenzene (g)	0.308	0.311	0.302	0.306
V-65 (mg)	39.5	67.4	35.7	60.5
CBr_4 (g)	0.278	0.282	0.141	0.139
Toluene (g)	3.079	3.141	2.753	2.800
Total mass (g)	4.411	4.502	3.938	4.021

V-65 (%)	3.0	5.0	3.0	5.0
CBr_4 (mmol)	0.838	0.850	0.425	0.419
Aminostyrene:CBr_4 (mol:mol)	1:1	1:1	1:0.5	1:0.5

In the second set of reactions (Table 2) much smaller amounts of CBr_4 were used. All the test tubes contained a soft polymer plug after approximately 20 hs in the oil bath. These polymer plugs were therefore analyzed further to determine the amount of aminostyrene that had actually been incorporated in the polymer matrix during the polymerization. FTIR, Elemental analysis, and Ninhydrin Colour Test showed that aminostyrene had been incorporated into the formed polymer.

***Table 2** - Amounts in polymerisation 2:*

	A2	B2	C2	D2
Aminostyrene (g)	0.115	0.106	0.102	0.102
Styrene (g)	0.640	0.600	0.619	0.623
Divinylbenzene (g)	0.328	0.312	0.324	0.316
V-65 (mg)	31.8	54.7	31.5	53.6
CBr_4 (g)	0.0287	0.0295	0.0132	0.0148
Toluene (g)	2.494	2.534	2.443	2.495
Total mass (g)	3.638	3.638	3.533	3.604
V-65 (%)	2.8	5.0	2.9	4.8
CBr_4 (mmol)	0.087	0.089	0.040	0.045
Aminostyrene:CBr_4 (mol:mol)	1:0.1	1:0.1	1:0.05	1:0.05

A third set of reactions (Table 3) was also carried out using even smaller amounts of the chain transfer agent. This time the results varied between the different test tubes. The two samples with the largest amount of CBr_4 had formed polymer plugs that visually looked acceptable. The sample with the lowest amount of CBr_4 in combination with the smallest amount of V-65 showed no signs of polymerization. The sample with the lowest amount of CBr_4 in combination with the higher amount of V-65 had polymerised, but some phase separation seemed to have taken place. No samples from this experiment were further analyzed.

Table 3 - Amounts in polymerisation 2:

	A3	B3	C3	D3
Aminostyrene (g)	0.102	0.101	0.111	0.104
Styrene (g)	0.612	0.606	0.614	0.606
Divinylbenzene (g)	0.315	0.325	0.301	0.300
V-65 (mg)	30.8	53.0	31.0	52.6
CBr_4 (g)	0.006	0.006	0.003	0.003
Toluene (g)	2.417	2.642	2.419	2.473
Total mass (g)	3.482	3.733	3.479	3.538
V-65 (%)	2.9	4.9	2.9	4.9
CBr_4 (mmol)	0.017	0.017	0.009	0.008
Aminostyrene:CBr_4 (mol:mol)	1:0.02	1:0.02	1:0.01	1:0.01

FTIR: Infrared spectra from the different crushed polymeric materials were recorded on a Perkin Elmer 16 PC FTIR using the diffuse reflectance technique (DRIFT).

Elemental Analysis: Samples were analyzed to determine the amount of nitrogen. The method used by Mikro Kemi AB is labeled MK 2006.

Pyrolysis GC-MS: A pyrolysis GC-MS analysis was carried out on the final reaction product of polymerisation 1. The GC-MS instrument was a Finnigan Trace GCQ, and the pyrolysis equipment was a Pyrola™ 2000. The pyrolysis was performed at 700°C.

Ninhydrine Colour Test: This test was performed according to the procedure described by Bunin (The combinatorial Index, Academic Press (1998) page 214). Three different stock solutions were prepared:

A - Ninhydrin (500 mg, 2,81mmol) in ethanol (10 mL)

B - Phenol (40 g, 425 mmol) in ethanol (10 mL)

C - KCN-solution (2 mL, 0,001 M) diluted with pyridine (up to a total volume of 100 mL)

Approximately 20 mg of the polymer fragments was weighed into a test tube. Then, 2-3 drops from every stock solution (A, B, and C) were added to the polymer fragments and the test tube was put in an oil bath holding a temperature of ~100°C for 5 minutes with occasional swirling. Materials containing amino groups turn dark blue when treated this way. Materials without amino groups do not change colour.

B. MANUFACTURE OF OPTIMIZED PARTICLES FOR USE IN OLIGONUCLEOTIDE SYNTHESIS.

Polymerisation principle: Seed particles around 0.5 µm in diameter was prepared by polymerising styrene in emulsion to give polymer chains of relatively short length. The seed particles were swelled in a two step procedure with addition of toluene as porogen plus monomers (technical grade of divinyl benzene, amino vinyl benzene, styrene) plus an azoinitiator, and polymerised. Appropriate stabilizators, initiators etc as discussed above were included. The system was selected to give monodisperse final particles of size 30 µm. The procedure was as outlined in US 4,336,173.

Results:

Lot	Divinyl benzene	Styrenes*	amino vinyl benzene			Porosity
	added % w/w	added % w/w	added % w/w	mmol /g**	Mmol /g***	% volume
1	64	16 + 0	20	1.67	1.74	70
2	50	12.5 + 27,5	10	0.84	0.88	70
3	64	16 + 10	10	0.84	0.92	70
4	40	10 + 30	20	1.67	1.69	70
5	30	7.5 + 42.5	20	1.67	1.72	70
6	30	7.5 + 52.5	10	0.84	0.97	70
7	20	5 + 55	20	1.67	1.57	70
8	64	16 + 10	10	0.84	0.98	78
9	40	10 + 40	10	0.84	0.94	78

* Styrenes means ethyl styrenes as part of technical grade of divinyl benzene plus technical grade of styrene.

** mmole amino groups added per total amount of monomers in grams.

*** mmole nitrogen (amino groups) of the obtained particles based on elemental analysis.

% w/w is based on total amount of monomers.

Porosity values are based on percentage of toluene included in the polymerisation mixture.

Analysing pore size distribution by the use of polystyrenes in tetrahydrofuran, it was found that 48-61 % of the pore volumes of lots 1-7 were in pores with pore size diameters within the interval of 115-880 Å constituting more than 30 % of the pore volume.

OLIGONUCLEOTIDE SYNTHESIS:

Introduction of 5'-protected deoxynucleotide on the support matrices: This was done by first succinylating the selected deoxynucleotide at its 3'-position (DMTr-protected at the 5'-position and appropriately protected at the amino group, if present). The obtained mono ester of succinic acid was activated with isobuturyl chloro formiate and coupled to a support matrix containing amino groups. The loading was determined by release of the DMTr-compound and measurement of this compound by UV/VIS. Initially it was found that support matrices (particles) of lot 1 (70% porogen) could be loaded with up to 0.344 mmole nucleotide per gram particles. The remaining amino groups are probably inaccessible for coupling possibly by being present in too small pores or embedded within the polymeric material.

Synthesis of oligonucleotides

This was performed automatically on Oligo PilotII (Amersham Pharmacia Biotech AB, Uppsala, Sweden). Starting from DMTr-protected nucleotide support, the synthesis comprises the steps of:

1. Hydrolysis of dimethoxytrityl by 3% (v/v) dichloroacetic acid in dichloromethane;
2. Acetonitrile wash;
3. Addition of 0.45 M tetrazole in acetonitrile and amidite in acetonitrile (1.5 equivalents with regard to starting nucleotide on the support);
4. Acetonitrile wash;
5. Oxidation of phosphite by 50 mM I_2 in 10% water and 90 % pyridine (v/v%);
6. Acetonitrile wash;
7. Capping of unreacted hydroxy groups that have not reacted with the amidite. Equal amounts of solution A (20 % of N-methylimidazole in acetonitrile) and solution B (20 % acetic acid anhydride in 30 % 2,6-dimethylpyridine and 50% acetonitrile.
8. Acetonitrile wash.

For synthesis of a 20-mer, 19 cycles of steps 1-8 was run before the oligonucleotide was fully deprotected and released from the support matrix.

When weighing together all features of the produced particles (swellability, rigidity, loading capacity, yield of oligonucleotide synthesis, costs etc) the support matrix of lot 6 was considered optimal at the priority filing and used as the first prototype. There were indications that still improved oparticles could be obtained by further increasing the content of porogen (toluene).

CLAIMS:

1. A support matrix comprising a polyvinyl backbone [(-CH_2CH_2)$_n$] to which a nucleoside/nucleotide, a deoxynucleoside/deoxynucleotide or an oligonucleotide is attached via an available 3'-position while having the available 5'-position protected or unprotected and, if present, having remaining hydroxy or amino groups protected, wherein said support matrix is porous and the pore size diameters are within the interval of 10-2000 Å for more than 10 % of the pore volume.

2. The support matrix according to claim 1, wherein said pore size diameters are within the interval of 100-1000 Å for more than 10 % of the pore volume.

3. The support matrix according to claim 2, wherein the pore size diameters are within the interval of 100-1000 Å for more than 30 % of the pore volume.

4. The support matrix according to any one of claims 1 to 4, wherein said matrix is in form of a monodisperse particle population.

5. The support matrix according to any one of claims 1 to 4, wherein said matrix has a loading of > 100 µmole of a single monomer unit of a nucleotide/nucleoside or deoxynucleotide/deoxynucleoside per gram dry matrix.

6. The support matrix according to claim 5, wherein said matrix has a loading of ≥ 150 µmole of a single monomer unit of a nucleotide/nucleoside or deoxynucleotide/deoxynucleoside per gram dry matrix.

7. The support matrix according to any one of claims 1 to 6, which has been obtained by copolymerization

of one or more mono vinyl monomers (monomer I) with one or more di-, tri- or polyvinyl monomers (monomer II) in the presence of a vinyl monomer which carries an amino-(C_{0-10}) hydrocarbon group attached to an aromatic ring.

8. Use of the support matrix as defined in any one of claims 1 to 7 for solid phase synthesis of an oligo/poly-nucleotide or an oligo/polypeptide.

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PATENT AGENTS