

## SYNTHESIS OF DNA FRAGMENTS LINKED TO A SOLID SUPPORT

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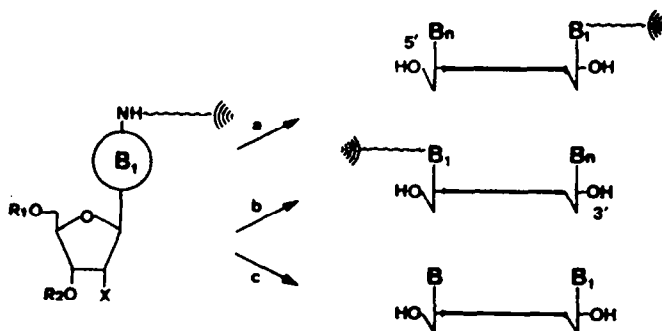
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**Abstract:** Two simple procedures for the preparation of DNA fragments covalently and specifically linked to a solid support are presented. The first method consists of the preparation of a nucleoside primer which serves as the initiative site for conventional synthesis of oligomers in either 3' or 5' direction. The second procedure involves the direct attachment of independently synthesized and purified oligomers to a functionalized solid support. The accessibility of such supported oligodeoxynucleotides to enzymes is checked with restriction endonucleases.

Several physical and chemical methods for coupling nucleic acids to a solid support<sup>1</sup> have been described. Polynucleotides can be immobilized by adsorption or entrapment within a matrix<sup>2</sup>, or covalently linked to an activated support by N-hydroxysuccinimide, carbonyldiimidazole or cyanogen bromide<sup>3</sup>. These methods involve multipoint attachment of long fragments (100 bp) by the aromatic bases rather than by a specific linkage<sup>4</sup>. Therefore, major regions of the DNAs are unavailable for base hybridization and other reactions. Another method consists of the chemical polymerisation of 5'-phosphate mononucleotides with DCC and esterification via their 5'-phosphate ends to cellulose powder<sup>5</sup>. Such supports are used as solid state primers or templates for ligases and polymerases, allowing the covalent attachment of homopolymers to a matrix<sup>6,7</sup>; the loading capacity of such supports is about 10-100 nMol of chain ends per mg of cellulose. Recently, an enzymatic method for linking (dA)-tailed ssDNA to oligo(dT)-cellulose and its application for in vivo synthesis of DNA probes have been reported<sup>8</sup>.

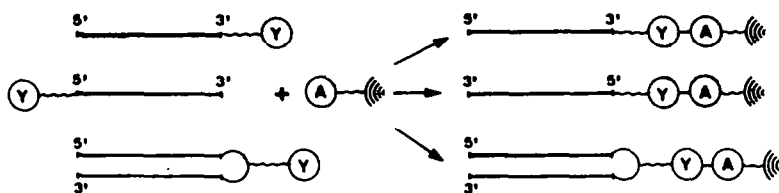
We report in this paper two procedures allowing simple access to oligonucleotides covalently attached to a solid support by a single specific linkage<sup>9,10</sup>.

The first method involves oligomers classically synthesized from a nucleoside primer (Scheme 1).



The first nucleoside is attached by the heterocyclic moiety to the polymer support via a spacer arm and serves as the initiation site for conventional synthesis in either 5' or 3' direction (routes a and b). Unlike the conventional supports, the spacer link is stable to deblocking conditions and the deprotected synthesized oligonucleotide remains attached by a covalent linkage to the solid support. The use of a ribonucleoside as anchor allows the release of the oligomer from the support by a chemical or enzymatic reaction with substantial yields (route c).

In a second procedure, we describe the direct attachment of independently synthesized and purified oligomers to a functionalized solid support via a long spacer link (Scheme 2)



Scheme 2

This technique is suitable for the linkage of double-stranded DNA on a solid support, unlike the previously reported methods for coupling oligonucleotides.

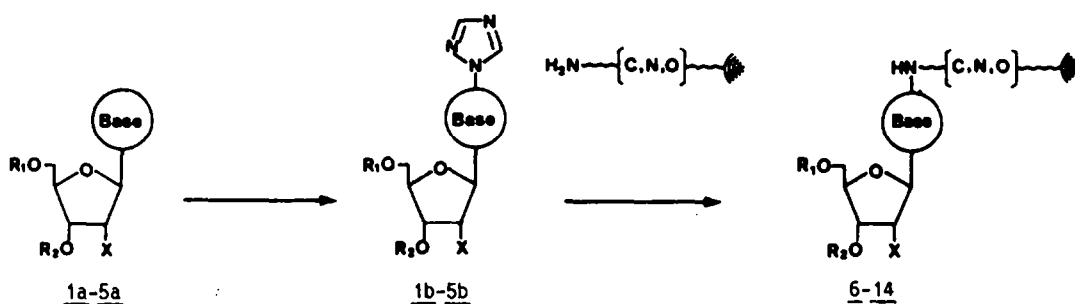
Such supported oligonucleotides provide convenient tools for the molecular biologist and could serve as affinity hybridization columns for both detection and isolation of complementary polynucleic acids or binding proteins.

## RESULTS AND DISCUSSION

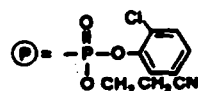
### 1 - PREPARATION OF OLIGONUCLEOTIDES BOUND TO A SOLID SUPPORT BY CHEMICAL SYNTHESIS FROM A PRIMER

#### 1.1 Attachment of the initial nucleoside to a polymer support

The solid support system described in Scheme 1 requires the attachment of an initial nucleoside, the primer, via its heterocyclic moiety (Scheme 3).



|    |                      |                    |      |         |            |
|----|----------------------|--------------------|------|---------|------------|
| 1a | R <sub>1</sub> : DMT | R <sub>2</sub> : P | X: H | Base: T | yield: 70% |
| 2a | DMT                  | Bz                 | H    | T       | 73%        |
| 3a | DMT                  | Bz                 | H    | G       | 68%        |
| 4a | Bz                   | P                  | OBz  | U       | 82%        |
| 5a | DMT                  | P                  | OBz  | U       | 79%        |



Scheme 3

The preparation of such a primer requires two preliminary steps: the activation of nucleoside and the functionalization of the polymer support with amino groups. Preliminary experiments in solution with model alkyl diamines (ethylenediamine, 1,10-diaminodecane, spermine) and base

activated nucleotides led to monosubstitution products.

Activation of the heterocyclic moiety at the C-4 positions of thymine and uracil and the C-6 position of guanine was performed with 1,2,4-triazole and O-chlorophenyl-phosphodichloridate in anhydrous pyridine using a reported procedure<sup>11-12</sup>. The protected nucleosides 1a to 5a were converted into the 4-(or 6-)-triazolyl derivatives 1b to 5b with good yields (Scheme 3).

We have tested different polymers commonly used in oligonucleotide synthesis: silica gel, polyacrylamide and controlled pore glass (CPG)<sup>13,14</sup>. The functionalization of these supports with spacer arms of different lengths bearing an amino group was carried out by standard methods: reaction of silica gel with either triethoxysilylpropylamine or dichlorodimethylsilane followed by aminoethanol<sup>15</sup>; treatment of cross-linked polyacrylmorpholide (Enzacryl gel K2) with ethylenediamine, 1,10-diaminodecane and spermine<sup>16</sup>; or, in the case of glass beads, use of a commercial support, the long chain alkylamine controlled pore glass (LCAA-CPG, 500 Å).

The next step is the substitution of these different functionalized supports with activated ribo- or deoxyribo-nucleotides. This reaction was performed in dioxane or pyridine. All non-reacted silanol groups (after functionalization with spacer arms) and residual amino groups were then capped with isobutylchloroformate followed by trimethylsilylchloride before any coupling step. The absence of primary amino groups was verified by the ninhydrine assay. The loading with nucleosides 1b to 5b was estimated by the spectroscopic analysis of the dimethoxytrityl cation (DMT) released by acidic treatment (2% BSA in CH<sub>3</sub>OH/CH<sub>2</sub>Cl<sub>2</sub> or 3% TCA in CH<sub>3</sub>CN) of an aliquot of the support. The loading of supports 6-14 varied between 9 and 94 µMol/g (Table).

TABLE: Functionalization of supports 6 to 14 with activated nucleosides

| Support n° | Polymer                             | Spacer arm             | Nucleoside                         | Substitution µmole/g |
|------------|-------------------------------------|------------------------|------------------------------------|----------------------|
| <u>6</u>   | Polyacryl-morpholide                | 1,10-diaminodecane     | DMT <sub>T</sub> Bz                | 63                   |
| <u>7</u>   | Polyacryl-morpholide                | 1,10-diaminodecane     | DMT <sub>T</sub> <sub>(P)</sub>    | 71                   |
| <u>8</u>   | Polyacryl-morpholide                | 1,10-diaminodecane     | BzU <sub>Bz</sub> <sub>(P)</sub>   | 29*                  |
| <u>9</u>   | Polyacryl-morpholide                | 1,2-diaminoethane      | DMT <sub>T</sub> <sub>(P)</sub>    | 32                   |
| <u>10</u>  | Polyacryl-morpholide                | spermine               | DMT <sub>T</sub> Bz                | 94                   |
| <u>11</u>  | Silica gel 7734 Merck (63-200 µ)    | 3-amino-1-propanol     | DMT <sub>T</sub> Bz                | 85                   |
| <u>12</u>  | Silica gel porasil Waters (37-75 µ) | 3-amino-1-propanol     | DMT <sub>G</sub> Bz                | 16                   |
| <u>13</u>  | LCAA-CPG 500 Å Pierce (125-177 µ)   | long chain alkyl-amine | DMT <sub>T</sub> Bz                | 25                   |
| <u>14</u>  | LCAA-CPG 500 Å Pierce (125-177 µ)   | long chain alkyl-amine | DMT <sub>U</sub> <sub>(P)</sub> Bz | 10                   |

\* The loading was estimated after coupling with DMT<sub>TOH</sub>.

### 1.2. Stability of the nucleoside-support

The stability of the primer-support linkage under deblocking conditions (0.3M N<sup>1</sup>,N<sup>2</sup>,N<sup>3</sup>,N<sup>4</sup>-tetramethylguanidinium 2-pyridine-aldoxime in 1:1 dioxane-water overnight followed by concentrated ammonia hydroxyde at 50° for 5h) was checked for each support by spectroscopic analysis of the released DMT. After such a treatment, no change in the loading of the CPG and polyacrylamide supports was detected. However after an overnight treatment at 50° with concentrated NH<sub>4</sub>OH, a loss of capacity (about 10%) was observed for the polyacrylamide supports as compared to the CPG support. These results were confirmed by analysis of the supernatants by HPLC. Thus, CPG appears to be the best support and was chosen for further experiments.

### 1.3. Oligodeoxynucleotides synthesized from the primer-CPG support

The proposed linkage leaves the 5' and 3' OH available for chain elongation from either end.

We have tested the CPG support for the synthesis of a 18-mer (15) following the phosphotriester method with a classical 3'-5' elongation<sup>14</sup> (Figure 1). The support 13 (200 mg, 5 µMol) loaded

with 2b was detritylated with 2% BSA. The synthesis was performed from the 5' OH ends with trimer blocks with a 82% average coupling yield. At the end of the elongation, the support bound oligomer was submitted to deblocking conditions (TMG-PAO,  $\text{NH}_4\text{OH}$ ). The presence of the elongated chain on the support was checked by acid hydrolysis; HPLC analysis of the supernatant indicated that the three bases are present in the expected ratio.

We have also synthesized two long fragments from support 13 (Figure 1) using two chemical methods: a 35-mer (16) prepared according to the phosphotriester method with manual addition of trimer blocks and a 34-mer (17) with the phosphoramidite method using an automatic synthesizer. The overall yield was about 25%. The presence of endonuclease sites RsaI and HpaII, in the 35- and 34-mer sequences respectively, allows the control of the elongated chain instead of using acid hydrolysis (cf § 3)

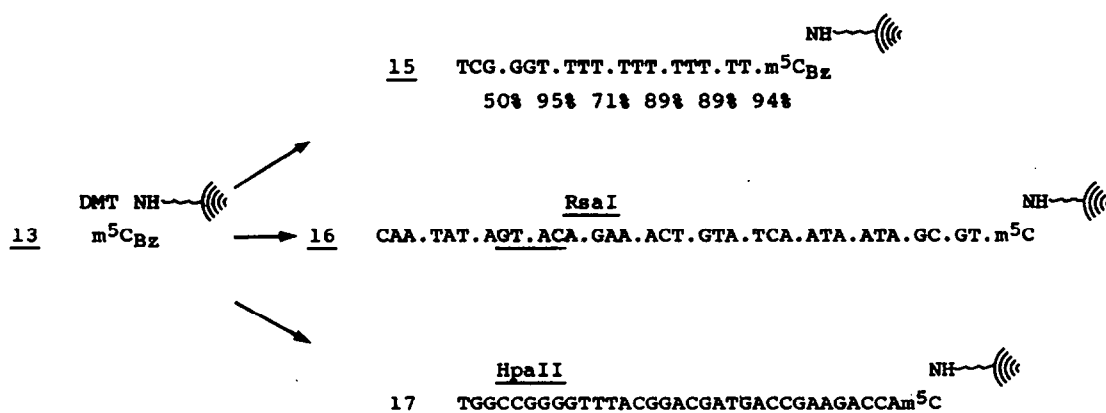


Figure 1

#### 1.4 Release of the synthesized oligodeoxynucleotide from the solid support

In order to check whether a covalently bound fragment could be cleaved from our support by a specific chemical reaction, we have used as a primer on CPG a protected uridine (support 14). We presume that, if the elongation is performed from the 3'-end of a ribose residue, an alkaline hydrolysis should permit the release of the anchored oligomer<sup>17</sup>.

In order to test this hypothesis, we have synthesized a thymidyl-tetramer from support 14 (35 mg) following the phosphotriester method. The first coupling with 5'-dimethoxytrityl thymidine gives a 3'-3' linkage with the 5'-end available for a classical 3'-5' elongation. The next step is the condensation of a trimer of T's. After complete deprotection, the supported oligomer 18 is treated with 1N NaOH at 50°C for 10 minutes. HPLC analysis reveals the presence of the tetramer (Tp)<sub>3</sub>T in the supernatant, identical to the same oligomer synthesized with the standard succinyl linkage.

#### 1.5 Elongation in either the 3' or 5' direction

As described before, the proposed linkage leaves free the 3' and 5' OH and allows chain elongation from both ends. In order to investigate this possibility, we decided to synthesize a 15-mer from the 3'-end of a primer nucleoside (support 14) in either 3'-5' (oligomer 19) or 5'-3' (oligomer 20) direction (Scheme 4). The use of a ribonucleoside<sup>17</sup> as primer allows visualization of the synthesized oligomers. Thus, after removal of the protecting groups, the two solid supported oligomers 19 and 20 were subjected to an alkaline hydrolysis. The supernatants were analyzed by polyacrylamide gel electrophoresis (Figure 2).

Scheme 4

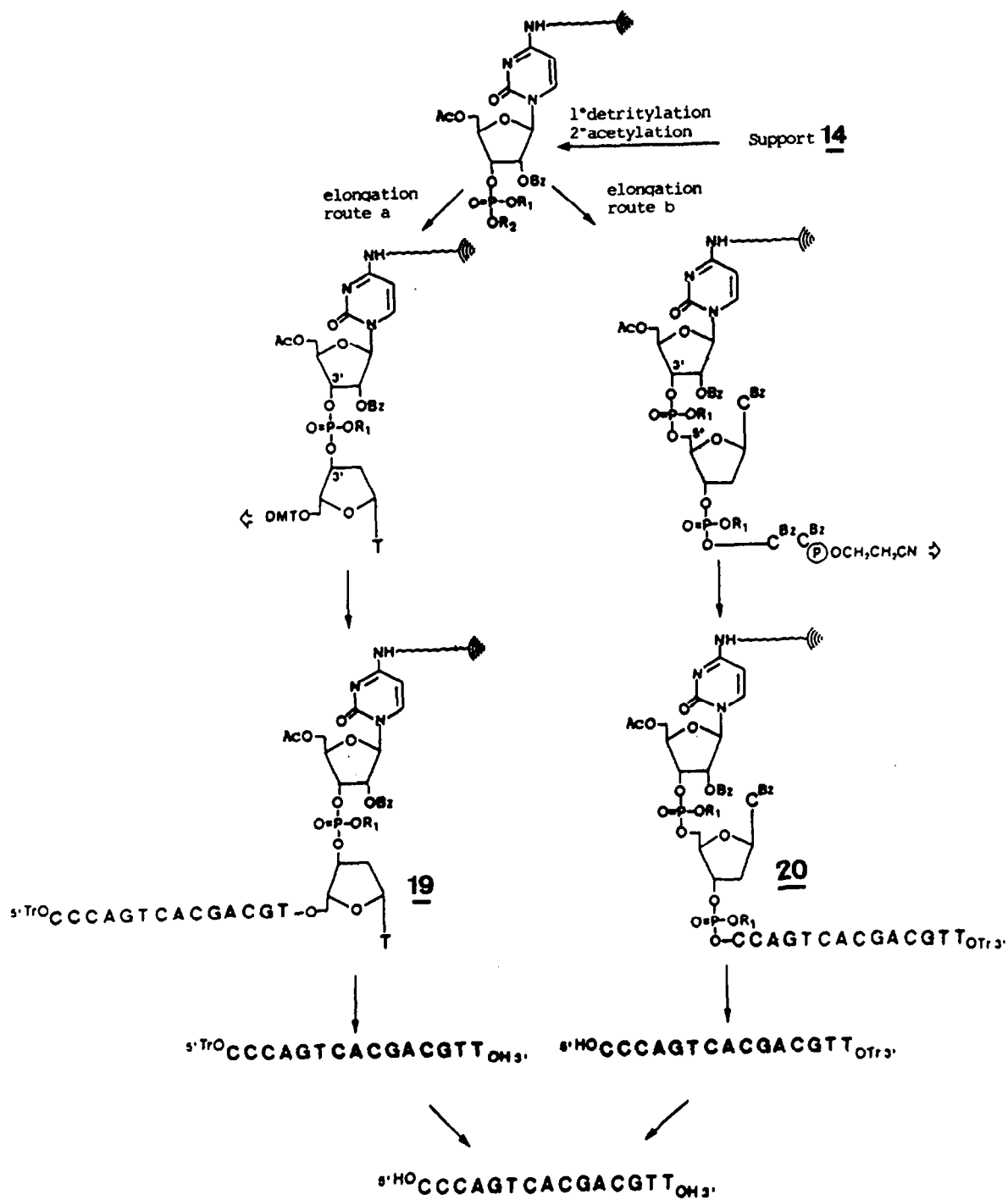




Figure 2: Autoradiogram of 20% polyacrylamide gel electrophoresis (8 M urea) showing crude oligomers obtained after alkaline treatment of supports 19 (lane 1) and 20 (lane 2).

In 3'-5' elongation (route a), coupling steps with the trimer blocks were followed by a capping reaction (acetic anhydride in pyridine). In the 5'-3' elongation (route b), the synthesis was carried out in absence of capping the unreacted phosphate groups and led to the formation of many by-products, as shown on Figure 2. Purification of the crude oligomers was performed by HPLC. The 5'-tritylated oligomer was obtained with 22% yield from the support 19 and the 3'-tritylated oligomer was obtained with 12% yield from the support 20. Detritylation of the two tritylated oligomers with 80% acetic acid for 4 hours led to the identical 15-mer.

This first procedure described above allows access to long fragments anchored to a solid support with good yields following the phosphotriester or phosphoramidite approach and the machine synthesis using such supports. The use of a cytidine residue as anchor permits the release of the partially or fully deblocked synthesized oligonucleotide from the support under mild conditions independently of the deblocking reactions.

Recently, a new solid support using a long spacer arm terminating in a primary hydroxyl has been described<sup>18</sup>. The proposed linkage is stable under deblocking steps and allows the release of the fully blocked oligomers. However this system does not permit chain elongation in both directions, since the 3' or 5' terminus is linked to the support.

## 2 - DIRECT ATTACHMENT OF A UNIQUE OLIGONUCLEOTIDE TO A SOLID SUPPORT

The method described above suffers one drawback: accumulation of shorter chains in addition to the desired product. If these by-products are too numerous, they could constitute a handicap for the use of such supports as affinity columns for binding proteins.

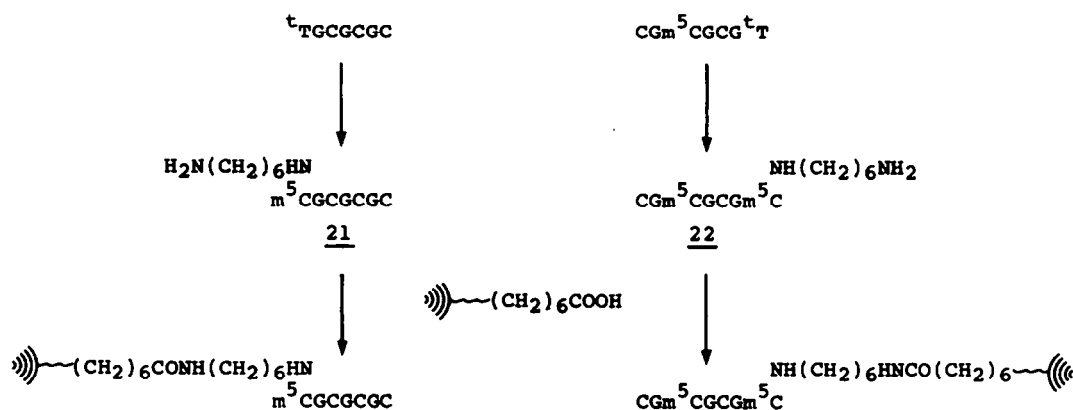
To avoid the presence of truncated failure sequences and the possible resort to a solid supported enzymatic digestion<sup>18,19</sup>, we have investigated another method for direct attachment to a solid support of an oligomer independently synthesized and purified.

This procedure consists of synthesizing oligomers which contain a reactive arm (Y) at any site of the sequence, followed by the formation of a covalent bond between this arm (Y) and a support functionalized with a complementary reactive group (A) (Scheme 2).

The incorporation of a modified nucleoside such as the 5-methyl-4-triazolyl-pyrimidin-2-one-1- $\beta$ -D-2'-deoxyribofuranoside<sup>10,21,22</sup> in the sequence occurs at any C site by manual or automatic synthesis. The reaction with a bifunctional chain such as 1,6-diaminohexane gives the expected reactive arm. The synthesized oligomer is then deprotected and purified according to classical technique (HPLC or gel electrophoresis). The reaction of this spacer arm with a convenient support can be performed in aqueous phase or organic mixture.

The choice of the activated support depends on the kind of bond to be formed, as well as the coupling medium. We have chosen the Sepharose-4B support bearing an hexanoic acid arm (CH-Sepharose-4B). The activation by the N-hydroxysuccinimide group is accomplished with 1,3-dicyclohexylcarbodiimide in anhydrous dioxane<sup>3</sup>. The coupling reaction is performed at 4° overnight in 0.1 M NaHCO<sub>3</sub> pH 8 with 0.5 M NaCl according to described procedures.

We first investigated the attachment of heptamers 21 and 22 containing a reactive arm at the 5' and 3'-termini, respectively (Scheme 5).



Scheme 5

To the triazolyl heptamers were added 5 equiv. of 1,6-diaminohexane in dioxane. The functionalized oligomers were then subjected to deblocking conditions (0.3 M TMG-PAO overnight; conc. NH<sub>4</sub>OH at 50° for 5h) and purified by HPLC on Zorbax ODS. The two tritylated heptamers 21 and 22 were dissolved in the coupling buffer (0.1 M NaHCO<sub>3</sub> pH 8, 0.5 M NaCl) and added to the activated CH-Sepharose-4B. A blank assay was carried out in the same conditions with an hexamer without the alkylamine chain, d(CG)<sub>3</sub>. After stirring overnight at 4°, the supports were washed with the coupling buffer. The coupling reaction was monitored by UV measurement of the DMT cation released by acid treatment of loaded supports. The efficiency of coupling for the heptamers 21 and 22 was about 80% whereas less than 2% of the hexamer d(CG)<sub>3</sub> remained in the blank (non specific linkage). The loading was estimated to 2  $\mu$ Mol of heptamers per g of Sepharose. The presence of the oligomers was checked by HPLC analysis after acid and enzymatic hydrolysis (PDE I) of the loaded supports.

We also investigated the attachment of a long fragment (35-mer) bearing the same amino spacer, annealed with its complementary strand (34-mer), to the activated CH-Sepharose-4B. The coupling reaction was monitored with the <sup>32</sup>P labeled 35-mer as tracer. The efficiency of coupling was about 35% in this experiment. The presence of these oligomers linked to Sepharose was controlled by enzymatic cleavage of the anchored duplex according protocol described in § 3.

Recently, DNA affinity supports using modified oligonucleotides covalently attached to nylon membranes have been reported in a congress by Ruth et al.<sup>23</sup>. The anchorage between the support and the oligonucleotide occurs at the C-5 position of a pyrimidine ring via a spacer arm.

### 3 - RESTRICTION ENDONUCLEASE CLEAVAGES

The use of restriction endonuclease allows the control of the presence of long sequences anchored to the support and, using the resulting fragment from the enzymatic cleavage, the estimation of their accessibility to these enzymes.

Figure 3 illustrates the cleavage by *HpaII* of the anchored 34-mer on CPG (16). In a first step, the supported 34-mer (16) was 5'-<sup>32</sup>P labeled using  $\gamma$ -<sup>32</sup>P-ATP and T4-polynucleotide kinase<sup>24</sup>. After incubation at 37° for 30 min. and heat denaturation, the support was washed with kinase buffer until no radioactivity was detected in supernatant. After hybridization with the complementary 33-mer, the reaction mixture was incubated with the corresponding endonuclease. The efficiency of cleavage was estimated by counting the radioactivity in the supernatant (60% yield). Polyacrylamide gel analysis confirmed the presence of the expected 4-mer in the supernatant, resulting from the cleavage by *HpaII* of the anchored 34-mer.

### CONCLUSION

We have presented the preparation of DNA fragments specifically attached to a matrix via a new link between a nucleotide base and a solid support.

The anchored oligomer can be obtained by chemical synthesis from a primer following standard procedures. The use of a ribonucleoside as primer allows the release of the synthesized anchored oligomer from the support under mild chemical conditions, independently of the deblocking reactions. Automation of the synthesis is easily achieved.

The solid-supported DNA fragment can also be obtained by direct attachment of a modified oligonucleotide to an activated support.

The supported DNA system is accessible to enzymes such as T4 polynucleotide kinase and restriction endonucleases.

The use of such supports as a primer for ligase allowing access to long fragments or as an anchored complementary strand for the detection and the purification of polynucleic acids will be presented elsewhere. The use of these solid supported DNA fragments as affinity columns for the purification of binding proteins is in progress.

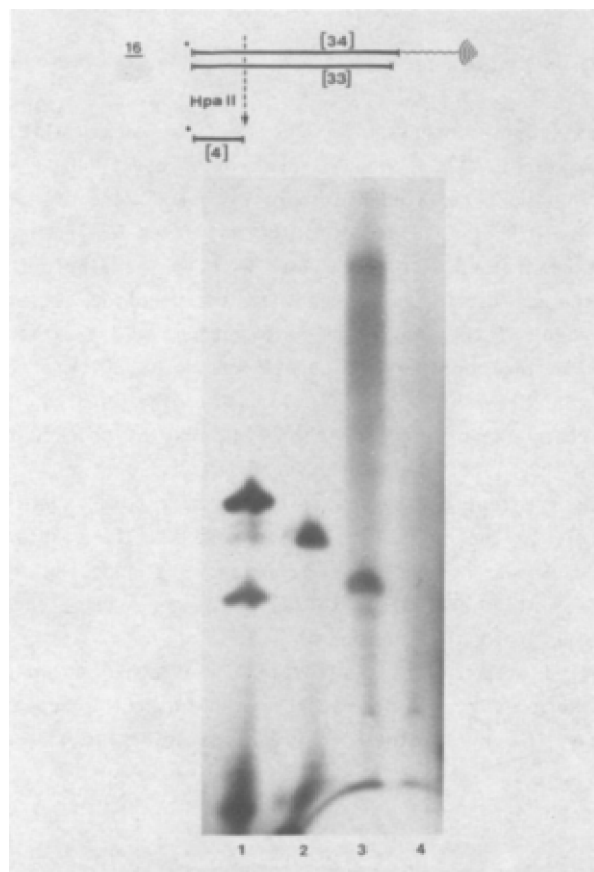


Figure 3: Autoradiogram of 20% polyacrylamide gel electrophoresis (8 M urea) showing fragment resulting from the cleavage by *HpaII* of the anchored 34-mer (16) hybridized with the complementary 33-mer (lane 3); the same reaction without the 33-mer (lane 4); fragments of 8,4 (lane 1) and 6 (lane 2) units as length markers.

## EXPERIMENTAL

### Materials

Enzacryl Gel K2 was purchased from Aldrich, LCAA-CPG (500 Å pore size) from Pierce and Sepharose-4B from Pharmacia. Machine synthesis of oligodeoxynucleotides were performed using an Applied Biosystems Model 380A Synthesizer with phosphoramidites. High Pressure Liquid Chromatography was carried out using Zorbax ODS 9.3 mm ( $B = 10^{-2}$  M triethylammonium acetate pH 7.5 and  $A = 50\%$   $B + 50\%$  acetonitrile, gradient 10-50%  $A$  in 20 min.) and HS5 C18 (gradient 5-25% of acetonitrile in  $B$  in 20 min.) columns. T4 polynucleotide kinase, restriction endonuclease HpaII and T4 DNA ligase were purchased from Amersham.

Functionalization of solid supports (6 to 14) with activated nucleosides (1b to 5b). The preparation of the support 6 is given as an example; 200 mg of Enzacryl functionalized by 1,10-diaminodecane (0.44 mMol/g)<sup>10</sup> and 150 mg (0.25 mMol) of 5-methyl-4-triazolyl-pyrimidin-2-one-5'-O-dimethoxytrityl-3'-O-benzoyl- $\beta$ -D-2'-deoxyribofuranoside<sup>11</sup> were left in suspension in dioxane or pyridine at room temperature for 2-10 days. The support was filtered and abundantly rinsed with pyridine, methanol and dichloromethane. The residual amino groups of the support were capped by treatment with acetic anhydride (200  $\mu$ l) in pyridine (2 ml). In the case of glass beads (500 mg), isobutylchloroformate (200  $\mu$ l) in pyridine (3 ml) for 15 min. were used according to standard procedure<sup>25</sup>. The loading of each support was estimated by spectroscopic measurement at 507 nm of DMT released by detritylation with 2% BSA of an aliquot (Table).

Synthesis of an anchored oligodeoxynucleotide from a primer nucleoside. As an example, the synthesis of a 15-mer from the 3'-ends of the cytidine primer 14 was described (Scheme 4).

In the 3'-5' elongation (route a), the first step consisted of a 3'-3' coupling with the 3'-end of the support and the 3'-OH of a 5'-dimethoxytritylated thymidine. 100 mg (1.0  $\mu$ Mol) of 14 were detritylated with 2% BSA, then acetylated with acetic anhydride. The support was then 3'-decyanoethylated with 1 ml *t*-butylamine-pyridine (1/9, v/v) for 20 min. and reacted with 60 equiv. DMT in presence of 100 equiv. of TPSNT (1,3,5-trisopropylbenzenesulfonyl nitrotriazole) in anhydrous pyridine (61% yield). The following coupling steps with <sup>DMT</sup>TG, <sup>DMT</sup>GAC, <sup>DMT</sup>CAC, <sup>DMT</sup>AGT, <sup>T</sup>CCC were accomplished with the classical 3'-5' elongation. Each coupling reaction was followed by a capping step of the unreacted 5'-ends. The supported oligomer was then subjected to deblocking conditions (2 ml 1 M TMG-PAO overnight, 2 ml conc.  $\text{NH}_4\text{OH}$  at 50° for 5 hours).

In the 5'-3' elongation (route b), the oligomer was prepared by 5'-3' coupling with trimer blocks. 150 mg (1.5  $\mu$ Mol) of support 14 were decyanoethylated with 1 ml *t*-butylamine-pyridine and then reacted successively with 28 equiv. of <sup>HO</sup>CCC, <sup>HO</sup>AGT, <sup>HO</sup>CAC, <sup>HO</sup>GAC and <sup>HO</sup>GTTT<sub>r</sub> in the presence of an excess of TPSNT (90 equiv.). Each coupling step was preceded by the decyanoethylation of the anchored intermediate oligomer. At the end of the synthesis, the anchored oligomer was deprotected (1 M TMG-PAO overnight, conc.  $\text{NH}_4\text{OH}$  at 50° for 5 h).

The expected sequences remained 3' or 5'-tritylated in order to facilitate HPLC purification of the released oligomers or to achieve solid supported enzymatic purification (unreported results).

Release of the synthesized oligomer from the support. After completion of the synthesis, the partially deprotected oligomers anchored to the support were submitted to an alkaline hydrolysis. 50 mg of supports 19 and 20 were treated with 0.5 ml 2 N NaOH at 60° for 10 min. After neutralization with Dowex 50W-pyridinium, 1.6 OD and 2.0 OD of the released products were isolated from 19 and 20 respectively. The crude oligomers were then purified on Zorbax ODS (gradient of acetonitrile in  $10^{-2}$  M triethylammonium acetate pH 7.2). 0.35 OD of the 5'-tritylated 15-mer (22%) and 0.23 OD of the 3'-tritylated 15-mer (11%) were isolated.

Synthesis of the modified oligodeoxynucleotides 21, 22 and 23. The oligomers bearing a linker arm were obtained by incorporation of a 5-methyl-4-triazolyl-pyrimidin-2-one-1- $\beta$ -D-2'-deoxyribofuranoside during the elongation of the chain.

The synthesis of the heptamers 21 and 22 was performed in solution following the phosphotriester method<sup>14</sup>. The incorporation of <sup>T</sup> as monomer was accomplished in the last coupling step. After purification on silica gel, the heptamers were treated with 5 equiv. of 1,6-hexanediamine in dioxane (0.15 to 0.20 M). After completion of the reaction (CCM), the oligomers were subjected to deblocking conditions (0.3 M TMG-PAO overnight, conc.  $\text{NH}_4\text{OH}$  at 50° for 5 h). The dimethoxytritylated oligomer were then purified by HPLC on Zorbax ODS.

The synthesis of the 35-mer was performed following the phosphoramidite chemistry using an Applied Biosystems Model 380A synthesizer. The modified nucleoside  $\epsilon$ T was incorporated as a extra step from a functionalized cartridge with the phosphotriester chemistry. The machine elongation was accomplished from this primer. To the synthesized supported oligomer (30 mg, 0.2  $\mu$ Mol) was added 10 equiv. of 1,6-diaminohexane in pyridine (0.15 M) for 48 h. The oligomer was then deprotected (TMG-PAO,  $\text{NH}_4\text{OH}$ ,  $\text{CH}_3\text{COOH}$ ) and purified by electrophoresis on 15% polyacrylamide gel (15 OD). The complementary strand was prepared using the synthesizer and purified according to standard methods<sup>14</sup>.

Attachment of a modified oligonucleotide to an activated support. To 200 mg of swollen activated CH-Sepharose-4B (Pharmacia) were added 500 nMol (2 mg) of heptamer 21 or 22 dissolved in 1 ml of the coupling buffer (0.1 M  $\text{NaHCO}_3$ , 0.5 M NaCl). After stirring overnight at 4°, the gel suspension was treated according to described procedure<sup>3</sup>. The efficiency of the reaction was estimated by UV measurement of the DMT cation released by acid treatment of loaded Sepharose (about 80%). The same quantity (3 OD) of strands 23 and 24 was measured and mixed in 50  $\mu$ l 0.5 M NaCl. After heating at 90°, the hybridized oligomers were added to activated CH-Sepharose-4B according to described procedure (Pharmacia). The efficiency of the attachment was estimated by adding an aliquot of  $^{32}\text{P}$ -labeled 35-mer to the hybridized oligomers (35% yield). The presence and the accessibility of the anchored duplex were checked by enzymatic cleavage (Figure 3).

Cleavage by a restriction endonuclease. An aliquot of deprotected oligomer bound to the support (1 mg of CPG-support, 1-10 nMol 5'-ends) was labeled in a 30  $\mu$ l reaction mixture containing  $\gamma$ - $^{32}\text{P}$ -ATP (14 pMol) and T4 polynucleotide kinase (13 U) at 37° for 30 min. according to established procedures<sup>24</sup>. The enzyme was denaturated by heating at 68° for 15 min and the support was washed abundantly with kinase buffer. To the the 5'-labeled anchored oligomer were added 200 pMol of the complementary strand in 20  $\mu$ l TA buffer (33 mM Tris-acetate pH 7.9, 10 mM magnesium acetate, 66 mM potassium acetate and 0.5 mM DTT). The reaction mixture was heated at 90° for 2 min. and cooled slowly to room temperature over a 1 h period. The restriction endonuclease (18 U) was then added. After incubation at 37° for 3 h, the supernatant (6  $\mu$ l) was loaded onto a 20% polyacrylamide gel (8 M urea) for analysis.

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