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Steric factors influencing hybridisation of nucleic acids to oligonucleotide arrays

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ABSTRACT

We have investigated the use of spacer molecules to reduce steric interference of the support on the hybridisation behaviour of immobilised oligonucleotides. These spacers are built up from a variety of monomeric units, using phosphoramidite chemistry, by condensation onto an amine-functionalised polypropylene support. The optimal spacer length was determined to be at least 40 atoms in length, giving up to 150-fold increase in the yield of hybridisation. The effects of different charged groups in the spacer were also examined, and it was shown that both positively and negatively charged groups in the spacer diminish the yield of hybridisation. Steric hindrance in hybridisation can also be a problem if the oligonucleotides attached to the support are too close to each other. Surface coverage was varied using a combination of cleavable and stable linkers, giving the highest hybridisation yields for surfaces containing ~50% of the maximum concentration of oligonucleotides.

INTRODUCTION

The combinatorial approach to synthesising and screening very large numbers of ligands attached to a solid support has many applications in the life sciences including new drugs development, enzyme/substrate reactions (1,2) and nucleic acid sequence analysis (3–6). Techniques using solid support-bound oligonucleotides as probes are finding a wide range of applications (7). For all these applications, the hybridisation of the oligonucleotides to target nucleic acids should be as free as possible from interference from the solid support.

In this study we used combinatorial techniques for simultaneous analysis of different factors influencing oligonucleotide duplex formation. We have previously used such methods to study the effects of base composition, length and structure on the hybridisation properties of oligonucleotides (8–11). A variety of ways of synthesis of combinatorial oligonucleotide arrays have been described (4–6). We used a physical masking method developed in our laboratory (13) to direct the synthesis of spacers to specific regions.

Though spacers have been used to mitigate the effects of the solid support (5,7,14), there has been no systematic study of the various factors affecting hybridisation behaviour. Properties of the spacer which are likely to be important include length, charge, hydrophobicity and solvation. We made novel monomers which can be used to build spacers with different chemical composition. Combinatorial methods were used to make arrays in which a probe oligonucleotide was attached to the surface by different spacer molecules. The arrays were hybridised with labelled complementary targets of high and low molecular weight. In the following we present the results of our study of the optimal composition and length of spacer in between oligonucleotides and a polypropylene support and of the concentration of oligonucleotides on the support.

We found that the spacers have a large effect on hybridisation yield, the most important property of the spacer being its length.

MATERIALS AND METHODS

Array synthesis was carried out as previously described (13) adapting phosphoramidite chemistry (15). Oligonucleotides used for hybridisation were made in an Applied Biosystems 392 DNA/RNA synthesiser using the same standard chemistry. 3′-DMTr-5′-phosphoramidites of base-protected nucleosides and aminated polypropylene were a gift from Beckman Instruments. Oligonucleotides and $tRNA^{Phe}$ (Sigma) were labelled according to standard methods using radioisotopes purchased from Amersham International. Mass spectra were recorded on Voyager™-Elite Biospectrometry™ Research Station, PerSeptive Biosystems. ^{1}H-NMR spectra were recorded on a Varian Gemini 200 MHz spectrometer. PhosphorImages were obtained using Molecular Dynamics PhosphorImager Model 400A. All chemicals were purchased from Aldrich Chemical Company. Silica gel for column chromatography and solvents were purchased from BDH/Merck.

O^1-DMTr-*O*^{n}-cyanoethoxydiisopropylamino-phosphoramidites

O^1-DMTr-O^n-cyanoethoxydiisopropylaminophosphoramidites of diols (type **1–3** spacers) and the cleavable reagent, (2-cyanoethoxy)-2-(2′-*O*-DMTr-oxyethylsulfonyl)ethoxy-*N*,*N*-diisopropylaminophosphine (**4**), were synthesised as described (16–18).

N-Fmoc-*O*^{1}-DMTr-*O*^{3}-cyanoethoxydiisopropylaminophosphinyl-2-amino-1,3-propanediol (6)

Serinol (2.05 g, 22.5 mmol) was dried by evaporation with 10 ml dry pyridine, dissolved in 120 ml dry pyridine and ice-cooled. FmocCl (5.7 g, 22.5 mmol, dissolved in 5 ml dry dioxane) was

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slowly added to this solution during 1 h under stirring. The solution was then stirred at room temperature for 4 h, ice-cooled and DMTrCl (7 g, 20.1 mmol) was slowly added during 30 min together with 3 ml *N,N′*-diisopropylethylamine. The reaction mixture was left overnight, then evaporated to an oil, taken up to 100 ml non-acidic chloroform, washed with water (3 × 250 ml) and brine (250 ml), evaporated to an oil, dissolved in 7 ml toluene and purified by flash chromatography on a 6 × 12 cm column using mixtures: methylene chloride–hexane (3:1, 2:1, 1:1, 300 ml of each, all containing 0.5% Et_3N) as eluents. Two main fractions were collected, evaporated to foam and dried *in vacuo*. The first fraction (R_f 0.56, CH_2Cl_2, 0.5% Et_3N) appeared to be a ditrytilated derivative of *N*-Fmoc-serinol. Yield 5.0 g (25%). ^{1}H-NMR: (DMSO-D6): 3.32 (m, 1H), 3.69 (s, 12H, OCH_3), 4.23 (m, 4H), 5.7 (m, 1H), 6.21 (m, 2H), 6.7–7.6 (m, 30H, arom.), 7.7 (d, 2H), 7.89 (d, 2H).

The second fraction (R_f 0.22) was determined to be monotritylated *N*-Fmoc-serinol (**5**). Yield: 6.0 g (46%). ^{1}H-NMR (DMSO-D6): 3.41 (m, 1H), 3.69 (s, 6H, OCH3), 4.2 (m, 2H), 4.63 (m, 2H), 5.71 (m, 1H), 6.26 (m, 2H), 6.7–7.5 (m, 17H, arom.), 7.7 (d, 2H), 7.89 (d, 2H). Both fractions, when treated with piperidine, showed decrease of R_f (cleavage of Fmoc-group) and positive test with fluorescamine (NH2- group). The monotritylated *N*-Fmoc-serinol (2 g, 3.6 mmol) was dried for 24 h *in vacuo* over P_2O_5 and transferred under argon into 35 ml dry acetonitrile, and 250 mg (3.6 mmol) dry tetrazole and 1.12 ml (3.6 mmol) bis-*N,N*-diisopropylamino-2-cyanoethoxyphosphite were added and the reaction was stirred under argon at room temperature. After 4 h the reaction mixture was diluted with 100 ml ethyl acetate, washed with saturated sodium bicarbonate solution (2×200 ml) and brine (200 ml), dried over anhydrous Na_2SO_4, evaporated, dissolved in 5 ml toluene and rapidly purified by flash-chromatography (dichloromethane:hexane 3:1, then dichloromethane, all with 0.5% Et_3N). Product-containing fractions were evaporated, dissolved in 5 ml toluene and precipitated into 500 ml ice-cold pentane. The solvent was decanted, and the residue was dried in vacuo over P_2O_5 to give 2.4 g (82%) of a yellowish powder. R_f 0.83 (CH_2Cl_2, 0.5% Et_3N), Calculated for $C_{48}H_{54}N_3O_7P$: 815.044. Found: (mass-spectrum, m/z): 836.92 (MI + Na^+).

N-(*N*-Fmoc-glycyl)-*O*^{1}-DMTr-*O*^{3}-cyanoethoxydiisopropyl-aminophosphinyl-2-amino-1,3-propanediol (8)

N-Fmoc-glycine pentafluorophenyl ether (6 g, 12.9 mmol) was added to a stirred solution of serinol (1.17 g, 12.8 mmol) in 100 ml dried pyridine and left overnight at room temperature. The solution was then ice-cooled, and DMTrCl (4.2 g, 12.5 mmol) was slowly added during 30 min. The reaction mixture was left overnight, then evaporated to an oil, taken up to 100 ml non-acidic chloroform, washed with water (3 × 250 ml) and brine (250 ml), evaporated to an oil, dissolved in 7 ml toluene and purified by flash chromatography on a 6 × 12 cm column using mixtures: methylene chloride–hexane (3:1, 2:1, 1:1, 300 ml of each, all containing 0.5% Et_3N) as eluents. Two main fractions were collected, evaporated to foam and dried *in vacuo*. The first fraction (R_f 0.54, $CHCl_3$, 0.5% Et_3N) appeared to be a ditrytilated derivative of *N*-Fmoc-glycyl-serinol. Yield: 2.2g (46%). ^{1}H-NMR ($CDCl_3$): 3.19 (m, 2H), 3.52 (m, 2H), 3.73 (s, 12H), 3.80 (m, 1H), 4.08 (m, 2H), 4.12 (m, 1H), 4.30 (m, 2H), 5.25 (m, 1H), 5.94 (m, 1H), 6.7–7.9 (m, 34H). Calculated for $C_{62}H_{58}N_2O_9$: 975.149. Found: (mass-spectrum, m/z): 997.494 (MI + Na^+), 1012.75 (MI + K^+).

The second fraction (R_f 0.08) was determined to be monotritylated *N*-Fmoc-glycyl-serinol (**7**). Yield: 2.08 g (25%). ^{1}H-NMR ($CDCl_3$): 3.34 (m, 2H), 3.78 (s, 6H), 3.81 (m, 1H), 3.87 (m, 2H), 4.12 (m, 2H), 4.2 (m, 1H), 4.39 (d, 2H), 5.41 (m, 1H), 6.45 (m, 1H) 6.7–7.9 (m, 21H). Calculated for $C_{41}H_{40}N_2O_7$: 672.776. Found: (mass-spectrum, m/z): 696.708 (MI + Na^+), 713.518 (MI + K^+).

Both fractions, when treated with piperidine, showed decrease of R_f (cleavage of Fmoc-group) and positive test with fluorescamine (NH_2- group). The monotritylated *N*-Fmoc-glycyl-serinol (2 g, 3 mmol) was phosphitylated and dried as described above to give 2.03 g (78%) of a yellowish powder. R_f: 0.49 ($CHCl_3$, 0.5% Et_3N). Calculated for $C_{50}H_{57}N_4O_8P$: 872.995. Found: (mass-spectrum, m/z): 896.197 (MI + Na^+).

5′-(α,α-bis-(4-methoxyphenyl)[ring-U-^{14}C]benzyl)-thymidine-3′-phosphoramidite (9)

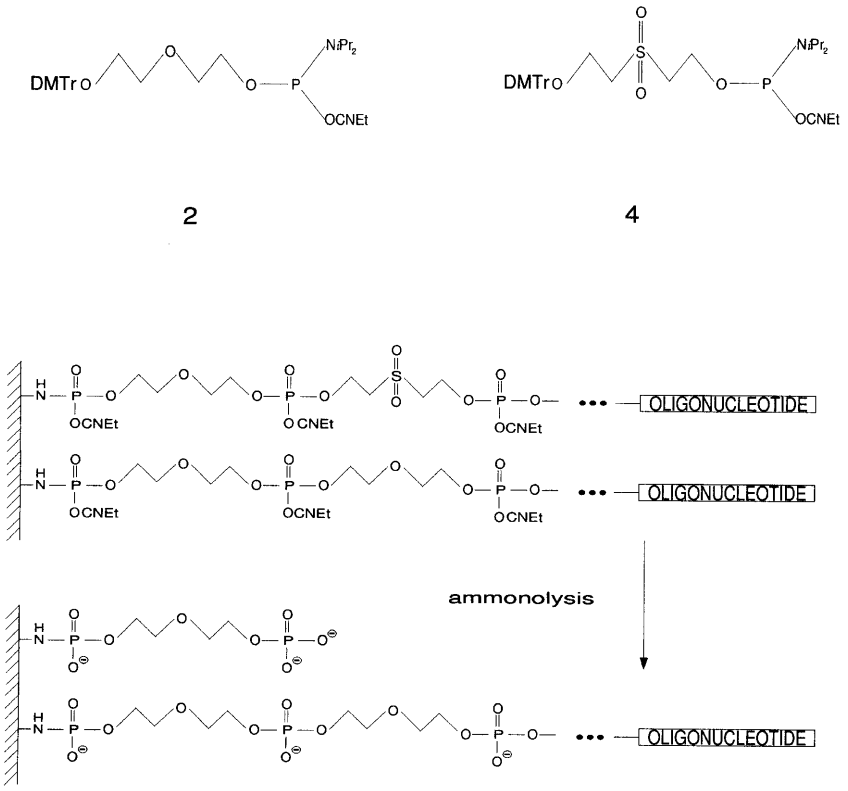
α,α-bis-(4-methoxyphenyl)[ring-U-^{14}C]benzyl alcohol (Amersham, 777 MBq/mmol, 21 mCi/mmol, 150 mg) was mixed with 4.8 g DMTrCl and dissolved in 40 ml toluene. Acetyl chloride (5 ml) was added and the mixture was boiled with stirring in a 100 ml round-bottom flask fitted with vertical condenser with drying tube on the oil bath for 3 h. After distilling off about half the solvent, the rest was cooled to room temperature and poured into 600 ml cold (–20°C) hexane to precipitate the DMTr*Cl which was collected by filtration and dried *in vacuo* to give 4.4 g (87%) of slightly pink solid. This ^{14}C-labelled DMTrCl was used to synthesise the ^{14}C-labelled T phosphoramidite as described (19).

The following oligonucleotides were used for hybridisation to arrays: 1, 5′-GGT.GCG.AAT.TCT; 2, 5′-GAC.CTC.CAG.ATT. In all cases, ‘reversed’ means 5′-immobilised oligonucleotide. Unless otherwise specified, oligonucleotides are covalently attached to the solid support through their 3′-phosphate.

RESULTS AND DISCUSSION

Effect of surface density of active sites on oligonucleotide synthesis and hybridisation yield

Phosphoramidite reagents for oligonucleotide synthesis are bulky enough to inhibit coupling to reactive groups on the support. The aminated polypropylene support contains ~0.3 nmol/cm^2 of NH_2 groups (20), which, assuming the surface is flat, gives a spacing between adjacent amino groups of ~8 Å. This distance is smaller than the size of tritylated nucleoside phosphoramidites (20–25 Å) or the diameter of the DNA helix (~18–20 Å); clearly these groups cannot be accommodated on the surface at the full density of the amino groups and the density of coupled groups will be determined by the dimensions of the monomers used to synthesise oligonucleotides. We have carried out a detailed physical study of the surface before and after coupling the spacer, and of the reaction of the spacer with phosphoramidites (21). An area of a sheet of aminated polypropylene was derivatised by reaction with a mixture of all four phosphoramidites (A, C, G and T) under standard conditions, but with a slightly longer period (2 min) of condensation step. Without removing the DMTr protecting group, a second, radiolabelled phosphoramidite (^{14}C-DMTrT phosphoramidite) was applied to an area which included that which had been coupled previously, and an adjacent area of untreated aminated polypropylene. This reaction was carried out over an extended period of 1 h. The ^{14}C-DMTr group



Scheme 1. Stable (**2**) and cleavable (**4**) synthons of similar length used for the synthesis of arrays of different concentrations of oligonucleotides. The two synthons were applied to the aminated polypropylene as a mixture (see text for ratios, and combinatorial protocol). Following oligonucleotide synthesis, those oligonucleotides synthesised on spacers coupled through **4** are removed during ammonolysis by alkali cleavage of the spacer.

was not removed. The polypropylene was first exposed to a storage Phosphor screen and then treated with ammonia under conditions normally employed in oligonucleotide synthesis. Radioactivity was measured using a PhosphorImager. The coupling of the radioactive monomer was inhibited by only 15–25% by the previous coupling of non-radioactive monomers. This surprising result suggests that coupling may be a much slower process than has been previously assumed. It is possible that the first couplings are rapid, but that subsequent couplings are inhibited by steric crowding of the surface. Comparing the strips of this polypropylene support before and after ammonia treatment, it was also noted that the area which contained only ^{14}C-DMTrT lost ~20% of the ^{14}C during ammonolysis, indicating that the bond formed after phosphitamide condensation onto the surface of the aminated polypropylene support is not completely stable or that there is something other than alkylamine groups on the support which can act as functional groups for phosphoramidite coupling to give a labile linkage.

Previous data obtained in our laboratory show that even at the concentration produced by a short coupling reaction oligonucleotides are too crowded for efficient hybridisation (22). To alter the concentration of ODNs on the polypropylene supports we used combinations of cleavable and stable linkers. This method relies on the synthesis of ODNs at the maximal concentration with subsequent loss of part of them by cleavage of the labile linker by ammonolysis. The cleavable linker was based on sulfonyldiethanol [structure **4** (12), Scheme 1] which undergoes beta-elimination in the basic conditions of the post-synthetic ammonolysis procedure, liberating phosphate groups. The stable component was

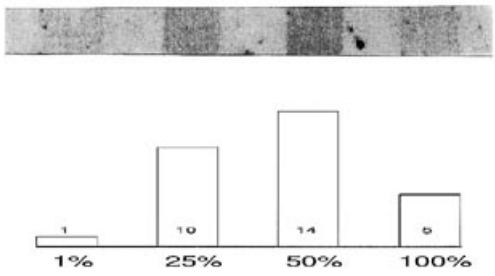
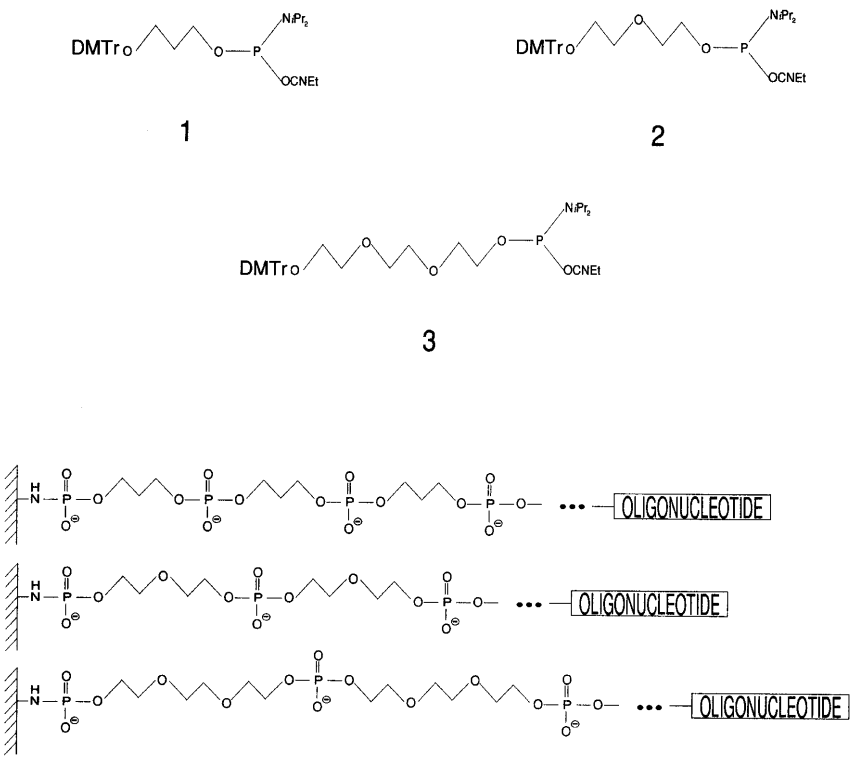


Figure 1. Hybridisation of complementary oligonucleotide to reversed oligonucleotide no. 2 at 36°C. Patches with concentration of stable spacer: 1, 25, 50 and 100%.

glycol synthon **2** (Scheme 1); as these two synthons are equal in length we assume the yields and rates of their condensation with the support are similar and, by premixing them in defined proportion, we produce similar proportions of cleavable and stable moieties on the support after condensation (Scheme 1).

We used stable and cleavable linkers mixed in different proportions: 100:0, 50:50, 20:80 and 1:99 mixtures of phosphoramidite synthons **2** and **4**. After capping (to make sure we do not have any reactive groups on the support) we synthesised oligonucleotides across the strip and subjected it to ammonolysis (Fig. 1). The augmentation of yield of hybridisation at 36°C for 50% concentration of stable spacer compared to that of 100% was ~50%. Oligonucleotides synthesised on lower concentrations gave lower yields.

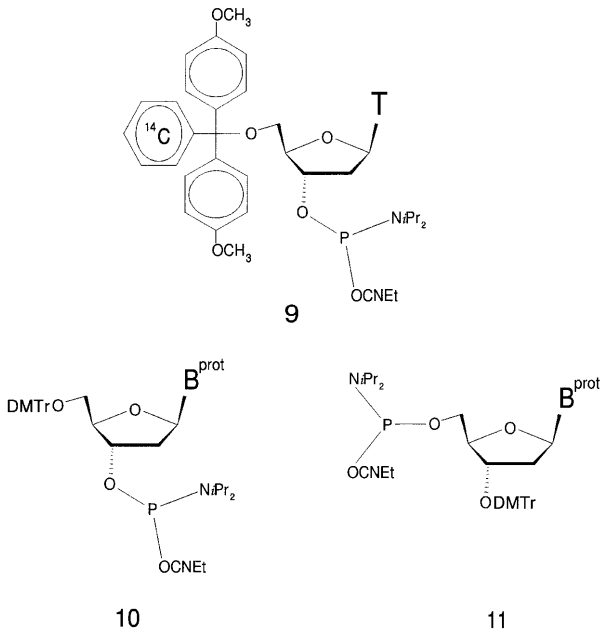


Scheme 2. Phosphoramidite synthons of different unit length and corresponding hydrophilic spacers synthesised directly on the surface of aminated polypropylene.

Effects of spacer length and charge on hybridisation

The more an immobilised molecule is spatially removed from the solid support the closer it is to the solution state and the more likely it is to react freely with dissolved molecules. This is especially important in the case of hydrophobic supports such as polypropylene (20), which are still rather solvent repellant despite amination. To assess the influence of hydrophilic spacers of different length on the hybridisation properties of immobilised oligonucleotides, we used appropriately protected phosphitamide derivatives of aliphatic diols (obtained by dimethoxytritylation of one hydroxy group of diols of different length and subsequent phosphorylation of the other). To examine the effect of the length of the uncharged part of the spacer attached to two phosphate groups on the properties of the whole spacer, synthons of different length were synthesised. We did not use longer $(OCH_2CH_2)_nO$-units, because it has been shown (23) that the yield of phosphoramidite condensation with long ($n > 5$) PEG phosphoramidite synthons does not exceed 60%. This would lead to an uncontrolled decrease of oligonucleotide yield with increasing length of spacer. Therefore we derivatised only propanediol, di- and triethyleneglycols (**1–3**). These synthons allowed us to produce three different types of spacers. We used previously described techniques (13) to synthesise arrays of spacers and oligonucleotides directly on the aminated polypropylene support (Scheme 2).

Oligonucleotides were synthesised in both 3′ to 5′ and 5′ to 3′ directions using appropriate monomers **10** and **11** (Scheme 3). Synthon **11** allows one to synthesise oligonucleotides with free 3′-ends, which is important for some array applications where enzymatic elongation is employed. Arrays synthesised only for hybridisation experiments can be built up using conventional



Scheme 3. Phosphoramidite derivatives of protected nucleosides: with ^{14}C-DMTr- group (**9**), for conventional 3′ to 5′ (**10**) and reversed 5′ to 3′ (**11**) oligonucleotide synthesis.

synthons **9**, which are slightly more effective in the oligonucleotide synthesis due to the higher reactivity of the primary hydroxyl group. Sequences used for hybridisation experiments were complementary to selected regions of $tRNA^{Phe}$; for use as a hybridisation target, this tRNA was labelled at the 3′-end by

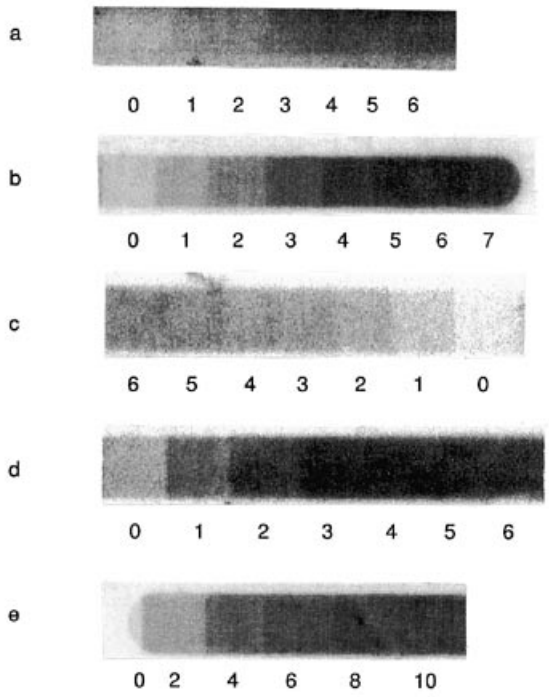


Figure 2. (**a**) Hybridisation of $tRNA^{Phe}$ to oligonucleotide no. 1 attached to the support through up to 6 units of **1**; (**b**) hybridisation of complementary oligonucleotide to oligonucleotide no. 1 attached to the support through up to 7 units of **1**; (**c**) hybridisation of complementary oligonucleotide to reversed oligonucleotide no. 1 attached to the support through up to 6 units of **1**; (**d**) hybridisation of complementary oligonucleotide to oligonucleotide no. 2 attached to the support through up to 6 units of **2**; (**e**) hybridisation of complementary oligonucleotide to reversed oligonucleotide no. 2 attached to the support through up to 10 units of **3.**

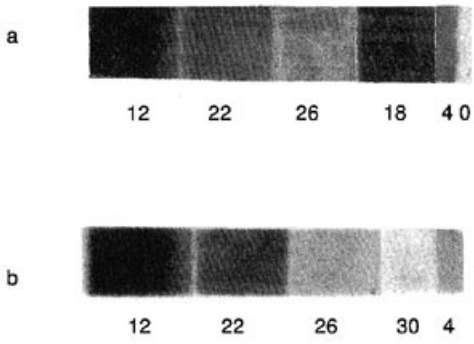
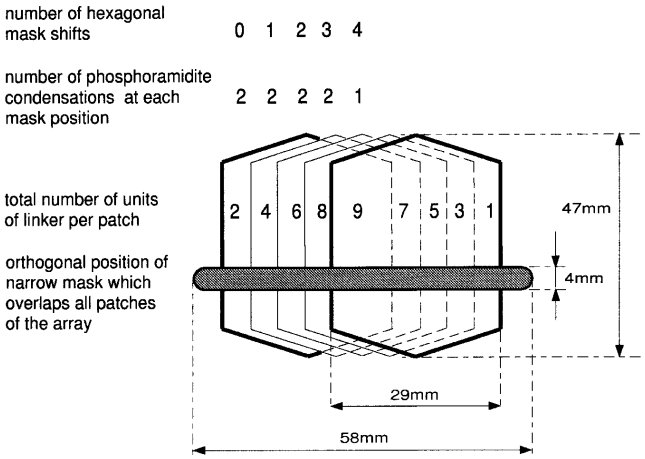


Figure 3. (**a**) Hybridisation of complementary oligonucleotide to oligonucleotide no. 1 attached to the support through 0, 4, 12, 18, 22 and 26 units of **1**; (**b**) hybridisation of complementary oligonucleotide to reversed oligonucleotide no. 1 attached to the support through 4, 12, 22, 26 and 30 units of **1**.



Scheme 4. Principle of combinatorial array synthesis: having done *n* mask shifts, one can produce an array of $(2n-1)$ different patches. The method used for making arrays of spacers of different length is as described in (8) with a hexagonal stainless steel cell, as illustrated. The inlet and outlet ports were at the lower and upper points of the hexagon. The cell was damped against the polypropylene surface, and solutions introduced from the reagents line of an ABI 381A DNA synthesiser. After each coupling, the cell was moved, if necessary, before applying the next reagent. Using the coupling schedule illustrated it was possible to make spacers of length 1–9 in five stages. Oligonucleotides were synthesised on spacer arrays using similar protocols, with the hexagonal reaction cell, or with a narrow rectangular cell placed with the long axis orthogonal to the array of spacers.

ligation of ^{32}P-5′,3′-ribocytidine diphosphate according to (24). In all cases hybridisation of arrays to labelled oligonucleotides gave greater discrimination than hybridisation to tRNA, but the relative intensities were similar, so for the greater part of the experiments we hybridised arrays to oligonucleotides.

Unless otherwise specified, hybridisations were carried out at 36°C to achieve the maximum differentiation of spacer influence by minimizing non-specific interactions. For both 3′- and 5′-immobilised oligonucleotides, a steady increase of hybridisation yield was observed with increasing length of spacer up to 8–10 units of glycol synthons **1–3** (Fig. 2, hybridisation to tRNA and oligonucleotides) in a preliminary experiment.

To determine the optimum length of spacer **1**, an array containing 12, 18, 22, 26 and 30 units was synthesised using a procedure similar to that shown in Scheme 4. Synthesis was carried out without capping, as capping would reduce the spacer concentration at each step, introducing a second variable (Fig. 3, hybridisation to tRNA and oligonucleotides). Combining these data with those shown in Figure 2 it is clear that the optimal length of glycol spacer **1** is ~10 units (60 atoms); surprisingly, duplex yield declined with further increase in the length of spacer until at 30 units the yield of hybridisation was the same as that with no spacer at all. The fact that a strip from an array containing a gradient of spacer from 0 to 80 atoms, when treated with ^{14}C-DMTrT phosphoramidite and tetrazole, is almost uniformly covered with the ^{14}C-label, shows that the phosphoramidite condensation is not strongly affected by the spacer length. We and others have shown that spacers increase the phosphoramidite coupling yield (14). We have also investigated this using the cleavable spacer **4** to allow us to cleave oligonucleotides from the polypropylene support and have found that the use of a spacer improves the coupling yield and forms a product of higher purity. Thus the decrease of hybridisation yield when using very long (n > 20, Scheme 3) spacers can not be explained by simply lower yield of the oligonucleotide synthesis.

We note that the optimal yield of duplex is achieved with spacers comprising 8–10 units of all three types of glycol synthons. In the case of type **1**, this gives a spacer 45–60 atoms in length; in the type **2**, 60–75 atoms; and for type **3**, 70–90 atoms. Thus the yield of hybridisation is not determined simply by the

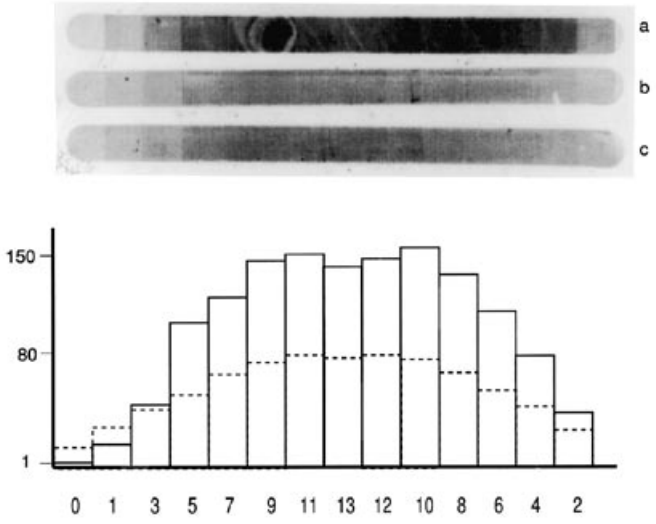


Figure 4. (**a**) Hybridisation of complementary oligonucleotide to oligonucleotide no. 1 attached to the support through up to 13 units of **3** (solid line on the graph); (**b**) hybridisation of complementary oligonucleotide to reversed oligonucleotide no. 1 attached to the support through up to 13 units of **3** (dotted line on the graph); (**c**) hybridisation of complementary oligonucleotide to reversed oligonucleotide no. 2 attached to the support through up to 13 units of **3**.

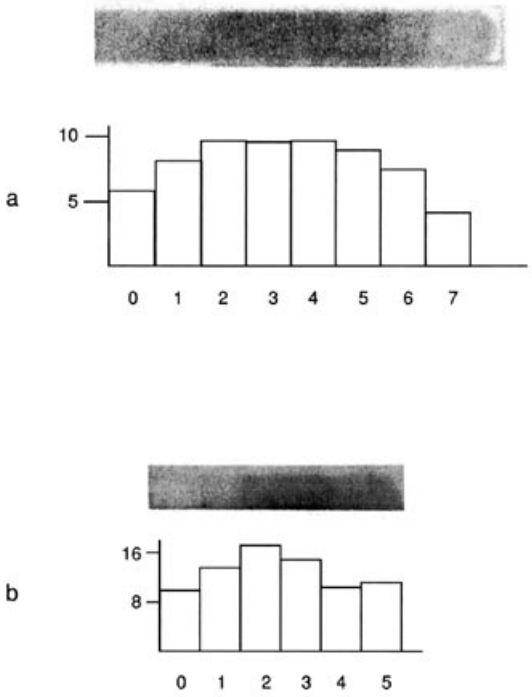


Figure 5 . (**a**) Hybridisation of complementary oligonucleotide to oligonucleotide no. 1 attached to the support through up to 7 units of **8**; (**b**) hybridisation of complementary oligonucleotide to oligonucleotide no. 1 attached to the support through up to 5 units of **6**.

length of spacer. The main difference between the spacer strands built up from synthons **1** and **3** is in the charge density: type **1** has one negatively charged dialkylphosphate group for each six atoms in the backbone, whereas type **3** has one for each 11 atoms.

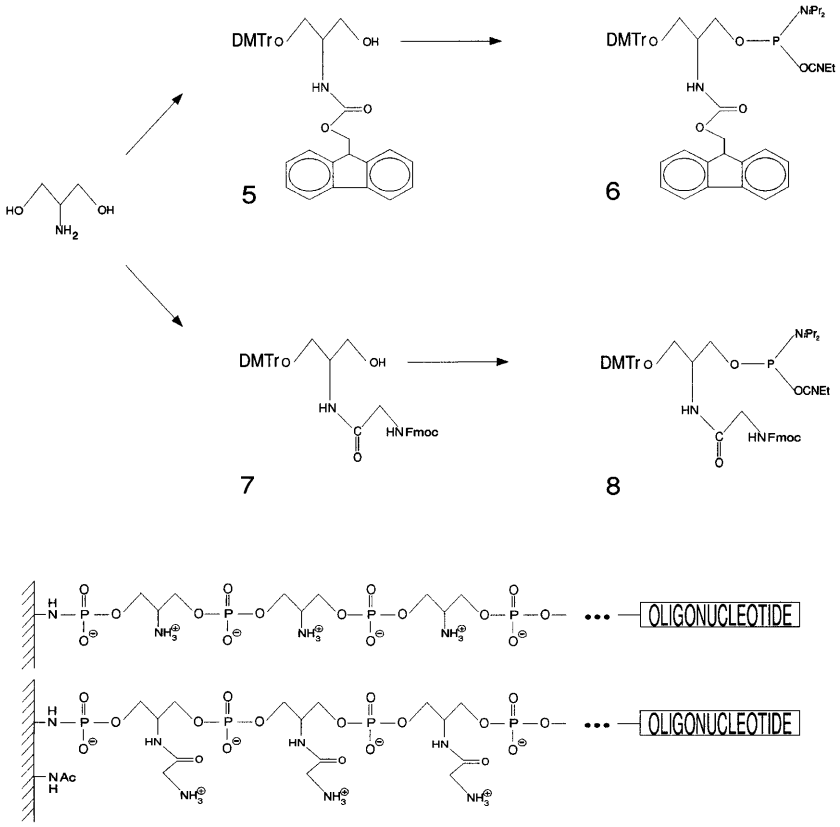
The greatest increase of the yield of hybridisation of oligonucleotides attached to the support through the 8mer chain of type **1** spacer was ~50-fold compared to oligonucleotide directly attached to the support (Fig. 2); the corresponding increase for the 8mer of type **3** was 100–150-fold (Fig. 4). Similar results showing optimum yield for 8mer spacer based on glycol synthon **2** were obtained. It seems likely that a high negative charge in the spacer could act to repel the target and hence to reduce the rate of duplex formation. This may explain why the yield decreases with further increase in spacer length and also why the highest yield with glycol synthon **3** is about three times higher than that with **1**. We note, however, that the oligo-T spacers used by Guo *et al.* (5), which produce ~20-fold enhancement of hybridisation, have a phosphodiester group each six atoms of the backbone. Our results suggest that the optimal spacer for both 5′ and 3′ immobilised oligonucleotides should have low negative charge density and have a length of 30–60 atoms, and this chain should be still amphiphilic to give the spacer hydrophilic properties.

Charge effects were further investigated using two phosphoramidite reagents based on a 3-carbon serinol structure containing an appropriately protected primary amino group (Scheme 5). These synthons give hydrophilic spacers containing both negatively charged phosphodiester groups and positively charged protonated amino groups. Both reagents were synthesised in three steps from serinol with high yield. Amino-synthons **6** and **8** differ in the distance of the amino group from the stem of the spacer, which in **8** is separated from the stem by a glycyl insert. Surprisingly, for both spacers maximum yield of hybridisation was observed for ~3–4 units of each monomer in the spacer (Fig. 5); further increase in number of linker units decreased the yield of hybridisation. A possible explanation for this decrease could be interaction of immobilised oligonucleotides with the spacer chain, which might lead to the formation of loop-type structures, similar to described in (25), stabilised by RNH_3^+–$(RO)_2P(O)O^-$ interactions.

CONCLUSION

There is growing interest in the use of solid state devices for tests which involve molecular interactions. Attachment of one of the ligands to a solid phase introduces problems which are not encountered when the interactions take place in a homogeneous solution as it constrains the ways in which the ligands can interact with each other. The bound ligand is not so free to diffuse as it would be in solution; this must reduce the reaction rate. The dissolved ligand may be prevented from making a close approach to the bound ligand by a number of steric factors: clearly, it cannot make an approach from the direction of the solid support unless there is a spacer between it and the bound ligand; it may also be hindered in its approach from the solution phase if the ligands on the solid support are too close together. Close approach will also be affected by the nature of the surface to which the bound ligands are attached; its charge, hydrophobicity and degree of solvation are all likely to influence significantly the environment in which the interaction of the ligands takes place.

We devised new spacer chemistries and procedures for making arrays of spacers which allowed us to study these factors systematically, and applied the method to the behaviour of oligonucleotides in molecular hybridisation. We found that the surface charge density had a small effect on duplex yield when varied between 6 and 11 atoms per charge. We found that, as expected, the surface density of the oligonucleotides was determined by the bulk of the units used for oligonucleotide synthesis;



Scheme 5. Synthesis of amino group-containing phosphoramidite synthons of different length and corresponding amino group-containing hydrophilic spacers synthesised directly on the surface of aminated polypropylene.

the amino groups on the surface were only ~20% occupied by oligonucleotides. However, this packing is close enough to inhibit duplex formation with target oligonucleotides by a factor of ~50%. Increasing the length of the spacer between the oligonucleotides and the solid support had by far the largest effect on duplex yield, with a maximum of 150-fold increase. Surprisingly, increasing the length of the spacer beyond 10–12 units diminished duplex yield until at ~25–30 units it equalled that found with no spacer.

These results suggest that the main effects of tethering a ligand to the solid support are the prevention of diffusion of the bound ligand, and steric constraints on the approach of the dissolved ligand. The combinatorial approach using arrays of spacer molecules described in this paper is one which could be adapted to other systems using solid state devices to study the interaction of ligands or to the efficiency of chemical coupling to solid supports.

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