Preparation of a novel psoralen containing deoxyadenosine building block for the facile solid phase synthesis of psoralen-modified oligonucleotides for a sequence specific crosslink to a given target sequence

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ABSTRACT

4,5',8 -Trimethylpsoralen was attached to the C8- position of deoxyadenosine via a sulfur atom and a five carbon atom linker. The modified deoxyadenosine was then converted to a protected phosphoramidite and used as usual as a building block for solid phase oligodeoxyribonucleotide synthesis. The efficiency of the photoreaction of a psoralen-modified oligonucleotide to a complementary matrix strand reached more than 90% within a 1hour irradiation time at a wavelength of 345 nm.

INTRODUCTION

Psoralens or furocoumarins are a class of natural occuring heterocyclic compounds, which can intercalate into double stranded regions of nucleic acids due to their planar and aromatic structure (1).

Psoralens are bifunctional photoreactive reagents which are able to form covalent bonds to nucleic acids, with a high preference for thymidine and uridine residues in the presence of near UV- light (320−360 nm) (2). The photoreaction takes place in a 2+2 cycloaddition with the (5'-4')- furan double bond or with the (3-4)-pyrone double bond or in case of opposite neighbouring pyrimidine bases with both double bonds leading to an interstrand crosslink (3). Their high specificity for nucleic acids, low toxicity in the absence of UV-light and their ability to form crosslinks has made them useful for the study of nucleic acids structure and function *in vitro* and *in vivo* (4). Psoralens are also used in clinical photochemotherapy in the treatment of psoriasis (5), a widespread skin disorder, and in the treatment of cutaneous T-cell lymphoma (6).

In the last few years there has been a rapid growing interest in the use of 'antisense' oligonucleotides in clinical applications for the treatment of different viral diseases (7). These oligonucleotides with a complementary sequence to messenger RNAs can inhibit translation and cellular proliferation very efficiently. To increase the nuclease stability the phosphate backbone could be modified in different ways, e.g. methylphosphonates (8) and thiophosphates (9), and to increase the hybrid stability, especially in the case of short oligonucleotide sequences intercalating agents e.g. acridines (10) could be attached to the 3' or 5' end of the oligonucleotides. In the latter case psoralens are very useful. As shown by several groups, psoralen modified oligonucleotides could be covalently coupled to given target sequences leading to a very effective inhibition of the translation of those target sequences (11). The coupling of the psoralen residue could be made in different ways. As a 'site specific engineered' monoadduct (12), it could be attached to the 5' end of oligonucleotides using a psoralen phosphoramidite linker compound in the last cycle of

the solid phase synthesis (13) or after the deblocking steps to an appropriately activated oligonucleotide (14).

In this paper we wish to report a new very efficient route for the synthesis of modified oligonucleotides by incorporating a psoralen base modified adenosine building block during the solid phase synthesis.

EXPERIMENTAL

Materials and methods

4,5',8-Trimethylpsoralen, 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane and silica gel 60 were purchased from Fluka (Buchs CH), bromomethylmethylether from Aldrich (Steinheim, FRG), 2'-deoxyadenosine from Pharma Waldhof (Düsseldorf FRG) [γ-^{32}P] ATP from Amersham Buchler (Braunschweig FRG), T4 polynucleotide kinase from Biolabs (Beverly, MA, USA), urea from BRL (Bethesda, MD, USA), acrylamide and bisacrylamide from BDH (Poole, UK). All other reagents were purchased in p.a. grade. Solid phase oligodeoxyribonucleotide synthesis was performed on an Applied Biosystems DNA synthesiser model 380 B-02 (Foster City, California USA). Protected phosphoramidites and solid supports were purchased from the same company. The oligodeoxynucleotides were purified using an h.p.l.c.-system model 510 and a C_{18}-cartridge from Waters (Bedford, MA, USA).

^{13}C and ^{31}P n.m.r. spectra were recorded on a Bruker AM 250 spectrometer using tetramethylsilane and external trimethyl phosphate as the respective references. The ^{13}C n.m.r. data were recorded using broad band proton noise decoupling. Assignments were made using off resonance data.

For the photoreactions an Aminco fluorescencespektrometer Modell 95 SLM (Urbana, USA) was used at a wavelength range from 330−350 nm.

8-Bromo-2'-deoxyadenosine and 8-mercapto-2'-deoxyadenosine were synthesised as described by Ikehara et al. (15,16).

Synthesis of the Building Block and Intermediates

4'- Bromomethyl-4,5',8-trimethylpsoralen (I): 4,5',8-Trimethylpsoralen (5 g, 21.9 mmol) was bromomethylated similar to the procedure for the synthesis of 4'-chloromethyl,-4,5,8-trimethylpsoralen described by Isaacs et al. (17). Instead of the highly toxic, cancer suspect and non commercially available chloromethylmethylether, the commercially available and less toxic bromomethylmethylether was used.The title compound was obtained as a white solid (5.3 g, 75.7%) of R_f 0.38 on t.l.c. in dichloromethane.^{13}C n.m.r. spectrum ($CDCl_3$) δ: 161.09 (C2), 155.10 (C7), 154.35 (C9), 152.98 (C4), 149.15 (C5'), 123.71 (C6), 116.24 (C10), 113.0 (C3), 112.34 (C4'), 111.16 (C5), 109.40 (C8), 22.62 (4' CH_2Br), 19.28,12.26 and 8.40 p.p.m. (CH_3s).

Compound (II): The introduction of the spacer arm was performed as described by Pieles and Englisch (13). Compound (I) (5g,15.62 mmol) was reacted with an excess of 1,5-pentanediol at 100°C .The desired product was obtained as an oil (5.01 g, 95%) of R_f 0.8 on t.l.c. in methanol/dichloromethane (1:9 v/v).^{13}C n.m.r. spectrum ($CDCl_3$) δ: 160.79 (C2), 153.97 (C7), 153.78 (C9), 152.97 (C4), 148.13 (C5'),124.49 (C6), 115.15 (C10), 111.59 (C5), 111.01 (C3 and C4'), 107.90 (C8) 69.67 (C1 spacer), 62.39 (pso-CH_2OCH_2-), 61.61 (C5 spacer), 31.90 (C4 spacer), 28.92 (C2 spacer), 22.04 (C3 spacer), 18.57,11.67 and 7.68 p.p.m. (-CH_3s).

Compound (III): Psoralen compound (II) (1g, 3.19 mmol) was dissolved in dry pyridine (20 ml). p-Toluenesulphonylchloride (607 mg, 6.38 mmol) was added and the mixture

was then stirred for 4 hours at room temperature. After evaporation of the solvent the crude mixture was directly applied to a silica gel column and eluted with dichloromethane. The desired product was obtained as a pale yellow oil (1.15g, 76.5%) of R_f 0.46 on t.l.c. in methanol/dichloromethane (1:9 v/v). ^{13}C n.m.r. spectrum ($CDCl_3$) δ: 160.90 (C2), 154.37 (C7), 154.21 (C9), 148.69 (C5'), 144.38 (tosyl C-S), 132.85 (-*C*-CH_3 tosyl), 129.48 (C-H tosyl), 127.41 (C-H tosyl), 124.79 (C6), 115.64 (C10), 112.23 (C3), 111.84 (C4'), 111.28 (C5), 108.40 (C8), 70.12 (C5 spacer), 69.45 (C1 spacer), 62.72 (pso-*C*H_2OCH_2), 28.78 (C2 spacer), 28.39 (C4 spacer), 22.01 (C3 spacer), 21.36 (C-*C*H_3 tosyl), 18.95, 12.00 and 8.08 p.p.m. (CH_3s pso).

Compound (IV): Psoralen compound (III) (2g, 3.6 mmol) and 8-mercapto-2'-deoxyadenosine (1.124 g, 3.6 mmol) were dissolved in dry dimethylformamide (50 ml). After adding 1.5 g of potassium carbonate the mixture was stirred under an argon atmosphere for 3−4h at 80°C. The reaction was monitored by t.l.c. After completion of the reaction the mixture was evaporated to dryness, residual dimethylformamide was removed by coevaporating two times with ethanol and the residue suspended in dichloromethane and directly applied to a silica gel column without further workup. The product was eluted with a linear gradient of methanol in dichloromethane. The methanol concentration was increased in 0.5% steps up to 5%. The product was obtained as a white solid (1.8 g, 75%) of R_f 0.55 in methanol/dichloromethane (1:9 v/v). ^{13}C n.m.r. spectrum ($CDCl_3$) δ: 161.51 (C2 pso), 154.63 (C6 ade), 154.60 (C9 pso), 153.88 (C4 pso), 153.34 (C7 pso), 150.39 (C2 ade), 150.38 (C8 ade), 149.72 (C5'pso), 149.00 (C4 ade), 125.07 (C6 pso), 120.75 (C5 ade), 115.99 (C10 pso), 112.55 (C5 pso), 111.97 (C3 pso), 111.49 (C4' pso), 108.95 (C8 pso), 89.53 (C4'ade), 86.61 (C1'ade), 73.04 (C3' ade), 69.64 (C1 spacer), 63.39 (C5' ade), 62.97 (pso-*C*H_2OCH_2), 40.02 (C2'ade), 32.58 (C5 spacer), 29.06 (C4 spacer), 28.88 (C2 spacer), 25.36 (C3 spacer), 19.22, 12.23 and 8.35 p.p.m. (CH_3s pso).

Compound (V): The modified 2'-deoxyadenosine compound (IV) (1.6 g,2.54 mmol) was dried by coevaporation from dry pyridine. The residue was dissolved in dry pyridine (20 ml) and 1,1-dichloro-1,1,3,3-tetraisopropyldisiloxane (936 mg, 2.79 mmol) was added and the mixture was stirred for 2h at room temperature. Benzoylchloride (892 mg, 6.33 mmol) was added and the mixture stirred over night. The reaction mixture was evaporated to dryness, and residual pyridine was removed by evaporation of toluene. The resulting gum was dissolved in dichloromethane (50 ml) and washed with 5% sodium hydrogen carbonate solution (2×30 ml). The organic layer was dried over sodium sulphate, filtered and evaporated to dryness. The residue was dissolved in dichloromethane (5 ml) and the compound was purified on a silica gel column. The product was eluted with 3% methanol in dichloromethane and obtained as a white foam (2.4 g,91.4%) of R_f 0.48 on t.l.c. in methanol/dichloromethane (3:97 v/v).^{13}C n.m.r. spectrum ($CDCl_3$) δ: 171.98 (carbonyl benzoyl), 161.27 (C2 pso), 156.81 (C6 ade), 154.42 (C9 and C4 pso), 153.20 (C5'pso), 150.17 (C8 ade), 149.10 (C2 ade), 148.86 (C5 pso), 147.99 (C4 ade), 134.28 (benzoyl), 132.43 (benzoyl), 129.03 (benzoyl), 128.29 (benzoyl), 125.00 (C6 pso), 123.62 (C5 ade), 115.83 C10 (pso), 112.39 (C5 pso), 111.39 (C3 pso), 111.44 (C4' pso), 108.72 (C8 pso), 85.07 (C4' ade), 82.40 (C1' ade), 71.69 (C3' ade), 69.57 (C1 spacer), 62.79 (C5' ade), 62.70 (pso-*C*H_2-O-CH_2), 38.12 (C2' ade), 31.99 (C5 spacer), 28.89 (C4 spacer), 28.67 (C2 spacer), 25.27 (C3 spacer), 19.02 (-CH_3 pso), 17.17−16.95 (isopropyl CH_3s), 13.02, 12.65, 12.44 and 12.08 (isopropyl CHs and -CH_3 pso), 8.20 p.p.m. (-CH_3 pso).

Compound (VI): The fully protected 2'-deoxyadenosine derivative (V) (2.4 g, 2.27 mmol) was dissolved in dry tetrahydrofuran (10 ml) and a 1 molar solution of tetrabutylammonium

fluoride (5.7 ml, 5.7 mmol) was added. The mixture was stirred for 15 min at room temperature, then evaporated to dryness, dissolved in dichloromethane (20 ml) and washed with a 5% sodium hydrogen carbonate solution (2 × 10 ml). After drying the organic layer with sodium sulphate and filtration, the solvent was evaporated and the residue dissolved in pyridine (20 ml). To this solution was added concentrated aqueous ammonia and the mixture was kept at room temperature for 30 min. The reaction mixture was then evaporated to dryness followed by an evaporation of toluene. The residue was taken up in dichloromethane (50 ml) and the mixture was worked up as described above. The compound was purified on silica gel and eluted with 3% methanol in dichloromethane.The desired product was obtained as a white foam (0.99 g, 60.1%) of R_f 0.48 on t.l.c. in methanol/dichloromethane (3:97 v/v).^{13}C n.m.r. spectrum ($CDCl_3$) δ: 164.54 (carbonyl benzoyl), 161.53 (C2 pso), 154.60 (C6 ade), 154.40 (C9 pso), 153.95 (C4 pso), 153.34 (C5′ pso), 152.35 (C8 pso), 150.05 (C2 ade), 148.80 (C8 ade), 146.99 (C5 ade), 133.64 (benzoyl), 132.49 (benzoyl), 128.60 (benzoyl), 127.65 (benzoyl), 124.82 (C6 pso), 123.92 (C5 ade), 115.85 (C10 pso), 112.43 (C5 pso), 11.41 (C4′ pso), 108.51 (C8 pso), 89.42 (C4′ ade), 86.60 (C1′ ade), 72.74 (C3′ ade), 69.71 (C1 spacer), 63.21 (C5′ ade), 62.20 (pso-CH_2OCH_2), 39.62 (C2′ ade), 32.19 (C5 spacer), 28.97 (C4 spacer), 28.71 (C2 spacer), 26.27 (C3 spacer), 19.15, 12.15 and 8.29 p.p.m. (-CH_3s pso).

Compound (VII): The adenosine compound (VI) (800 mg, 1.10 mmol) and 4, 4′-dimethoxytritylchloride (495 mg,1.4 mmol) were dissolved in dry pyridine 10 ml). The mixture was stirred under an argon atmosphere for 1 hour. After completion of the reaction methanol (5 ml) was added and the mixture was stirred for another 10 min at room temperature. After removal of the solvent, the remaining yellow gum was dissolved in 5 ml of dichloromethane and directly applied to a silica gel column. The product was eluted with 3% methanol in dichloromethane containing 0.5% of triethylamine. The product was obtained as a white foam (500 mg,42%) of R_f 0.53 on t.l.c. in methanol/dichloromethane (3:97 v/v).

Compound (VIII): The adenosine compound VII (500 mg 0.46 mmol) was dissolved in dry 1,2 dichloroethane (5ml). Diisopropylethylamine (100 μl, 0.92 mmol) and 2-cyanoethoxy-N,N-diisopropylaminochlorophosphine (100 μl, 0.51 mmol) were added and the mixture was stirred 1.5 h at room temperature. The reaction was quenched by adding 5% sodium hydrogen carbonate solution (5 ml) and stirred for another 15 min. The mixture was diluted with dichloromethane (10 ml) and the organic layer washed with 5% sodium hydrogen carbonate solution (15 ml). The organic layer was dried with sodium sulphate, filtered and the solvent was removed. The product was purified on a silica gel column using the following solvent system for packing and elution. Solvent A: Dichlormethane/triethylamine 10:1. Solvent B: n-Hexane/ triethylamine 10:1. Solvent A and B were mixed in equal amounts. The product was obtained as a white foam (400 mg, 72.5%) of R_f 0.77 and 0.85 for the two isomers on t.l.c. in ethyl acetate/ dichloromethane/triethylamine (45:45:10 v/v).^{31}P n.m.r. spectrum ($CDCl_3$) δ: 145.62, 145.35 p.p.m.

Oligonucleotide synthesis

Oligonucleotide synthesis was carried out on a 0.1 μmol scale on an automatic DNA-synthesiser using standard procedures (18). The psoralen modified base was coupled in the presence of 5-(4-nitrophenyl)-1H-tetrazole (19) using a reaction time of 8 min. After the synthesis the oligonucleotides were deprotected with aqueous ammonia at 60°C for 8 hours and purified by using reversed phase h.p.l.c.. For the elution a linear gradient from 0−100 % buffer B in 38 min of the following buffers was used.

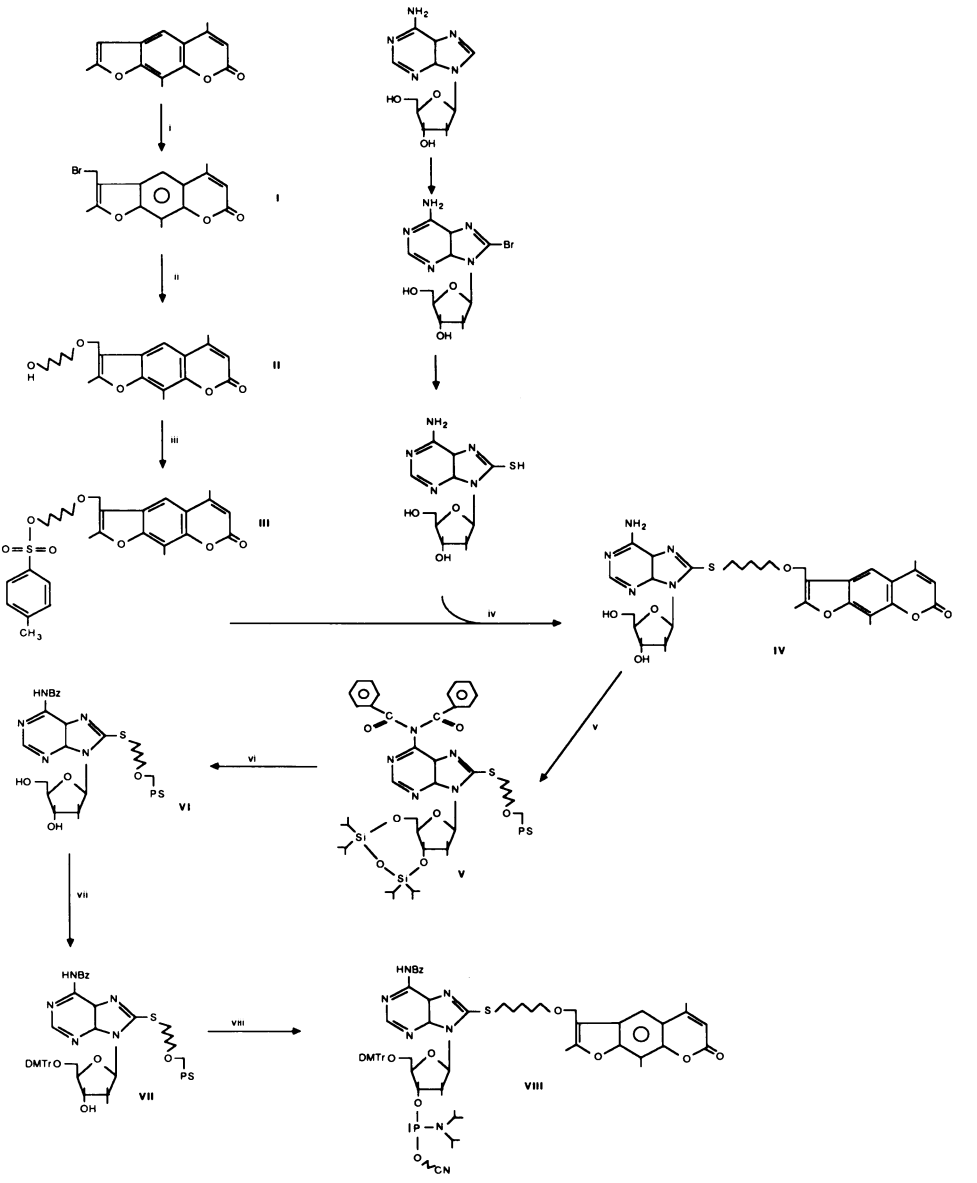


Figure 1. Reaction scheme for the synthesis of the psoralen modified adenosine derivative. Reagents: i, bromomethylmethylether in glacial acetic acid for 24 hours; ii, 1,5-pentandiol at 100°C for 2 hours; iii, tosyl chloride in pyridine for 4 hours at room temperature; iv, 8-mercapto-2′-deoxyadenosine and compound III in dimethylformamide at 80°C in the presence of potassium carbonate for 2–3 hours; v, 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane in pyridine and benzoyl chloride in pyridine; vi, tetrabutylammonium fluoride in tetrahydrofuran and aqueous ammonia in pyridine; vii, 4,4′-dimethoxytritylchloride in pyridine; viii, 2-cyanoethoxy-N,N-diisopropylaminochlorophosphine in 1,2-dichloroethane in the presence of diisopropylethylamine.

Buffer A: 5% acetonitrile in 100 mM triethylammoniumacetate pH 7.0.
Buffer B: 70% acetonitrile in 100 mM triethylammoniumacetate pH 7.0
After the purification the remaining 5'-O-dimethoxytrityl group was cleaved by treatment with 80% acetic acid for 15 min at room temperature. The oligonucleotides were precipitated by adding 1/10 volume of a 3M sodium acetate solution and 3 volumes of cold ethanol. The solution was kept at −20°C for 2−3 hours, the precipitate was centrifuged and dissolved in 300 μl of autoclaved water after the removal of the supernatant. The yield was determined by measuring the OD_{260}.

Radioactive labelling of oligonucleotides and photoexperiments

300 pmol of the oligonucleotides were radioactive labelled at the 5' end using T4-polynucleotide kinase and [γ-^{32}P] ATP under standard conditions (20). The total volume of the labelling reaction was 20 μl. For the photoexperiments only the unmodified matrix strand was labelled. The psoralen-modified oligonucleotides were dissolved in the same concentration as the labelled one in the kinase buffer.

2μl of the unmodified radioactive labelled matrix strand and 6 μl of the unlabelled psoralen-modified oligonucleotide were mixed in a total volume of 20 μl of autoclaved water, heated for 10 min to 85°C then cooled during 20 min to 37°C. The hybridized oligonucleotides were transferred to a quartz test-tube and were irradiated for 50 min at

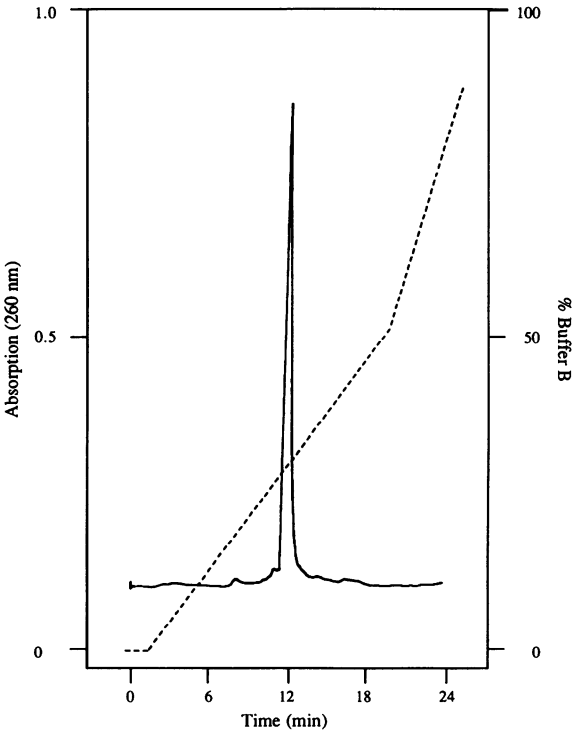


Figure 2. H.p.l.c.trace of the fully deblocked modified oligodeoxyribonucleotide from the photo experiment shown in Figure 3b. Conditions: Buffer A 5% acetonitrile in 100mM triethylammonium acetate pH 7.0. Buffer B 70% acetonitrile in 100 mM triethylammonium acetate pH 7.0. Gradient 0−100% buffer B in 38 min.

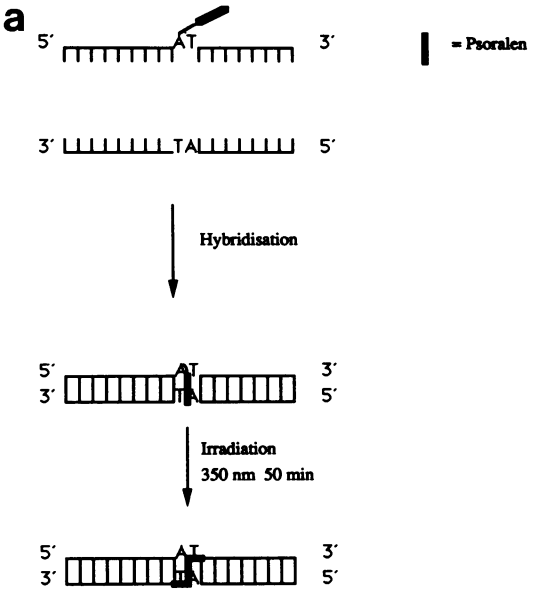
a wavelength of 345 nm. After the irradiation the samples were mixed with 8μl of dye-mix (95% deionised formamide, 0.1% bromophenol blue, 0.1% xylene cyanol). The probes were heated to 85°C transferred to ice and then loaded on a 20% 8M urea containing polyacrylamide gel. The analysis of the photoreaction product was performed by using autoradiography.

RESULTS AND DISCUSSION

The psoralen modified 2′-deoxyadenosine building block was synthesised as illustrated in Figure 1. 4,5′8-Trimethylpsoralen was halomethylated at the 4′ position with bromomethylmethylether using the procedure published by Isaacs et al. (15). This new derivative avoids the use of the dangerous, highly carcenogenic and non commercially available chloromethylmethylether.The advantage of the corresponding bromo derivative is, that it could be obtained in higher yields, the reaction time is much shorter, and the product is much more reactive so that nucleophilic displacement reactions take place more easily.

The introduction of the spacer arm was performed by reaction of the 4′-bromomethyl-4,5′,8-trimethylpsoralen derivative with 1,5-pentanediol under the same conditions published by Pieles and Englisch (16). From computer simulations it was estimated that a spacer arm with 5 carbon atoms has the optimal length for the intercalation of the psoralen residue within the AT site. The corresponding derivative with 2 carbon atoms showed only very minor photocrosslinking capability.

The C5 psoralen derivative was then tosylated in pyridine to obtain a good leaving group for the following substitution reaction with 8-mercapto-2′-deoxyadenosine, prepared form 2′-deoxyadenosine as described by Ikehara et al. (17,18). The condensation of the psoralen derivative to the heterocycle of adenosine was newly developed in the course of this synthesis. The tosylated psoralen derivative and 8-mercapto-2′-deoxyadenosine were



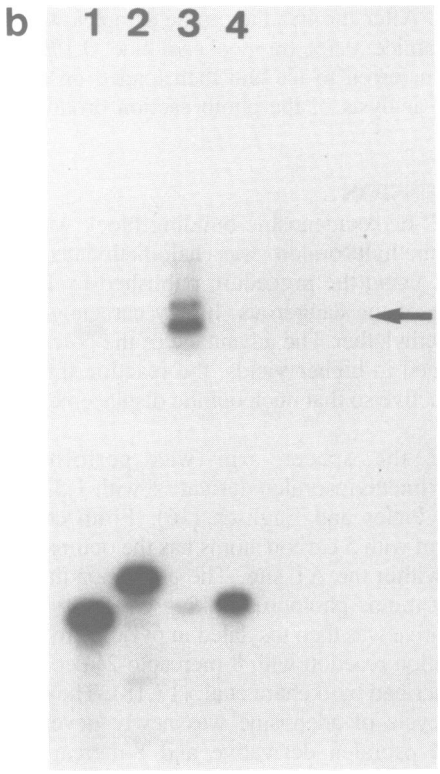


Figure 3a. Reaction scheme for the hybridisation and the photo crosslinking reaction of the two oligonucleotides.
Figure 3b. Gel analysis of the photo crosslinking experiment. Lane 1 unmodified labelled matrix strand alone. Lane 2 modified labelled oligonucleotide alone. Lane 3 crosslinking reaction with irradiation at 345 nm for 50 min. Lane 4 control without irradiation. The arrow marks the crosslinked products.

dissolved in dimethylformamide and heated in the presence of potassium carbonate which functioned as an activator for the thiol group.For the protection of the N^6 amino function of the adenosine, the 3′ and 5′ hydroxyl groups of the sugar moiety were first blocked with 1,3-dichloro-1,1,3,3-tetraisopro-pyldisiloxane.Using the normal transient protection route with trimethylchlorosilane and removal of these groups with aqueous ammonia gave a product which could not be converted to the corresponding 5′-O-dimethoxytrityl compound.

The N^6 amino function of adenosine was protected using benzoyl chloride under standard conditions. In this case the only observed product was the double acylated compound from which the additional benzoyl group had to be removed by treatment with aqueous ammonia in pyridine after the removal of the siloxane group with tetrabutylammonium fluoride. Finally the deprotected adenosine (VI) compound was converted to the 5′-O-protected derivative using dimethoxytritylchloride and subsequently reacted with 2-cyanoethoxy-N,N-diisopropylaminochlorophosphine to yield the desired phosphoramidite building block (VIII).

The modified adenosine derivative was introduced in the course of a solid phase oligonucleotide synthesis using standard reaction protocols. The coupling of the modified base was performed in the presence of the more reactive 5-(4- nitrophenyl)-1H-tetrazole with a prolonged coupling time, instead of the normal tetrazole.

After the ammonia deprotection step the oligonucleotide was purified by h.p.l.c. on a reversed phase column. The 5'-O- dimethoxytrityl group was removed with acetic acid and the fully deprotected oligonucleotide was precipitated in cold ethanol. Figure 2 shows the h.p.l.c. trace of the fully deprotected oligonucleotide.

The first photo crosslinking experiments were performed with an oligonucleotide which contains a multiple AT site with the modified base in its centre.

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5' GGCTGTATATATCGTAC 3'
3' CCGACATATATAGGATG 5'
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The asterisk marks the position of the modified base. For the experiment only the unmodified matrix strand was radioactive labelled at the 5'end. The oligonucleotides were hybridised by heat denaturation and slow cooling to room temperature. A molar ratio of 3:1 modified/ unmodified oligonucleotide was used. The hybridized sample was then exposed to near UV-light of 345 nm for 50 min, then mixed with the gel loading buffer, denaturated by heating and loaded on a denaturing 20% polyacrylamide gel containing 8M urea. Figure 3a shows a scheme of the photo crosslinking reaction and Figure 3b shows the result of the crosslinking experiment.

The extent of crosslinking in this experiment was more than 90% and the crosslinked products showed bands with different mobilities which correspond to the different crosslinking possibilities of the intercalated psoralen in the multiple AT site. There is a major crosslinking product, implying sequence specificity.

In order to determine the specificity of the crosslinking reaction of the intercalated psoralen, a new set of modified oligonucleotides was synthesised which contained the modified base directly 5' or 3' to a thymidine residue, with a separation of one base from

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1.) Mod 21   5' CTG CAT GCG CTA GCC GAC GCA CTA C 3'
             3' GAC GTA CGC GAT CGG CTG CGT GAT G 5'

                      *
2.) Mod 14   5' CTG CAT GCG CTA GCC GAC GCA CTA C 3'
             3' GAC GTA CGC GAT CGG CTG CCT GAT G 5'

                                *
3.) Mod 5    5' CTG CAT GCG CTA GCC GAC GCA CTA C 3'
             3' GAC GTA CGC GAT CGG CTG CGT GAT G 5'

                                 *
4.) Mod 9    5' CTG CAT GCG CTA GCC GAC GCA CTA C 3'
             3' GAC GTA CGC GAT CGG CTG CGT GAT G 5'
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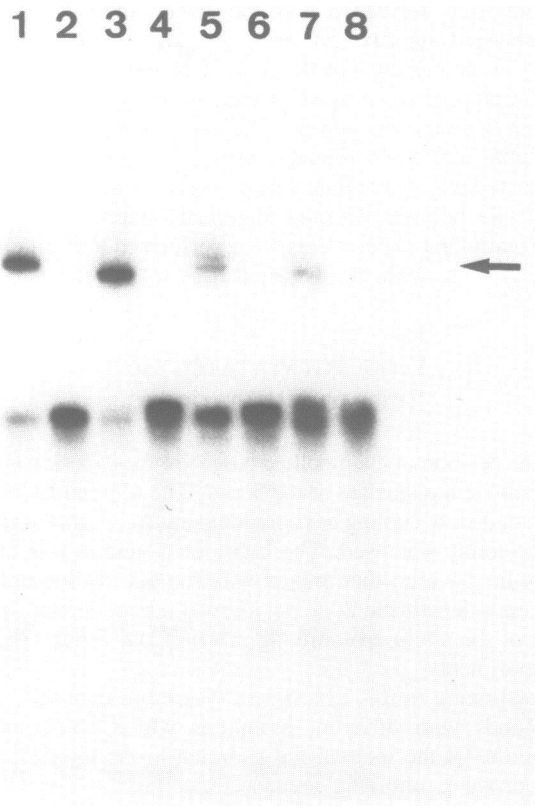

Figure 4. Results of the photo crosslinking experiments with oligonucleotides which carry the psoralen modification at different positions. Lane 1,mod.21 with 50 min irradiation at 345 nm. Lane 2, mod.21 control without irradiation. Lane 3, mod.14 with 50 min irradiation at 345 nm. Lane 4, mod.14 control without irradiation. Lane 5, mod. 5 with 50 min irradiation at 345 nm. Lane 6, mod.5 control without irradiation. Lane 7, mod.9 with 50 min irradiation at 345 nm. Lane 8, mod.9 control without irradiation. The arrow marks the crosslinked products.

a TA sequence to the 3'side, and with no AT or TA sequence nearby. Corresponding to the position of the modified base the oligonucleotides were assigned as 1). mod 21, 2). mod 14, 3). mod 5, 4). mod 9.

Figure 4 shows the results of these crosslinking experiments. Lane 1 (mod 21) and lane 3 (mod 14) show the highest extent of crosslinked product ($>$ 90%). Lane 5 shows the result of the experiment with the oligonucleotide (mod 5) which contains a TA site one base pair apart from the psoralen modified base. In this case only a very minor photo crosslinking reaction took place. From these results it is obvious that the psoralen residue covalently attached to adenosine intercalates and photoreacts with very high preference to a neighbouring thymidine and to the thymidine in the opposite strand. Lane 7 shows also very minor crosslink formation. In this case the psoralen derivative could only form a thymidine monoadduct or a C-T or T-C crosslink. These results could also explain the observed extra bands of crosslinked products in lane 5, because the same possibility for the formation of C-T or T-C crosslinks or thymidine monoadducts exists.

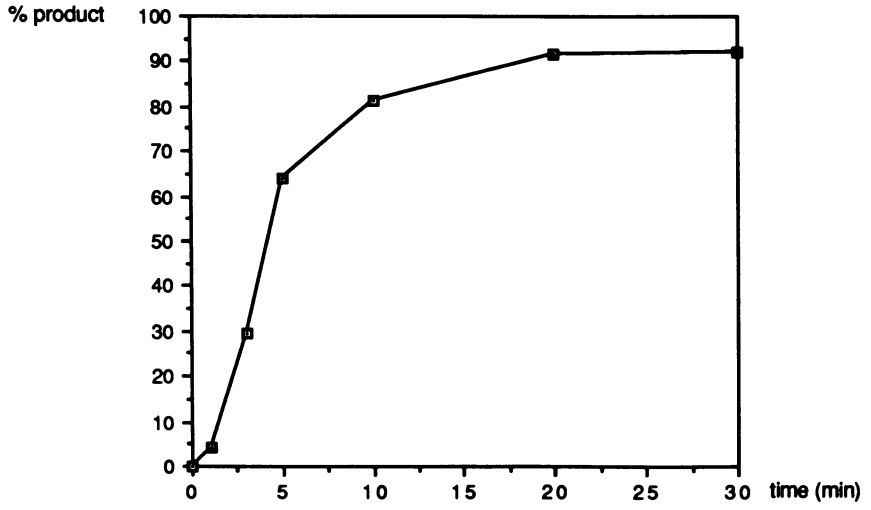

Figure 5. Kinetics of the photo crosslinking experiment of the oligonucleotide mod.14 within an irradiation time from 0 to 30 min.

Figure 5 shows the kinetic data of the crosslinking experiment of oligonucleotide mod 14. The extent of crosslinking reached the highest level of more than 90% after 20 min of irradiation time with the given facilities.

The advantage of this new class of modified building blocks is the facile way of synthesis and incorporation into oligonucleotides.This newly developed chemistry could also easily be adapted to RNA analogs.The modified oligonucleotides could then be used in a great variety of applications, due to their free 5′ and 3′ hydroxyl groups they could be radioactive labelled or incorporated enzymatically into DNA. These free hydroxyl groups also allow multiple labelling or functionalising with fluorescence groups like acridines (21), chelating agents like EDTA (22) etc.

These modified oligonucleotides could also be very useful as 'antisense oligo-nucleotides' in clinical applications for diagnostics or combined chemotherapy and photochemotherapy of several viral diseases.

ACKNOWLEDGEMENTS

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Abbreviations used: Pso, psoralen; ade, adenosine.

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