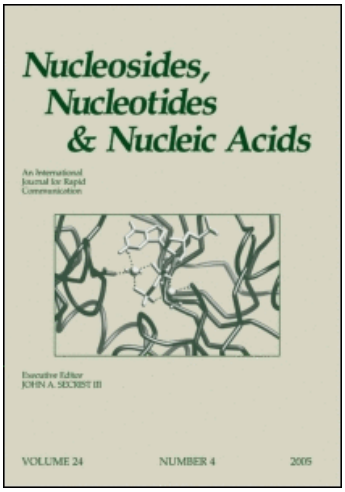

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FLUORESCENT LABELLING OF OLIGODEOXYRIBONUCLEOTIDES BY THE OXYAMINO-ALDEHYDE COUPLING REACTION

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ABSTRACT: We describe the reaction of oligonucleotides containing an aldehydic group at the 5'-end or inside the sequence with an oxyamino label. The reaction was found to be highly selective and represents an efficient method for derivatization of oligonucleotides.

INTRODUCTION

Oligonucleotides are used extensively in molecular biology as linkers, probes, and primers for sequencing, amplification, secondary structure determination... For therapeutic applications, "antisense" oligonucleotides can be used for inhibiting the expression of viral mRNA or for blocking gene transcription through triple helix formation. For these purposes, a number of modifications including incorporation of fluorescent, lipophilic, intercalating, crosslinking, alkylating or DNA cleaving entities into oligonucleotides have been reported.[1-3] The wide range of applications has thus spurred the development of new procedures for derivatizations which in most cases require site-specific modification of the oligonucleotide.[1-3] The most common strategies for generating conjugated oligonucleotides involve post-synthetic modification by preliminary incorporation of a modified nucleoside bearing a nucleophilic function, into DNA by automated synthesis, and subsequent linkage of the reporter to the tethered nucleophile. The nucleophiles most generally used are primary amines or thiols.[1] Using linkers bearing the same nucleophile (NH_2 or SH), modifications at the 5'-OH of the terminal residue have also been performed.[1] However, these approaches in which the nucleophile is incorporated into synthetic

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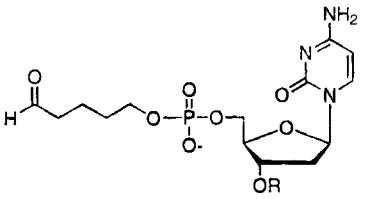
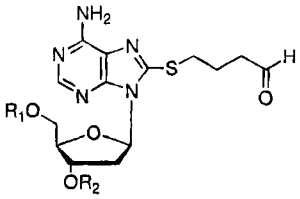
DNA generally require large excess of the electrophilic reporter molecule (isothiocyanates, activated esters...) to bring the conjugation reaction to completion.

Post functionalization involving conjugation of a nucleophilic ligand reacting with an electrophilic site in the DNA oligomer has been less studied. Oligonucleotides have been modified at the 5'-end with a carboxylic acid or an aldehyde and coupled with hydrazide residues.[4] Jones and Coll. have reported more recently the use of an aldehyde at the 5'-end to prepare an oligonucleotide-immunogenic protein (KLH) conjugate by reductive amination.[5] A di-aldehyde has been introduced at the 3'-end of an oligonucleotide by sodium periodate oxidation of a terminal ribonucleotide.[6] For functionalization at preselected positions inside the sequence, the aldehydic function of the abasic site[7, 8] or an abasic site analogue bearing a linker with a terminal masked aldehyde[9] have been used. However, strong destabilisation of the double helix was induced by the abasic site. In all those cases, involving an aldehydic function in DNA, the ligands was introduced as a primary amine by reductive amination. This presents the major drawback that a further reduction step is required after reaction to stabilize the conjugate. Furthermore, in the case of derivatization *via* an abasic site using an amine, DNA cleavage through β-elimination was observed.

We have previously reported the use of the oxyamino-aldehyde coupling reaction as an efficient method for derivatization at any preselected position of oligodeoxyribonucleotides through the abasic site[10] or via an aldehydic chain linked at the C-8 position of an adenine moiety inside a DNA sequence.[11] However, study of the duplex stability (by measuring the melting temperature) indicated destabilisation of the double helix induced by the abasic site or by the C_8 modified base. We thus decided to test the oxyamino-aldehyde coupling reaction for derivatization at the 5'-extremity of oligonucleotides in order to avoid duplex destabilisation. We report in this paper the general procedure to introduce the aldehydic function at the 5'-extremity of oligodeoxyribonucleotides and describe synthesis of the modified undecamer **1** as an example (figure 1). We show that this DNA fragment is efficiently and selectively labeled with a fluorophore containing the oxyamino function to give conjugate **14** (scheme 4). We show that the modification does not destabilize duplex formation by contrast to what is observed for the analog undecamer **2** in which the reactive aldehydic group has been introduced through a linking chain at the C-8 position of an adenine residue (figure 1). In addition, we describe full details of the synthesis of the latter oligonucleotide **2** that were not presented in our preliminary communication.[11]



1: R = GCACACACGC



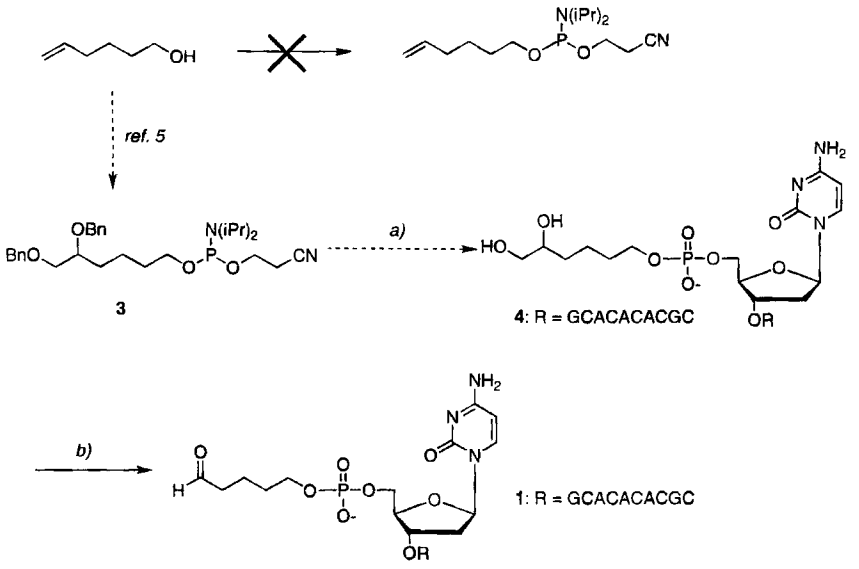
2: R_1=CGCAC, R_2=CACGC

FIGURE 1: Structure of the aldehyde containing oligonucleotides **1** and **2**.

RESULTS AND DISCUSSIONS

1) Introduction of an aldehyde at the 5'-terminus - Synthesis of the undecamer **1** (scheme 1):

We previously noted that an aldehydic group must be masked during oligonucleotide synthesis and we proposed the use of an alkene as a precursor for the aldehyde.[11] Attempts to prepare the phosphoramidite derivative of 5-hexen-1-ol was found unsuccessfull. We thus prepared the corresponding protected diol phosphoramidite **3** as described.[5] The undecamer **4** containing a 5'-linker bearing the diol was then synthesized according to standard β-cyanoethyl phosphoramidite chemistry. After deprotection with concentrated ammonia for 24 h at 50°C, the



a) automated solid phase synthesis, b) $NaIO_4$

SCHEME 1: Synthesis of undecamer **1** - modification at 5'-extremity.

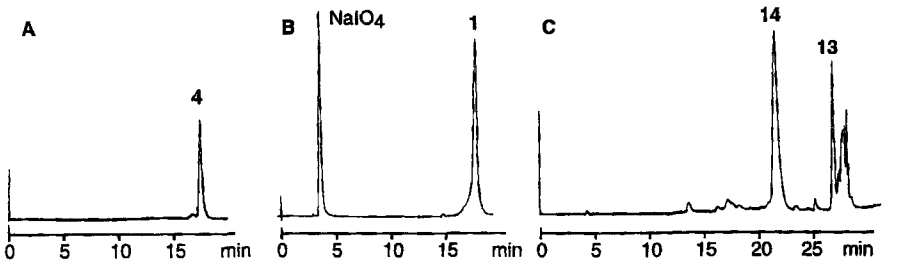
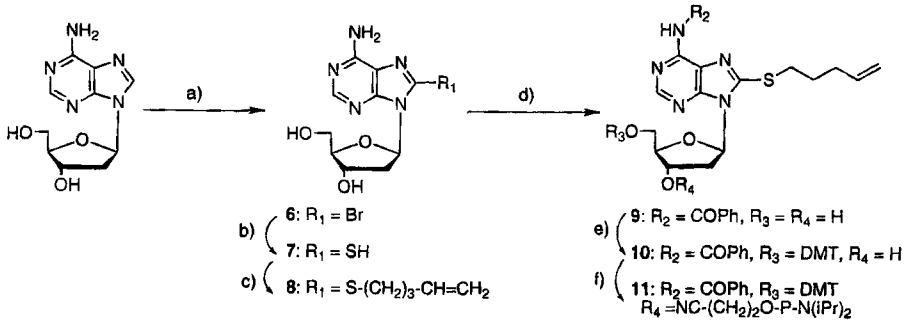


FIGURE 2: HPLC profiles (detection at 260 nm): (**A**) undecamer diol **4**; (**B**) crude reaction mixture of undecamer diol **4** with $NaIO_4$; (**C**) crude reaction mixture of fluorophore **13** with the undecamer **1**. For the HPLC conditions, see experimental part.

oligonucleotide **4** was purified by reverse phase HPLC (figure 2A). The structure of diol **4** was established by ESMS spectrum that showed the multicharged ions $[M-3H]^{3-}$ at m/e = 1154.6 and $[M-4]^{4-}$ at m/e = 865.7 corresponding to a mass of 3466.2. Cleavage of the diol was performed using 50 eq. of $NaIO_4$ at room temperature for 30 minutes. Oxidation of the diol was very clean leading to selective formation of the target aldehyde **1** (figure 2B).

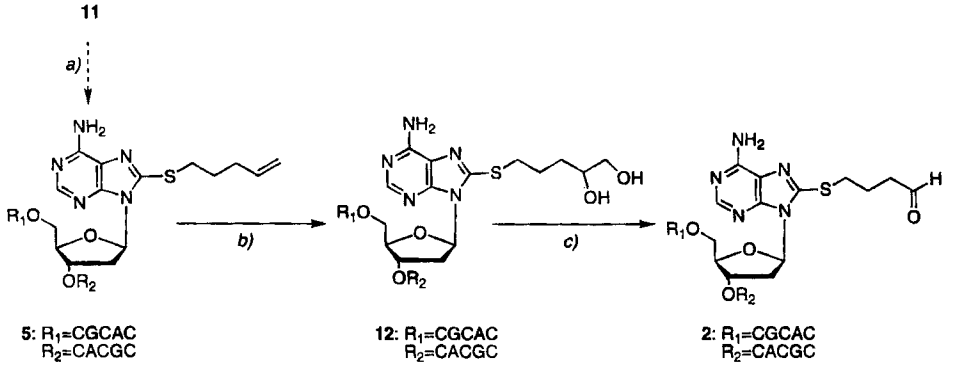
2) Introduction of an aldehyde on the adenine moiety - Synthesis of the undecamer **2** (schemes 2 and 3):

The aldehyde containing oligonucleotide **2** was prepared by oxidation of the corresponding alkenyl oligonucleotide d(CGCACXCACGC) **5** in which X represents the C_8 modified adenine.



a) Br_2, AcO^-Na^+ buffer pH = 5 b) NaSH c) Br-$(CH_2)_3$-CH=CH_2, K_2CO_3, DMF d) i) Bn-Cl, pyridine ii) NaOH 2N, pyridine, dioxan e) DMTr Cl, pyridine f) NC-$(CH_2)_2$O-P(Cl)-N(iPr)$_2$, NEt(iPr)$_2$

SCHEME 2: Preparation of the phosphoramidite **11**.



a) automated solid phase oligonucleotides synthesis b) OsO_4, NMMO, H_2O_2, c) $NaIO_4$, H_2O, dark

SCHEME 3: Synthesis of undecamer **2** - modification inside the sequence.

Oligonucleotide **5** was synthesized according to standard β-cyanoethyl phosphoramidite chemistry. The modified base X was incorporated using the corresponding phosphoramidite derivative **11** which was prepared in 5 steps from 2'-deoxyadenosine, as depicted in scheme 2. Bromination at position C-8 of 2'-deoxyadenosine was performed as described.[12] Introduction of the thio group was accomplished by treating the bromide **6** with hydrogen sulphide in DMF under argon to prevent oxidation of the thiol **7**.[13] Alkylation of **7** with 1-bromo-4-pentene in basic conditions gave the thioether **8**. Subsequent protection of the exocyclic amino group as a benzamide and of the 5'-hydroxyl as a DMT group using standard procedures afforded the protected nucleoside **10** which was then phosphitylated at the 3'-OH. The resulting phosphoramidite **11** was used as a building block to prepare the undecamer **5** by conventional automatic solid phase synthesis. Coupling of the modified nucleoside occurred with satisfactory yield (over 90%).

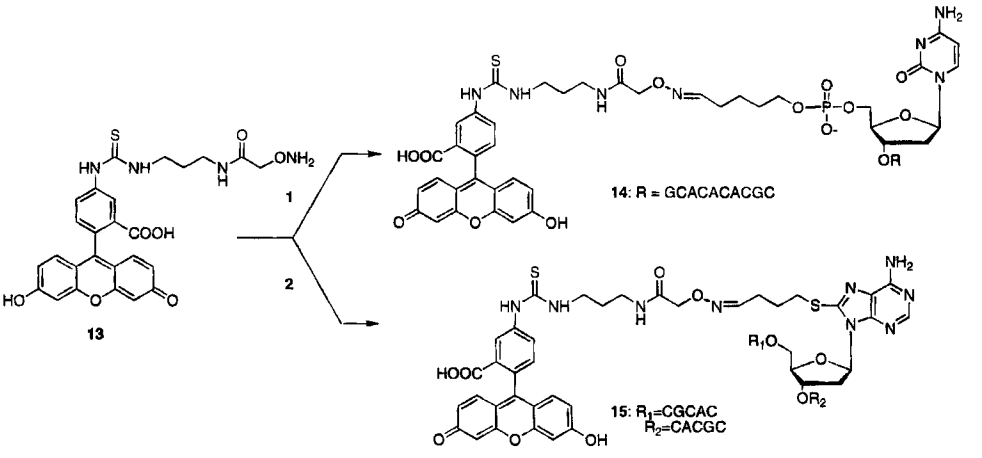
After deprotection with concentrated ammonia for 2 days at room temperature, the alkenyl oligonucleotide **5** was purified by reverse phase HPLC, detritylated using 80% aqueous AcOH and characterized by ESMS spectra. Bishydroxylation of the double bond was then carried out in water in the presence of OsO_4 (0.4 eq) and *N*-methylmorpholine *N*-oxyde at room temperature overnight leading to selective formation of the vicinal diol **12** (scheme 3). The structure of **12** was confirmed by ESMS. The diol was then oxidised with sodium periodate to give the target aldehyde **2**. It can be noted that a ten fold increase in the concentration of OsO_4 and $NaIO_4$ reagents was necessary to complete oxidation, as compared to the conditions required to oxidize the corresponding monomer. However, no oxidation at sulfur could be detected using this excess of reagent.

3) Derivatization of oligonucleotides **1** and **2** (scheme 4):

Results at the nucleoside level indicated that the aldehydic group reacted quite efficiently with a fluorescent oxyamine to afford the corresponding oxime ether in good yield (80%). We applied this reaction to derivatize the oligonucleotides inside the sequence in the case of undecamer **2**, and at the 5'-end using undecamer **1**. Reaction of the fluorescein derivative **13** with oligonucleotides **1** and **2** was carried out at room temperature in water at pH = 7 for 30 minutes using a slight excess of the oxyamino fluorophore (1.2 eq.). In both cases the reaction was highly selective affording respectively the oxime ethers **14** and **15**. As an example, the HPLC profile of the crude reaction mixture of oligonucleotide **1** with the label **13** is depicted in figure 2C. The structure of the conjugated oligonucleotides **14** and **15** was confirmed by ESMS. For example in the case of **15** we observed the multicharged ions $[M-6H]^{6-}$ at *m/e* = 647.3 and $[M-5H]^{5-}$ at *m/e* = 776.9 corresponding to a mass of 3891.5.

4) Stability of the functionalized duplexes (figure 3 and table 1):

The consequences on the hybridization properties of the oligonucleotides introduced by the linker, inside the sequence at the C-8 position of an adenine moiety as in **5** (**5** contains the *n*-pentenyl chain) and at the terminal 5'-phosphate as in **4** (**4** contains the diol functionality) were evaluated by melting temperature (Tm) measurements. Undecamers **4** and **5** were hybridized with their complementary strand d(GCGTGTGTGCG), and the melting temperatures of the resulting duplexes were determined. For comparison, the parent oligonucleotide and the tetrahydrofuranyl as an analog of abasic site containing oligonucleotide were equally studied. For the duplex formed



SCHEME 4: Synthesis of the conjugated oligonucleotides **14** and **15**.

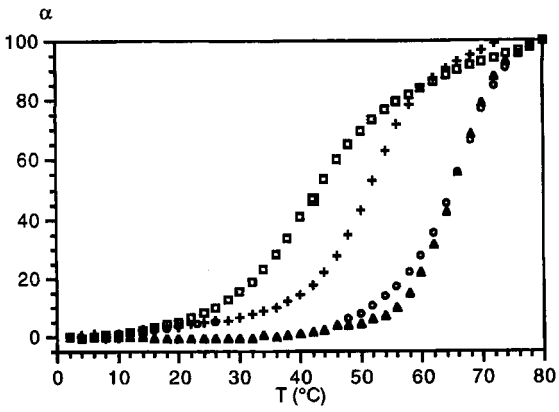


FIGURE 3: Tm curves for natural duplex (○), for duplex containing the abasic site (□), for oligonucleotides **4** (▲) and **5** (+) in phosphate buffer (0.01 M sodium phosphate, 0.001 M EDTA, 100 mM NaCl, pH = 7).

TABLE 1: Melting temperatures of duplexes formed by hybridization of indicated oligonucleotides with the complementary strand d(GCGTGTGTGCG). (a) *X* represents the C_8 modified adenine, (b) This strand contains the stable tetrahydrofuranyl analog (Tf) of the abasic site.[14]

Parent oligonucleotide	5'-CGCACACACGC-3'	Tm = 65°C
Oligonucleotide **4**	5'-^{diol}CGCACACACGC-3'	Tm = 65°C
Oligonucleotide **5** (a)	5'-CGCAC*X*CACGC-3'	Tm = 51°C
Abasic site oligonucleotide (b)	5'-CGCAC*Tf*CACGC-3'	Tm = 42.7°C

with undecamer **5**, a melting temperature Tm = 51°C was determined, corresponding to a destabilization introduced by the adenine C-8 linker ΔTm = 14°C as compared to the parent duplex (Tm = 65.0°C). Nevertheless, the destabilisation is less important than that induced by an abasic site (ΔTm = 22.3°C). On the other hand, a melting temperature Tm = 65.0°C was determined for the duplex with undecamer **4** indicating that the presence of the linker at the 5'-OH terminus does not modify the hybridization properties.

5) Spectral properties of the conjugate **14** and **15**:

In order to determine whether the spectral properties of the fluorescent moiety were modified in labeled undecamers **14** and **15**, we measured their absorption and fluorescence in Tris HCl buffer (pH = 9) for comparison with the fluorescent probe **13**. The three compounds **13**, **14** and **15** showed the same excitation and emission maxima respectively at 492 nm and 514 nm

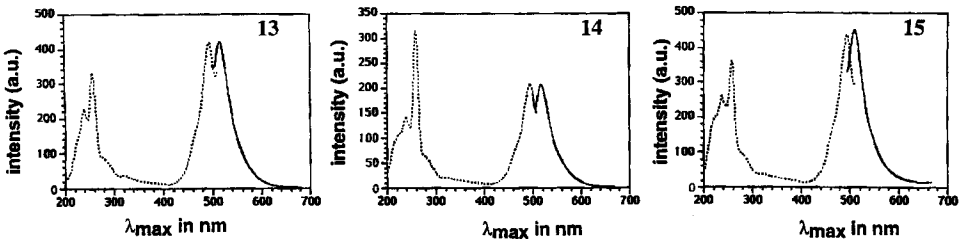


FIGURE 4: Fluorescence spectra of **13**, **14** and **15** in Tris HCl buffer (pH = 9). excitation spectra (----), emission spectra (——).

(figure 4). A slight quenching of fluorescence estimated to less than 10% was observed for conjugate **15**.

CONCLUSION

In conclusion, labelling of oligonucleotides by reaction of oxyamino fluorophores with an aldehydic function present on the polymer was found to be very convenient. The coupling reaction is stoechiometric, highly selective and rapid. The aldehydic precursor, i.e., a diol or an alkene can be incorporated at any preselected position inside the sequence or at the 5'-end of the oligonucleotide. The conjugated oligonucleotides were found to be stable in these conditions. No reduction step of the oxime ether is necessary to stabilize the conjugate. This coupling reaction has been used recently for labelling of a fragment of 16S RNA from *Mycobacteria tuberculosis*.[15]

EXPERIMENTAL PART

General: All commercially available chemical reagents were used without purification. Preparation of compounds **3**, **13** was previously described.[5,16] Analytical TLC were performed on 0.2 mm silica 60 coated aluminium foils with F-254 indicator (Merck). Prep. column chromatographies were done using silica gel (Merck 60, 200-63 μm). High-Performance Liquid Chromatography (HPLC) purification was carried out using a Waters chromatograph consisting of two M510 pumps, a M490E detector and a M680 system controller. A C18 column (Macherey-Nagel Nucleosil: 10 x 250 mm, 7 μm) was used. Melting points were measured on a Electrothermal Serie IA9100 apparatus. UV spectra were performed on a Perkin-Lambda 15UV/VIS and Fourier Transform Infrared spectra on a Perkin-Elmer Impact 400 spectrophotometers. NMR spectra were recorded on Bruker AC200 and WP250. Spectra were referenced to the residual proton solvent peaks. The mass spectra were recorded on a Delsi-

Nermag R10-10 spectrometer. For ES-MS spectra, analysis were performed on a VG Platform II (Micromass) in the negative ion mode. The eluant was 50% aqueous acetonitrile and the flow rate was 5µL/min. The oligonucleotides were dissolved in 50% aqueous acetonitrile and 1% of NEt_3 was added for the measure. Elemental analyses were performed by "Service Central de Microanalyse du CNRS". In several cases correct elemental analysis could not be obtained due to the polar and hygroscopic character of the compounds

2'-Deoxy-8-mercaptoadenosine **7**: To a solution of 8-bromo-2'-deoxyadenosine **6** (15 g, 45 mmol) in DMF (200 mL), was added under argon a 40% aqueous solution of sodium hydrogenosulfide. The reaction was stirred for 4 h at room temperature and the solvent was then evaporated. The residual oil obtained was washed with hexane and Et_2O then dissolved in water (80 mL). Compound **7** was precipitated by addition of 1N HCl aqueous solution. After filtration and washing with cold water, compound **7** was obtained as a pale sandy powder (10.8 g, 85%). Mp. 141-142°C. ^{1}H-NMR (DMSO d_6, 200 MHz): δppm = 12.55 (1H, br s, S<u>H</u>), 8.18 (1H, s, C_2-<u>H</u>), 6.95 (2H, br s, N<u>H</u>$_2$), 6.75 (1H, t, J = 6.4 Hz, $C_{1'}$-<u>H</u>), 5.35 (1H, br t, $C_{3'}$-O<u>H</u>), 5.23 (1H, br t, $C_{5'}$-O<u>H</u>), 4.46 (1H, m, $C_{3'}$-<u>H</u>), 4.35 (1H, m, $C_{4'}$-<u>H</u>), 3.68-3.53 (2H, m, $C_{5',5''}$-<u>H</u>), 2.95 (1H, m, $C_{2'}$-<u>H</u>), 2.07 (1H, m, $C_{2''}$-<u>H</u>). MS (FAB(+), glycerol matrix): m/e = 284 $[M+H]^+$. UV (NaH_2PO_4 100 mM, pH = 7): $\lambda_{max}(\varepsilon)$ = 297 (22600). IR (KBr disk): 3425, 2920, 1657, 1458, 1367, 1104 cm^{-1}. Anal. Calcd. for $C_{10}H_{13}N_5O_3S$: C 39.86 H 5.02 N 23.24 S 10.64. Found: C 40.09 H 5.05, N 22.90 S 10.58.

2'-Deoxy-8-(pent-4-enylsulfanyl)adenosine **8**: To a mixture of 7 (4.72 g, 16.7 mmol) and potassium carbonate (4.55 g, 33 mmol) in dry DMF (50 mL), 1-bromopent-4-ene (2.2 mL, 18.4 mmol) was added under argon. The reaction mixture was stirred for 3 h at room temperature then filtered off. The solvent was evaporated to dryness and the oily residue obtained was purified by silica gel column chromatography (CH_2Cl_2/MeOH: 95/5 then 90/10, v/v) to give **8** as a white powder (4.15 g, 71%). Mp. 130-131°C. ^{1}H-NMR (DMSO d_6, 250 MHz): δppm = 8.05 (1H, s, C_2-<u>H</u>), 7.20 (2H, br s, N<u>H</u>$_2$), 6.20 (1H, dd, $C_{1'}$-<u>H</u>), 5.80 (1H, m, C<u>H</u>=CH_2), 5.50 (1H, br t, $C_{5'}$-O<u>H</u>), 5.35 (1H, br d, $C_{3'}$-O<u>H</u>), 5.00 (2H, m, CH=C<u>H</u>$_2$), 4.40 (1H, m, $C_{3'}$-<u>H</u>), 3.88 (1H, m, $C_{4'}$-<u>H</u>), 3.60-3.40 (2H, m, $C_{5',5''}$-<u>H</u>), 3.30 (2H, t, S-C<u>H</u>$_2$), 3.10 (1H, m, $C_{2'}$-<u>H</u>), 2.18 (2H, m, C<u>H</u>$_2$-CH=CH_2), 2.10 (1H, m, $C_{2''}$-<u>H</u>), 1.80 (2H, m, S-CH_2-C<u>H</u>$_2$). ^{13}C-NMR (DMSO d_6, 75 MHz): δppm = 154.5 (quat), 151.0 (<u>C</u>H), 148.1 (quat), 137.3 (<u>C</u>H), 119.6 (quat), 115.3 (<u>C</u>H$_2$), 88.0 (<u>C</u>H), 84.6 (<u>C</u>H), 71.1 (<u>C</u>H), 62.0 (<u>C</u>H$_2$), 37.1 (<u>C</u>H$_2$), 31.7 (<u>C</u>H$_2$), 30.5 (<u>C</u>H$_2$), 27.8 (<u>C</u>H$_2$). MS (FAB(+), NBA matrix): m/e = 352 $[M+H]^+$, 236 $[M\text{-dribose}+2H]^+$. UV (EtOH): $\lambda_{max}(\varepsilon)$ = 280 (8035). IR (KBr disk): 3321, 3191 (large), 3075, 2917, 1653, 1598, 1573, 1468, 1338, 1295,

1098 cm^{-1}. Anal. Calcd. for $C_{15}H_{21}N_5O_3S$, 0.25 H_2O: C 50.62 H 6.09 N 19.68. Found: C 50.47 H 6.06 N 19.98.

N6-(Benzoyl)-2'-deoxy-8-(pent-4-enylsulfanyl)adenosine **9**: Compound **8** (2 g, 5.7 mmol) was dissolved in dry pyridine (50 mL) and the solution was cooled at 4°C. Benzoyle chloride (7 mL, 41 mmol) was added dropwise and the mixture was stirred overnight at room temperature. Water (1 mL) was then added and the solvent was evaporated under vacuum. The residual oil obtained was diluted in CH_2Cl_2 and the organic layer was washed with a 5% aqueous $NaHCO_3$ solution then with brine, dried ($MgSO_4$) and evaporated affording the tetrabenzoylated intermediate which was used in the next step without further purification. The tetrabenzoylated intermediate compound was dissolved in a dioxane-pyridine solution (180 mL, 1/1, v/v) and a NaOH 2N aqueous solution (100 mL) was added. The mixture was vigorously stirred for 1 h and then neutralised by addition of a 1N HCl aqueous solution. The aqueous layer was extracted with CH_2Cl_2 (3 X 150 mL). The organic layers were washed with 5% $NaHCO_3$ aqueous solution, dried (Na_2SO_4) and evaporated to dryness. The oily residue obtained was purified by silica gel column chromatography (AcOEt/MeOH: 99/1, v/v) to give compound **9** as a white powder (2 g, 76%). Mp. 150°C. ^{1}H-NMR ($CDCl_3$, 200 MHz): δppm = 8.98 (1H, s, NH), 8.60 (1H, s, C_2-H), 7.99-7.54 (5H, m, ArH Bn), 6.36 (1H, dd, J = 5.9 Hz, $C_{1'}$-H), 6.20 (1H, m, $C_{5'}$-OH), 5.78 (1H, m, CH=CH_2), 5.10-4.95 (2H, m, CH=CH_2), 4.77 (1H, d, $C_{3'}$-H), 4.19 (1H, m, $C_{4'}$-H), 3.90 (2H, m, $C_{5',5''}$-H), 3.36 (2H, m, S-CH_2), 3.01 (1H, m, $C_{2'}$-H), 2.25 (3H, m, $C_{2''}$-H and CH_2-CH=CH_2), 1.86 (2H, m, S-CH_2CH_2). ^{13}C-NMR ($CDCl_3$, 75 MHz): δppm = 164.4 (Ph-CO), 154.0 (quat), 152.5 (CH), 150.4 (quat), 147.2 (quat), 136.9 (CH), 133.8, 132.7 (CH), 128.9 (CH), 127.7 (CH), 124.1 (quat), 115.8 (CH_2), 89.5 (CH), 86.7 (CH), 73.3 (CH), 63.4 (CH_2), 40.1 (CH_2), 32.5 (CH_2), 31.8 (CH_2), 28.1 (CH_2). MS (FAB (+), NBA matrix): m/e = 456 $[M+H]^+$, 340 [M+2H-deoxyribose]$^+$. UV (CH_2Cl_2): $\lambda_{max}(\varepsilon)$ = 240 (23950), 304 (20750). IR (KBr disk): 3327, 3250, 3083, 2928, 2864, 1675, 1604, 1583, 1504, 1458, 1312, 1254, 1104 cm^{-1}. Anal. Calcd. for $C_{22}H_{25}N_5O_4S$: C 58.01 H 5.53 N 15.37 S 7.04. Found: C 58.28 H 5.50 N 15.31 S 7.19.

5'-O-(4,4'-dimethoxytrityl)-N6-(benzoyl)-2'-deoxy-8-(pent-4-enylsulfanyl) adenosine **10**: A solution of compound **9** (1 g, 2.2 mmol) in dry pyridine (10 mL) was cooled at 0°C and 4,4'-dimethoxytrityl chloride (1.4 g, 4.13 mmol) was added under argon. The reaction was stirred for 7 h and MeOH (1 mL) and CH_2Cl_2 (300 mL) were added. The organic layer was washed with brine then dried ($MgSO_4$) and evaporated under vacuum. The oily residue obtained was purified by silica gel column chromatography (CH_2Cl_2/AcOEt/NEt_3: 90/8/2, v/v) affording compound **10** as a white powder (1 g, 60%). ^{1}H-NMR ($CDCl_3$, 250 MHz): δppm = 8.84 (1H, s, NH), 8.43 (1H, s,

C_2-H), 7.98-7.55 (5H, m, ArH Bn), 7.38-7.11 (9H, m, ArH DMT), 6.78 (4H, m, ArH DMT), 6.34 (1H, t, J = 5.4 Hz, $C_{1'}$-H), 5.79 (1H, m, CH=CH_2), 5.06-4.93 (2H, m, CH=CH_2), 4.89 (1H, d, $C_{3'}$-OH), 4.11 (1H, m, $C_{3'}$-H), 3.78 (6H, s, CH_3O), 3.62-3.42 (3H, m, $C_{4'}$-H and $C_{5',5''}$-H), 3.38 (2H, t, S-CH_2), 2.55 (1H, m, $C_{2'}$-H), 2.32 (1H, m, $C_{2'}$-H), 2.22 (2H, q, CH_2-CH=CH_2), 1.84 (2H, m, S-CH_2CH_2). ^{13}C-NMR: ($CDCl_3$, 75 MHz): δppm = 158.4 (Ph-CO), 154.6 (quat), 152.8 (CH), 150.8 (quat), 144.1 (quat), 137.3 (CH), 137.1 (quat), 135.9 (quat), 135.7 (quat), 134.1 (quat), 132.6 (CH), 130.0 (CH), 129.8 (CH), 128.8 (CH), 128.1 (quat), 127.7 (CH), 126.8 (CH), 124.1 (quat), 115.7 (CH_2), 113.0 (CH), 86.3 (quat), 85.8 (CH), 84.4 (CH), 72.8 (CH), 63.7 (CH_2), 55.2 (CH_3), 36.8 (CH_2), 32.6 (CH_2), 31.7 (CH_2), 31.6 (quat), 28.2 (CH_2). MS (FAB (+), NBA matrix): m/e = 759 $[M+H]^+$, 454 $[M\text{-}DMT]^+$, 340 $[M+H\text{-}DMT\text{-}dribose]^+$, 303 $[DMT]^+$. UV (CH_2Cl_2): $\lambda_{max}(\varepsilon)$ = 236 (20810), 303 (10000). IR (KBr disk): 3414, 3271, 2961, 2936, 2875, 1703, 1612, 1515, 1454, 1336, 1255, 1174, 1082 cm^{-1}.

***Phosphoramidite* 11**: Compound **10** (1 g, 1.3 mmol) was dissolved under argon in a solution of CH_2Cl_2 (10 mL) and *N,N*-diisopropylethylamine (1.68 mL, 13 mmol). 2-Cyanoethyl-*N,N*-diisopropylchlorophosphoramidite (0.4 mL, 1.76 mmol) was added dropwise and the solution was stirred for 30 min at room temperature. A 5% $NaHCO_3$ aqueous solution (3 mL) and CH_2Cl_2 (60 mL) were then added. The organic layer was evaporated and the oily residue obtained was purified by silica gel column chromatography (CH_2Cl_2/AcOEt/NEt_3: 90/8/2, v/v) to give compound **11** as a pale yellow powder (1.1 g, 87%). Mp. 55°C. ^{1}H-NMR ($CDCl_3$, 250 MHz): δppm = 8.84 (1H, s, NH), 8.46 (1H, dd, C_2-H), 8.02 (2H, m, ArH Bn), 7.60-7.11 (9H, m, ArH DMT and Bn), 6.78 (4H, m, ArH DMT), 6.34 (1H, dd, $C_{1'}$-H), 5.81 (1H, m, CH=CH_2), 5.15-4.99 (3H, m, CH=CH_2 and $C_{3'}$-H), 4.26 (1H, m, $C_{4'}$-H), 3.82-3.40 (4H, m, OCH_2CH_2CN and $C_{5',5''}$-H), 3.78 (6H, s, OCH_3), 3.38-3.29 (3H, m, SCH_2 and $C_{2'}$H), 2.63 (1H, t, CH_2CN), 2.47 (1H, t, CH_2-CN), 2.38 (1H, m, $C_{2'}$H), 2.22 (2H, q, CH_2-CH=CH_2), 1.87 (2H, m, SCH_2CH_2), 1.72 (2H, m, $CHCH_3$), 1.09 (12H, m, $CHCH_3$). ^{31}P-NMR ($CDCl_3$, 80 MHz): δppm = 147.1 and 146.7 (2s, 2 diast.). MS (FAB (+), NBA matrix): m/e = 959 $[M+H]^+$, 340, 303.

Oligonucleotides synthesis: DNA synthesis was performed on a Expedite DNA synthesiser (Perkin-Elmer) following the manufacturer's protocols for DNA phosphoramidite synthesis. After treatment with concentrated ammonia and lyophilisation, the oligonucleotides **4** and **5** were purified by HPLC. Gradient elution was performed by building up a linear gradient starting with solvent A (ammonium acetate buffer pH = 6, 20 mM/ CH_3CN, 95/5 (v/v)) and applying solvent B (CH_3CN) up to 30% for 20 min with a flow rate of 4 mL/min. In case of aldehydes, we used a

phosphate buffer pH = 7, 20 mM/MeOH, 95/5 (v/v) for solvent A and MeOH for B. In case of oligonucleotide **5**, a treatment by 80% AcOH aqueous solution for 1 h was performed to cleave the trityl protection. The residue after lyophilisation was then dissolved in water and the aqueous layer was extensively washed with Et_2O. After lyophilisation, the oligonucleotides were stocked at -18°C. ESMS for **4**: Calcd mass: 3467.21, found 3466.2; ESMS for **5**: Calcd mass: 3371.4, found 3371.3.

***Aldehydic oligonucleotide* 1**: To a solution of oligonucleotide **4** (200 nmol) in water (500 µL), $NaIO_4$ (50 eq., 2.2 mg) was added and the solution was stirred at room temperature for 15 min. The oligonucleotide **1** was then purified by HPLC (150 nmol, 75%).

***Undecamer diol* 12**: To a solution of oligonucleotide **5** (340 nmol) in water (500 µL), a 0.02% OsO_4 aqueous solution (170 µL, 137 nmol), NMMO (2 µL) and 3% H_2O_2 aqueous solution (2 µL) were added. The mixture was stirred overnight at room temperature then lyophilised. The pellet was dissolved in water (1 mL) and the modified oligonucleotide **12** was purified by HPLC (220 nmol, 65%). ESMS (negative mode) Calcd mass: 3405.4, found 3404.3.

***Aldehydic oligonucleotide* 2**: A 200 µL aliquot (11 µmol) of a 54 mM solution of $NaIO_4$ was added to a solution of oligonucleotide **12** (110 nmol) in water (200 µL), and the mixture was stirred for 15 min at room temperature in the dark. The crude mixture was then purified by HPLC affording the oligonucleotide **2** (95 nmol, 86%).

***Coupling reaction with oligonucleotide* 1**: To a solution of oligonucleotide **1** (151 nmol) in water (500 µL), a solution of **13** (181 nmol) in DMF (100 µL) was added. The reaction mixture was stirred at room temperature at darkness for 1 hour then purified by HPLC to give quantitatively the conjugate oligonucleotide **15**. ESMS (negative mode) Calcd mass: 3985.2, found 3985.5.

***Coupling reaction with oligonucleotide* 2**: To a solution of oligonucleotide **2** (95 nmol) in water (200 µL), a solution of the fluorescein probe **13** (100 nmol) in DMF (100 µL) was added. The reaction mixture was stirred at darkness for 30 min. at r.t. The crude mixture was then purified by HPLC affording quantitatively the conjugate oligonucleotide **15**. ESMS (negative mode) Calcd mass: 3890.4, found 3891.5.

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