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Tetrahedron 61 (2005) 6138–6142
Tetrahedron

# A novel phosphoramidite for the synthesis of α-oxo aldehyde-modified oligodeoxynucleotides

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Received 3 February 2005; revised 10 March 2005; accepted 17 March 2005

Available online 13 May 2005

**Abstract**—The synthesis and use of a novel phosphoramidite derivatized by a bis[2-(*tert*-butyldisulfanyl)ethoxycarbonylamino]acetyl moiety for the synthesis of oligodeoxynucleotides modified at the 5′-end by an α-oxo aldehyde functionality is presented. Incorporation of the phosphoramidite reagent was performed after the automated solid-phase oligonucleotide synthesis. Simultaneous cleavage/deprotection of the oligodeoxynucleotides and unmasking of the α-oxo aldehyde group could be achieved using NaOH in aqueous methanol in the presence of dithiothreitol.


## 1. Introduction

Synthetic tools allowing the site-specific modification of oligodeoxynucleotides (ODN) have many applications such as the synthesis of conjugates,[1] of biomaterials or of miniaturized devices.[2] In this context, the unique reactivity of aldehyde functionality has been exploited for the modification of ODNs using thiazolidine, oxime, hydrazone or semicarbazone ligation chemistries.[1–5] Not surprisingly, the efficiency of aldehyde Schiff-base chemistry has stimulated the last few years many synthetic efforts toward the synthesis of aldehyde–ODNs. Most of the time, the aldehyde functionality was obtained by oxidative cleavage of a vicinal diol with periodate.[6–11] The direct introduction of a protected aldehyde moiety during solid phase elongation is less common.[1] Usually, ODNs were modified by aliphatic,[9] aromatic[12] or glycol aldehyde groups.[10] Little attention was given to α-oxo aldehydes despite the numerous applications of glyoxylyl group in the peptide field.[13–17] Recent results demonstrating the good stability of the α-oxo semicarbazone bond toward hydrolysis and the utility of the glyoxylyl group in either the synthesis of peptide–ODN conjugates,[11] or the preparation of ODN microarrays[18] should stimulate the use of this ligation chemistry.

To date, α-oxo aldehyde–ODNs were prepared in solution by oxidative cleavage of tartaramide[11] or seryl[19] deprotected precursors with periodate. However, these valuable two-step procedures are not ideally suited for the parallel synthesis of α-oxo aldehyde–ODNs as is usually required for ODN microarray projects. In addition, the use of periodate in the final step may be harmful to moieties sensitive to oxidants such as thiol or biotin as already documented in the peptide field.[20] Thus, the preparation of a phosphoramidite that could generate the α-oxo aldehyde group during the final basic treatment used for the deprotection of the ODN should simplify the synthesis of COCHO–ODNs and stimulate their use for the preparation of bioconjugates.

## 2. Results and discussion

We report in this article the synthesis of a novel phosphoramidite derivative[21,22] incorporating a bis[2-(*tert*-butyldisulfanyl)ethoxycarbonylamino]acetyl group as a masked α-oxo aldehyde functionality. This phosphoramidite was coupled to the 5′-terminus of CPG supported oligodeoxynucleotides using standard protocols. Unmasking of the COCHO group and cleavage/deprotection of the ODN could be realized in one step using mild and non-oxidative experimental conditions.

Recently, bis-(9*H*-fluoren-9-ylmethoxycarbonylamino)-acetic acid had been prepared in one step from glyoxylic acid and introduced into the peptide chain after solid-phase peptide elongation. Deprotection and cleavage of the

*Keywords*: Alpha-oxo aldehyde; Oligodeoxynucleotide; Phosphoramidite; DTT.
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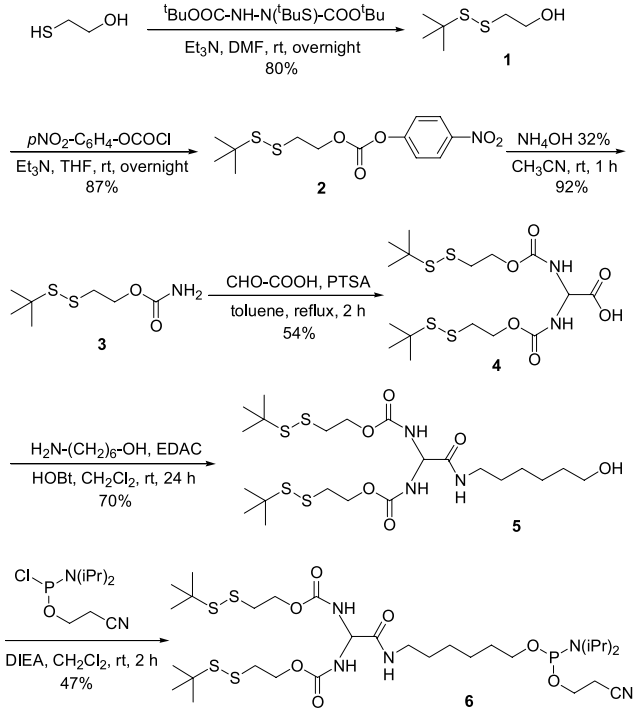
doi:10.1016/j.tet.2005.03.144

peptide from the solid support using trifluoroacetic acid was followed by unmasking of the glyoxylyl group in the presence of a base.[23,24] Replacement of Fmoc groups by 2-(*tert*-butyldisulfanyl)ethoxycarbonyl groups led to a novel derivative that could be cleanly deprotected in the presence of a phosphine.[25] The *tert*-butyldisulfanyl moiety was already used in the context of solid phase phosphoramidite chemistry,[26,27] thus the utility of the bis[2-(*tert*-butyldisulfanyl)ethoxycarbonylamino]acetyl group as a way to introduce a masked glyoxylyl group during solid phase ODN synthesis on CPG support was examined.

The synthesis of phosphoramidite **6** is depicted in Scheme 1. In a first step, *tert*-butylsulfenyl group was introduced on 2-mercaptoethanol using di-*tert*-butyl 1-(*tert*-butylthio)-1,2-hydrazine dicarboxylate[28] to afford disulfide **1** in 80% yield. This latter was converted to carbamate **3** by reaction with *p*-nitrophenyl chloroformate and ammonia successively. Reaction of solid glyoxylic acid hydrate with 2 equiv of carbamate **3** in refluxing toluene and in the presence of a catalytic amount of *p*-toluenesulfonic acid afforded bis-[2-(*tert*-butyldisulfanyl)ethoxycarbonylamino] acetic acid **4** in 54% yield following purification by silica gel chromatography. Acid **4** was then coupled to 6-aminohexan-1-ol using *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride/HOBt activation. Phosphitylation of the resulting alcohol **5** using 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite afforded the desired phosphoramidite **6** in 47% yield. $^{1}$H, $^{13}$C, $^{31}$P NMR and high-resolution mass spectrometry confirmed the identity of phosphoramidite **6**.

Next, the coupling of phosphoramidite **6** to the 5′-end of the 5′-dT$_7$-3′-CPG solid support[29] was examined (Fig. 1). This supported ODN was obtained by coupling 6 times β-cyanoethyl phosphoramidite DMT-dT to DMT-dT-CPG




**Scheme 1.** Synthesis of phosphoramidite **6**.

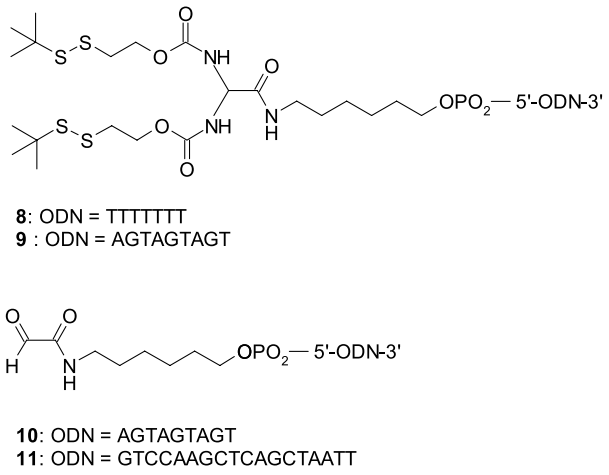



**Figure 1.** 5′-Modified ODNs synthesized in this study.

support. The coupling of **6** was performed using a 0.5 M solution in acetonitrile (10 min, twice). After the coupling, the formed phosphite group was oxidized into phosphate diester moiety using iodine in acetonitrile as for standard ODN synthesis. The product was separated from the CPG support with 32% aqueous $NH_4OH$ at rt during 1 h and analyzed by RP-HPLC. The retention time of ODN **8** (Fig. 2, 16.35 min) was significantly higher than those of unmodified ODN **7** (9.10 min) as the result of the presence of two *tert*-butylsulfanyl moieties on the molecule. The structure of ODN **8** was confirmed by MALDI-TOF mass spectrometry (ODN **8**: *m*/*z* $[M-H]^-$ Calcd 2700.52, found 2700.91).

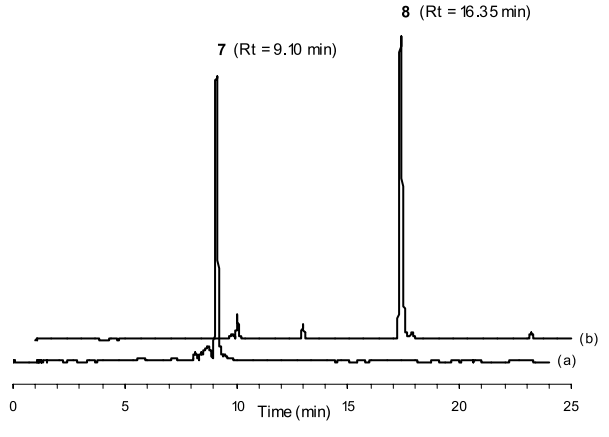



**Figure 2.** RP-HPLC traces of (a) crude trityl off ODN **7** (retention time: 9.10 min) and (b) crude ODN **8** (retention time: 16.35 min). C18 nucleosil column (4.6×250 mm), eluent A: 10 mM TEAA, eluent B: $CH_3CN$; linear gradient 5–50% B in 20 min, 1 mL/min, detection at 260 nm.

These results show the stability of the bis[2-(*tert*-butyldisulfanyl)ethoxycarbonylamino]acetyl group in the presence of iodine (oxidation step), the efficiency of the coupling reaction and the stability of the masked aldehyde moiety toward concentrated ammonia at room temperature.

Next, synthesis of glyoxylyl ODN **10** was examined using mild deprotection conditions (0.4 M NaOH in MeOH/$H_2O$

4/1 by vol at rt for 17 h).[30,31] RP-HPLC analysis of the crude desalted product revealed a single peak. However, the MALDI-TOF spectrum of the product displayed a peak at *m/z* 3262.45 (negative ion mode) which did not correspond to the calculated *m/z* ratio for ODN **10** nor **9**. The loss of 148.22 mass units relative to **9** ($[M-H]^-$: *m/z* Calcd 3410.67) was suspected to be due to an instability of the *tert*-butylsulfanyl groups in the presence of NaOH/MeOH. Model ODN **8** obtained by coupling phosphoramidite **6** to 5′-dT$_7$-3′-CPG led to similar results. Thus, simultaneous reduction of the disulfide bonds and deprotection of the ODN was attempted. Addition of an excess of DTT to the mild deprotection mixture led successfully to the desired ODN **10** (MALDI-TOF $[M-H]^-$: *m/z* Calcd 3010.57, found 3010.65). The usefulness of this procedure was confirmed with compound **11** using acetyl-protected dC for solid phase ODN synthesis (Fig. 3).

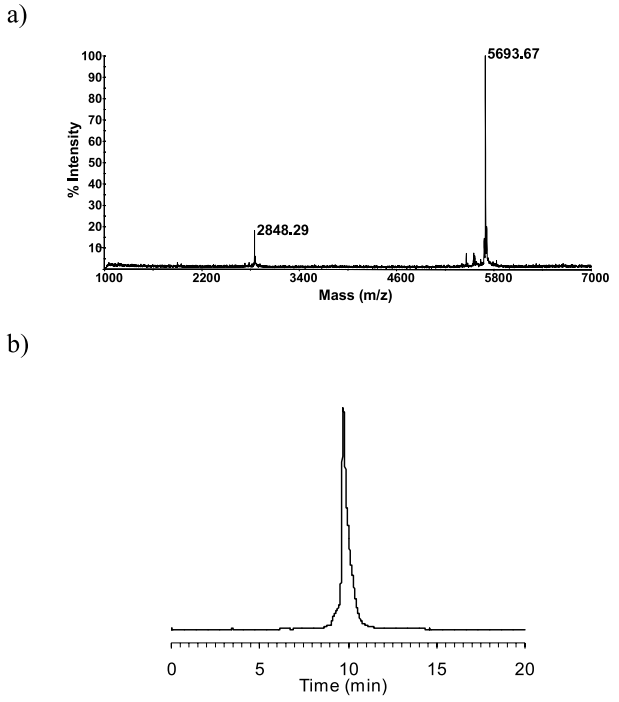



**Figure 3.** (a) MALDI-TOF spectrum of ODN **11**. Positive ion mode, 3-hydroxypicolinic acid was used as matrix; $[M+H]^+$: *m/z* Calcd 5693.02, found 5693.67; (b) RP-HPLC trace of ODN **12** purified on a C18 Sep-Pak cartridge. C18 nucleosil column (4.6×250 mm), eluent A: 10 mM TEAA, eluent B: $CH_3CN$; linear gradient 5–50% B in 20 min, 1 mL/min, detection at 260 nm.

In conclusion, we describe in this article that a phosphoramidite derivatized by a bis[2-(*tert*-butyldisulfanyl)ethoxycarbonylamino]acetyl moiety allows the introduction of a glyoxylyl group at the 5′-end of ODNs. The phosphoramidite was coupled using standard protocols. The bis[2-(*tert*-butyldisulfanyl)ethoxycarbonylamino]acetyl moiety was found to be stable in the presence of iodine. Clean unmasking of the α-oxo aldehyde group and deprotection of the ODN chain (acetyl protection for dC) was performed simultaneously using NaOH/MeOH in the presence of an excess of DTT.

## 3. Experimental

### 3.1. General

All commercially available chemical reagents were used without purification. Reactions were monitored by thin layer chromatography (TLC) on silica gel sheets containing UV fluorescent indicator (Macherey Nagel).

### 3.2. Experimental procedures

NMR spectra were recorded on a Bruker DRX 300 spectrometer at 300 MHz for $^1$H NMR, 75 MHz for $^{13}$C NMR and 121 MHz for $^{31}$P NMR. Chemical shifts were reported in ppm and tetramethylsilane (TMS) was used as an internal reference. MALDI-TOF spectra were recorded on a PerSeptive Biosystems Voyager-DE STR spectrometer, using either 3-hydroxypicolinic acid (3-HPA) or 6-aza-2-thiothymine (ATT) matrices, in positive or negative ion mode, respectively. IR spectra were recorded on a Perkin–Elmer Spectrum 1000 FT-IR spectrometer. Melting points were taken on a Büchi 530 apparatus in open capillary tubes and are uncorrected. RP-HPLC analyses were performed on a Shimadzu LC10-A, using a C18 Nucleosil column (4.6×250 mm) and the following conditions: eluent A: 10 mM TEAA, eluent B: acetonitrile; linear gradient 5–50% B in 20 min, 1 mL/min, detection at 260 nm. Automated oligonucleotide syntheses were performed on an Applied Biosystems 392 synthesizer. CPG columns were purchased from Applied Biosystems (France). β-Cyanoethyl-protected phosphoramidites Bz-dC, Bz-dA, iBu-dG and dT were purchased from Proligo (Germany) and Ac-dC from Eurogentec (France). All automated oligonucleotide synthesis reagents were purchased from Proligo (Germany), except anhydrous acetonitrile, from VWR (France).

**3.2.1. 2-(*tert*-Butyldisulfanyl)ethanol 1.** To a stirred solution of di-*tert*-butyl 1-(*tert*-butylthio)-1,2-hydrazinedicarboxylate (12.5 g, 39 mmol) and triethylamine (4.53 mL, 32.5 mmol) in DMF (150 mL) was added dropwise 2.28 mL (32.5 mmol) of 2-mercaptoethanol under argon. After stirring overnight at room temperature, the reaction mixture was evaporated to dryness. Petroleum ether was added to the resulting oily residue under vigorous stirring. The white solid that precipitates was eliminated by filtration and the filtrate was evaporated in vacuo. The residue was purified by chromatography on silica gel ($CH_2Cl_2$/AcOEt: 9/1). The pure compound **1** was obtained as a pale yellow oil (4.32 g, 26 mmol) in 80% yield. IR (NaCl) $\nu$ ($cm^{-1}$) 3350 (OH), 2960 (CH), 1046 (C–O); $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 1.35 (s, 9H, $3CH_3$), 2.09 (t, 1H, OH), 2.86 (t, 2H, $J$=6.0 Hz, $CH_2$–S), 3.87 (t, 2H, $J$=6.0 Hz, $CH_2$–O); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 29.9 ($CH_3$), 42.7 ($CH_2$–S), 48.0 ($C$–$(CH_3)_3$), 60.7 ($CH_2$–O); MS (CI, $NH_3$) *m/z* 184 $[M+NH_4]^+$; HRMS (CI, $NH_3$) *m/z* Calcd for $[M+NH_4]^+$ $C_6H_{18}NOS_2$ 184.0830, found 184.0826.

**3.2.2. 2-(*tert*-Butyldisulfanyl)ethyl 4-nitrophenyl carbonate 2.** Compound **1** (4.0 g, 24 mmol) and 4-nitrophenyl chloroformate (7.26 g, 36 mmol) were dissolved in anhydrous THF (50 mL) under argon and the mixture was cooled to 0 °C. Triethylamine (5.02 mL, 36 mmol) was added

dropwise to the stirred solution. The reaction mixture was stirred at 0 °C for 10 min then at rt overnight. The solution was filtered and the filtrate was evaporated in vacuo to give a yellow oil which was purified by chromatography on silica gel (cyclohexane/AcOEt: 9/1). The pure compound **2** was obtained as a clear oil (6.96 g, 21 mmol) in 87% yield. IR (NaCl) $\nu$ ($cm^{-1}$) 3085 (CH Ar), 2962 (CH), 1769 (C=O), 1617 (C=C Ar), 1526 ($NO_2$), 1347 ($NO_2$), 1215 (C–N), 1165 (C–O); $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 1.36 (s, 9H, $3CH_3$), 3.01 (t, 2H, $J$=6.8 Hz, $CH_2$–S), 4.52 (t, 2H, $J$=6.8 Hz, $CH_2$–O), 7.39 (d, 2H,, $J$=9.2 Hz, 2CH Ph), 8.27 (d, 2H, $J$=9.2 Hz, 2CH Ph); $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 29.8 ($CH_3$), 37.9 ($CH_2$–S), 48.2 (*C*–$(CH_3)_3$), 67.3 ($CH_2$–O), 121.8 (CH Ar), 125.3 (CH Ar), 145.5 (C Ar), 152.7 (CO), 155.9 (C Ar); MS (CI, $NH_3$) *m/z* 349 $[M+NH_4]^+$; HRMS (CI, $NH_3$) *m/z* Calcd for $[M+NH_4]^+$ $C_{13}H_{21}N_2O_5S_2$ 349.0892, found 349.0888. Anal. Calcd for $C_{13}H_{17}NO_5S_2$: C, 47.11; H, 5.17; N, 4.23; S, 19.35. Found: C, 47.01; H, 5.37; N, 4.33; S, 19.07.

**3.2.3. 2-(*tert*-Butyldisulfanyl)ethyl carbamate 3.** To a suspension of compound **2** (6.5 g, 19.6 mmol) in acetonitrile (80 mL) was added dropwise 32% aqueous ammonia solution (16 mL) under stirring. The reaction mixture was stirred at room temperature for 1 h. Ethyl acetate (800 mL) was added to the reaction mixture and the resulting solution was washed with water (5×400 mL) and saturated NaCl (200 mL). The organic layer was dried on $MgSO_4$ and evaporated in vacuo. After drying on $P_2O_5$ under reduced pressure, carbamate **3** (3.77 g, 18 mmol) was obtained as a white solid in 92% yield. Mp 80–82 °C; IR (KBr) $\nu$ ($cm^{-1}$) 3402 ($NH_2$), 2957 (CH), 1682 (C=O), 1168 (C–O); $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 1.34 (s, 9H, $3CH_3$), 2.91 (t, 2H, $J$=6.6 Hz, $CH_2$–S), 4.30 (t, 2H, $J$=6.6 Hz, $CH_2$–O), 4.73 (br, 2H, $NH_2$); $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 29.8 ($CH_3$), 38.9 ($CH_2$–S), 47.9 (*C*–$(CH_3)_3$), 63.3 ($CH_2$–O), 156.5 (CO); MS (CI, $NH_3$) *m/z* 227 $[M+NH_4]^+$; HRMS (CI, $NH_3$) *m/z* Calcd for $[M+NH_4]^+$ $C_7H_{19}N_2O_2S_2$ 227.0888, found 227.0890. Anal. Calcd for $C_7H_{15}NO_2S_2$: C, 40.17; H, 7.22; N, 6.69; S, 30.63. Found: C, 39.89; H, 7.36; N, 6.71; S, 31.18.

**3.2.4. Bis[2-(*tert*-butyldisulfanyl)ethoxycarbonylamino]acetic acid 4.** Carbamate **3** (3.5 g, 16.7 mmol), glyoxylic acid monohydrate (0.77 g, 8.35 mmol) and PTSA (15.8 mg, 83 μmol) were dissolved in toluene (500 mL) and refluxed for 2 h (Dean-Stark trap). The reaction mixture was cooled to rt and allowed to precipitate overnight. The resulting precipitate was filtered and washed with toluene. The crude product was purified by silica gel chromatography ($CHCl_3$/MeOH/AcOH: 36/1/0.5). The product was concentrated, co-evaporated three time with toluene and dried in vacuo on $P_2O_5$. Compound **4** (2.14 g, 4.51 mmol) was obtained as a white solid in 54% yield. Mp 112–114 °C; IR (KBr) $\nu$ ($cm^{-1}$) 3313 (OH), 2961 (CH), 1731 (C=O), 1690 (C=O); $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 1.33 (s, 18H, $6CH_3$), 2.90 (t, 4H, $J$=6.7 Hz, $2CH_2$–S), 4.07 (br, 2H, 2NH), 4.32 (t, 4H, $J$=6.7 Hz, $2CH_2$–O), 5.42 (t, 1H, $J$=7.0 Hz, C*H*–COOH); $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 30.2 ($CH_3$), 39.0 ($CH_2$–S), 48.4 (*C*–$(CH_3)_3$), 60.2 (*C*H–COOH), 64.4 ($CH_2$–O), 156.2 (OCO–NH), 170.3 (COOH); MS (FAB) *m/z* 497.1 $[M+Na]^+$; HRMS (FAB) *m/z* Calcd for $[M+Na]^+$ $C_{16}H_{30}N_2O_6S_4Na$ 497.0884, found 497.0881. Anal. Calcd for $C_{16}H_{30}N_2O_6S_4$: C, 40.49; H, 6.37; N, 5.90; S, 27.02. Found: C, 40.41; H, 6.48; N, 6.00; S, 26.79.

**3.2.5. Compound 5.** A mixture of compound **4** (2.0 g, 4.21 mmol), *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (1.21 g, 6.31 mmol) and HOBt (0.852 g, 6.31 mmol) was dissolved in $CH_2Cl_2$ (100 mL) under argon. 6-Aminohexan-1-ol (0.739 g, 6.31 mmol) was added and the reaction mixture was stirred at room temperature for 24 h. The reaction mixture was evaporated to dryness and the crude residue was purified by chromatography on silica gel ($CH_2Cl_2$/MeOH: 95/5). The pure compound **5** was obtained as a white solid (1.69 g, 2.95 mmol) in 70% yield. Mp 93–95 °C; IR (KBr) $\nu$ ($cm^{-1}$) 3294 (OH), 2938 (CH), 1714 (C=O), 1651 (C=O); $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 1.20–1.40 (m, 22H, $6CH_3$+$2CH_2$ alkyl chain), 1.47 (m, 4H, $2CH_2$ alkyl chain), 1.60 (br, 1H, OH), 2.83 (t, 4H, $J$=6.6 Hz, $2CH_2$–S), 3.21 (m, 2H, $CH_2$ alkyl chain), 3.57 (m, 2H, $CH_2$ alkyl chain), 4.26 (t, 4H, $J$=6.6 Hz, $2CH_2$–OCO), 5.43 (t, 1H, $J$=7.0 Hz, C*H*–CONH), 5.96 (br, 2H, 2NH), 6.70 (br, 1H, NH); $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 25.6 ($CH_2$), 26.7 ($CH_2$), 29.5 ($CH_2$), 30.2 ($CH_3$), 32.8 ($CH_2$), 39.1 ($CH_2$–S), 40.1 ($CH_2$), 48.4 (*C*–$(CH_3)_3$), 60.7 (*C*H–CONH), 63.0 ($CH_2$), 64.1 ($CH_2$–OCO), 156.0 (OCONH), 167.8 (CONH); MS (FAB) *m/z* 596.2 $[M+Na]^+$; HRMS (FAB) *m/z* Calcd for $[M+Na]^+$ $C_{22}H_{43}N_3O_6S_4Na$ 596.1932, found 596.1935. Anal. Calcd for $C_{22}H_{43}N_3O_6S_4$: C, 46.05; H, 7.55; N, 7.32; S, 22.35. Found: C, 45.97; H, 7.45; N, 7.34; S, 22.40.

**3.2.6. Phosphoramidite 6.** DIEA (1.82 mL, 10.44 mmol) and 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (872 μL, 3.91 mmol) were added to a solution of compound **5** (1.50 g, 2.61 mmol) in freshly distilled $CH_2Cl_2$ (20 mL) at room temperature under argon. After stirring the mixture for 2 h, methanol (105 μL, 2.61 mmol) was added and the mixture was stirred for an additional 30 min. The reaction mixture was evaporated to dryness and cyclohexane/AcOEt/$Et_3N$ (60/40/2) was added. The resulting precipitate was eliminated by filtration and the filtrate was evaporated in vacuo. The crude product was purified by silica gel chromatography (cyclohexane/AcOEt/$Et_3N$ 60/40/2). The product was concentrated, coevaporated three time with toluene and dried in vacuo. Compound **6** was obtained as a clear viscous oil (0.952 g, 1.23 mmol) in 47% yield. IR (KBr) $\nu$ ($cm^{-1}$) 3293 (NH), 2966 (CH), 2252 (C≡N), 1713 (C=O), 1652 (C=O), 975 (P–O); $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 1.05–1.15 (m, $6CH_3$ *i*Pr), 1.20–1.35 (m, 22H, $6CH_3$ $^t$Bu+$2CH_2$ alkyl chain), 1.51 (m, 4H, $2CH_2$ alkyl chain), 2.57 (t, $J$=6.4 Hz, C*H*$_2$–CN), 2.83 (t, 4H, $J$=6.6 Hz, $2CH_2$–S), 3.19 (m, 2H, $CH_2$ alkyl chain), 3.45–3.65 (m, 4H, 2CH *i*Pr+$CH_2$ alkyl chain), 3.70–3.85 (m, 2H, C*H*$_2$–$CH_2$CN), 4.26 (t, 4H, $J$=6.6 Hz, $2CH_2$–OCO), 5.41 (t, 1H, $J$=7.0 Hz, C*H*–CONH), 5.86 (br, 2H, 2NH), 6.55 (br, 1H, NH); $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ (ppm) 20.7–20.8 (*C*$H_2$–CN), 25.0–25.1 ($CH_3$ *i*Pr), 26.0 ($CH_2$), 26.9 ($CH_2$), 29.6 ($CH_2$), 30.2 ($CH_3$ $^t$Bu), 31.4–31.5 ($CH_2$), 39.1 ($CH_2$–S), 40.3 ($CH_2$), 43.3–43.4 (CH *i*Pr), 48.4 (*C*–$(CH_3)_3$), 58.5–58.8 (*C*$H_2$–$CH_2$CN), 60.7 (*C*H–CONH), 63.8–64.0 ($CH_2$), 64.1 ($CH_2$–OCO), 117.9 (CN), 156.0 (CO), 167.6 (CO); $^{31}P$ NMR (121 MHz, $CDCl_3$) $\delta$ 147.4; MS (FAB) *m/z* 796.3 $[M+Na]^+$; HRMS (FAB) *m/z* Calcd for $[M+Na]^+$ $C_{31}H_{60}N_5O_7PS_4Na$ 796.3011, found 796.3019.

### 3.3. Typical experimental procedure, synthesis of ODN 11

The 5′-modified oligonucleotides were assembled on an Applied Biosystems 392 synthesizer in 1.0 μmol scales, using the standard phosphoramidite protocol. Bz-dA, iBu-dG and Ac-dC β-cyanoethyl-5′-DMT protected phosphoramidites were used. Phosphoramidite **6** was introduced at the last cycle using an extended coupling time with a double delivery (2×10 min) and a phosphoramidite concentration of 0.5 M. Simultaneous cleavage/deprotection of the 5′-modified oligonucleotide and unmasking of the α-oxo aldehyde function was performed by treating the solid support with 0.4 M NaOH in MeOH/$H_2O$ (4/1 by vol, 1 mL) containing DTT (100 equiv) at room temperature for 17 h, using the double syringe method. The mixture was then transferred on a flask and 1.5 mL of 2 M TEAA was added. The methanol was eliminated from the mixture by evaporation on a rotary evaporator and the product was desalted on a NAP-5 column (Sephadex G25, Amersham Biosciences), and then purified on a C18 Sep-Pak cartridge (Waters). Overall yield 29% (determined by UV at 260 nm).

## Acknowledgements

We gratefully acknowledge le Ministère de l'Economie, des Finances et de l'Industrie (GenHomme, Coquepuce Project), CNRS, Université de Lille 2 and Institut Pasteur de Lille for financial support. We thank Gérard Montagne for NMR experiments and Anthony Romieu, David Hot, Ludovic Huot and Yves Lemoine for useful discussions.

## Supplementary data

Supplementary data associated with this article can be found, in the online version, at 10.1016/j.tet.2005.03.144

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