Novel Non-Nucleosidic Phosphoramidite Building Blocks for Versatile Functionalization of Oligonucleotides at Primary Hydroxy Groups

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Synthesis of two non-nucleosidic phosphoramidite building blocks, **1a**, **b**, that enable attachment of various tether groups to oligonucleotides at their 5′-terminus (or 1′-OH of 3′-deoxypsico-nucleoside units) is described. Introduction of these linkers during the oligonucleotide assembly on a solid support, and their subsequent derivatization upon deprotection, afforded amino-, carboxy-, and sulfanyl-alkyl-tethered oligonucleotides.

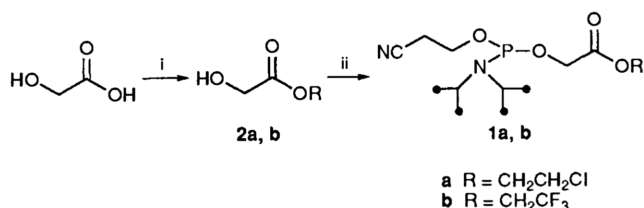
Oligonucleotides bearing either a reporter group or a chemical cross-linker at their 5′-terminus are widely used in molecular biology as diagnostic probes[1] and regulators of gene expression.[2] Usually, a non-nucleotidic building block containing an aliphatic spacer arm with a protected functional group is introduced either as a phosphoramidite[3-10] or H-phosphonate[11] at the last cycle of the oligonucleotide synthesis, and this additional functionality is released for labelling upon deprotection. Alternatively, the 5′-hydroxy function may be phosphorylated either chemically[10,12-16] or enzymically,[17] followed by carbodiimide-assisted condensation with an alkane-α,ω-diamine and introduction of the conjugate group in solution.[18]

Building blocks containing a readily displaceable group offer a more versatile approach to the preparation of oligonucleotide conjugates, since a single building block may be exploited to introduce a variety of tether groups. The applicability of this methodology has been demonstrated with base-modified nucleotidic building blocks. Sung[19] originally inserted 4-triazolopyrimidinone nucleosides into oligonucleotides, and ammonolysed them to cytosine derivatives. More recently, the triazole leaving group has been displaced with a variety of nucleophiles to prepare non-radioactive DNA probes.[20] MacMillan and Verdine[21] used O^4-(2,4,6-trimethylphenyl)-2′-deoxyuridine and Markiewicz *et al.*[22] N^4-tosyl-2′-deoxycytidine for the same purpose; upon completion of the chain assembly, the leaving group was displaced with alkanediamines. The same approach has also been used for 5′-labelling. Gildea *et al.*[23] prepared, by phosphoramidite strategy, oligonucleotides bearing a 5′-terminal 4,4′-dimethoxy-4″-succinimidooxycarbonyltrityl group instead of the conventional 4,4′-dimethoxytrityl group. Displacement of the succinimidooxy group with ω-substituted alkylamines then gave the desired oligonucleotide conjugates. We now report on an alternative, straightforward method for the 5′-derivatization of oligonucleotides that enables introduction of amino-, carboxy-, or sulfanylalkyl tether groups.

Non-nucleosidic phosphoramidite building blocks, **1a**, **b**, were prepared and used at the last cycle of oligonucleotide synthesis. The desired functional group was introduced by displacing the 2-chloroethoxy or 2,2,2-trifluoroethoxy group with an alkane-α,ω-diamine, hydroxide ion or cystamine (containing a masked thiol group) as the first step of oligonucleotide deprotection. The same approach was also used to functionalize the nonterminal primary hydroxy groups of oligonucleotides containing 3′-deoxypsicothymidine units. The latter units enable nonterminal derivatization of the oligonucleotide without base or phosphate backbone modifications.

Results and Discussion

Synthesis of Phosphoramidites.—The halogen-substituted alkyl glycolates, **2a**, **b**, were obtained by esterification of glycolic acid with 2-chloroethanol or 2,2,2-trifluoroethanol preactivated with thionyl dichloride (Scheme 1). After purification by recrystallization (**2a**) or sublimation (**2b**), glycolates **2a**, **b** were converted into the corresponding phosphoramidites, **1a**, **b**, by treatment with 2-cyanoethyl *N,N,N′,N′*-tetraisopropylphosphorodiamidite in the presence of 1*H*-tetrazole. The applicability of phosphoramidites **1a**, **b** as building blocks in the machine-assisted oligonucleotide synthesis was examined by attaching them to the 5′-position of a commercial thymidine-derivatized solid support. After standard oxidation with iodine, the solid support was ammonolysed, and the product distribution was determined by reversed-phase HPLC. According to the HPLC analysis, the coupling yields under standard conditions (0.1 mol dm^{-3} **1a**, **b** in acetonitrile; coupling time 20 s) were compatible with those for normal nucleoside phosphoramidites.



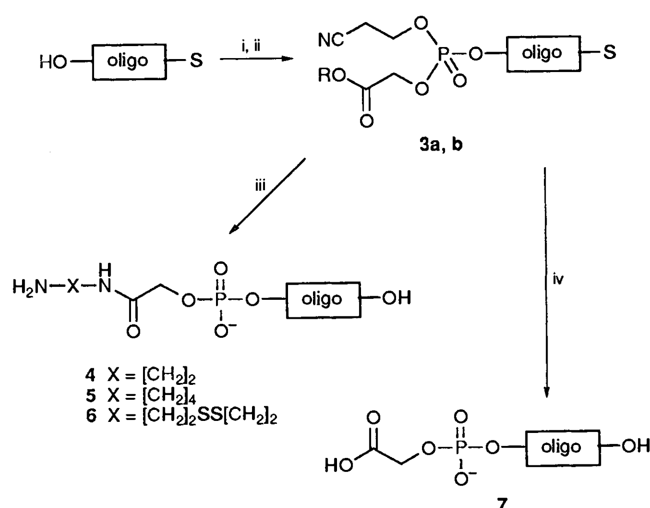
Scheme 1 *Reagents:* i, HOR, $SOCl_2$; ii, 2-cyanoethyl *N,N,N′,N′*-tetraisopropylphosphorodiamidite, tetrazole, acetonitrile

Synthesis of 5′-Modified Oligonucleotides.—To demonstrate the usefulness of compounds **1a**, **b** in the preparation of 5′-modified oligonucleotides, a model sequence d(TCCGTGGA-GTCGTG) bearing various functionalities at its 5′-terminus was synthesized (Scheme 2).The phosphoramidites **1a**, **b** were attached to the 5′-end of the oligonucleotide at the last coupling step, followed by oxidation with iodine. After the oligonucleotide chain assembly was completed, the protected oligonucleotides, **3a**, **b**, were treated with various nucleophiles as described in Table 1. We noted on preparing 3′-functionalized oligonucleotides[24] that the side reactions, such as transamination of the N^4-benzoylated cytosine residues, are negligible as long as the concentration of the aqueous diamine used as cleaving agent is less than 2 mol dm^{-3}. Accordingly, the 5′-ω-aminoalkylated oligonucleotides, **4–6**, may be obtained by cleavage of the reactive ester bond of phosphates **3a**, **b** with an appropriate alkane-α,ω-diamine or cystamine in water. The 5′-carboxymethyl phosphate oligonucleotide **7** was obtained by

Table 1 Deprotection procedures applied to obtain the 1′- and 5′-modified oligonucleotides

Modified phosphoramidite	Protected oligonucleotide	Product	Deprotection procedure[a]
1a	**3a**	**4**	(i) 0.5 mol dm^{-3} aq. ethane-1,2-diamine for 12 h, (ii) ammonolysis[b]
1b	**3b**	**4**	(i) 0.1 mol dm^{-3} aq. ethane-1,2-diamine for 6 h, (ii) ammonolysis[b]
1a	**3a**	**5**	(i) 0.5 mol dm^{-3} aq. butane-1,4-diamine for 12 h, (ii) ammonolysis[b]
1b	**3b**	**5**	(i) 0.1 mol dm^{-3} aq. butane-1,4-diamine for 6 h, (ii) ammonolysis[b]
1a	**3a**	**6**	(i) 0.5 mol dm^{-3} aq. cystamine for 12 h, (ii) ammonolysis[b]
1b	**3b**	**6**	(i) 0.1 mol dm^{-3} aq. cystamine for 6 h, (ii) ammonolysis[b]
1a	**3a**	**7**	(i) 0.1 mol dm^{-3} aq. NaOH for 4 h, (ii) ammonolysis[b]
1b	**3b**	**7**	As above
1b	**10**	**12**	(i) 0.1 mol dm^{-3} aq. butane-1,4-diamine for 6 h, (ii) ammonolysis[b]
1b	**10**	**13**	(i) 0.1 mol dm^{-3} aq. cystamine for 6 h, (ii) ammonolysis[b]
1b	**11**	**14**	(i) 0.5 mol dm^{-3} aq. butane-1,4-diamine for 12 h, (ii) ammonolysis[b]
1b	**11**	**15**	(i) 0.5 mol dm^{-3} aq. cystamine for 12 h, (ii) ammonolysis[b]

[a] At room temperature, if not otherwise mentioned. [b] Conc. aq. ammonia, 7 h at 55 °C.



Scheme 2 *Reagents:* i, **1a, b**, tetrazole, acetonitrile; ii, iodine, pyridine, water; iii, aq. diamine, then aq. ammonia; iv, aq. NaOH, then aq. ammonia; S = solid support

treatment of phosphates **3a, b** with aq. sodium hydroxide. Conventional treatment with conc. ammonia gave complete deprotection in all cases.

The difference in the leaving-group ability of chloroethyl and trifluoroethyl groups is reflected in the reaction times and diamine concentrations needed to introduce the desired 5′-tethers. While 12 h of treatment with a 0.5 mol dm^{-3} solution of diamine was necessary to convert compound **3a** into compounds **4–6**, the same tether was introduced into compound **3b** by 0.1 mol dm^{-3} aqueous diamine in 6 h. In both cases the yields of the target oligonucleotides were about 90%.*

Synthesis of 1′-Modified Oligonucleotides.—The methodology described above was applied to 1′-derivatization of oligonucleotides containing 3′-deoxypsicothymidine units. Accordingly, two sequences, bearing either one (**8**) or two (**9**) 3′-deoxypsicothymidine units (Π) were prepared in a fully protected form, and the 1′-*O*-laevulinyl (4-oxopentanoyl) protection was selectively removed with hydrazine acetate as reported previously[25,26] (Scheme 3). Compound **1b** was then coupled to the 1′-*O*-deprotected material in a standard manner. Oxidation of the 1′-phosphite group and subsequent detritylation of the 5′-terminus yielded the oligonucleotides containing one (**10**) or two (**11**) trifluoroethoxycarbonylmethyl phosphate groups at C-1′ (Π^1).

Treatment of compounds **10** and **11** with butane-1,4-diamine or cystamine introduced the desired 1′-tethers (Π^2). When a single 1′-spacer arm was attached (**12, 13**), the reaction time and diamine concentration used were the same as those given above for the 5′-modified oligonucleotides (Table 1). No difference in the yields of 1′- and 5′-modified oligonucleotides was observed. For the attachment of two 1′-tethers (**14, 15**) higher diamine concentrations (0.5 mol dm^{-3}) and longer reaction times (12 h) were preferred. Under these conditions the desired functional groups were introduced in moderate yield (85%).*

Characterization of the Oligonucleotides Prepared.—The oligonucleotides prepared were isolated by ion-exchange HPLC, purified on an RP column, and desalted by gel filtration.[24] Their HPLC retention times are given in Table 2. The ion-exchange HPLC profiles of the crude 5′-carboxyalkylated oligonucleotide **7** and the oligonucleotides bearing cystamine spacers (**6, 13, 15**) are shown in Figs. 1 and 2 as illustrative examples.

Digestion of compounds **4–7** with phosphodiesterase I and alkaline phosphatase gave the natural nucleosides in expected ratios, whereas the same oligomers were resistant toward phosphodiesterase II. These observations agree with the results reported for the other 5′-modified oligonucleotides.[5,27,28] The structures of the tethered oligonucleotides were further verified by labelling them with a reporter group. Accordingly, the oligonucleotides bearing ω-aminoalkyl or cystamine tethers, **4–6, 12–15**, were treated with 4-(dimethylamino)azobenzene-4′-sulfonyl (dabsyl) chloride to give sulfonamides **16–21** (Scheme 4). UV–VIS spectra of the labelled oligonucleotides exhibited the characteristic dabsyl absorption at 470 nm and oligonucleotide absorption at 260 nm (Table 3). The ratio $A(470)/A(260)$ observed with compounds **20** and **21** was approximately twice that observed with analogues **16–19**. The presence of the disulfide bond in compounds **18, 20** and **22** was additionally verified by allowing them to react with 1,4-dithio-DL-threitol.[29] HPLC analysis of the reaction mixture showed quantitative conversion of the labelled oligonucleotide into two products, *i.e.* the sulfanylalkyl oligonucleotide, **23–25**, and the liberated dye.

Experimental

General.—Reagents for the machine-assisted oligonucleotide synthesis were purchased from Cruachem. Cystamine dihydrochloride (Aldrich) was converted into the free-base form[30] and

* Peaks of capped sequences are not included in calculations.

8 TCCGПGGAGTCGTG

9 TCCGПGGAGПCGTG

10 TCCGП[1]GGAGTCGTG

11 TCCGП[1]GGAGП[1]CGTG

12 TCCGП[2]GGAGTCGTG X = $[CH_2]_4$

13 TCCGП[2]GGAGTCGTG X = $[CH_2]_2SS[CH_2]_2$

14 TCCGП[2]GGAGП[2]CGTG X = $[CH_2]_4$

15 TCCGП[2]GGAGП[2]CGTG X = $[CH_2]_2SS[CH_2]_2$

Scheme 3 *Reagents:* i, hydrazine acetate; ii, **1a**, **b**, tetrazole, acetonitrile; iii, iodine, pyridine, water; iv, aq. diamine, then aq. ammonia. Lv = laevulinyl ($MeCOCH_2CH_2CO$–)

Table 2 HPLC retention times of the oligonucleotides prepared

Column	Retention time of oligonucleotide (t_R/min)							
	4	**5**	**6**	**7**	**12**	**13**	**14**	**15**
Ion exchange[a]	35.6	35.6	35.6	42.7	34.6	34.7	30.9	32.7
Rev. phase[b]	23.2	23.5	24.2	22.1	23.6	23.9	21.8	23.0

[a] System 2. [b] System 3. For structures, consult Schemes 2 and 3.

Table 3 Properties of the labelled oligonucleotides

Compound	$A(470)/A(260)$	t_R/min[a]	t_R/min[a,b]
16	0.22	34.9	
17	0.22	39.5	
18	0.21	45.4	24.5 **23**
19	0.21	36.2	
20	0.21	40.8	23.5 **24**
21	0.37	53.8	
22	0.36	61.2	23.0 **25**

[a] Reversed-phase column (System 3). [b] Retention time of the oligonucleotide after reduction with 1,4-dithio-DL-threitol. For structures, consult Scheme 4.

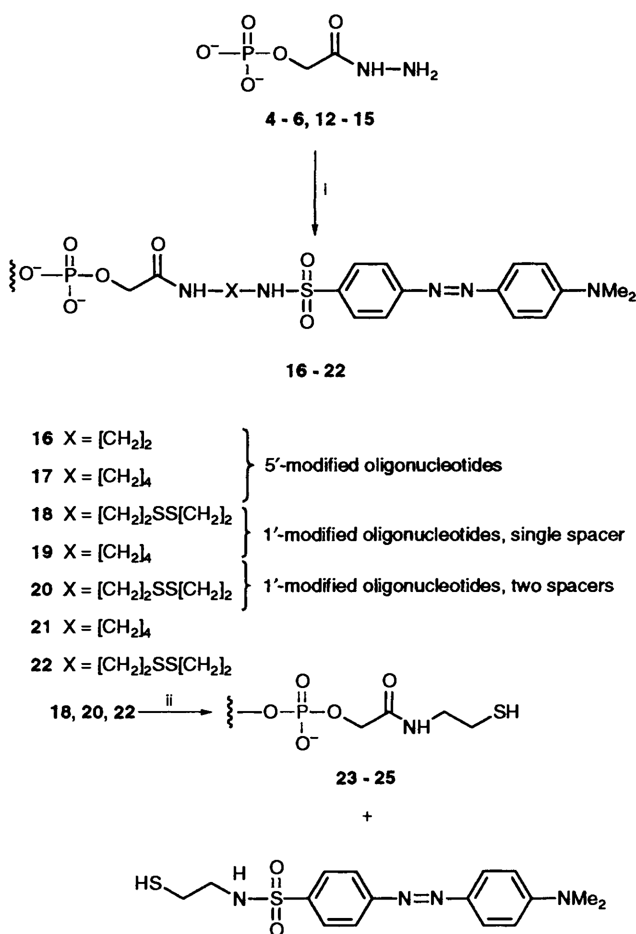
used without purification. 2-Cyanoethyl *N,N,N',N'*-tetraisopropylphosphorodiamidite was synthesized according to the published procedure.[31] NMR spectra were recorded on a JEOL GX-400 spectrometer operating at 399.8 and 161.9 MHz for ^1H and ^{31}P, respectively. $SiMe_4$ was used as internal (^1H) and H_3PO_4 as external (^{31}P) reference. *J* Values are given in Hz. IR spectra were recorded for films on a Perkin-Elmer 1600 FT-IR spectrometer. M.p.s were measured on a Büchi 510 apparatus and are uncorrected.

HPLC Techniques.—HPLC analyses were carried out as described previously in detail.[24] Chromatographic conditions

Fig. 1 Ion-exchange HPLC profiles of crude oligonucleotides **6** and **7** prepared with the aid of phosphoramidite **1a** (gradient: System 1). The large peak at t_R ~ 5 min refers to non-nucleosidic material.

Fig. 2 Ion-exchange HPLC profiles of crude oligonucleotides **13** and **15** prepared with the aid of phosphoramidite **1b** (gradient: System 2). The large peaks at t_R ~ 5 min refer to non-nucleosidic material.

in the present work were: System 1: ion exchange (SynChropak AX-300, 4.6 × 250 mm, 6 µm), flow rate 1 cm^3 min^{-1}, buffer A = 0.05 mol dm^{-3} KH_2PO_4 in 50% (v/v) aq. formamide, pH 5.60; buffer B = A + 0.6 mol dm^{-3} $(NH_4)_2SO_4$, pH 5.60; from 10 to 80% B in 30 min. System 2: as above, but from 0 to 60% B in 60 min. System 3: reversed-phase (Nucleosil 300-5C18, 4.0 × 250 mm, 5 µm) flow rate 1 cm^3 min^{-1}; buffer A = 0.05 mol dm^{-3} NH_4OAc; buffer B = A + 50% (v/v) MeCN; from 0 to 70% B in 70 min. System 4: as above, but A = 0.10 mol dm^{-3} triethylammonium acetate (TEAA), pH 6.2; B = A + 50% MeCN, pH 6.2; from 0 to 70% B in 40 min. System 5: reversed-



Scheme 4 *Reagents:* i, dabsyl chloride; ii, 1,4-dithio-DL-threitol

phase (Hypersil C-18; 4.6 × 240 mm, 6 μm) 0.05 mol dm^{-3} NH_4OAc + 2% MeCN, flow rate 1 cm^3 min^{-1}.

2-Chloroethyl Glycolate **2a**.—Thionyl dichloride (4.8 cm^3, 66 mmol) was poured cautiously into 2-chloroethanol (25 cm^3, 0.37 mol) after which glycolic acid (3.0 g, 39 mmol) was added, and the mixture was stirred overnight at ambient temperature. All volatile materials were evaporated off under reduced pressure. The residue was crystallized from a mixture of hexane and diethyl ether to give *compound* **2a** (2.20 g, 41%) as a glittering powder, m.p. 77 °C (Found: C, 34.5; H, 5.0. $C_4H_7ClO_3$ requires C, 34.7; H, 5.1%); ν_{max}/cm^{-1} 1743 (C=O) and 3436 (OH); $\delta_H(CDCl_3)$ 4.47 (2 H, t, *J* 5.9, CO_2CH_2), 4.24 (2 H, br s, $HOCH_2$), 3.72 (2 H, t, *J* 5.9, CH_2Cl) and 2.44 (1 H, br, OH).

2,2,2-Trifluoroethyl Glycolate **2b**.—Thionyl dichloride (0.80 cm^3, 11.0 mmol) was mixed with 2,2,2-trifluoroethanol (25 cm^3, 0.28 mol). Glycolic acid (0.55 g, 7.40 mmol) was added, and the mixture was stirred overnight at ambient temperature. All volatile materials were evaporated off under reduced pressure. The remaining solid was sublimed to give *compound* **2b** (0.40 g, 34%) as hygroscopic needles, m.p. 42 °C (Found: C, 30.4; H, 3.1. $C_4H_5F_3O_3$ requires C, 30.4; H, 3.2%); ν_{max}/cm^{-1} 1766 (C=O) and 3392 (OH); $\delta_H(CDCl_3)$ 4.58 (2 H, q, $J_{H,F}$ 8.3, CH_2CF_3), 4.31 (2 H, d, *J* 4.9, $HOCH_2$) and 2.27 (1 H, br t, *J* 4.9, OH).

Synthesis of Phosphoramidites **1a**, **b**.—Predried chloroethyl glycolate **2a** (2.0 mmol, 276 mg) or **2b** (2.0 mmol, 316 mg) and 2-cyanoethyl *N,N,N',N'*-tetraisopropylphosphorodiamidite (3.0 mmol, 904 mg) were dissolved in dry acetonitrile (1.0 cm^3). Tetrazole (4.5 cm^3, 0.45 mol dm^{-3} solution in acetonitrile) was added and the reaction mixture was shaken for 30 min. The mixture was poured into 5% aq. $NaHCO_3$ (100 cm^3), and extracted with methylene dichloride (2 × 50 cm^3), and the extract was dried over Na_2SO_4 and concentrated to an oil. Cold hexane (−80 °C) was added to the residue, and the mixture was shaken vigorously. After decantation of the hexane layer, the phosphoramidites **1a**, **b** were obtained as oils. For compound **1a**, $\delta_P(CDCl_3)$ 150.8; for **1b**, $\delta_P(CDCl_3)$ 151.3.

Determination of Coupling Efficiency.—The modified phosphoramidite **1a**, **b** (0.1 mol dm^{-3} in acetonitrile) was coupled (20 s) to the thymidine-derivatized solid support on an Applied Biosystems 392 DNA Synthesizer (ABI). After oxidation with iodine the solid-support-bound material was treated with conc. aq. ammonia for 4 h at room temperature. The reaction mixture was concentrated, and the product distribution was determined by HPLC (System 5). The coupling yield was calculated from the ratio of the integral areas of thymidine and thymidine-5'-carbamoylmethyl phosphate (~97%). The extinction coefficient of the 5'-derivative was assumed to be the same as for thymidine.

Preparation of 5'-Carboxymethylphosphate-derivatized Oligonucleotide 7.—The protected oligonucleotide d(TCCGTG-GAGTCGTG) was synthesized on a 0.20 μmol scale on the ABI instrument using phosphoramidite chemistry and recommended protocols. The modified phosphoramidite **1a**, **b** was attached to its 5'-end at the last coupling step. After the chain assembly was complete, the oligonucleotide **3a**, **b** was treated with aq. NaOH (0.1 mol dm^{-3}; 200 mm^3) for 4 h at room temperature. The solution was diluted with conc. aq. ammonia (2 cm^3) and kept for 7 h at 55 °C before being concentrated to half volume and the pH adjusted to 7 with acetic acid. After evaporation to dryness, the product was purified on HPLC (Systems 1 and 3) and desalted by gel filtration.[24]

Introduction of the Alkylamine and Cystamine Spacer Arms to the 1'- and 5'-Position of Oligonucleotides.—Synthesis of compounds **3a**, **b** is described above. Oligonucleotides bearing psicothymidine units in their structure were prepared, and their 1'-*O*-protecting groups were cleaved as described previously.[25,26] Coupling of compound **1b** to the 1'-*O*-deprotected material was done by using standard protocols to give products **10** and **11**. Introduction of the spacer arms was performed by keeping esters **3a**, **b** or **10**, **11** in aq. diamine (*i.e.*, ethane-1,2-diamine, butane-1,4-diamine or cystamine; 200 mm^3) for an appropriate time period (for reaction times and concentrations, see Table 1), after which the mixture was ammonolysed as described above. When the deprotection was complete, the solution was concentrated under reduced pressure. The excess of ethane-1,2-diamine was removable by co-evaporations with water, and butane-1,4-diamine and cystamine were neutralized with acetic acid. Purification was performed by HPLC [System 1 (**4–6**), 2 (**12–15**) and 3]. Salts were removed by gel filtration.[24]

Labelling of Amino Groups with Dabsyl Chloride.—Oligonucleotides with ω-amino spacer arms, **4–6**, **12–15**, were allowed to react with 4-(dimethylamino)azobenzene-4'-sulfonyl (dabsyl) chloride by using a modification of a published procedure.[32] Accordingly, the oligonucleotide (0.5–1.0 absorbance units) was converted into the triethylammonium salt by passing it through an RP-column using a mixture of TEAA–MeCN as eluent (System 4). The solvent was evaporated off and the remaining material was dissolved in a $NaHCO_3/Na_2CO_3$ buffer (50 mm^3; 0.10 mol dm^{-3}; pH 10.3). Dabsyl chloride [0.5 mg in 50 mm^3 of freshly distilled *N,N*-dimethylformamide (DMF)] was added, and the reaction mixture was kept for 1 h (**4–6**, **12**, **13**) or 6 h (**14**, **15**) at ambient temperature. The reaction was quenched with

conc. aq. ammonia (10 mm^3), and the mixture was stored for an additional 30 min before being diluted with aq. ammonium acetate (50 mm^3; 0.05 mol dm^{-3}) and extracted with ethyl acetate (4 × 200 mm^3). The pH of the aqueous layer was adjusted to 7 with acetic acid and centrifuged. The oligonucleotides bearing a single label (**16–20**) were purified by reversed-phase HPLC (System 3), and those oligonucleotides with two labels (**21, 22**) first by ion-exchange HPLC* (System 2, t_R **21** 44.1 min, t_R **22** 46.2 min) and then by reversed-phase HPLC (System 3). Their reversed-phase HPLC retention times and UV–VIS spectral properties are listed in Table 3. As a control reaction, the unmodified sequence d(TCCGTGGAGTCGTG) was treated (6 h) with dabsyl chloride as described above. HPLC analyses (Systems 2 and 3) of the reaction mixture showed the presence of a single oligonucleotide peak co-eluting with the starting material.

Acknowledgements

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* Oligonucleotides **21** and **22** co-eluted with the unchanged dye on the reversed-phase column.

References

1 N. Sinha and S. Striepeke in *Oligonucleotides and Analogues: A Practical Approach*, ed. F. Eckstein, IRL Press, Oxford, 1991, pp. 185–210.
2 N. T. Thuong and C. Hélène, *Angew. Chem., Int. Ed. Engl.*, 1993, **32**, 666.
3 B. A. Connolly, *Nucleic Acids Res.*, 1985, **13**, 4485.
4 S. Agrawal, C. Christodoulou and M. J. Gait, *Nucleic Acids Res.*, 1986, **14**, 6227.
5 J. M. Coull, H. L. Weith and R. Bischoff, *Tetrahedron Lett.*, 1986, **27**, 3991.
6 J. N. Kremsky, J. L. Wooters, J. P. Dougherty, R. E. Meyers, M. Collins and E. L. Brown, *Nucleic Acids Res.*, 1987, **15**, 2891.
7 B. A. Connolly, *Nucleic Acids Res.*, 1987, **15**, 3131.
8 R. Bischoff, J. M. Coull and F. E. Regnier, *Anal. Biochem.*, 1987, **164**, 336.
9 P. S. Nelson, R. Sherman-Gold and R. Leon, *Nucleic Acids Res.*, 1989, **17**, 7179.
10 For additional references, see: S. L. Beaucage and R. P. Iyer, *Tetrahedron*, 1993, **49**, 1925; J. Goodchild, *Bioconjugate Chem.*, 1990, **1**, 165.
11 N. D. Sinha and R. M. Cook, *Nucleic Acids Res.*, 1988, **16**, 2659.
12 E. Uhlman and J. Engels, *Tetrahedron Lett.*, 1986, **27**, 1023.
13 T. Horn and M. S. Urdea, *DNA*, 1986, **5**, 421.
14 M. Bower, M. F. Summers, B. Kell, J. Hoskins, G. Zon and W. D. Wilson, *Nucleic Acids Res.*, 1987, **15**, 3531.
15 W. Bannwarth and E. Küng, *Tetrahedron Lett.*, 1989, **30**, 4219.
16 J. E. Celebuski, C. Chan and R. A. Jones, *J. Org. Chem.*, 1992, **57**, 5535.
17 A. Chollet and E. Kawashima, *Nucleic Acids Res.*, 1985, **13**, 1529.
18 J. Teare and P. Wollenzien, *Nucleic Acids Res.*, 1989, **17**, 3359.
19 W. L. Sung, *Nucleic Acids Res.*, 1981, **9**, 6139.
20 S. Le Brun, N. Duchange, A. Namane, M. M. Zakin, T. Huynh-Dinh and J. Igolen, *Biochimie*, 1989, **71**, 319.
21 A. M. MacMillan and G. L. Verdine, *Tetrahedron*, 1991, **47**, 2603.
22 W. T. Markiewicz, G. Gröger, R. Rösch, A. Zebrowska and H. Seliger, *Nucleosides, Nucleotides*, 1992, **11**, 1703.
23 B. D. Gildea, J. M. Coull and H. Köster, *Tetrahedron Lett.*, 1991, **32**, 7095.
24 J. Hovinen, A. Guzaev, A. Azhayev and H. Lönnberg, *Tetrahedron*, 1994, **50**, 7203.
25 A. Azhayev, A. Guzaev, J. Hovinen, E. Azhayeva and H. Lönnberg, *Tetrahedron Lett.*, 1993, **34**, 6435.
26 A. Guzaev, E. Azhayeva, J. Hovinen, A. Azhayev and H. Lönnberg, *Bioconjugate Chem.*, in the press.
27 R. K. Gaur, *Nucleosides, Nucleotides*, 1991, **10**, 895.
28 J. S. Mann, Y. Shibata and T. Meehan, *Bioconjugate Chem.*, 1992, **3**, 554.
29 R. Zuckermann, D. Corey and P. Schultz, *Nucleic Acids Res.*, 1987, **15**, 5305.
30 J. Fidanza and L. W. McLaughlin, *J. Org. Chem.*, 1992, **57**, 2340.
31 J. Nielsen and O. Dahl, *Nucleic Acids Res.*, 1987, **15**, 3626.
32 J. L. Ruth, in ref. 1, p. 269.

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