Short Communication

A Highly Reactive, Odourless Substitute for Thiophenol/ Triethylamine as a Deprotection Reagent in the Synthesis of Oligonucleotides and their Analogues

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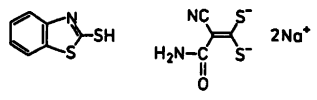
During the solid-phase synthesis of oligonucleotides by phosphite or phosphoramidite methods, the phosphate groups are protected by alkyl groups. These must be selectively removed before the final treatment with hot, aqueous ammonia which removes the base-protecting groups.[1] The methyl group is widely used for this purpose; it can be selectively removed by a mixture of thiophenol and triethylamine.[2] The latter reagent mixture, however, is easily oxidized in air and has an obnoxious smell, so many researchers prefer to use the 2-cyanoethyl protecting group instead; this is easily removed with aqueous ammonia.[3] The higher reactivity of methyl compared with 2-cyanoethyl phosphoramidites,[4] which is important for, e.g., oligoribonucleotide synthesis,[5] and the use of methyl or substituted benzyl protecting groups in the preparation of modified oligonucleotides, e.g., nucleoside phosphorodithioates,[6,7] makes it highly desirable to have an alternative reagent to thiophenol/triethylamine without its unpleasant properties.

Andrus and Beaucage have recently proposed a mixture of ethyldiisopropylamine and 2-mercaptobenzothiazole (**1**) for this purpose.[8] The reagent **1** is odourless and



1 **2**

demethylates methyl phosphates cleanly, but the rate is ca. ten times lower than that of thiophenol. We report here that the easily prepared compound disodium 2-carbamoyl-2-cyanoethylene-1,1-dithiolate (**2**) is stable, odourless and only weakly basic (pK_B 8.4), yet removes methyl or 2,4-dichlorobenzyl groups from phosphates or dithiophosphates considerably faster than thiophenol/triethylamine.

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Experimental

The reagent **2** was made from commercially available cyanoacetamide, carbon disulfide and aqueous sodium hydroxide.[9] Compound **2** was isolated as the yellow, crystalline trihydrate from 80 % aqueous MeOH by precipitation with EtOH. It is soluble up to at least 2 M in polar solvents such as water, methanol and *N*,*N*-dimethylformamide (DMF), and the solutions are stable in contact with air for at least one week. Compounds **3**[10] and **4**[11] were prepared by published methods, **5** (δ_P 93.5, 93.3 ppm in $CDCl_3$) and **6** (δ_P 71.8, 71.2 ppm in DMF) from $MeSP(S)Cl_2$ and 5′-DMTdT, followed by 3′-acetyl dT or H_2O, respectively.[12]

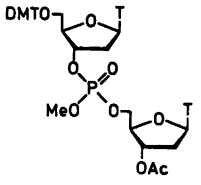
The dealkylation reactions were monitored by ^{31}P NMR spectroscopy on a JEOL FX90Q spectrometer at 27–28 °C in 5 mm tubes. A DMF solution of **3** or **4** (0.4 M, 0.2 ml) was mixed with a DMF solution of **2** or PhSH/Et_3N 1:1 mol/mol (0.4 M, 0.2 ml) at zero time, and ^1H-decoupled ^{31}P NMR spectra were obtained at 1–5 min intervals. A nitrogen blanket and degassed solutions were necessary when PhSH/Et_3N was used in order to minimize oxidation of PhS^-. The concentrations were calculated from the integral values, and second-order rate constants k_2 calculated from the equation $k_2T = (A - A_0)/AA_0$, where A = [**2**] or [PhSH]. Half-lives under pseudo-first-order conditions (1 M in **2** or PhSH/Et_3N) were calculated from the equation $t_{1/2} = \ln 2/k_2$. Experiments under pseudo-first-order conditions were performed similarly by mixing DMF solutions of **4** or **5** (0.2 M, 0.2 ml) with solutions of **2** or PhSH/Et_3N (2.0 M, 0.2 ml).

A series of oligodeoxyribonucleotides (16–28-mers) were synthesized in 0.2 μmol scale on a Biosearch 8750 synthesizer, using protected nucleoside methyl *N*,*N*-diisopropylphosphoramidites (Applied Biosystems) and a standard coupling cycle. Demethylation was performed by suspending the support in a solution of **2** (ca. 1.7 M, prepared

from 20 mmol of **2** in 10 ml of DMF) for 10 min at 22 °C; this was followed by standard deacylation with 32 % aqueous ammonia at 55 °C for 12–16 h. Degradation with snake venom phosphodiesterase and alkaline phosphatase (Pharmacia) was performed as described.[13] HPLC analysis of the degraded products was performed on a Kontron gradient HPLC system using a MinoRPC S 5/20 column (Pharmacia) and linear gradients of 0 % B for 30 min, 0–20 % B for 30–65 min, with solvent A: 0.1 M aqueous NH_4Ac, 1 % CH_3CN, pH 5.5, solvent B: 0.1 M aqueous NH_4Ac, 80 % CH_3CN, pH 5.5.

Results and discussion

Dealkylation of dinucleoside phosphortriesters **3** and **4** were monitored by ^{31}P NMR spectroscopy in DMF so-



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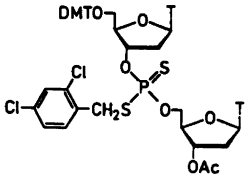

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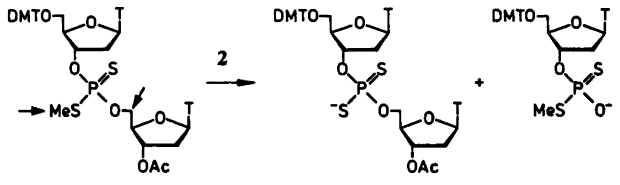
lutions at 27–28 °C.[14] The reactions were generally too fast to obtain precise $t_{1/2}$ values for 1–2 M solutions of **2** under the usual pseudo-first-order conditions. Instead, second-order rate constants were calculated from ^{31}P NMR data on 0.2 M solutions. From these rate constants $t_{1/2}$ values were calculated for 1 M solutions of **2** or PhSH/Et_3N. The results are given in Table 1.

The data in Table 1 show that **3** or **4** is dealkylated 3–5 times faster with **2** than with PhSH/Et_3N at 0.2 M concentrations in DMF. The difference at higher concentrations of dealkylating reagent however may be less, as indicated by the two estimated $t_{1/2}$ values at 1 M (0.7 versus 1.1 min). The data further shows that the dealkylation of **4** in DMF is more difficult than that of **3**. Earlier data indicated similar dealkylation rates with PhSH/Et_3N/dioxane 1:1:2 v/v ($t_{1/2}$ 3–4 min for **3**,[15] 3 min for **4**[6]). Taken together the results show that **2** in DMF dealkylates **3** or **4** 10–60 times faster than PhSH/Et_3N in dioxane at similar concentrations (1 M).



5 **6**

Scheme 1.

We have also examined the dealkylation rate of **5** with a 1.7 M solution of **2** (Scheme 1) in DMF and found a $t_{1/2}$ value of ca. 40 min at 27 °C. However, in contrast with **3** and **4**, where no by-products were observed during the dealkylation reactions (^{31}P NMR spectroscopy and TLC), **5** gave, besides the dithioate, ca. 35 % of a by-product (δ_P 71.8 and 71.2 ppm in DMF), identified as **6**. The formation of **6** is ascribed to attack by **2** at the 5′-carbon because the *S*-methyl group is only slowly removed. A similar by-product has been described during demethylation of a dinucleoside *S*-methyl phosphorothioate with PhSH/Et_3N/dioxane.[16] These results show that a methyl group is not a suitable *S*-protecting group for the preparation of either nucleoside phosphorothioates[16] or phosphorodithioates.[17]

Having established the usefulness of **2** as a dealkylating agent for dimers in solution, the reagent was tested in solid-support syntheses of oligonucleotides prepared from methoxy phosphoramidites. The crude oligonucleotides were demethylated on the support by treatment with a solution of **2** (ca. 1.7 M) for 10 min at ca. 25 °C. The products, after normal deblocking with conc. aqueous ammonia and EtOH precipitation, had purities indistinguishable (FPLC, PAGE) from those of products prepared from 2-cyanoethyl phosphoramidites, and the yields were similar. After degradation with snake venom phosphodiesterase and alkaline phosphatase HPLC analysis showed that no modified bases were present (detection limit 0.1 %).

In conclusion, we have shown that **2** is an attractive alternative reagent to PhSH/Et_3N for deprotection of nucleoside methyl phosphortriesters because it is odourless, stable, and efficient. The high dealkylation rates makes **2** useful also for dealkylation of modified phosphortriesters which are difficult to dealkylate, e.g. alkyl phosphorodithioates.

Table 1. Dealkylation rates of dinucleoside phosphortriesters **3** or **4** with **2** or PhSH/Et_3N in DMF, 27–28 °C.

Reagent	Solvent	k_2/M^{-1} s^{-1} [a]		$t_{1/2}$/min [b]	
		3	**4**	**3**	**4**
2	DMF	0.09	0.020	0.1	0.6 (0.7)[c]
PhSH/Et_3N	DMF	0.029	0.0042	0.4	2.7 (1.1)[c]
PhSH/Et_3N	Dioxane			6–8 [d]	6 [d]

[a]Second-order rate constant, determined on equimolar 0.2 M solutions. [b]Calculated from k_2 under pseudo-first-order conditions, 1 M in **2** or PhSH/Et_3N. [c]Estimated for reactions 1 M in **2** or PhSH/Et_3N, 0.1 M in **4**. [d]Estimated as twice the values at ca. 2 M conc.[6,15]

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