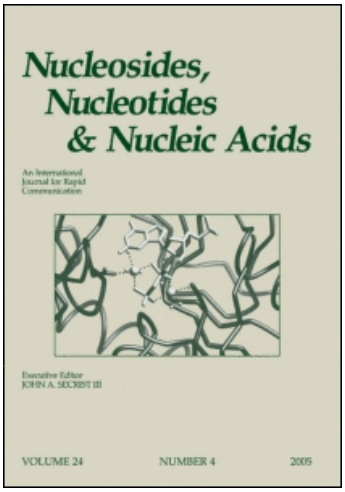

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A NOVEL PROTECTING STRATEGY FOR INTERNUCLEOSIDIC PHOSPHATE AND PHOSPHOROTHIOATE GROUPS

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A NOVEL PROTECTING STRATEGY FOR INTERNUCLEOSIDIC PHOSPHATE AND PHOSPHOROTHIOATE GROUPS

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ABSTRACT

The utility of 2–(*N*–isopropyl–*N*–anisoylamino)ethyl group for protection of internucleosidic phosphate linkages in oligonucleotide synthesis was studied. The group demonstrated high coupling yields, favorable deprotection kinetics and a high hydrolytic stability of phosphoramidite building blocks. The mechanism of deprotection was established using a model phosphate triester.

Current oligonucleotide synthesis employs a phosphoramidite method and is carried out automatically by stepwise coupling of nucleoside building blocks to a solid support. This allows an efficient preparation of natural and modified DNA fragments on a routine basis. Phosphoramidite building blocks are most often protected with a 2–cyanoethyl group at the phosphite moiety (1). Treatment of the oligonucleotide with ammonia effects β–elimination in the 2–cyanoethyl group releasing internucleosidic phosphodiester moieties and acrylonitrile as a side product. Recently, acrylonitrile has been demonstrated to alkylate nucleic bases under conditions of deprotection (2). Increasing interest in manufacturing oligonucleotides as drugs motivated our search for alternate protecting groups, whose removal is governed by mechanisms different from β–elimination.

We reported recently the use of 2–[(1–naphthyl)carbamoyloxy]ethyl group for protection of internucleosidic phosphates (3). Here we report the use of a novel 2–(*N*–isopropyl–*N*–anisoylamino)ethyl protection in the oligonucleotide synthesis.

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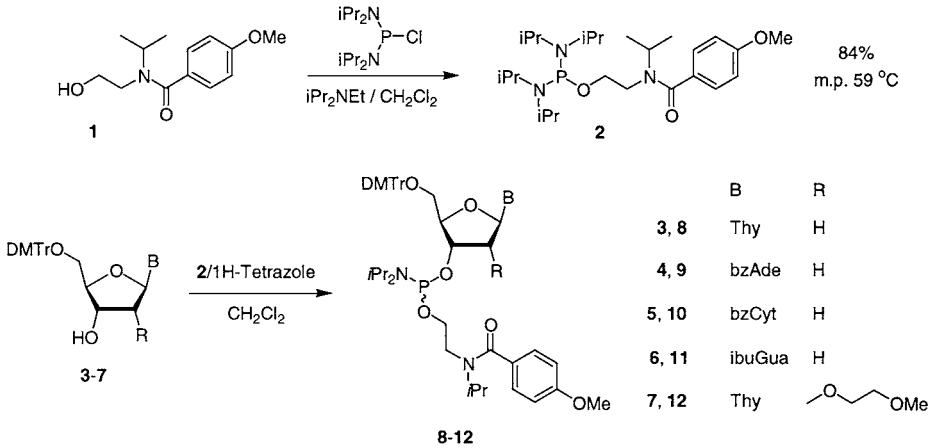

Figure 1. Synthesis of phosphoramidite building blocks **8**–**12**.

For the preparation of novel phosphoramidite building blocks,**1** was converted to a bisamidite **2**, which was isolated as a crystalline compound on silica gel. Nucleosides **3**–**7** were treated with **2** in the presence of 1*H*–tetrazole to give **8**–**12** (Fig. 1). These were isolated by column chromatography in 75–89% yield and characterized. The phosphoramidites **8**–**12** were stable compounds whose shelf life exceeded 9 months. Moreover, as measured by ^{31}P NMR, the half-life of disappearance of **8** in 95% aqueous MeCN was 73 h, which indicated its high hydrolytic stability.

Next, the removal of the proposed protecting group (PG) was studied. Model compounds, **13**–**15**, were synthesized either on a solid support (**13** and **14**) or in solution (15, Fig. 2). Half-lives of deprotection of **13** and **14** under various conditions were measured by a gel phase ^{31}P NMR. In phosphite triester **13**, the PG was indefinitely stable in MeCN. In contrast, it was rapidly removed from phosphate triester **14** ($\tau_{1/2} = 50$ min in MeCN). In a similar manner, a model T_{15} phosphorothioate was assembled on a polystyrene solid support (20 μmol) using phosphoramidite **8**. As revealed by ^{31}P NMR in gel phase, the PG was removed to *ca*. 90% extent when the synthesis of the oligonucleotide was complete.

Apparently, the extent of deprotection of the oligonucleotide attached to a solid support should depend on the duration of the synthesis and hence on the synthetic

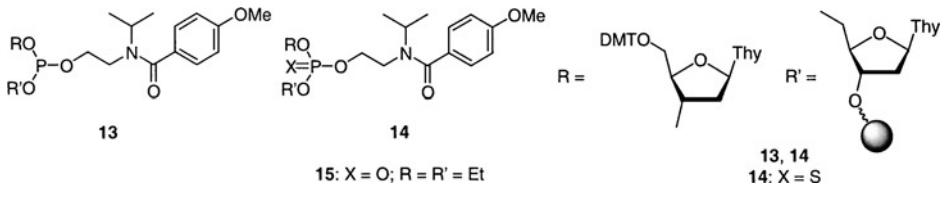

Figure 2a. Structures of model compounds **13**–**15**.

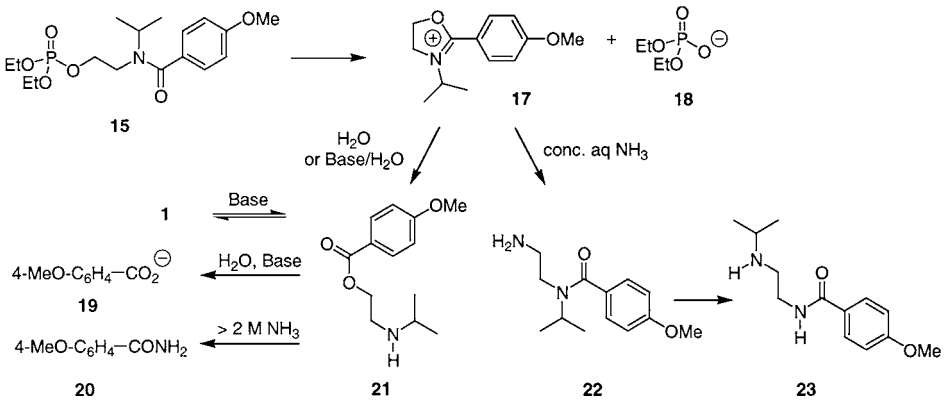

Figure 2b. The fate of **15** under neutral and basic aqueous conditions.

scale employed. On a small scale, the synthesized oligonucleotide remains largely protected. In this case, the remaining protecting groups are removed at the final deprotection step. In order to illustrate the deprotection mechanism under neutral and basic aqueous conditions, the fate of **15** was next studied (Fig. 2). The products, **1** and **19–23**, formed from **15** do not react with nucleic bases. To confirm this, mixtures of **15** (10 equiv) and each of the 5′–DMTr protected nucleosides (dA, dC, dG, and T; 1 equiv) were treated with ammonium hydroxide. As demonstrated by HPLC, each of the nucleosides remain unchanged for at least 48 h at 55°C.

It is known that nucleoside phosphoramidites efficiently and selectively couple at the 5′–position of solid support-bound oligonucleotides with deprotected backbone (4). Phosphoramidites **8–12** were used for preparation of oligodeoxynucleotides and their phosphorothioate and 2′–*O*–(2–methoxyethyl) analogs, **24–30**, on 1 to 150 μmol scale (Table). In agreement with previous observations (7), **8–12** were capable of an efficient chain elongation. Analysis of the deprotection mixtures revealed no apparent modifications of nucleic bases or oligonucleotide backbone. The yields of isolated oligonucleotides were similar to those obtained with the standard 2–cyanoethyl phosphoramidites.

Table 1. Oligonucleotides **24–30** Synthesized with the Aid of **8–12**

Compound	Sequence (5′ → 3′)	Backbone	Target
24	GCC CAA GCT GGC ATC CGT CA	P=O	Human ICAM–1
25	TCC CGC CTG TGA CAT GCA TT	P=O	Human C–*raf*
26	GCC CAA GCT GGC ATC CGT CA	P=S	Human ICAM–1
27	TCC CGC CTG TGA CAT GCA TT	P=S	Human C–*raf*
28	ATG CAT TCT GCC CCC AAG GA	P=S	Mouse C–*raf*
29	AGC TTC TTT GCA CAT GTA AA	P=S	Human MDM–2
30	$(T^*T^*T^*)_6$ T^*T^*[a]	P=S	–

a. T* stands for 2′–*O*–(2–methoxyethyl)–5–methyluridine residue.

In conclusion, the 2–(*N*–isopropyl–*N*–anisoylamino)ethyl is a novel, convenient phosphodiester protecting group. It is quantitatively removed either on solid support by treatment with a polar organic solvent or under standard deprotection conditions.

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