

# Protection of 5'-Hydroxy Functions of Nucleosides

The 5'-OH group is the primary hydroxy group of nucleosides. It is the least influenced by the electron-withdrawing effects of the other substituents on the sugar moiety. Moreover, it is the least sterically hindered hydroxy function, and shows the highest reactivity of all nucleoside hydroxy groups in nucleophilic substitutions. Although nucleobases can eventually be left unprotected, and nucleosides with free 3'-hydroxy groups have been used in some triester syntheses, it is mandatory to protect 5'-hydroxyls in all methods of oligonucleotide synthesis that require nucleoside synthons.

For chemical oligonucleotide synthesis, the blocking groups for the 5'-hydroxy function must be integrated into an orthogonal protection system. Several such systems have been proposed and are in use for deoxyribo- and ribooligonucleotide synthesis as well as for the preparation of structurally modified oligonucleotide analogs. These protection schemes, details of which are described elsewhere (e.g., *UNITS 2.1-2.5* and *3.1-3.4*), can be distinguished by requiring, in principle, three alternative methods for 5'-deprotection: (1) acid conditions, (2) alkaline or ammoniacal conditions, or (3) selective deblocking reagents applied essentially in the absence of acid or base. Protecting groups for 5'-hydroxy functions can be broadly classified into these three categories, and the subsequent sections of this unit will follow this division.

Additional criteria governing the choice of 5'-hydroxyl-protecting groups include (1) the direction of chain lengthening, (2) the use of polymer supports and/or other purification handles, and (3) additional features related to molecular instability or chemical reactivity in the case of oligonucleotides deviating from biological structure.

In particular, the direction of chain extension determines whether a 5'-hydroxyl-protecting group will be permanent (i.e., remain attached to the growing oligonucleotide chain) or intermediary (i.e., will have to be removed prior to each chain extension). If lengthening occurs from the 5' to the 3' end, a permanent protecting group is used. If chain extension is done from the 3' to the 5' end, which is currently most common, the 5'-hydroxy function must be substituted by an intermediary protecting group. In polymer support synthesis, the poly-

meric carrier assumes the role of a permanent protecting group and is now usually attached to the 3'-hydroxy end. However, there are a number of mostly earlier publications that describe carrier fixation through the 5' end (see *Miscellaneous Acid-Labile 5'-Substituents* and see *5'-Hydroxyl-Protecting Groups Cleaved Under Nonacidic and Nonalkaline Conditions*).

In polymer support synthesis and in some solution methods, it is desirable to simplify the workup of the crude product obtained after oligonucleotide chain extensions. A variety of 5'-hydroxyl-protecting groups have been designed that serve as "purification handles" for this purpose (see *Triaryl-methyl Groups as Affinity Ligands*). The underlying idea is to single out the product chain from a complex admixture of truncated and failure sequences, although this may still be an unattainable goal. Nevertheless, such "handle" methods may significantly reduce the time and effort for oligonucleotide purification, especially on a preparative scale.

A final point in these introductory remarks is that there is no protecting group exclusively in use for the 5'-hydroxy function. Only the conditions of the reaction determine whether the same group will serve for the protection of the 5', the 3', or the 2' hydroxyl, or even of functional groups at nucleobases, because there are only subtle differences in the reactivity of all of these functions. To master these subtle differences is an art of regioselective substitution and comprises much of the challenge of oligonucleotide synthesis. Additionally, there are differences in the approach used for deoxyribo- versus ribooligonucleotide synthesis, and these will be discussed in the subsequent sections.

## SCOPE OF THIS OVERVIEW

This unit will deal with substituents for the 5'-hydroxy function that fulfill the following criteria of protecting groups. (1) The substituents can be affixed to/removed from the 5'-hydroxy function of a growing oligonucleotide chain before/after chain elongation or in the context of other reactions of the oligonucleotide chain. (2) The substituents remain bound during chain elongation and do not interfere with other oligonucleotide reactions. Substituents that serve to permanently modify

the 5' position are not in the scope of this unit. This includes chemical modifications of the 5'-hydroxy function as well as the substitution of the 5'-hydroxy function by linkers, labels, spacers, and other groups that are meant to remain an integral part of the completed oligonucleotide chain (e.g., UNITS 4.2 & 4.3). Also, protecting groups used specifically for the preparation of certain oligonucleotide analogs will not be treated here, as such syntheses are discussed in further units (e.g., see Chapter 4).

Protected nucleoside-5'-phosphates have played a role especially in the early days of oligonucleotide synthesis. Essentially, a 5'-phosphate residue can be used for protection of the terminus, because enzymatic hydrolysis can easily revert to a 5' hydroxyl. However, only those cases when this was the declared reason for 5'-phosphorylation will be mentioned in the following text (see examples given in Fig. 2.3.10).

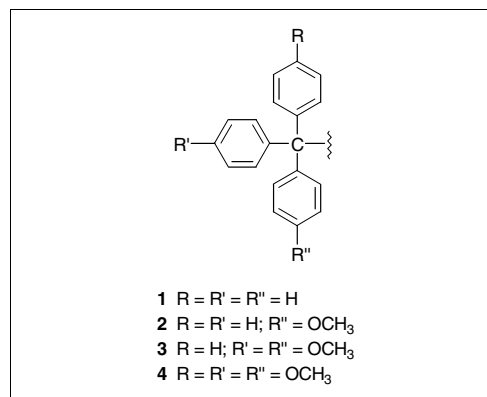
The topic of 5'-hydroxyl protection is essential to oligonucleotide synthesis and, therefore, is generally included in all textbooks on preparative nucleotide chemistry, as well as in books and articles dealing specifically with the field of protective groups. A few recent reviews have dealt more extensively with groups for protection of 5' hydroxyls, including articles by Sonveaux (1986) and Beaucage and Iyer (1992, 1993). More extensive reviews in the earlier literature are given by Kössel and Seliger (1975) and Reese (1978).

## ACID-LABILE PROTECTING GROUPS

### Triarylmethyl and Related Substituents

#### *Introduction of trityl and substituted trityl groups*

The triphenylmethyl (trityl or Tr) group (S.1; Fig. 2.3.1), a well-known sugar protecting group, was first used for nucleoside 5'-protection in Lord Todd's laboratory (Andersen et al., 1954) during hydrogenolytic removal. However, this work had limited success. The breakthrough came from H.G. Khorana's group, who discovered that detritylation in acidic medium is greatly facilitated if the trityl group is modified with one to three *p*-methoxy substituents (Gilham and Khorana, 1958; Smith et al., 1962). In 80% acetic acid, the rate of hydrolysis increased roughly 10-fold with each introduction of an additional methoxy group.



**Figure 2.3.1** Trityl and *p*-methoxy-substituted trityl protecting groups.

Since their introduction as acid-labile protecting groups for ribo- (Smith et al., 1962) and deoxyribonucleotide (Schaller et al., 1963) chemistry, the mono- and dimethoxytrityl groups (MMTr, S.2, and DMTr, S.3, respectively; Fig. 2.3.1) have become standard for the protection of the 5'-hydroxy function.

The DMTr group has especially proven its value in automated solid-phase deoxyribooligonucleotide synthesis, for five main reasons (Sonveaux, 1986). (1) It can be introduced regiospecifically and in high yield at the 5'-hydroxy function of (base-protected) nucleosides. (2) It can be readily and quantitatively removed from the growing oligonucleotide chain by nonaqueous acid. (3) It is sufficiently stable to tetrazole, which is used as an activator in the chain extension step. (4) Its deprotection in nonaqueous acid gives an intense color reaction (ascribed to a cationic species), which can be monitored by spectroscopy to estimate yields of chain elongation. (5) A terminal 5'-DMTr group conveys a certain hydrophobicity to the longest oligonucleotide chain in the crude product released from the polymer support. This hydrophobicity is often used to isolate the target oligonucleotide from the mixture of truncated and failure chains. These considerations (1 to 5) will be elaborated on further throughout this unit.

Other trityl protecting groups are less useful for automated synthesis. Unsubstituted trityl and MMTr require conditions that are too harsh for multistep removal with the complication of depurination (see below). The trimethoxytrityl group (TMTr; S.4; Fig. 2.3.1) is extremely sensitive. It can be introduced readily and in high yield at the 5'-position of deoxyribonucleosides and was found to be completely stable when stored at -20°C for ~6 months; however, it is partially removed in a mixture of tetrazole

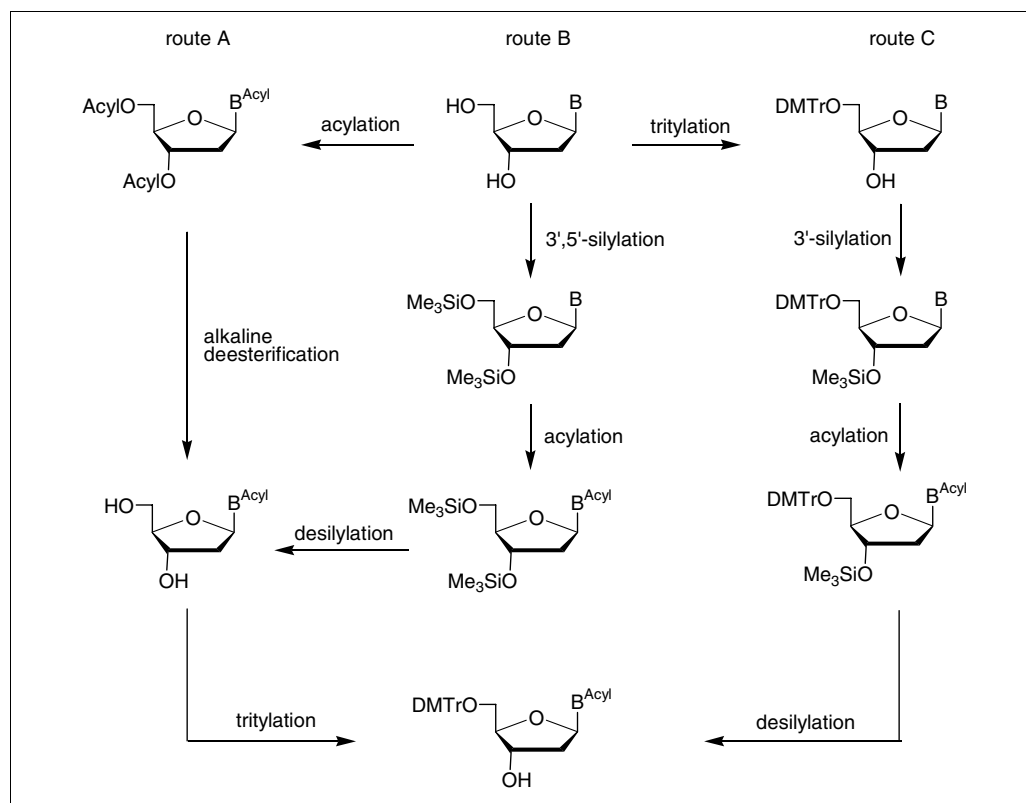
and acetonitrile (Kotschi, 1987). This detritylation occurred more readily with purine than with pyrimidine nucleosides. Also, an oligoadenylate prepared with TMTTr-deoxyadenosine contained a significant admixture of longer chains, obviously arising from multiple monomer addition.

The trityl protecting groups are generally introduced by treatment of nucleosides with the respective trityl chloride. The reactivity of these trityl chlorides increases with increasing number of *p*-methoxy substituents. If the reaction is run at room temperature and with not more than a slight excess of trityl chloride, the substitution will be highly regiospecific at the 5' hydroxyl. If the reagent is in higher excess and the temperature is raised, the substitution also occurs on the more sterically hindered 3' hydroxyl. Exocyclic amino groups are usually protected prior to tritylation, because they would otherwise react with trityl chlorides.

Three routes to base-protected, 5'-tritylated nucleosides are described in Figure 2.3.2. The classical approach (route A) from Khorana's laboratory uses per-acylation of all hydroxy and amino functions, followed by treatment with strong alkali, which selectively cleaves acyl esters and thus liberates 5' and 3' hydroxyls

for tritylation. This method is no longer in use. The more elegant route B relies on transient silyl protection to apply acyl groups regioselectively to the nucleobases in a procedure that can be carried out in a single reaction tube (Ti et al., 1982; also see "silylation first" procedure in Fritz et al., 1982). The alternative route C uses initial 5'-tritylation followed by silylation and then acylation ("tritylation first" procedure, Fritz et al., 1982; for a recent report see Wada et al., 1998a,b). In most cases, pyridine serves both to dissolve the reactants and to neutralize the ensuing hydrochloric acid.

If necessary, trityl groups can be substituted at the unprotected 5'-hydroxy function of oligonucleotide chains, either postsynthesis or in exchange for other protecting groups. This can be done by treating a support-bound oligonucleotide with 4,4'-DMTr chloride in pyridine/4-dimethylaminopyridine for 1 hr, which restores ~95% of the previously removed DMTr (Kotschi, 1987; Reddy et al., 1987). This reaction could even be performed in the presence of unprotected internucleotidic bonds due to the lability of phosphoric acid trityl esters (Reddy et al., 1987). With support-bound nucleosides, however, the reaction was more sluggish and had to be



**Figure 2.3.2** Three routes to *N*-protected, 5'-*O*-tritylated 2'-deoxyribonucleosides (modified from Fritz et al., 1982, with permission from Verlag Chemie).

activated by addition of tetra-*n*-butylammonium nitrate and 2,4,6-collidine in dimethylformamide (DMF). The retritilation procedure has recently been applied to the preparation of oligonucleotide-polyamide conjugates (Tong et al., 1993) and to the postsynthetic introduction of the 4-(17-tetra-benzo(*a,c,g,i*)fluorenylmethyl)-4',4''-dimethoxytrityl protecting group (TBF-DMTr; **S.25**; Fig. 2.3.8; Ramage and Wahl, 1993; see Triarylmethyl Groups as Affinity Ligands).

Other methods of tritilation can be applied if this is required by the sensitivity of modified nucleoside or oligonucleotide reactants. Reports have described the application of powdered molecular sieves as acid scavengers (Kohli et al., 1980), and the use of 4-*N,N*-dimethylaminopyridine in a mixture of triethylamine and DMF as an alternative solvent (Chaudhary and Hernandez, 1979). As alternatives to trityl chlorides, *N*-tritylpyridinium fluoroborate (Fersht and Jencks, 1970) and DMTr tetrafluoroborate (Lakshman and Zajc, 1996) have been described.

#### Removal of trityl and substituted trityl groups

The removal of trityl groups was initially performed with 80% aqueous acetic acid (e.g., Smith et al., 1962; Schaller et al., 1963), and such aqueous media are still in use if the deprotection is carried out in the last step of oligonucleotide workup. Nuclear magnetic resonance (NMR) studies have shown that the chemical shifts of all nucleoside protons change upon removal of 5'-trityl groups. On this basis, rate constants were determined for the hydrolysis of different methoxy-substituted trityl groups from the 5' and 3' positions of deoxythymidine in aqueous tetrahydrofuran solution brought to different pHs with HCl; a linear relationship between pH and hydrolysis rate was established (Regel et al., 1974).

If detritylations are done as intermediate steps during machine-aided polymer-support synthesis, nonaqueous acidic media are generally applied. The most common are solutions of strong protic acids, such as trichloro- or dichloroacetic acid (Adams et al., 1983; for a recent survey of detritylation conditions see Habus and Agrawal, 1994). Methylene chloride is routinely used as a solvent, although there is a tendency to avoid chlorinated hydrocarbons. For this reason, toluene has been recommended as an alternative for detritylation with dichloroacetic acid (Krotz et al., 1999; Table 2.3.1). This was the result of extensive kinetic studies that showed that the rate of detritylation decreases in the series DMTrdG<sup>*i*-Bu</sup> > DMTrdA<sup>Bz</sup> > DMTrdC<sup>Bz</sup> > DMTrdT (where Bz is benzoyl), and is highest not only in haloaliphatic but also in aromatic solvents, but is slow in DMF, hexane, ethylacetate, tetrahydrofuran, or *tert*-butylmethyl ether.

Unwanted retritilation can occur through reversal of the equilibrium generated by nonaqueous detritylation, between the colored cationic species and the DMTr-oligonucleotide (Fig. 2.3.3; Dellinger et al., 1998). The same problem was reported for the 9-phenylxanthen-9-yl group (see discussion of pixyl and related protecting groups, below; Reese et al., 1986). This is usually not a problem in solid-support oligonucleotide synthesis, since this equilibrium is shifted by washing and filtration; however, it can lead to incomplete deblocking in solution-phase synthesis.

Detritylation steps in solid-phase oligonucleotide synthesis are generally believed to be complete when appropriate treatment with nonaqueous acid and extensive washing leave resins and wash solutions colorless. Minute deviations from this scheme of quantitative deblocking, which can occur, for example, through trace impurities in the deblocking solution, are listed among the reasons for the occurrence of truncated sequences in the crude

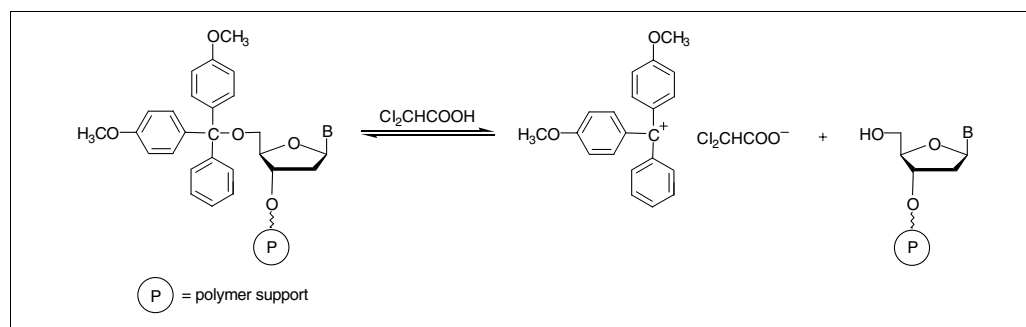


Figure 2.3.3 Detritylation equilibrium.

products released from the polymer support (e.g., Fearon et al., 1995). Whether this is a potential reason for failure cannot be decided without distinguishing between the efficiencies of the individual steps in the elongation cycle. The literature appears undecided about whether such failures occur statistically throughout all cycles (Fearon et al., 1995) or with higher probability during the initial chain elongation (Temsamani et al., 1995).

#### ***Depurination as a side reaction during detritylation***

The most stringent problem, however, is the avoidance of depurination on removal of trityl groups. N-Acylated nucleosides and N-acylated units in oligonucleotides are especially susceptible to deglycosidation. This leads to the formation of apurinic sites, with subsequent chain cleavage during ammoniacal deprotection. This side reaction becomes more and more problematic with longer oligonucleotide chains or larger-scale preparations. The incentive to overcome the depurination problem has led to the development of a wide variety of finely tuned acid-deprotection conditions; examples are given in Table 2.3.1. Although most publications list only the optimum detritylation conditions without giving background data, recent studies, stimulated by large-scale oligonu-

cleotide support synthesis, have been accompanied by extensive analyses of detritylation versus depurination kinetics (Paul and Royappa, 1996; Septak, 1996). The essence of their findings is that haloacetic acids bind strongly to immobilized growing oligonucleotide chains. If, as usual, very dilute acid solutions are applied, detritylation is slowed by depletion of acid from the medium, whereas depurination is allowed to proceed through acid saturation. The authors, therefore, recommend using a short pulse of more concentrated acid (e.g., 15% dichloroacetic acid in methylene chloride) and avoiding any acetonitrile contamination. The length of the acid treatment must be adjusted to the length of the growing oligonucleotide chain.

In some cases, ion exchange resins in the H<sup>+</sup> form may be a good choice for detritylation (Patil et al., 1994), especially to substitute for acetic acid in large-scale preparations (Iyer et al., 1995). A long treatment with silica gel was found advantageous in the preparation of sensitive nucleosides (Rosowsky et al., 1989).

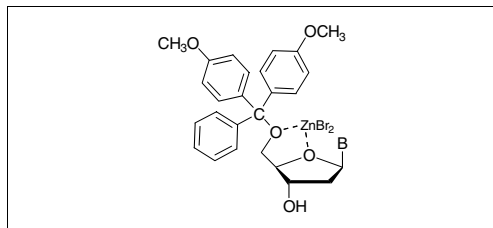
#### ***Lewis acid deprotection of trityl and substituted trityl groups and miscellaneous detritylation methods***

Great expectations to solve the depurination problem had accompanied the introduction of

**Table 2.3.1** Examples of Acidic Deprotection Conditions of 5'-Trityl Groups<sup>a</sup>

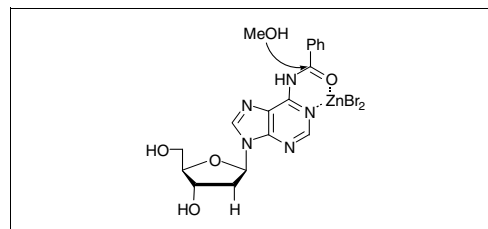
Protecting group	Deprotection conditions	Application	Reference
DMTr	Benzene sulfonic acid in 9:1 (v/v) DMF/DCM	ODN solid phase	Patel et al. (1982)
DMTr	3% (w/v) TCA in DCM	A-rich ODN solid phase	Tanaka and Oishi (1985)
DMTr	3% (w/v) TCA in 95:5 (v/v) DCM/CH <sub>3</sub> OH	Protected dA	Takaku et al. (1983)
DMTr	0.1 M <i>p</i> -toluene sulfonic acid in THF	ODN solid phase	Matteucci and Caruthers (1981)
DMTr	0.1 M <i>p</i> -toluene sulfonic acid in acetonitrile	ODN solid phase	Seliger et al. (1987); Septak (1996)
DMTr	2% (v/v) DCA/0.1% (v/v) CH <sub>3</sub> OH in DCM	ODN solid phase	Habus and Agrawal (1994)
DMTr	3% (v/v) DCA in 1,2-dichloroethane	ODN solid phase	Sproat and Gait (1984)
DMTr	3% (v/v) DCA in toluene	ODN solid phase	Krotz et al. (1999)
DMTr	3% (w/v) TCA in 1% CH <sub>3</sub> OH/nitromethane	ODN solid phase	Sinha et al. (1984)
DMTr	2% (w/v) benzene sulfonic acid in 7:3 (v/v) DCM/CH <sub>3</sub> OH	ODN solution	Gaffney et al. (1984)
DMTr	15% (v/v) DCA in DCM	ODN solid phase	Habus and Agrawal (1994)

<sup>a</sup>Abbreviations: DCA, dichloroacetic acid; DCM, dichloromethane; DMF, dimethylformamide; DMTr, dimethoxytrityl; ODN, conditions applied in oligodeoxynucleotide synthesis; TCA, trichloroacetic acid; THF, tetrahydrofuran.



**Figure 2.3.4** Proposed mechanism for the selective removal of 5'-O-DMTr groups by ZnBr<sub>2</sub> in organic solvent (according to Matteucci and Caruthers, 1980).

zinc bromide as a detritylating agent (Kohli et al., 1980; Matteucci and Caruthers, 1980). The mechanism, described for β-methoxyethoxymethyl ethers by Corey et al. (1976), involves the formation of a bidentate complex with O-C5' and intracyclic oxygen (Fig. 2.3.4). This explains the selectivity for 5'-hydroxy over 3'-hydroxy detritylation, which was found to disappear upon addition of an alcohol (Waldmeier et al., 1982). This does not play a role in oligonucleotide support synthesis, since the 3'-hydroxy function, conventionally, is anchored to a support. Here the objective is to ensure the most efficient and quantitative deblocking of immobilized growing chains. Initially, a saturated (~0.1 M) solution of zinc bromide in nitromethane was applied (Matteucci and Caruthers, 1980, 1981). Under these conditions, depurination of N<sub>6</sub>-benzoyl-deoxyadenosine was found to be insignificant over a 24-hr period. However, the formation of an unreactive chain end (presumably a zinc compound) was observed under anhydrous conditions, requiring a hydrolytic wash after detritylation. Additionally, the time required for complete deblocking was relatively long (~15 min for purine, ~30 min for pyrimidine; Matteucci and Caruthers, 1981). Therefore, it was found advantageous to add 1% water (Seliger et al., 1982; Winnacker and Dörper, 1982) or 5% methanol (Caruthers, 1982) to the nitromethane solution, which serves to increase the zinc bromide concentration. Applying zinc bromide in a number of protic solvents, Itakura and colleagues (Kierzek et al., 1981) found that a 0.7 M solution of zinc bromide in 9:1 (v/v) chloroform/methanol would lead to complete detritylation within 1 min. Depurination was not detected; however, on prolonged treatment, *N*-acyl groups were found to be removed, especially from the adenine moiety (Fig. 2.3.5). Since this was also attributed to a nucleophilic attack on a chelate



**Figure 2.3.5** Deacylation of nucleobases as a side reaction in the removal of 5'-O-trityl protecting groups with zinc bromide (Kierzek et al., 1981).

of zinc bromide with the protected base (Kierzek et al., 1981; Beaucage and Iyer, 1992), sterically hindered alcohol components were found to suppress this side reaction. A 1 M solution of zinc bromide in 85:15 (v/v) dichloromethane/isopropanol was found to be an optimal deblocking reagent (Kierzek et al., 1981; Ito et al., 1982; Itakura et al., 1984). Addition of zinc bromide to amide groups linking the oligonucleotide chains to the support was also postulated (Ito et al., 1982; Adams et al., 1983).

In spite of these extensive investigations, neither zinc bromide nor other Lewis acids—such as TiCl<sub>4</sub>, AlCl<sub>3</sub> (Matteucci and Caruthers, 1981), diethyl and diisopropyl aluminum chloride (Köster and Sinha, 1982), or boron trifluoride, applied as etherate (Engels, 1979) or methanol complex (Mitchell et al., 1990)—are of importance in current automated solid-phase deoxyribooligonucleotide synthesis. One of the reasons may be the deposition or adsorption of reagents and by-products (e.g., zinc salts) within the oligonucleotide/solid support system, resulting in the necessity for extensive and time-consuming washes.

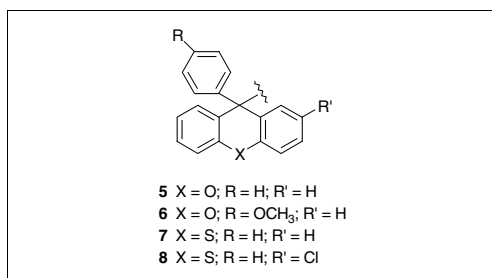
Outside the mainstream, a number of studies have reported the application of unusual detritylation reagents. Examples are formic acid (Bessodes et al., 1986), 1,1,1,3,3,3-hexafluoro-2-propanol (Leonard and Neelima, 1995), chlorine/chloroform solution (Fuentes et al., 1994), or diethyl oxomalonate/methanol solution (Sekine, 1994); some earlier reports are summarized in other reviews (e.g., Beaucage and Iyer, 1992). Other noteworthy detritylation alternatives, such as reductive cleavage with radical anion (Letsinger and Finnan, 1975) or electrochemical deblocking (Mairanovsky, 1976), may not be easily adaptable to automated oligonucleotide synthesis.

In routine solid-phase oligonucleotide synthesis, the detritylation solution usually goes to waste after yield monitoring. However, this is

not tolerable for syntheses scaled up to kilogram dimensions and beyond, because the DMTr group comprises ~35% of the total weight of constituent-protected nucleoside phosphoramidites. Recently, a process has been reported for the workup and neutralization of the detritylation solution, coupled with the re-conversion of the ensuing dimethoxytrityl alcohol (DMTrOH) to DMTr chloride. This process resulted in the recycling of 89% of the weight of DMTr residues. At the same time, >90% of the hazardous solvent dichloromethane was recovered with >95% purity (Guo et al., 1998).

### Pixyl and related protecting groups

As a structural analog to the trityl group, the 9-phenylxanthen-9-yl (pixyl or Px) protecting group (S.5, Fig. 2.3.6) has been described (Chattopadhyaya and Reese, 1978; Chattopadhyaya, 1980). This protecting group is removed by acid at approximately the same rate as the DMTr group; however, the pixyl derivatives of nucleosides can be more readily purified by crystallization. The preparation of monomeric (Christodoulou and Reese, 1983) and all sixteen dimeric (Balgobin et al., 1981b) building blocks for deoxyribooligonucleotide synthesis in solution has been done using the pixyl group, and the combination of such blocks to deoxyribooligonucleotides of biological interest has been described (Josephson and Chattopadhyaya, 1981; Balgobin and Chattopadhyaya, 1982b). A more acid-labile variant is the 9-(*p*-anisyl)xanthen-9-yl (MOX) group (S.6; Kwiatkowski et al., 1983; Kwiatkowski and Chattopadhyaya, 1984; Tanimura et al., 1988, 1989; Tanimura and Imada, 1990). Alternatively, the 9-phenylthioxanthen-9-yl (*S*-pixyl; S.7) and 9-phenyl-7-chlorothioxanthen-9-yl (S.8) groups were introduced to modulate deprotection (Balgobin and Chattopadhyaya, 1982a). The recent finding that the pixyl substituent is susceptible to photochemical cleav-



**Figure 2.3.6** 9-Phenylxanthen-9-yl and related 5'-hydroxyl-protecting groups.

age (Misetic and Boyd, 1998) may revive interest in this 5'-protecting group.

### Trityl and related groups for 5'-hydroxyl protection in oligoribonucleotide synthesis

Somewhat different considerations apply to the use of trityl and related groups for 5'-hydroxyl protection in oligoribonucleotide synthesis. Depurination is not very problematic in this case, which allowed the application of MMTr as the preferred protecting group in earlier studies focusing on the preparation of relatively short sequences by solution methods (for reviews, see Reese, 1978, 1989; Ohtsuka and Iwai, 1987). Detailed procedures for the chemical preparation of small oligoribonucleotides were described by van Boom and Wreesmann (1984). In such oligoribonucleotide syntheses where the conditions of acid deprotection are not of great concern, essentially all of the previously described trityl-derived protecting groups should be applicable in RNA synthesis. This has, in fact, been shown in a number of publications cited earlier; however, for simplicity, most protecting groups have been tested first (and often only) in DNA chemistry.

Nonetheless, modern strategies of oligoribonucleotide synthesis, in particular the preparation of long sequences on solid phase, require orthogonality of the complete set of protecting groups used for all functionalities of the RNA synthons. In particular, the intermediate protecting group for the 5' hydroxyl must blend with the groups used for 2'-hydroxyl protection. The latter must be stable throughout all chain elongations, and their removal after completion of synthesis, in the presence of unprotected phosphodiester internucleotide bonds, should not be done in an alkaline medium for risk of isomerization (e.g., Beaucage and Iyer, 1992). Therefore, 2'-hydroxyl-protecting groups should be removed in acidic solution or by a specific reagent in near-neutral medium. Hence, acid-labile protecting groups of the trityl type can be used for 5'-protection of ribonucleotide synthons only under the following conditions. (1) If an acid-labile protecting group is used for the 2' hydroxyl, there must be a significant difference in the deprotection rates for the 5' and 2' functionalities. (2) If the 2'-hydroxyl-protecting groups are stable to acid and released by a specific reagent in a close-to-neutral medium, there is free choice of acid-labile 5'-hydroxyl protection. (3) An acid-labile protecting group can be used for 2'-hydroxyl protection in combination with a substituent for

5'-hydroxyl protection that can be removed under nonacidic conditions. This alternative will be discussed below (see Blocking Groups Labile to Nonacidic Conditions).

It is beyond the scope of this unit to give credit to all publications that have described solutions to the above protection alternatives (1) and (2); these are treated in detail in recent reviews (Beaucage and Iyer, 1992, 1993). As a general guideline, it can be said that the combination of the most common acid-labile protecting groups (i.e., DMTr or pixyl for 5' hydroxyls, and tetrahydropyranyl or 4-methoxytetrahydropyranyl for 2' hydroxyls) is not fully compatible with approach (1). This can be remedied by changing the conditions or properties of the 5'-hydroxyl-protecting groups or by changing the nature of the 2'-hydroxyl-protecting group. For instance, it was found that the 5'-DMTr group could be deprotected selectively at 0°C without cleavage of 2'-tetrahydropyranyl (Seliger et al., 1983; Caruthers et al., 1986). Since this condition does not lend itself to automated support synthesis, the use of the 4,4',4''-trimethoxytrityl protecting group was investigated for the 5' hydroxyl (Seliger et al., 1986); however, the lability of this substituent caused substantial loss on chromatographic purification of the synthons.

A number of 2'-hydroxyl-protecting groups with modulated or increased acid stability have been described that are compatible with 5'-DMTr protection. Examples are the 1-(2-chloro-4-methylphenyl)-4-methoxypiperidin-4-yl (CTMP) and 1-(2-fluorophenyl)-4-methoxypiperidin-4-yl (FPMP) groups (Reese et al., 1986; Reese and Thompson, 1988), and the 1-(2-chloro-ethoxy)ethyl (Yamakage et al., 1989) and 3-methoxy-1,5-dicarbomethoxypentan-3-yl (Sandström et al., 1985) groups. However, none of these approaches is of widespread application in current solid-support oligoribonucleotide synthesis.

The approach that has currently won the competition for a DMTr-compatible system of 2'- and 5'-hydroxyl protection comes from alternative (2) above, namely the introduction of appropriate silyl groups at the 2' hydroxyl. This approach, which was mainly developed in the laboratory of Ogilvie (Usman et al., 1987, and references therein; Ogilvie et al., 1988), has been extensively investigated during recent years (for discussion, see Beaucage and Iyer, 1992) and is at present the most commonly used method for automated solid-phase oligoribonucleotide preparations.

## Triarylmethyl Groups as Affinity Ligands

Up to now, the modification of trityl protecting groups has been discussed only with respect to steering the acid lability by introduction of one, two, or three *p*-methoxy substituents. In an attempt to simplify the workup of the crude product of polymer-support synthesis, it was first shown that MMTr or DMTr groups can serve to single out the target chains by hydrophobic chromatography if they remain bound to the last monomer unit after completion of chain elongations ("trityl-on purification"; Seliger et al., 1977a, 1978). Subsequent to purification, these groups are cleaved with 80% acetic acid to generate the unprotected oligonucleotide. Such groups are referred to as "purification handles."

More recently, a number of trityl groups substituted with longer alkyl chains, ranging from C<sub>8</sub>H<sub>17</sub> to C<sub>16</sub>H<sub>33</sub> (S.9 to S.13; Table 2.3.2), have been described as lipophilic protecting groups (Görtz and Seliger, 1981) and used for separation on reversed-phase columns (Seliger and Görtz, 1981). The structures of substituted trityl groups tailored for purification assistance and other special applications are summarized in Table 2.3.2. The 4-decyloxytrityl (C<sub>10</sub>Tr or DTr) group (S.10, Fig. 2.3.7; Seliger and Schmidt, 1987) was found to be especially useful for the purification of genes and gene fragments with lengths up to 147 bases (Seliger et al., 1987; Schmidt et al., 1988). The C<sub>16</sub>Tr group (S.13) has the highest lipophilic affinity; however, the solubility of C<sub>16</sub>Tr-nucleosides in acetonitrile and other solvents common for oligonucleotide synthesis is somewhat decreased. In order to minimize the risk of depurination during terminal acid deprotection, a series of more labile 4-methoxy-4'-alkoxytrityl groups (S.14 to S.20; Table 2.3.2) was proposed for purification assistance (Gupta et al., 1991). Of these, the 4-methoxy-4'-octyloxytrityl group (S.19; MOTr; Fig. 2.3.7) was found most suitable. The 4,4'-dianisyl-2''-hexadecyloxyphenyl group (S.21; Table 2.3.2) has mainly been used for oligoribonucleotide purification (van Boom and Wreesman, 1984). The pixyl (S.5), MOX (S.6; Kwiatkowski et al., 1983; Kwiatkowski and Chattopadhyaya, 1984; Tanimura et al., 1988, 1989; Tanimura and Imada, 1990), and especially the 9-(4-octadecyloxyphenyl)xanthin-9-yl (C<sub>18</sub>Px; S.22; Fig. 2.3.7; Welch et al., 1986) groups have been used as purification handles in solution- and solid-phase oligonucleotide synthesis.



**Table 2.3.2** 5'-Hydroxyl-Protecting Groups (R<sub>1</sub>, R<sub>2</sub>, and R<sub>3</sub>) of the Substituted Triarylmethyl Type as Purification Handles or for Other Special Applications<sup>a,b</sup>

Structure	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	Abbreviation	Deprotection	Reference
S.2	Phenyl	Phenyl	<i>p</i> -Anisyl	MMTr	Acid, ZnBr <sub>2</sub> , etc.	Seliger et al. (1978)
S.3	Phenyl	<i>p</i> -Anisyl	<i>p</i> -Anisyl	DMTr	Acid, ZnBr <sub>2</sub> , etc.	Seliger et al. (1978)
S.5	Phenyl	Xanthen-9-yl	Xanthen-9-yl	Px	Acid	Chattopadhyaya and Reese (1978)
S.6	<i>p</i> -Anisyl	Xanthen-9-yl	Xanthen-9-yl	C <sub>1</sub> Px	Acid	Kwiatkowski and Chattopadhyaya (1984)
S.9	Phenyl	Phenyl	4-Octyloxyphenyl	C <sub>8</sub> Tr	Acid	Seliger and Schmidt (1987)
S.10	Phenyl	Phenyl	4-Decyloxyphenyl	C <sub>10</sub> Tr or DTr	Acid	Seliger and Schmidt (1987)
S.11	Phenyl	Phenyl	4-Dodecyloxyphenyl	C <sub>12</sub> Tr	Acid	Seliger and Schmidt (1987)
S.12	Phenyl	Phenyl	4-Tetradecyloxyphenyl	C <sub>14</sub> Tr	Acid	Seliger and Schmidt (1987)
S.13	Phenyl	Phenyl	4-Hexadecyloxyphenyl	C <sub>16</sub> Tr	Acid	Seliger and Schmidt (1987)
S.14	Phenyl	<i>p</i> -Anisyl	4-Propyloxyphenyl		Acid	Gupta et al. (1991)
S.15	Phenyl	<i>p</i> -Anisyl	4-Butyloxyphenyl		Acid	Gupta et al. (1991)
S.16	Phenyl	<i>p</i> -Anisyl	4-Pentyloxyphenyl		Acid	Gupta et al. (1991)
S.17	Phenyl	<i>p</i> -Anisyl	4-Hexyloxyphenyl		Acid	Gupta et al. (1991)
S.18	Phenyl	<i>p</i> -Anisyl	4-Heptyloxyphenyl		Acid	Gupta et al. (1991)
S.19	Phenyl	<i>p</i> -Anisyl	4-Octyloxyphenyl	MOTr	Acid	Gupta et al. (1991)
S.20	Phenyl	<i>p</i> -Anisyl	4-Dodecyloxyphenyl		Acid	Gupta et al. (1991)
S.21	<i>p</i> -Anisyl	<i>p</i> -Anisyl	2-Hexadecyloxyphenyl		Acid	van Boom and Wreesman (1984)
S.22	4-Octadecyloxyphenyl	Xanthen-9-yl	Xanthen-9-yl	C <sub>18</sub> Px	Acid	Kwiatkowski and Chattopadhyaya (1984)
S.23	<i>p</i> -Anisyl	<i>p</i> -Anisyl	Pyrenyl	BMPM	Acid	Fourrey et al. (1987)
S.24	Phenyl	Phenyl	4-(17-Tetrabenzos( <i>a,c,g,i</i> )fluorenylmethyl)phenyl	TBF-Tr	Acid	Ramage and Wahl (1993)
S.25	<i>p</i> -Anisyl	<i>p</i> -Anisyl	4-(17-Tetrabenzos( <i>a,c,g,i</i> )fluorenylmethyl)phenyl	TBF-DMTr	Acid	Ramage and Wahl (1993)
S.26	<i>p</i> -Anisyl	<i>p</i> -Anisyl	4-[(Succinimidyl- <i>N</i> -oxy)carbonyl]phenyl		Acid	Gildea et al. (1990)
S.30	Phenyl	<i>p</i> -Anisyl	1-Naphthyl		Acid, ZnBr <sub>2</sub>	Fisher and Caruthers (1983)
S.31	<i>o</i> -Anisyl	<i>o</i> -Anisyl	1-Naphthyl		Acid, ZnBr <sub>2</sub>	Fisher and Caruthers (1983)
S.32	Phenyl	<i>p</i> -Fluorophenyl	1-Naphthyl		Acid, ZnBr <sub>2</sub>	Fisher and Caruthers (1983)
S.33	Phenyl	Phenyl	<i>p</i> -Tolyl		Acid, ZnBr <sub>2</sub>	Fisher and Caruthers (1983)
S.34	Phenyl	<i>o</i> -Anisyl	<i>o</i> -Anisyl		Acid, ZnBr <sub>2</sub>	Fisher and Caruthers (1983)
S.35	<i>p</i> -Anisyl	<i>p</i> -Anisyl	3-(Imidazolyl-1-methyl)phenyl	IDTr	Acid	Sekine and Hata (1987)
S.36	<i>p</i> -Anisyl	<i>p</i> -Anisyl	3-(Imidazolyl-1-ethylcarbonyl)phenyl	IETr	Acid	Sekine et al. (1993)

**Table 2.3.2** Continued

Structure	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	Abbreviation	Deprotection	Reference
<b>S.37</b>	<i>p</i> -Anisyl	<i>p</i> -Anisyl	3-(Imidazolyl-1-propylcarbamoyl)phenyl	IPTr	Acid	Sekine et al. (1993)
<b>S.38</b>	<i>p</i> -Anisyl	<i>p</i> -Anisyl	3-(Imidazolyl-1-butylcarbamoyl)phenyl	IBTr	Acid	Sekine et al. (1993)
<b>S.39</b>	<i>p</i> -Anisyl	<i>p</i> -Anisyl	3-(Imidazolyl-1-hexylcarbamoyl)phenyl	IHTr	Acid	Sekine et al. (1993)
<b>S.40</b>	<i>p</i> -Anisyl	<i>p</i> -Anisyl	3-( <i>N</i> -Methylimidazolyl-2-ethylcarbamoyl)phenyl	IMTr	Acid	Sekine et al. (1993)
<b>S.41</b>	<i>p</i> -Benzoyloxy	<i>p</i> -Benzoyloxyphenyl	<i>p</i> -Benzoyloxyphenyl		Alkali	Sekine and Hata (1983)
<b>S.42</b>	<i>p</i> -(4,5-Dichlorophthalimido)phenyl	<i>p</i> -(4,5-Dichlorophthalimido)phenyl	<i>p</i> -(4,5-Dichlorophthalimido)phenyl	CPTr	Hydrazine in pyridine/acetic acid	Sekine and Hata (1984); Happ and Scalfi-Happ (1988)
<b>S.43</b>	<i>p</i> -(Levulinyl)oxyphenyl	<i>p</i> -(Levulinyl)oxyphenyl	<i>p</i> -(Levulinyl)oxyphenyl		Hydrazine in pyridine/acetic acid	Sekine and Hata (1985)
<b>S.44</b>	<i>p</i> -Anisyl	<i>p</i> -Anisyl	<i>p</i> -(Fluorenyl-9-methoxycarbonyl)phenyl		β-elimination	Happ and Scalfi-Happ (1988)
<b>S.45</b>	<i>p</i> -Anisyl	<i>p</i> -Anisyl	<i>p</i> -(Fluorenyl-9-methoxycarbonyl)aminophenyl		β-elimination	Happ and Scalfi-Happ (1988)

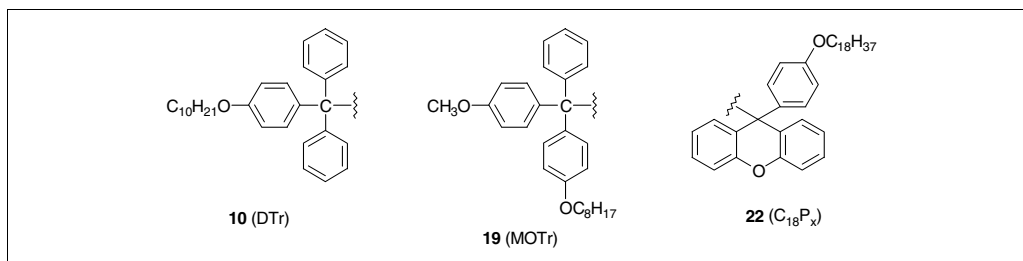
<sup>a</sup>Abbreviations: BMPM, 1,1-bis(4-methoxyphenyl)-1-pyrenylmethyl; CPTr, tris-(4,5-dichlorophthalimido)trityl; DMTr, dimethoxytrityl; DTr, 4-decyloxytrityl; IBTr, (imidazolyl-1-butylcarbamoyl)dimethoxytrityl; IDTr, (imidazolyl-1-methylcarbamoyl)dimethoxytrityl; IETr, (imidazolyl-1-ethylcarbamoyl)dimethoxytrityl; IHTr, (imidazolyl-1-hexylcarbamoyl)dimethoxytrityl; IMTr, *N*-methylimidazolyl-(*Z*-ethylcarbamoyl)dimethoxytrityl; IPTTr, (imidazolyl-1-propylcarbamoyl)dimethoxytrityl; MOTr, 4-methoxy-4'-octyloxytrityl; MMTr, monomethoxytrityl; Px, 9-phenylxanthen-9-yl (pixyl); Tr, triphenylmethyl (trityl).

<sup>b</sup>Other applications might include purification, fluorescent labeling, visible absorption, biotin substitution, color coding, 3'-phosphate activation, and nonacidic cleavage.

The 1,1-bis(4-methoxyphenyl)-1-pyrenylmethyl (BMPM) group (**S.23**; Fig. 2.3.8; Table 2.3.2; Fourrey et al., 1987) not only allows the easy purification of target sequences from the solid-phase preparation of deoxyribooligonucleotides and their methyl phosphonate analogs, but also allows their detection by thin-layer chromatography (TLC) or gel electrophoresis down to the picomole level by virtue of the fluorescence of the 5' substituent in the visible range. Ramage and Wahl (1993) have described the 4-(17-tetrabenz(*a,c,g,i*)fluorenylmethyl)trityl (TBF-Tr; **S.24**) and 4-(17-tetrabenz(*a,c,g,i*)fluorenylmethyl)-4',4''-dimethoxytrityl (TBF-DMTr; **S.25**; Fig. 2.3.8; Table 2.3.2) protecting groups. The latter group was especially found interesting for the purification of long oligonucleotides. Its acid hydrolysis is about twice as fast as that of DMTr, and the product is easily identified through the visible absorption of the substituent. The yields

of TBF-DMTr-protected synthons in phosphoramidite synthesis were only between 70% and 88%, but this problem could be circumvented by postsynthetic treatment of the 5'-deprotected, support-bound oligonucleotide with TBF-DMTrCl.

Gildea et al. (1990) have described a DMTr group substituted with a hydroxysuccinimide active ester residue (**S.26**; Fig. 2.3.9). This linker allowed the addition of, for example, biotin, allowing the possibility of purifying a target oligonucleotide from a crude solid-phase product on a streptavidin-agarose column (Fig. 2.3.9). The 5'-hydroxyl-protecting group could subsequently be released by acid treatment to elute the unprotected oligonucleotide. This trityl-on purification scheme can be coupled with chemical 5' phosphorylation, as demonstrated by Lönnberg and collaborators (see protecting group **S.27** in Figure 2.3.10; Guzaev et al., 1995). Bannwarth and Wippler have de-



**Figure 2.3.7** Examples of trityl or pixyl groups modified to serve as purification handles (for complete list see Table 2.3.2).

scribed an interesting approach to combined purification and phosphorylation by adding a protected uridine-5'-phosphate at the 5' terminus of an oligonucleotide chain. The uridine base was modified with a DMTr-protected thioether residue (**S.28** in Fig. 2.3.10). After detritylation, the target sequence was selectively retained and purified by disulfide formation with the activated thiol function of a resin. The oligonucleotide was released by periodate treatment with formation of a terminal 5' phosphate (Bannwarth and Wippler, 1990).

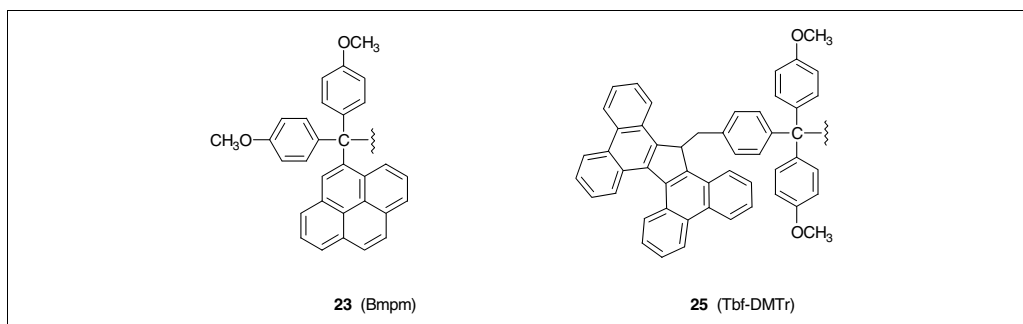
Of course, the "dual applications" described above for many modified or substituted trityl groups can be similarly found in oligoribonucleotide chemistry. The trityl-on purification, for instance, is adaptable in the ribonucleotide series, and a recent publication has established that the deprotection of DMTr does not cause migration of the internucleotidic linkages (Mullah and Andrus, 1996). The 4,4'-dimethoxy-2''-hexadecyloxytrityl group (**S.21**; van Boom and Wreesmann, 1984) and the 9-(4-octadecyloxyphenyl)xanthen-9-yl group (**S.22**; Welch et al., 1986) were alternatively introduced to promote the separation of RNA fragments.

A problem inherent to polymer-support synthesis is that the product is always a more or less complex mixture of the desired chain with

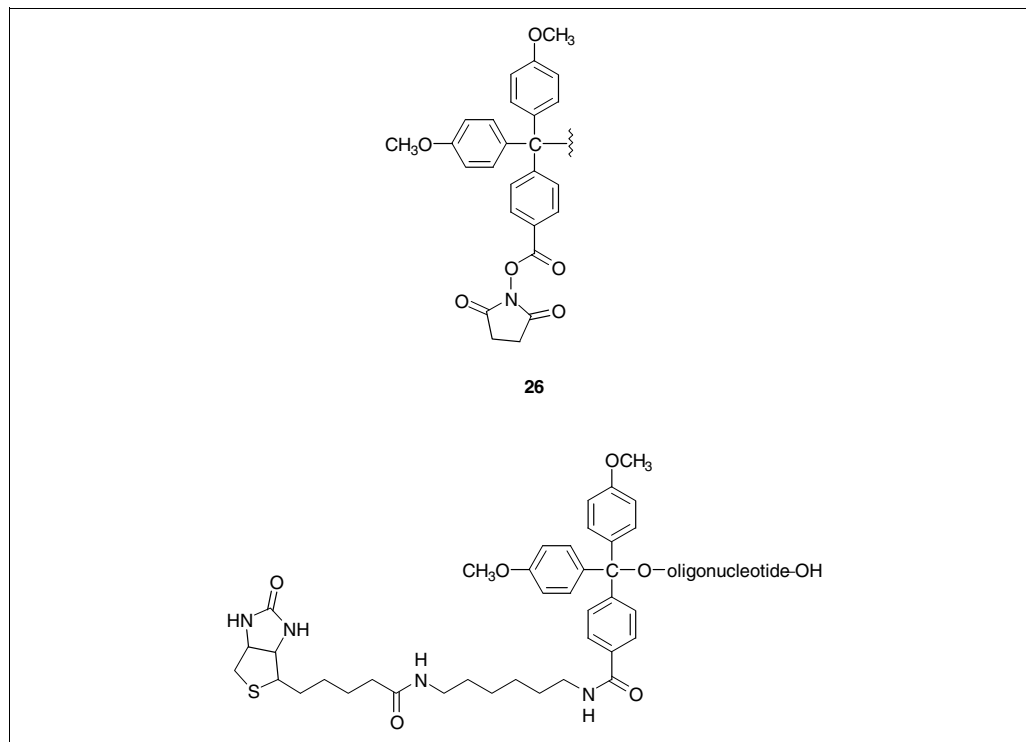
truncated and failure sequences (Földes-Papp et al., 1998, and references therein). In view of the efficiency and selectivity of affinity techniques, a solution-phase synthesis using "affinity protecting groups" would be an interesting alternative to solid-phase preparations (Seliger, 1993). An example of such an affinity separation-based solution synthesis was described (Seliger et al., 1977b) using a combination of the 5'-MMTr and 3'-lipoyl groups. Very recently, an approach to large-scale oligonucleotide synthesis was described in which the chain elongation was done in solution and the extended chain was intermediately anchored to a polymer via a Diels-Alder reaction, so as to allow filtration of educts and by-products (product-anchored sequential synthesis or PASS; Pieken, 1997). A purification-oriented oligonucleotide synthesis in solution has also been achieved through the use of a bridged bis-DMTr 5'-protecting group as soluble carrier (**S.29** in Fig. 2.3.11; Biernat et al., 1983).

### Color-Coded Triarylmethyl Groups and Triarylmethyl Groups with a Catalytic Function

In order to modulate the visible absorption of the species obtained after nonaqueous acid deprotection, a variety of groups of the triarylmethyl type has been constructed (see **S.30** to



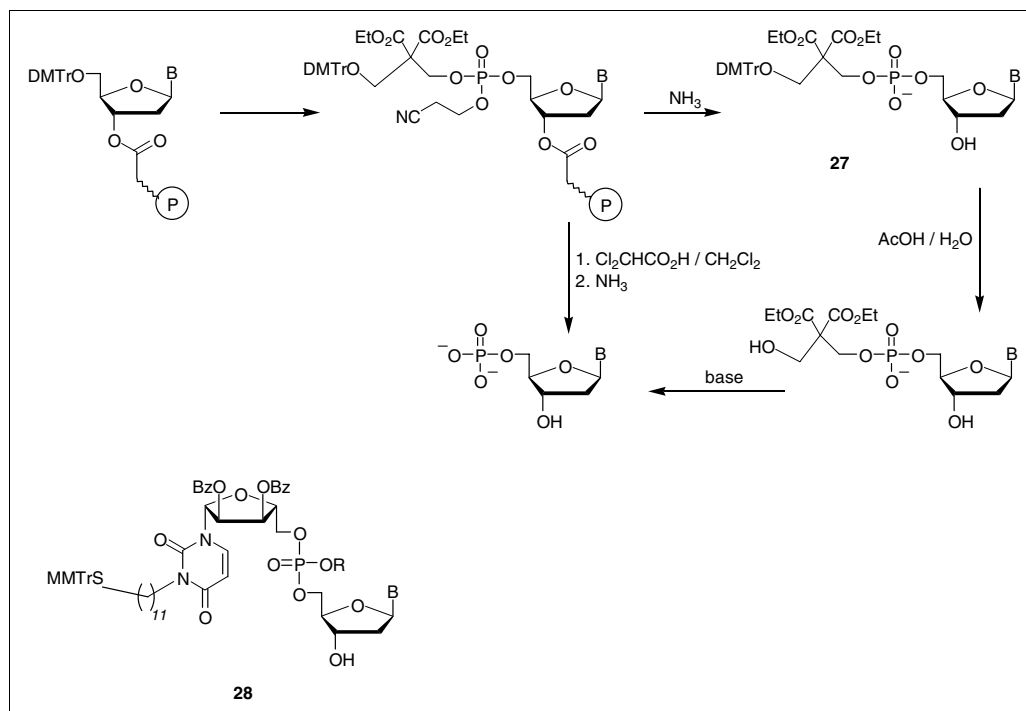
**Figure 2.3.8** Examples of trityl groups tailored to serve as combined purification handles and visible-absorbing fluorescent markers.



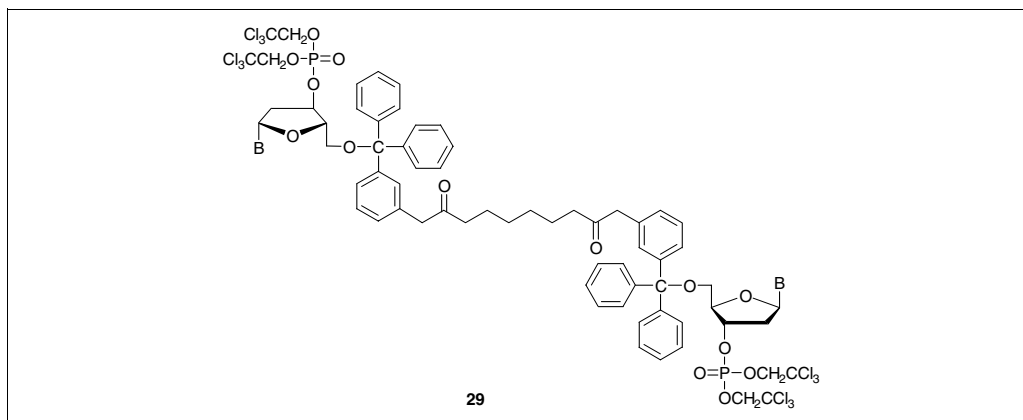
**Figure 2.3.9** A hydroxysuccinimide-substituted DMTr group and its biotinyl derivative for affinity purification of solid-phase oligonucleotide products.

S.34 in Table 2.3.2). This should allow the “color coding” of different synthons (Fisher and Caruthers, 1983), a principle that has not found application in routine automated oli-

gonucleotide synthesis, but may be useful to monitor the mixed simultaneous addition of two or more nucleotides.



**Figure 2.3.10** 5'-Phosphorylation via DMTr- or MMTr-protected protecting groups.



**Figure 2.3.11** Example of modified trityl protection for a purification-oriented solution synthesis (Biernat et al., 1983).

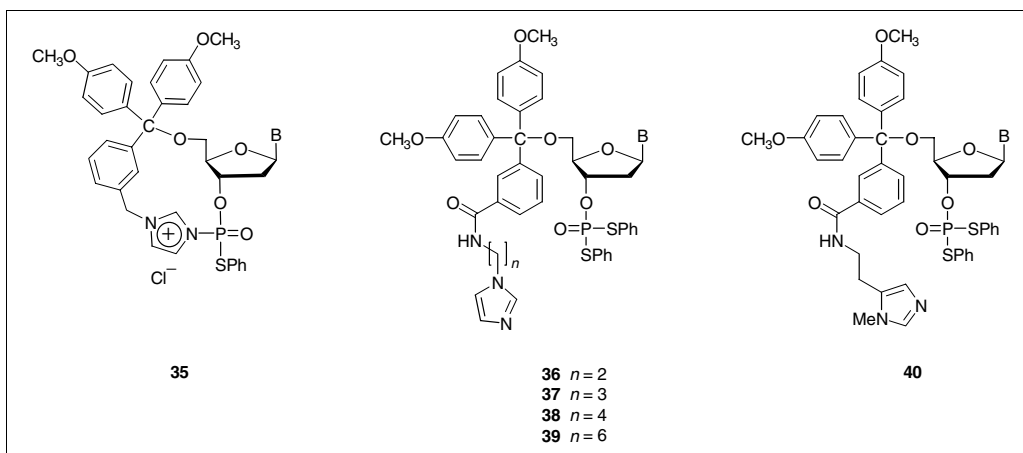
Through another structural modification, namely the introduction of an imidazol-1-yl-methyl residue, the DMTr substituent could be transformed into a protecting group that serves to activate a protected 3'-phosphate moiety (S.35; Fig. 2.3.12; Sekine and Hata, 1987). This concept was recently extended to the introduction of a number of 3-imidazolylalkylcarbamoylphenyl-4,4'-dianisylmethyl substituents (S.36 to S.39, Fig. 2.3.12), as well as a corresponding *N*-methylimidazolyl derivative (S.40; Sekine et al., 1993; Wada et al., 1998b). In addition to accelerating the rate of internucleotide bond formation, a shift in the ratio of diastereomeric triester was observed in some cases.

### Miscellaneous Acid-Labile 5'-Substituents

In view of the multiple advantages of trityl groups, as well as their modified and substi-

tuted derivatives, other acid-labile residues do not play a role as protecting groups in current oligonucleotide chemistry. Acetal groups have, for example, been used in early syntheses (e.g., Grams and Letsinger, 1970), and 5'-*O*-tetrahydropyranyl or 5'-*O*-methoxytetrahydropyranyl derivatives have been mentioned (Reese, 1978).

Although a thorough discussion is not in the scope of this unit, there are also approaches to solid-phase oligonucleotide synthesis that use a support anchored to the 5'-hydroxy function. A number of studies appeared, especially in the 1980s, in which support-bound trityl groups were used as anchors for oligonucleotide preparations in the 5'-to-3' direction (Shabarova, 1980; Belagaje and Brush, 1982; Birch-Hirschfeld et al., 1983; Rosenthal et al., 1983). The availability of 3'-protected nucleoside-5'-phosphoramidites or -*H*-phosphonates allows



**Figure 2.3.12** DMTr groups substituted with imidazolyl residues for 3'-phosphate activation (S.36 to S.40), and a proposed activated intermediate (S.35).

**Protection of Nucleosides for Oligonucleotide Synthesis**

## 2.3.13

the use of all timely methods of solid-phase synthesis in both directions of chain growth.

Finally, it may be noteworthy that Tr or DMTr groups present at the 5' terminus of deoxyribooligonucleotides were found to enhance their anti-HIV activity (Furukawa et al., 1994; Hotoda et al., 1994), a fact that was attributed to the enhancement of membrane permeability through the remaining protecting group.

## BLOCKING GROUPS LABILE TO NONACIDIC CONDITIONS

### Acyl Substituents and Base-Labile Triarylmethyl Groups

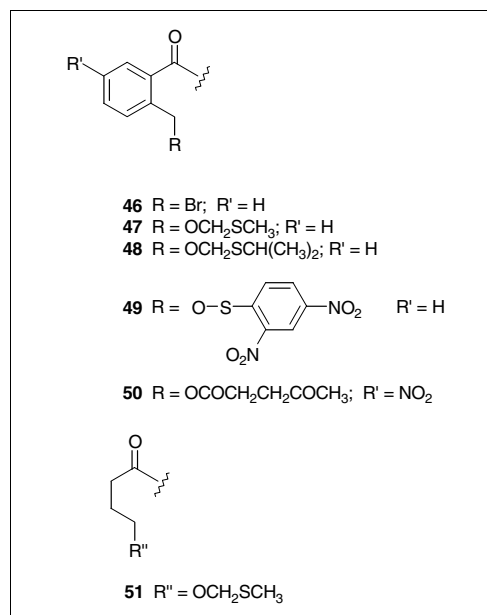
In spite of their obvious advantages, acid-labile protecting groups for the 5'-hydroxy function have some limitations to their application in oligonucleotide synthesis. In the deoxyribonucleotide series, even a small number of statistically distributed depurination sites may seriously impair the quality of a solid-phase or large-scale synthesis product. In the ribonucleotide series, multiple acid deprotection steps may interfere with the stability of 2'-hydroxyl-protecting groups. This has stimulated the search for new orthogonal protection schemes involving 5'-hydroxyl-protecting groups that can be deblocked under nonacidic conditions.

Of course, there are limitations to the use of simple acyl groups such as acetyl or benzoyl as long as the exocyclic amino groups of nucleobases are blocked by acyl residues as well. Thus, acetyl protecting groups are found in the recent literature only for syntheses at the monomer level—e.g., in the preparation of <sup>15</sup>N-labeled (Kamaike et al., 1995, 1996) or <sup>2</sup>H-labeled nucleosides (Kawashima et al., 1995, 1997), or of components for oligodeoxyribonucleotides with base-modified or mutagenic units (Matsuda et al., 1993; Ozaki et al., 1994; for earlier literature see, e.g., Kössel and Seliger, 1975; Reese, 1978; Sonveaux, 1986). From the preparative standpoint, methods for rapid O-acylation of nucleoside hydroxy groups through phase-transfer catalysis may be noteworthy (Sekine, 1993). Isobutyryl (Gaffney and Jones, 1982a,b) or methoxyacetyl groups (Reese and Skone, 1984) were introduced at sugar hydroxy functions in order to allow the additional protection of the 6-oxo group of deoxyguanosine as well as the 4-oxo group of thymidine.

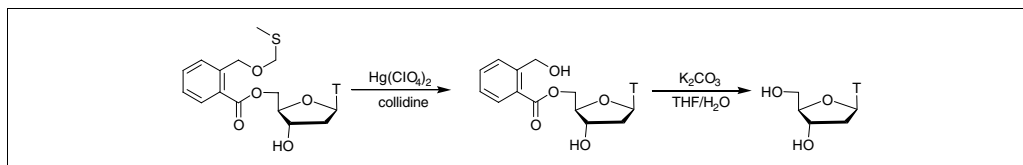
Acyl groups that can be removed under virtually neutral conditions are more attractive. Earlier examples, such as methoxy- and phenoxyacetyl (Reese and Stewart, 1968), did not

completely fulfill the expectations. A more interesting alternative was the *o*-bromomethylbenzoyl group (**S.46**; Fig. 2.3.13; Chattopadhyaya et al., 1979), which was used for the preparation of an SV40-specific deoxyribooligonucleotide (Chattopadhyaya and Reese, 1980). Derivatives of the 4-hydroxybutyryl and 2-hydroxymethylbenzoyl groups (Brown et al., 1984; van Boom and Wreesman, 1984; Reese, 1985; Brown et al., 1989a,b) are similarly of interest, since the neighboring participation of the hydroxy groups allows their hydrolysis under extremely mild conditions.

Of course, the hydroxy groups have to be protected during chain elongation. In case of the 4-(methylthiomethoxy)butyryl (MTMB; **S.51**), 2-(methylthiomethoxymethyl)benzoyl (MTMT; **S.47**), and 2-(isopropylthiomethoxymethyl)benzoyl (DTMT; **S.48**) groups (Fig. 2.3.13), the deblocking of the methylthiomethyl residue was done with mercury(II) perchlorate and 2,4,6-collidine within 3 hr at room temperature, and the subsequent treatment with K<sub>2</sub>CO<sub>3</sub> in tetrahydrofuran/water released the 2-hydroxymethylbenzoyl substituent within 30 sec (Fig. 2.3.14). As an alternative, the 2-(2,4-dinitrophenylsulfenyl)benzoyl (DNBSB) group (**S.49**; Fig. 2.3.13) was initiated by removal of the sulfenyl residue with *p*-toluenethiol (Christodoulou et al., 1987a,b). Although these “protected protecting groups” are appealing from their deprotection conditions, difficulties in in-



**Figure 2.3.13** Acyl substituents for removal at close-to-neutral pH.



**Figure 2.3.14** Example of two-step removal of an *ortho*-substituted benzoyl protecting group (Brown et al., 1984; Reese, 1985).

roduction and, in particular, the two-step procedure of removal make them less practical for current automated oligonucleotide synthesis.

The  $\beta$ -benzoylpropionyl (**S.52**; Letsinger et al., 1967) and the levulinyl (**S.53**; van Boom and Burgers, 1976; Iwai and Ohtsuka, 1988; Iwai et al., 1990) groups can be deprotected by hydrazine in a pyridine/acetic acid mixture (Fig. 2.3.15). However, a partial deprotection of acyl groups from the nucleobases was also observed under the conditions of hydrazinolysis of the  $\beta$ -benzoylpropionyl group (Letsinger and Miller, 1969). Especially mild conditions of hydrazinolytic deprotection apply to the 2-levulinylloxymethyl-5-nitrobenzoyl group (**S.50**; Fig. 2.3.13; Kamaike et al., 1997). Appropriate substitution with electron-donating substituents that stabilize a trityl cation may strongly modify the conditions of cleavage. Based upon the earlier finding that the 4-hydroxytrityl group is hydrolyzed much more easily than the corresponding 4-acetoxytrityl residue (Taunton-Rigby et al., 1972), the 4,4',4''-tris(benzoyloxy)trityl substituent (**S.41**; Table 2.3.2) was prepared as an acid-stable but base-labile "protected 5'-protecting group" (Sekine and Hata, 1983), the removal of which is shown in Figure 2.3.16. This principle was further extended to 4,4',4''-tris(4,5-dichlorophthalimido)trityl (**S.42**; Sekine and Hata, 1984) and 4,4',4''-tris(levulinylloxy)trityl (**S.43**; Table 2.3.2; Sekine and Hata, 1985) as protecting groups labile to hydrazine treatment in pyridine/acetic acid.

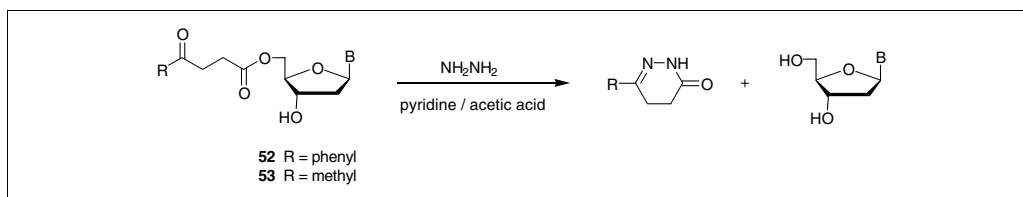
Based on the same general concept, the 4-(9-fluorenylmethoxycarbonyloxy)-4',4''-dimethoxytrityl (**S.44**) and 4-(9-fluorenyl-

methoxycarbonyl)amino-4',4''-dimethoxytrityl (**S.45**; Table 2.3.2) groups were introduced. Their release could be triggered through  $\beta$ -elimination (Scalfi-Happ et al., 1987; Happ and Scalfi-Happ, 1988). The usefulness of hydrazine-labile modified trityl groups such as 4,4',4''-tris(4,5-dichlorophthalimido)trityl (**S.42**; Sekine and Hata, 1984, 1986; Scalfi-Happ et al., 1987) and the **S.44** and **S.45** groups for  $\beta$ -elimination-triggered deprotection (Happ and Scalfi-Happ, 1988) was also demonstrated for large-scale oligoribonucleotide synthesis and for the preparation of 2'(3')-O-aminoacyl-oligoribonucleotides (Scalfi-Happ et al., 1987).

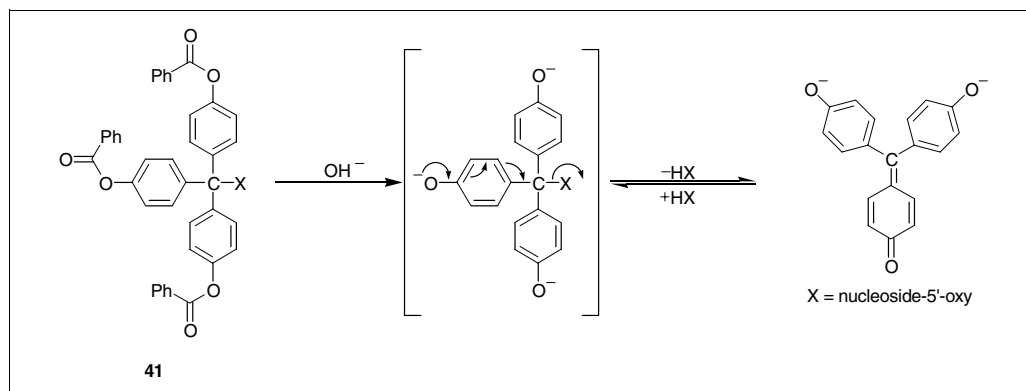
### Carbonate-Type Protecting Groups

Protecting groups of the carbonate type, most popular in peptide chemistry, have also received much attention in oligonucleotide synthesis. In earlier work, the isobutyloxycarbonyl group (**S.54**; Fig. 2.3.17; Ogilvie and Letsinger, 1967) and the *p*-nitrophenyloxycarbonyl (NPOC) group (**S.55**; Letsinger and Ogilvie, 1967) were shown to be introduced rather selectively at 5'-hydroxy functions en route to 3'-tritylated thymidine and uridine derivatives. However, their removal in dilute sodium hydroxide/dioxane would not be compatible with exocyclic acyl protection of nucleobases.

*p*-Nitrophenyloxycarbonyl and a number of other carbonate protecting groups could also be introduced into partially protected thymidine and uridine via the corresponding 5'-chloroformates (Seliger, 1972). These studies later led to the introduction of the 5'-*p*-phenylazophenyl-



**Figure 2.3.15** Hydrazinolytic deprotection of  $\beta$ -benzoylpropionyl (**S.52**) and levulinyl (**S.53**) groups.



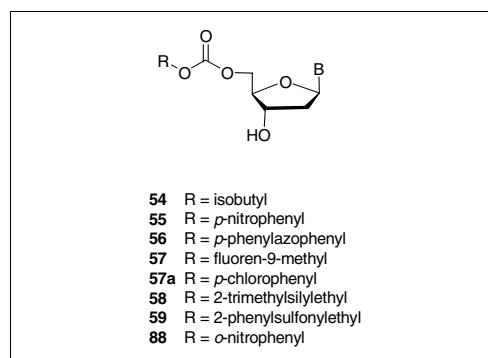
**Figure 2.3.16** 4,4',4''-Tris(benzoyloxy)trityl as an acid-stable, base-labile 5'-hydroxyl-protecting group.

oxycarbonyl (PAPOC) group (**S.56**; Kössel and Seliger, 1975), which was introduced via *p*-phenylazophenylchloroformate (Seliger and Kotschi, 1985; Seliger et al., 1986). This group could be released by a two-step treatment with  $\beta$ -cyanoethanol/triethylamine and diazabicycloundecene (DBU). This group retains some of the features of trityl- or pixyl-type substituents (i.e., its deprotection gives a visible-absorbing solution), and the PAPOC nucleosides are readily crystalline; however, the two-step deprotection procedure is less advantageous. More attention has been given to the 9-fluorenylmethoxycarbonyl (Fmoc) group (**S.57**, Fig. 2.3.17), a standard in peptide protection. Fmoc-protected synthons allowed an alternative protocol for solid-support deoxyribo- and ribooligonucleotide synthesis, retaining the standard acyl protection for the bases and avoiding acid deblocking steps (Gioeli and Chattopadhyaya, 1982; Balgobin and Chattopadhyaya, 1987; Ma and Sonveaux, 1987, 1989). The potential of 5'-Fmoc protection for oligoribonucleotide synthesis was demonstrated by Fukuda et al. (1988) and by the group of Gait (Lehmann et al., 1989). The Fmoc group was also used in acetal-linked solid-support oligonucleotide synthesis (Palom et al., 1993).

Recently, the *p*-chlorophenylloxycarbonyl group (**S.57a**, Fig. 2.3.17) was found to be removed in <2 min in a solution of peroxyanions buffered to pH 9.6 (10 ml LiOH·H<sub>2</sub>O, 12 ml 30% H<sub>2</sub>O<sub>2</sub>, 1.78 g *m*-chloroperbenzoic acid, 15 ml 1.5 M AMP buffer, pH 10.3, in 50 ml dioxane; Caruthers et al., 1994; Dellinger et al., 1998). This allowed the design of a solid-phase two-step chain-elongation cycle consisting of only a coupling step and the combined deprotection/oxidation step (excluding capping), reducing the phosphoramidite cycle time to 6 min.

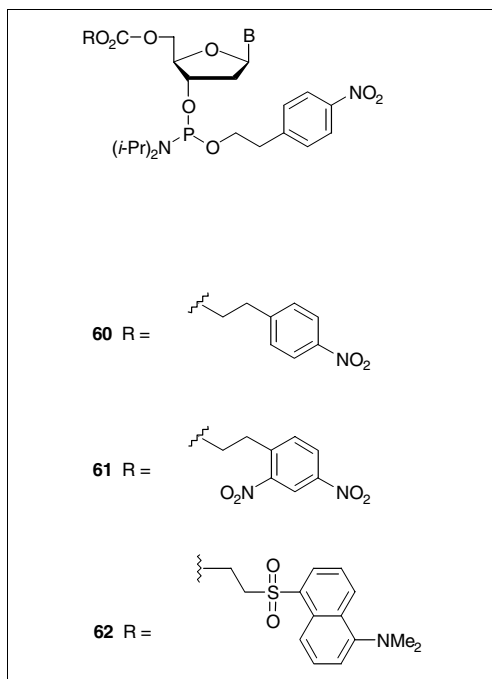
Other carbonate protecting groups that could be selectively released in the presence of base-protecting acyl groups include 2-(trimethylsilyl)ethoxycarbonyl (**S.58**; Fig. 2.3.17; Gioeli et al., 1981) and 2-(phenylsulfonyl)ethoxycarbonyl (**S.59**; Balgobin et al., 1981a).

More recently, Pfleiderer and colleagues have developed an orthogonal protection scheme for solution- and solid-phase deoxyribo- and ribooligonucleotide synthesis, with avoidance of acid-deprotection steps. The main feature is the exclusive use of protecting groups that can be cleaved by  $\beta$ -elimination. For instance, in combination with the *p*-nitrophenylethyl (NPE) phosphate protecting group, the 2-(4-nitrophenyl)ethoxycarbonyl (NPEOC; **S.60**) or 2-(2,4-dinitrophenyl)ethoxycarbonyl (DNPEOC; **S.61**) substituents were applied for 5'-protection (Schirmeister et al., 1993; Fig. 2.3.18). The NPE/NPEOC strategy was further extended to large-scale oligonucleotide synthesis (Weiler and Pfleiderer, 1995). Particularly attractive is the dansylethoxycarbonyl group (**S.62**; Bergmann and Pfleiderer, 1994a,b,c), which



**Figure 2.3.17** Carbonate protecting groups for the 5'-hydroxy function.





**Figure 2.3.18** Carbonate substituents for 5'-OH as part of an orthogonal scheme of protecting groups that can be cleaved by  $\beta$ -elimination.

can easily be cleaved with dilute DBU in aprotic solvent and allows yield monitoring by far UV or fluorescence detection at 530 nm, even in small-scale synthesis of long RNA molecules (Pfleiderer et al., 1995). A further advantage of this scheme of deprotection via  $\beta$ -elimination in solid-phase synthesis is the possibility of retaining a deprotected oligonucleotide product on the support, which facilitates purification of the target sequence.

### 5'-O-Silyl Protecting Groups

The use of silyl groups for 5'-hydroxyl protection does not have any major advantages over trityl in the deoxyribonucleotide series; examples in the literature are concerned with modeling the behavior of different silyl groups (e.g., **S.63** to **S.67**; Fig. 2.3.19; Ogilvie et al., 1974) or the preparation of structurally modified nucleosides (e.g., keto-nucleosides; Robins et al., 1990; McEldoon and Wiemer, 1995).

In oligoribonucleotide synthesis, however, silyl protecting groups are nearly essential in developing orthogonal protection systems. The alternative of 2'-silyl protection combined with 5'-MMTr or 5'-DMTr has been discussed above (see Triarylmethyl and Related Substituents). A new route to orthogonal ribonucleoside protection uses acid-labile groups for the 2'-hydroxy function in combination with silyl sub-

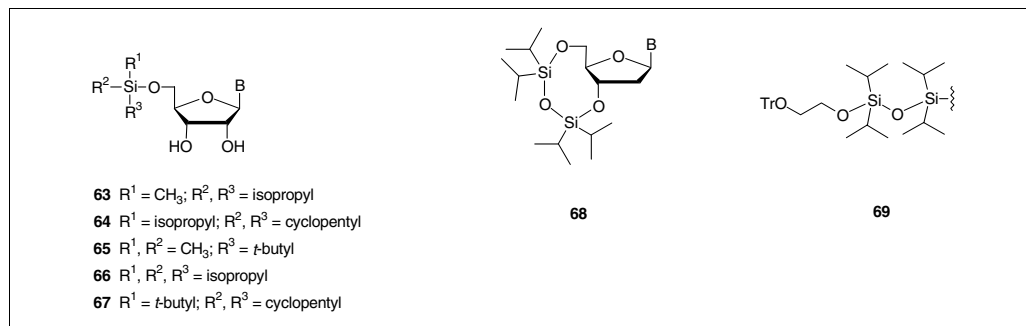
stituents at the 5'-hydroxy function. In this case, the tetra(isopropyl)disiloxane-1,3-diyl group (**S.68**; Fig. 2.3.19; Markiewicz, 1979, 1980) can be elegantly used as a transient bifunctional protecting group for 3' and 5' hydroxyls, which ensures the regioselective introduction of an acid-labile group at the 2' position. Subsequent to deblocking of the disiloxane-diyl group, another silyl substituent is selectively attached to protect the 5'-hydroxy function.

Two approaches of this kind have recently been described. The 1,1,3,3-tetra(isopropyl)-3-[2-(triphenylmethoxy)ethoxy]disiloxane-1-yl (TES) group (**S.69**; Fig. 2.3.19; Hirao et al., 1998), a "trityl-protected silyl protecting group," can be deblocked with 0.1 M tetrabutylammonium fluoride in tetrahydrofuran within 1 min at room temperature. The resultant deblocking solution will be colored on addition of acid. In solid-phase synthesis, using terephthalate as an anchor to the support, the silyl deprotection conditions do not lead to removal of other blocking groups except for the  $\beta$ -cyanoethyl residue at the internucleotidic linkages, which is lost during this treatment. A better solution to this orthogonality problem has recently been reported (Caruthers, 1998, and pers. comm.). A number of silyl substituents (**S.70** to **S.86**; Fig. 2.3.20) can be regioselectively introduced and deprotected in <30 sec with tetrabutylammonium fluoride in tetrahydrofuran. On testing for stability in acid and base (Table 2.3.3), this author found that the bis(trimethylsilyloxy)cyclooctyloxysilyl group (**S.83**) was best for 5'-hydroxyl protection, as the resulting fully protected phosphoramidite synthons (Fig. 2.3.21) could be prevented from oiling out on workup.

The internucleotidic bonds, in this case, were protected by the methoxy group, which was found to be resistant to fluoride cleavage, but is released with a thiol reagent. Alternatively, the bis(trimethylsilyloxy)-1,3-(trityloxy)propyl-2-oxysilyl group (**S.87**; Fig. 2.3.20) can be designated for monitoring the deprotection step.

### Photosensitive 5'-Hydroxyl-Protecting Groups

Groups that can be cleaved by photochemical methods have not been of relevance to 5'-hydroxyl protection in earlier times, although photolabile phosphate-protecting groups such as *o*-nitrobenzyl were in use (Hasan et al., 1997, and references therein). Only recently has the photochemical cleavage of 5'-hydroxyl-protecting groups become an

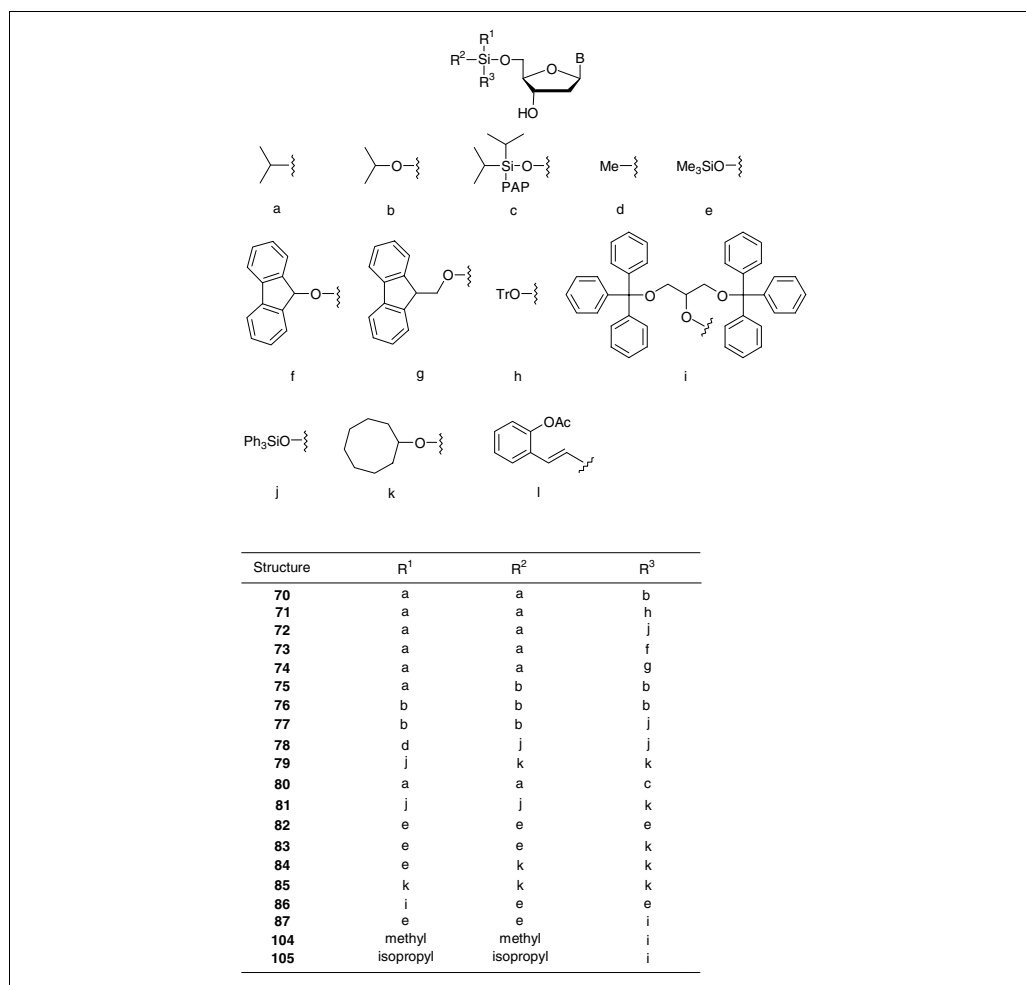


**Figure 2.3.19** Silyl protecting groups for the 5'-hydroxy function.

interesting option, since Fodor and collaborators pointed out the possibility of producing DNA arrays on glass substrates (DNA “chips”) by light-directed solid-phase synthesis (Fodor et al., 1991; Pease et al., 1994). Arrays containing up to  $10^6$  unique oligonucleotide sequences per square centimeter can be constructed by the elongation of each sequence triggered through photodeprotection of the 5'-hydroxy function

of relevant oligonucleotide spots in a technique similar to photolithography.

The efficiency of this technique relies very much on the ease of photochemical deprotection and the extent to which it can be quantified. Several new protecting groups been developed for this purpose. Based on the experience of investigations on carbonate protecting groups, the *o*-nitrophenyloxycarbonyl (NPOC; **S.88**;



**Figure 2.3.20** Silyl protecting groups that are rapidly deblocked by tetrabutylammonium fluoride (Caruthers, 1998). PAP, 4-phenylazophenyl.

**Table 2.3.3** Fluoride Deprotection and Acid/Base Degradation of Silyl Protecting Groups **S.70** to **S.86**<sup>a</sup>

Structure	R <sup>1</sup> , R <sup>2</sup> , R <sup>3</sup>	Fluoride deprotection <sup>b</sup>	Acid degradation (%) <sup>c</sup>	Base degradation (%) <sup>d</sup>
<b>S.70</b>	a,a,b	<1 min	1% 24 hr	0% 24 hr
<b>S.71</b>	a,a,h	<15 sec	100% 24 hr	75% 24 hr
<b>S.72</b>	a,a,j	<15 sec	1% 24 hr	0% 24 hr
<b>S.73</b>	a,a,f	<30 sec	1% 24 hr	0% 24 hr
<b>S.74</b>	a,a,g	<30 sec	5% 13 hr	5% 13 hr
<b>S.75</b>	a,b,b	<30 sec	NA	NA
<b>S.76</b>	b,b,b	<15 sec	5% 5 hr	5% 5 hr
<b>S.77</b>	b,b,j	<15 sec	1% 24 hr	0% 24 hr
<b>S.78</b>	d,j,j	<15 sec	NA	NA
<b>S.79</b>	j,k,k	<15 sec	NA	NA
<b>S.80</b>	a,a,c	<15 sec	NA	NA
<b>S.81</b>	j,j,k	<15 sec	NA	NA
<b>S.82</b>	e,e,e	<15 sec	NA	NA
<b>S.83</b>	e,e,k	<15 sec	NA	NA
<b>S.84</b>	e,k,k	<15 sec	NA	NA
<b>S.85</b>	k,k,k	<15 sec	NA	NA
<b>S.86</b>	i,e,e	<15 sec	NA	NA

<sup>a</sup>From Caruthers (1998 and pers. comm.). See Figure 2.3.20 for structures and substituents (R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup>).

<sup>b</sup>Time for complete removal of protecting group with 5 eq tetrabutylammonium fluoride in tetrahydrofuran.

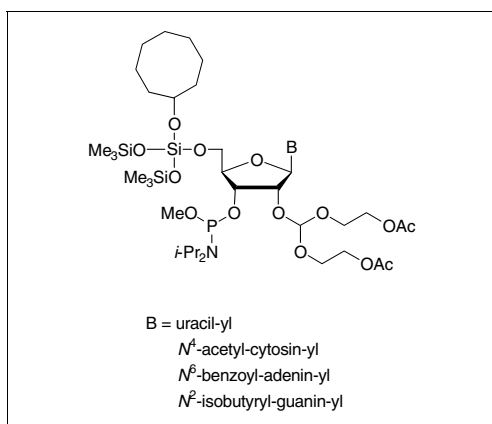
<sup>c</sup>Percent degradation in 0.01 M HCl within specified time. NA, data not available.

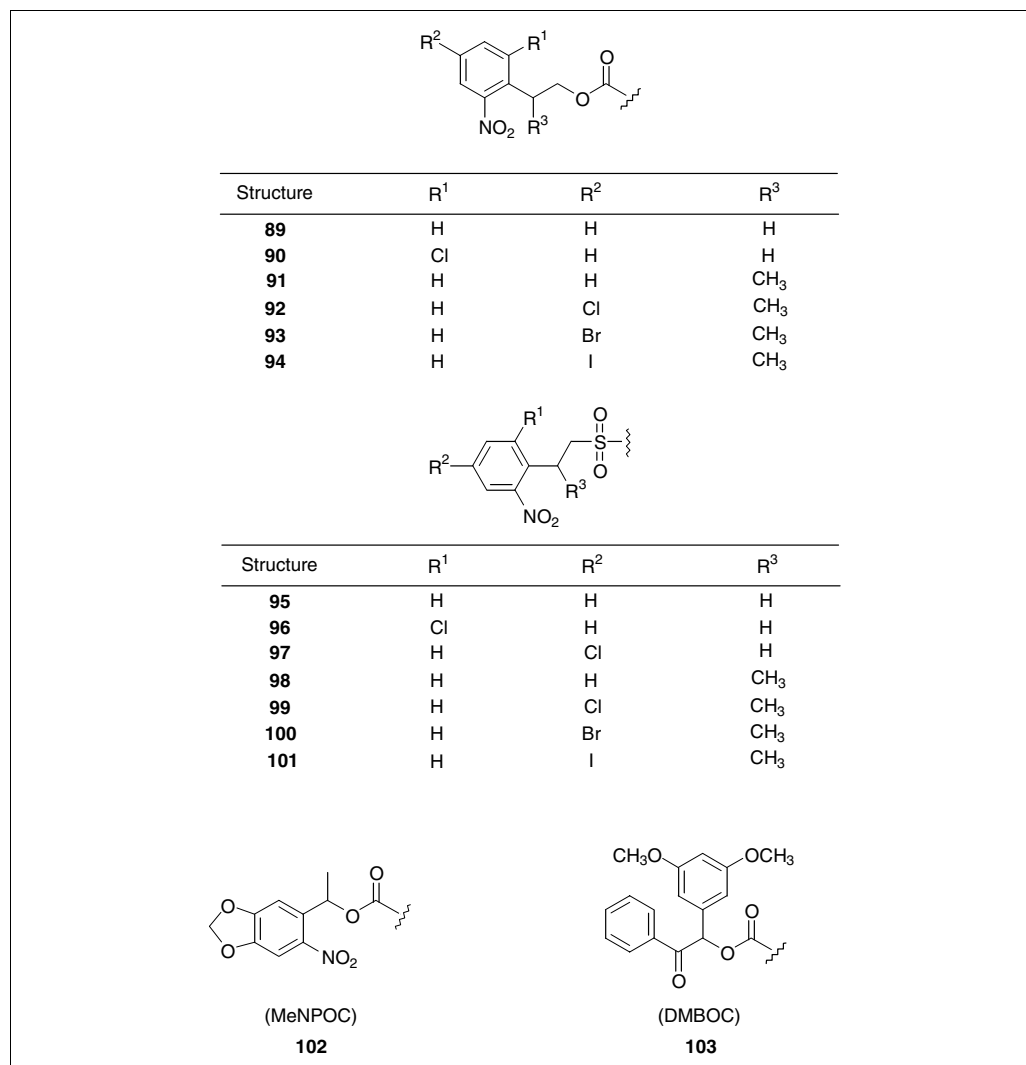
<sup>d</sup>Percent degradation in 10% aqueous triethylamine within specified time. NA, data not available.

Fig. 2.3.17) and *o*-nitrophenylethoxycarbonyl (NPEOC; **S.89**; Fig. 2.3.22) groups were reported from Pfeleiderer's laboratory (Hasan et al., 1997). Their photochemical cleavage leads to the formation of *o*-nitrobenzaldehyde and CO<sub>2</sub> in the case of NPOC, or *o*-nitrostyrene and CO<sub>2</sub> in the case of NPEOC (Fig. 2.3.23). The parent nucleosides were recovered in a clean reaction after irradiation at 365 nm, with a half-life of 7.5 min for NPEOC (Bühler et al., 1999; Table 2.3.4). Such long deprotection times are, of course, less suitable if a great

number of parallel oligonucleotide chain extensions are to be performed in a fast and efficient way. Therefore, a number of new protecting groups have been described that are either of the *o*-NPEOC type (**S.90** to **S.94**, Fig. 2.3.22) or of the *o*-nitrophenylethylsulfonyl type (**S.95** to **S.101**, Fig. 2.3.22) (Bühler et al., 1999), for which half-lives of photochemical deprotection are reported to be between 10 min and 50 sec (Table 2.3.4). Up to 85% of unprotected nucleosides were recovered after 10 min irradiation time.

Other protecting groups such as methylnitropiperonyloxycarbonyl (MeNPOC; **S.102**; Fig. 2.3.22; Pease et al., 1994) and 3',5'-dimethoxybenzoinoxycarbonyl (DMBOC; **S.103**; Fig. 2.3.22; Pirrung and Bradley, 1995a,b) have been tested in solid-support oligonucleotide synthesis. A comparison of results obtained with both protecting groups and with conventional DMTr protection showed that cycle yields are diminished with photochemical deprotection with respect to cycles including conventional detritylation, and some by-product was obtained due to damage of *N*-benzoyl cytosine bases. In a parallel study, the removal of the MeNPOC group (Fig. 2.3.24) was found to be independent of the length and terminal base of the growing oligonucleotide chain, and was most rapid in non-polar solvents or in absence of solvent ( $t_{1/2}$  =

**Figure 2.3.21** 5'-O-Silyl-protected synthons for oligoribonucleotide synthesis (Caruthers, 1998).



**Figure 2.3.22** Photochemically cleaved 5'-hydroxyl-protecting groups of the carbonate or sulfonate type. Taken in part from Bühler et al. (1999) with permission from Marcel Dekker.

10 to 13 sec at  $A = 365$  nm, irradiation intensity  $27.5$  mW/cm<sup>2</sup>). The average yield of chain elongation was 92% to 94% in solid-support reactions and ~96% on photolysis in solution, as compared to 98% in conventional cycles (McGall et al., 1997). A thorough investigation on the DMBOC group (Pirrung et al., 1998) showed half times of deprotection on a glass surface in the presence of dioxane (326.5 nm, irradiation with  $44.5$  mW/cm<sup>2</sup>) to be between 5 and 13 sec. The use of shorter wavelengths decreases the deprotection time, but is discouraged due to the risk of damage to the deoxyribo-oligonucleotide chain. The yields per chain extension cycle were found to be base dependent, ranging from 91% to 98% for T, 82% to 95% for C, 79% to 92% for G, and 74% to 84% for A.

Recently, McGall and collaborators have proposed a technique for the preparation of oligonucleotide arrays that combines photolithography with acid deprotection (McGall et al., 1996; Wallraff et al., 1997). In this case, spots of growing oligonucleotide chains are grafted to the surface of a functionalized carrier (chip), which is then covered by a photosensitive polymer coating (a “photoresist”). Oligonucleotide spots that are meant for chain lengthening are first exposed to further chain elongation by photoetching of the polymer coating. They can then be subjected to conventional 5'-deprotection and chain lengthening.

It is likely that, in further pursuit of light-directed combinatorial oligonucleotide synthesis, new protecting groups will have to be developed. Some of these may deviate from the

**Table 2.3.4** Photolabile 5'-Protecting Groups of the *o*-Nitrophenylethoxycarbonyl and *o*-Nitrophenylethylsulfonyl Types<sup>a</sup>

Structure	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>	<i>t</i> <sub>1/2</sub> <sup>b</sup>
<i>Carbonate groups</i>				
<b>S.89</b>	H	H	H	7.5 min
<b>S.90</b>	Cl	H	H	82 min
<b>S.91</b>	H	H	CH <sub>3</sub>	56 sec
<b>S.92</b>	H	Cl	CH <sub>3</sub>	62 sec
<b>S.93</b>	H	Br	CH <sub>3</sub>	60 sec
<b>S.94</b>	H	I	CH <sub>3</sub>	53 sec
<i>Sulfonate groups</i>				
<b>S.95</b>	H	H	H	10.9 min
<b>S.96</b>	Cl	H	H	16.3 min
<b>S.97</b>	H	Cl	H	10.9 min
<b>S.98</b>	H	H	CH <sub>3</sub>	82 sec
<b>S.99</b>	H	Cl	CH <sub>3</sub>	66 sec
<b>S.100</b>	H	Br	CH <sub>3</sub>	70 sec
<b>S.101</b>	H	I	CH <sub>3</sub>	60 sec

<sup>a</sup>Data from Bühler et al. (1999) with permission from Marcel Dekker. Deprotection conditions: irradiation with a 200 W mercury lamp (365 nm) in 1:1 (v/v) methanol/water. See Figure 2.3.22 for structures and substituents (R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup>).

<sup>b</sup>Values given for 5'-protected thymidine derivatives.

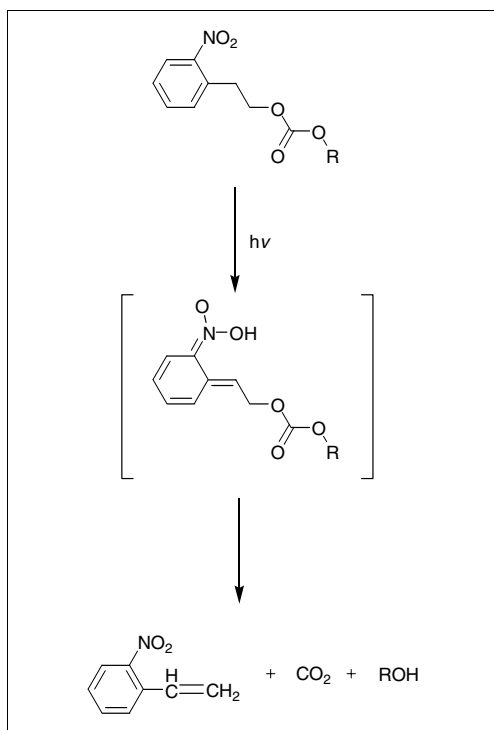
structural concept of carbonate esters, which are now the first choice. Examples include photochemically cleavable silyl groups such as hydroxystyryldimethylsilyl (HSDMS; **S.104**; Fig. 2.3.20) and hydroxystyryldiisopropylsilyl (HSDIS; **S.105**; Fig. 2.3.20; Pirrung and Lee, 1993). However, these do not seem to have been

extensively tested for oligonucleotide synthesis.

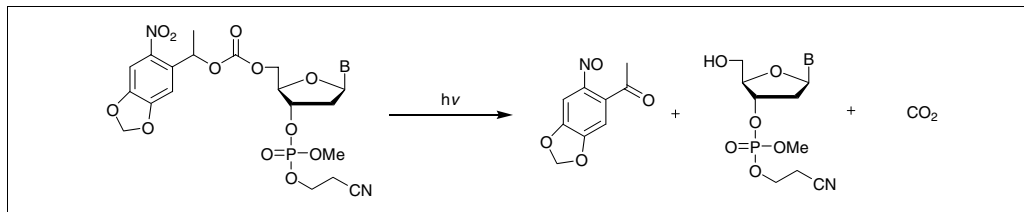
### 5'-Hydroxyl-Protecting Groups Cleaved Under Nonacidic and Nonalkaline Conditions

The general interest in 5'-hydroxyl-protecting groups that are cleaved under nonacidic and nonalkaline conditions has yielded a number of interesting solutions. Structural modifications of trityl that allow deprotection under nonacidic conditions (e.g., in a two-step procedure involving hydrazine in a near-neutral solution) have been discussed in the previous section (see Acyl Substituents and Base-Labile Triaryl-methyl Groups). 2,4-Dinitrobenzenesulfonyl residues (**S.106**, Fig. 2.3.25) have been introduced by reaction with the corresponding sulfonyl chloride (Grams and Letsinger, 1968). This protecting group can be removed by treatment with a nucleophilic reagent such as thiophenol in pyridine (Letsinger et al., 1964). Stereochemical and mechanistic aspects of introduction and release of sulfonyl groups have been investigated (Bazin et al., 1985).

The protection of hydroxy functions of sugar derivatives by *p*-methoxybenzyl (**S.107**) and 3,4-dimethoxybenzyl groups (**S.108**; Fig. 2.3.25), which are susceptible to oxidative cleavage with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ), has been described (Oikawa et al., 1982), but these groups are not in use in oligonucleotide chemistry. The *p*-methoxybenzyl group could also be released from the 5' hydroxyl in 68% yield by treatment with ceric



**Figure 2.3.23** Proposed mechanism for photodeprotection of the *o*-nitrophenylethoxycarbonyl group (Hasan et al., 1997).



**Figure 2.3.24** Proposed photocleavage of the MeNPOC protecting group.

ammonium nitrate in aqueous acetonitrile (Luzzio and Menes, 1994); attempts at hydrolytic removal of the benzyl group did not give satisfactory results.

The 1,1-dianisyl-2,2,2-trichloroethyl residue (**S.109**, Fig. 2.3.25) was introduced in the laboratory of Ugi as a protecting group that is stable to acid and base, but is readily removed by reductive fragmentation with the supernucleophile lithium cobalt(I)-phthalocyanine (Karl et al., 1995; Klösel et al., 1996). The application of this group in the preparation of a dinucleoside-trifluoromethyl-phosphonate was demonstrated (Karl et al., 1996).

Again, it is beyond the scope of this unit to review all publications that describe the linkage of the 5' hydroxyl to polymer supports with a non-acid-labile anchor. Examples of anchoring through ester linkage (e.g., Shimidzu and Letsinger, 1968) or, more often, through succinate groups (Ohtsuka et al., 1984; Weiss et al., 1984) are in the literature; in each case, the chain extension was carried out from the 5' to the 3' end.

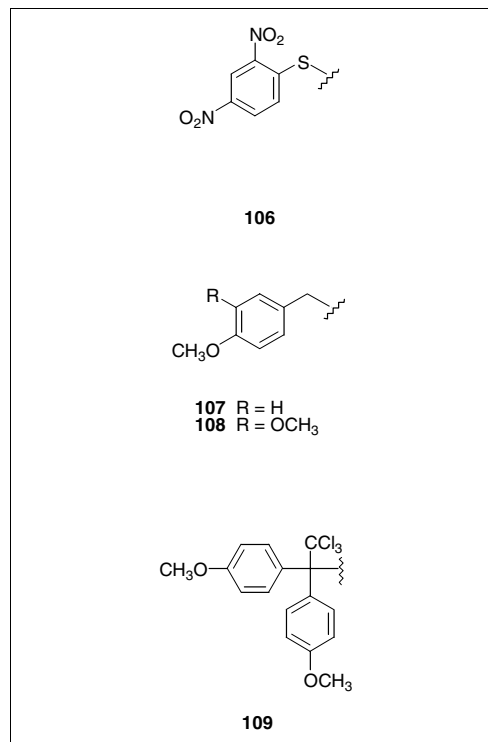
### Capping of the 5'-Hydroxy Terminus in Oligonucleotide Synthesis

Capping refers to the introduction of a protecting group at the 5'-hydroxy function as a postcondensation step during solid-phase oligonucleotide synthesis. The capping step is meant to mask growing chain ends that have escaped elongation and, therefore, will remain as truncated or failure sequences. For this reason, the groups substituted during capping should not be removed before the end of chain growth, if at all. Hence, capping groups should (1) be easily attached even to longer oligonucleotide chains through a highly reactive and sterically unhindered reagent, and (2) be compatible with the chemistry chosen for chain elongation.

For solid-phase preparations of oligonucleotides according to the phosphotriester method, phenylisocyanate has been used as a capping reagent in earlier procedures (e.g., Gait et al., 1982). Subsequently, capping was mostly

done with acetic anhydride/4-dimethylaminopyridine (e.g., Köster et al., 1983, 1984). In order to blend with a set of protecting groups removable under  $\beta$ -elimination conditions, Reiner and Pfeleiderer have proposed a number of  $\beta$ -substituted ethylsulfonylethyl chlorides as capping reagents. The resulting sulfonyl groups are removed with DBU together with the protecting groups for bases and internucleotide linkages, as well as the terminal 5'-NPEOC group (Reiner and Pfeleiderer, 1987).

In oligonucleotide synthesis with 5'-DMTr-protected phosphoramidite synthons, capping with acetic anhydride activated by 4-dimethylaminopyridine or *N*-methylimidazole has been the standard procedure from the beginning (Atkinson and Smith, 1984). Because the yields of chain extension in phosphoramidite support synthesis average 98% to 99%, only ~1% to 2% of unreacted ends are left over for capping



**Figure 2.3.25** Miscellaneous 5'-hydroxyl-protecting groups labile to nonacidic conditions.

during each cycle. The acetylation is generally assumed to be nearly quantitative, but there are no experimental methods to detect average capping yields. Recently, an estimation of capping efficiency has been available through the correlation of calculations using fractal mathematics with the results of capillary electrophoretic separation of the crude product of oligonucleotide preparations of up to 60 bases in length. For support syntheses using the common controlled-pore glass (CPG) or polystyrene supports, the best correlation between calculated and experimentally determined chain length distributions was observed if capping was assumed to proceed with not more than 70% to 80% of the non-elongated chain ends (Földes-Papp et al., 1996, 1998).

When the phenoxyacetyl group was used for the protection of exocyclic amino groups, partial replacement of this group by acetyl on the guanine base was observed during conventional capping. This could be avoided by using phenoxyacetic anhydride in place of acetic anhydride (Chaix et al., 1989).

The choice of capping procedure necessarily influences the workup of the solid support product. The capping procedures described so far have the common feature that the protecting groups attached to the truncated chains are hydrolyzed on removal of the polymer support and deprotection of bases and internucleotidic bonds. This, again, is prerequisite to the trityl-on purifications described in a previous section (see Triarylmethyl Groups as Affinity Ligands). Horn and Urdea have described an alternative route where bases, internucleotidic bonds, and truncated chain ends are deprotected while the crude product—with DMTr on the 5' end of the full-length oligonucleotide—remains bound to the support. This allows the use of phosphodiesterase to selectively digest the truncated chains and, thus, further simplify the workup (Horn and Urdea, 1985).

Other capping alternatives result in an irreversible modification of the truncated chains. This applies to the application of highly reactive phosphite derivatives, for instance, to a mixture of diethoxy-*N,N*-diisopropylphosphoramidite with tetrazole (Yu et al., 1994), or to diethoxytetrazolophosphane, a stable tetrazole derivative of diethoxyphosphorous acid (Berner et al., 1989). Also, bis(1,1,1,3,3,3-hexafluoro-2-propyl)-2-propylphosphite, activated by *N*-methylimidazole, has been described as a capping reagent to blend with oligonucleotide syntheses using deoxyribonucleoside-3'-bis(1,1,1,3,3,3-hexafluoro-2-pro-

pyl)phosphite synthons (Hosaka et al., 1991). In these cases, an adjustment of workup and purification procedures may be required.

Recently, a capping procedure that leads to complete reversal of the removal of truncated chains has been described (Natt and Häner, 1997). In this case, capping is done using  $\beta$ -cyanoethoxy-(*n*-octyloxy)-phosphoramidite/tetrazole as a lipophilic phosphitylating reagent ("lipocap"). If the synthesis is conducted to generate a crude "trityl-off" product, reversed-phase high-performance liquid chromatography (RP-HPLC) will lead to a stronger retention of the more lipophilic truncated chains. The target sequence was eluted first and found to be recovered in higher quantity than through "trityl-on" purification.

In oligonucleotide syntheses with *H*-phosphonate intermediates, the average yields of chain elongation are slightly lower due to "self-capping" with the acyl chloride activating agent. Therefore, an additional capping step is generally unnecessary. Yet, in order to avoid further elongation of reactive truncated chain ends, especially in large-scale preparations, some authors have recommended a capping procedure with, for example, triethylammonium isopropyl phosphite (Andrus et al., 1988) or  $\beta$ -cyanoethyl-*H*-phosphonate (Gaffney and Jones, 1988).

## ENZYMATIC METHODS FOR 5'-HYDROXYL PROTECTION AND DEPROTECTION

Compared to the efficiency of automated chemical solid-phase preparation techniques, enzymatic methods play only a minor role in current oligonucleotide synthesis. This is all the more true for 5'-hydroxyl protection or deprotection methods, which are often considered more or less trivial steps in the elongation cycle. Nevertheless, there are some noteworthy arguments in favor of performing protection/deprotection steps via enzyme catalysis. Enzymatic reactions are regio- and stereospecific and often proceed with good yields and high selectivity. Such points are particularly important, for instance, for large-scale oligonucleotide synthesis, an area where enzymatic routes may be worth consideration.

The state of enzymatic protecting-group techniques for biomolecules has been reviewed by Waldmann and Sebastian (1994). A specialized survey of enzymatic oligonucleotide acylation/deacylation reactions has recently been published by Prasad and Wengel (1996). A summary of enzymatic reactions leading to the

introduction of 5'-protecting groups is given in Table 2.3.5. Essentially, two classes of enzymes are known to introduce acyl or alkoxy-carbonyl substituents at the 5'-hydroxy function of nucleosides, namely subtilisin and lipases. The best results, so far, were obtained with subtilisin 8350, an enzyme prepared from subtilisin via site-specific mutagenesis.

Catalysis of acetyl-group transfer from isopropenyl acetate in anhydrous DMF occurred selectively to the 5'-hydroxy group of various deoxyribo- or ribonucleosides in yields ranging from 65% to nearly 100% (Wong et al., 1990). Somewhat lower yields were found for acyl transfer from trichloro- or trifluoroethylbutyrate to ribonucleosides catalyzed by subtilisin in DMF or pyridine (Riva et al., 1988; Singh et al., 1994).

Lipases from various sources have alternatively been applied, but the yields are generally lower and the conditions must be chosen very carefully to ensure the introduction of acyl or alkoxy-carbonyl groups selectively or predominantly at the 5'-hydroxy position (Nozaki et al., 1990; Moris and Gotor, 1992a,b, 1993; Garcia-Alles et al., 1993; Garcia-Alles and Gotor, 1995; Ozaki et al., 1995).

Enzyme-catalyzed deacylations were of interest in early times of nucleotide chemistry, when an enzymatic approach to oligonucleotide chain lengthening seemed to be a feasible alternative to the still cumbersome methods of phosphodiester chemistry. The dihydrocinnamoyl protecting group received some attention at that time, because 3'-dihydrocinnamoyl-nucleoside-5'-diphosphates were dis-

covered as monoaddition substrates for polynucleotide phosphorylase (Kaufmann et al., 1971), and the same protecting group could be removed by chymotrypsin (Sachdev and Starkovsky, 1969; Taunton-Rigby, 1973). If, however, a 3',5'-disubstituted nucleoside derivative was the substrate, a distinct preference for the hydrolysis of the 5'-O-acyl group was demonstrated (Taunton-Rigby, 1973). The regioselective 5'-O-deacetylation of 2',3',5'-triacyl nucleosides was described by Singh et al. (1993).

In recent times, the tendency has been to reverse the action of the before-mentioned enzymes, subtilisin and lipase. Whereas lipase from *Pseudomonas fluorescens* has a preference for the removal of secondary O-acyl groups, subtilisin attacks first the acyl substituent on the 5'-hydroxy moiety (Uemura et al., 1989a,b). In both cases, however, the reaction can proceed to complete deprotection. Selective deprotection of 5'-O-acyl was found with lipase from porcine pancreas, which yielded 3'-O-acetylthymidine from the 3',5'-disubstituted nucleoside in 98% yield (Wong et al., 1990). Similarly, 2',3',5'-tri-O-acetylated pyrimidine and purine ribonucleosides (A, C, G, U, and modified bases) were selectively 5'-deprotected by subtilisin to give 2',3'-di-O-acyl nucleosides in 40% to 92% yield (Singh et al., 1993).

In general, it appears that the potential of enzymatic protection/deprotection reactions for the 5'-hydroxy function remains to be further exploited. The isolation of enzymes from different sources, as well the adaptation of enzyme specificity through mutagenesis, protein

**Table 2.3.5** Enzymatic Introduction of Protecting Groups at the 5'-Hydroxy Function

Acyl group transferred	Enzyme	Substrate acyl donor	Product	Reference
Butyryl	Subtilisin	Trichloroethylbutyrate in DMF	5'-O-Acyl predominant	Riva et al. (1988)
Butyryl	Subtilisin	Trifluoroethylbutyrate in pyridine	67%-82% 5'-O-acyl	Singh et al. (1994)
Acetyl	Subtilisin 8350	Isopropenyl acetate	80%-100% 5'-O-acyl	Wong et al. (1990)
Acyl	Lipase SP 435 ( <i>Candida antarctica</i> )	Alkane carbonyl oxime esters r(d)A, G, T, U	5'-O-Acyl	Moris and Gotor (1992a,b, 1993)
Alkoxy-carbonyl	Lipase SP 435 ( <i>Candida antarctica</i> )	Alkoxy carbonyl oxime esters r(d)A, G, T, U	5'-O-Alkoxy-carbonyl	Moris and Gotor (1992a,b, 1993)
Acyl	Lipase ( <i>Pseudomonas fluorescens</i> )	Pentanoic acid anhydride, preferably dC	31%-75% 5'-O-Acyl	Nozaki et al. (1990)



engineering, and in vitro evolution and selection techniques, may provide tools tailored to perform highly specific reactions and, thus, to match chemical methods for introduction and removal of protecting groups.

### **PROTECTION OF 5'-HYDROXY FUNCTIONS: REMAINING PROBLEMS, CONSIDERATIONS, AND OPTIONS**

Protection and deprotection of the 5'-hydroxy group remain essential operations for oligonucleotide synthesis, independent of the chemistry used for the formation of the internucleotidic bond. To many researchers involved in routine small-scale oligonucleotide synthesis, questions connected with 5'-hydroxyl-protecting groups appear to be solved, although the protection chemistry used in >95% of all syntheses is now 35 years old. Failures that arise through incomplete deprotection or capping during the elongation cycles have been discussed above. Many attempts have been made to minimize these failures through the design of new protecting groups for the 5' hydroxyl, nucleobases, or other functional groups of the oligonucleotide, or through the development of an altogether new orthogonal protection scheme. However, no method exists, so far, that allows detection and quantitative analysis of, for example, small numbers of depurination sites statistically distributed within a population of long-chain oligonucleotide molecules. Techniques still have to be developed that might help assess such homogeneity problems, which are of importance especially when oligonucleotides are produced in large numbers, large scale, or extended length.

Generally, more consideration should be given to the avoidance of acid deprotection steps. A particularly interesting new deprotection chemistry comes from the application of photolabile protecting groups. Considerable efforts are still necessary to establish quantitative and selective removal of such groups, and new developments are under way at several laboratories. For solid-phase bulk synthesis, other deprotection alternatives that use neither acid nor alkaline/ammoniacal media may have to be investigated. The availability of silyl protecting groups that can be released in <1 min by fluoride treatment at room temperature may find application beyond oligoribonucleotide synthesis. Recent years have seen rapid progress in the preparation of structurally modified oligonucleotides, among them structures highly sensitive to acid or base. These syntheses often

require a new kind of protection chemistry; such developments may feed back into routine preparations of unmodified oligonucleotides.

One of the reasons that trityl groups have been the first choice for several decades is that, beyond their application for the protection of the growing chain, they can be endowed by appropriate substitution with additional properties useful for oligonucleotide synthesis and purification. Examples—such as the monitoring of chain extension yields, the simplification of the separation of support products, or the activation of a 3'-phosphate moiety—have been discussed above. There will be a general interest to also endow non-acid-labile protecting groups with similar properties; examples are in the literature, but there are still options for new developments. Such projects may also yield new solutions to simple practical questions, e.g., the choice of nontoxic, environmentally unproblematic coupling and deprotection solvents.

The development of synthetic oligonucleotides that are ready to move into the drug market has led to the budding of a new field: the technology of industrial oligonucleotide production. In this frame, protection and deprotection steps are part of a series of unit operations that have to be carefully analyzed and optimized with respect to technical performance. In the previous sections, reference has occasionally been given to investigations that have been performed on this technical background, with the idea in mind that these investigations may well add new experience to small-scale routine synthesis as well. Future reviews may have to give broader attention to technical aspects as this field develops into a science of its own.

In this context, it is interesting to see that oligonucleotide synthesis in the past years has developed in the direction of ever new chemistry, while chemical production in general looks more and more to biotechnology as a tool to solve problems connected with the utilization of fossil carbon sources, environmental pollution, or waste disposal. The impact of such problems is already recognized and will be increasingly felt as oligonucleotide synthesis moves more towards bulk production. Enzymatic methods, which may be in the foreground of future developments, necessarily require a new approach to questions of protection and deprotection.

## LITERATURE CITED

- Adams, S.P., Kavka, K.S., Wykes, E.J., Holder, S.B., and Galluppi, G.R. 1983. Hindered dialkylamino nucleoside phosphite reagents in the synthesis of two DNA 51-mers. *J. Am. Chem. Soc.* 105:661-663.
- Andersen, W., Hayes, D.H., Michelson, A.M., and Todd, A.R. 1954. Deoxyribonucleosides and related compounds. Part IV. The configuration at the glycosidic centre in deoxyadenosine and deoxycytidine. *J. Chem. Soc.* 1882-1887.
- Andrus, A., Efcavitch, J.W., McBride, L.J., and Giusti, B. 1988. Novel activating and capping reagents for improved hydrogen-phosphonate DNA synthesis. *Tetrahedron Lett.* 29:861-864.
- Atkinson, T. and Smith, M. 1984. Solid-phase synthesis of oligodeoxyribonucleotides by the phosphate triester method. In *A Practical Approach to Oligonucleotide Synthesis* (M.J. Gait, ed.) pp. 35-81. IRL Press, Oxford.
- Balgobin, N. and Chattopadhyaya, J.B. 1982a. Two sulfur containing protecting groups for alcoholic hydroxyl function. *Chem. Scr.* 19:143-144.
- Balgobin, N. and Chattopadhyaya, J.B. 1982b. An efficient chemical synthesis of a biologically functional DNA molecule,  ${}^5\text{d}(\text{A-T-G-G-G-T-T-T-C-T-T-C-G-C})_3$ , through the phospho-triester approach. *Chem. Scr.* 20:133-138.
- Balgobin, N. and Chattopadhyaya, J. 1987. Solid phase synthesis of DNA under a non-depurinating condition with a base labile 5'-protecting group (Fmoc) using phosphiteamidite approach. *Nucleosides Nucleotides* 6:461-463.
- Balgobin, N., Josephson, S., and Chattopadhyaya, J.B. 1981a. The 2-phenylsulfonyl ethyl-carbonyl (PSEC) group for the protection of the hydroxyl function. *Tetrahedron Lett.* 22:3667-3670.
- Balgobin, N., Josephson, S., and Chattopadhyaya, J.B. 1981b. A general approach to the chemical synthesis of oligodeoxyribonucleotides. *Acta Chem. Scand. B* 35:201-212.
- Bannwarth, W. and Wippler, J. 1990. A new combined purification/phosphorylation procedure for oligodeoxynucleotides. *Helv. Chim. Acta* 73:1139-1147.
- Bazin, H., Heikkilä, J., and Chattopadhyaya, J. 1985. Some aspects of the reaction of arene-sulfonyl chlorides with hydroxyl functions of ribonucleosides. *Acta Chem. Scand. B* 39:391-400.
- Beaucage, S.L. and Iyer, R.P. 1992. Advances in the synthesis of oligonucleotides by the phosphoramidite approach. *Tetrahedron* 48:2223-2311.
- Beaucage, S.L. and Iyer, R.P. 1993. The synthesis of modified oligonucleotides by the phosphoramidite approach and their applications. *Tetrahedron* 49:6123-6194.
- Belagaje, R. and Brush, C.K. 1982. Polymer supported synthesis of oligonucleotides by a phosphotriester method. *Nucl. Acids Res.* 10:6295-6303.
- Bergmann, F. and Pfeleiderer, W. 1994a. The 2-dansylethoxycarbonyl (= 2-{{[5-(dimethylamino)naphthalen-1-yl]sulfonyl}ethoxycarbonyl; Dnseoc) group for protection of the 5'-hydroxy function in oligodeoxyribonucleotide synthesis. *Helv. Chim. Acta* 77:203-215.
- Bergmann, F. and Pfeleiderer, W. 1994b. The 2-dansylethoxycarbonyl (= 2-{{[5-(dimethylamino)naphthalen-1-yl]sulfonyl}ethoxycarbonyl; Dnseoc) group for protection of the 5'-hydroxy function in oligoribonucleotide synthesis. *Helv. Chim. Acta* 77:481-501.
- Bergmann, F. and Pfeleiderer, W. 1994c. Solid-phase synthesis of oligoribonucleotides using the 2-dansylethoxycarbonyl (= 2-{{[5-(dimethylamino)naphthalen-1-yl]sulfonyl}ethoxycarbonyl; Dnseoc) group for 5'-hydroxy protection. *Helv. Chim. Acta* 77:988-998.
- Berner, S., Mühlegger, K., and Seliger, H. 1989. Studies on the role of tetrazole in the activation of phosphoramidites. *Nucl. Acids Res.* 17:853-864.
- Bessodes, M., Komiotis, D., and Antonakis, K. 1986. Rapid and selective detritylation of primary alcohols using formic acid. *Tetrahedron Lett.* 27:579-580.
- Biernat, J., Wolter, A., and Köster, H. 1983. Purification oriented synthesis of oligodeoxynucleotides in solution. *Tetrahedron Lett.* 24:751-754.
- Birch-Hirschfeld, E., Weiss, R., Rosenthal, A., and Cech, D. 1983. Festphasensynthese von Oligodesoxyribonucleotiden an Polystyren-Teflon-Trägern nach der Diestermethode. *J. Prakt. Chem.* 325:133-142.
- Brown, J.M., Christodoulou, C., Reese, C.B., and Sindona, G. 1984. Two new protected acyl protecting groups for alcoholic hydroxy functions. *J. Chem. Soc. Perkin Trans. 1* 1785-1790.
- Brown, J.M., Christodoulou, C., Jones, S.S., Modak, A.S., Reese, C.B., Sibanda, S., and Ubasawa, A. 1989a. Synthesis of the 3'-terminal half of yeast alanine transfer ribonucleic acid (tRNA<sup>Ala</sup>) by the phosphotriester approach in solution. Part 1. Preparation of the nucleoside building blocks. *J. Chem. Soc. Perkin Trans. 1* 1735-1750.
- Brown, J.M., Christodoulou, C., Modak, A.S., Reese, C.B., and Serafinowska, H.T. 1989b. Synthesis of the 3'-terminal half of yeast alanine transfer ribonucleic acid (tRNA<sup>Ala</sup>) by the phosphotriester approach in solution. Part 2. *J. Chem. Soc. Perkin Trans. 1* 1751-1767.
- Bühler, S., Giegrich, H., and Pfeleiderer, W. 1999. New photolabile protecting groups of the 2-(2-nitrophenyl)ethoxycarbonyl and the 2-(2-nitrophenyl)ethylsulfonyl type for the oligonucleotide synthesis. *Nucleosides Nucleotides* 8:1281-1283.

- Caruthers, M.H. 1982. Chemical synthesis of oligodeoxynucleotides using the phosphite triester intermediates. *In* Chemical and Enzymatic Synthesis of Gene Fragments: A Laboratory Manual (H.G. Gassen and A. Lang, eds.) pp. 71-79. Verlag Chemie, Weinheim, Germany.
- Caruthers, M.H. 1998. Synthesis of DNA, RNA, and various new analogs on polymer supports. Lecture, 8th International Conference on Polymer-Based Technology, Ma'ale Hachamisha, Israel.
- Caruthers, M.H., Dellinger, D., and Prosser, K. 1986. Nucleic acid synthesis and applications to molecular biology. *Chem. Scr.* 26:25-30.
- Caruthers, M.H., Wyrzikiewicz, T., and Dellinger, D.J. 1994. Synthesis of oligonucleotides and oligonucleotide analogs on polymer supports. *In* Innovation and Perspectives in Solid Phase Synthesis (R. Epton, ed.) pp. 39-44. Mayflower, Birmingham, U.K.
- Chaix, C., Molko, D., and Teoule, R. 1989. The use of labile base protecting groups in oligoribonucleotide synthesis. *Tetrahedron Lett.* 30:71-74.
- Chattopadhyaya, J.B. 1980. Synthesis of adenylyl-(2'-5')-adenylyl-(2'-5')-adenosine (2-5A core). *Tetrahedron Lett.* 21:4113-4116.
- Chattopadhyaya, J.B. and Reese, C.B. 1978. The 9-phenylxanthen-9-yl protecting group. *J. Chem. Soc., Chem. Commun.* 639-640.
- Chattopadhyaya, J.B. and Reese, C.B. 1980. Chemical synthesis of a tridecanucleoside dodecaphosphate sequence of SV40 DNA. *Nucl. Acids Res.* 8:2039-2053.
- Chattopadhyaya, J.B., Reese, C.B., and Todd, A.H. 1979. 2-Dibromomethylbenzoyl: An acyl protecting group removable under exceptionally mild conditions. *J. Chem. Soc., Chem. Commun.* 987-988.
- Chaudhary, S.K. and Hernandez, O. 1979. 4-Dimethylaminopyridine: An efficient and selective catalyst for the silylation of alcohols. *Tetrahedron Lett.* 20:99-102.
- Christodoulou, C. and Reese, C.B. 1983. Dealkylation of nucleoside arylmethyl 2-chlorophenyl phosphates: The 2,4-dinitrobenzyl protecting group. *Tetrahedron Lett.* 24:951-954.
- Christodoulou, C., Agrawal, S., and Gait, M.J. 1987a. A new 5'-protecting group for use in the solid-phase synthesis of oligoribonucleotides. *Nucleosides Nucleotides* 6:341-344.
- Christodoulou, C., Agrawal, S., and Gait, M.J. 1987b. Progress towards solid-phase synthesis of oligoribonucleotides. *In* Biophosphates and Their Analogues—Synthesis, Structure, Metabolism and Activity (K.S. Bruzik and W.J. Stec, eds.) pp 99-106. Elsevier/North-Holland, Amsterdam.
- Corey, E.J., Gras, J.-L., and Ulrich, P. 1976. A new general method for the protection of the hydroxyl function. *Tetrahedron Lett.* 11:809-812.
- Dellinger, D.J., Wyrzkiewicz, T.K., Betley, J.R., and Caruthers, M.H. 1998. Solid-phase synthesis of oligodeoxyribonucleotides using phosphoramidite synthons in a two step synthesis cycle. XIIIth International Round Table: Nucleosides, Nucleotides and their Biological Applications, Montpellier. Poster 209.
- Engels, J. 1979. Selektive elektrochemische Schutzgruppenabspaltung in der Nucleotidsynthese. *Angew. Chem.* 91:155-156
- Fearon, K.L., Stults, J.T., Bergot, B.J., Christensen, L.M., and Raible, A.M. 1995. Investigation of the 'n-1' impurity in phosphorothioate oligodeoxynucleotides synthesized by the solid-phase  $\beta$ -cyanoethyl phosphoramidite method using stepwise sulfurization. *Nucl. Acids Res.* 23:2754-2761.
- Fersht, A.R. and Jencks, W.P. 1970. The acetylpyridinium ion intermediate in pyridine-catalyzed hydrolysis and acyl transfer reactions of acetic anhydride. Observation, kinetics, structure-reactivity correlations, and effects of concentrated salt solutions. *J. Am. Chem. Soc.* 92:5432-5442.
- Fisher, E.F. and Caruthers, M.H. 1983. Color coded triarylmethyl protecting groups useful for deoxypolynucleotide synthesis. *Nucl. Acids Res.* 11:1589-1599.
- Fodor, S.P.A., Read, J.L., Pirrung, M.C., Stryer, L., Lu, A.T., and Solas, D. 1991. Light-directed, spatially addressable parallel chemical synthesis. *Science* 251:767-773.
- Földes-Papp, Z., Birch-Hirschfeld, E., Eickhoff, H., Baumann, G., Peng, W.-G., Biber, T., Seydel, R., Kleinschmidt, A.K., and Seliger, H. 1996. Fractals for multicyclic synthesis conditions of biopolymers; examples of oligonucleotide synthesis measured by high-performance capillary electrophoresis and ion-exchange high-performance liquid chromatography. *J. Chromatogr.* 739:431-447.
- Földes-Papp, Z., Baumann, G., Birch-Hirschfeld, E., Eickhoff, H., Greulich, K.O., Kleinschmidt, A.K., and Seliger, H. 1998. The analysis of oligonucleotide preparations by fractal measures. *Biopolymers* 45:361-379.
- Fourrey, J.L., Varenne, J., Blonski, C., Dousset, P., and Shire, D. 1987. 1,1-Bis-(4-methoxyphenyl)-1'-pyrenyl methyl (bmpm): A new fluorescent 5' protecting group for the purification of unmodified and modified oligonucleotides. *Tetrahedron Lett.* 28:5157-5160.
- Fritz, H.-J., Frommer, W.-B., Kramer, W., and Werr, W. 1982. Simplified preparations of blocked 2'-deoxyribonucleosides as starting materials for chemical oligonucleotide synthesis. *In* Chemical and Enzymatic Synthesis of Gene Fragments: A Laboratory Manual (H.G. Gassen and A. Lang, eds.) pp. 43-52. Verlag Chemie, Weinheim, Germany.
- Fuentes, J., Cuevas, T., and Pradera, M.A. 1994. A mild and efficient detritylation of some carbohydrate derivatives. *Synth. Commun.* 24:2237-2245.

- Fukuda, T., Hamana, T., and Marumoto, R. 1988. Synthesis of RNA oligomer using 9-fluorenylmethoxycarbonyl (Fmoc) group for 5'-hydroxyl protection. *Nucl. Acids Res. Symp. Ser.* 19:13-16.
- Furukawa, H., Momota, K., Agatsuma, T., Yamamoto, I., Kimura, S., and Shimada, K. 1994. Mechanism of inhibition of HIV-1 infection in vitro by guanine-rich oligonucleotides modified at the 5' terminal by dimethoxytrityl residue. *Nucl. Acids Res.* 22:5621-5627.
- Gaffney, B.L. and Jones, R.A. 1982a. Synthesis of O-6-alkylated deoxyguanosine nucleosides. *Tetrahedron Lett.* 23:2253-2256.
- Gaffney, B.L. and Jones, R.A. 1982b. A new strategy for the protection of deoxyguanosine during oligonucleotide synthesis. *Tetrahedron Lett.* 23:2257-2260.
- Gaffney, B.L. and Jones, R.A. 1988. Large-scale oligonucleotide synthesis by the H-phosphonate method. *Tetrahedron Lett.* 29:2619-2622.
- Gaffney, B.L., Marky, L.A., and Jones, R.A. 1984. The influence of the purine 2-amino group on DNA conformation and stability. II. Synthesis and physical characterization of d(CGT(2-NH<sub>2</sub>)ACG), d(CGU(2-NH<sub>2</sub>)ACG), and d(CGT(2-NH<sub>2</sub>)AT(2-NH<sub>2</sub>)ACG). *Tetrahedron* 40:3-13.
- Gait, M.J., Matthes, H.W.D., Singh, M., Sproat, B.S., and Titmas, R.C. 1982. Synthesis of oligodeoxyribonucleotides by a continuous flow, phosphotriester method on a kieselgur/polyamide support. In *Chemical and Enzymatic Synthesis of Gene Fragments: A Laboratory Manual* (H.G. Gassen and A. Lang, eds.) pp. 1-42. Verlag Chemie, Weinheim, Germany.
- Garcia-Alles, L.F. and Gotor, V. 1995. Synthesis of 5'-O- and 3'-O-nucleoside carbamates. *Tetrahedron* 51:307-316.
- Garcia-Alles, L.F., Moris, F., and Gotor, V. 1993. Chemo-enzymatic synthesis of 2'-deoxynucleoside urethanes. *Tetrahedron Lett.* 34:6337-6338.
- Gildea, B.D., Coull, J.M., and Köster, H. 1990. A versatile acid-labile linker for modification of synthetic biomolecules. *Tetrahedron Lett.* 31:7095-7098.
- Gilham, P.T. and Khorana, H.G. 1958. Studies on Polynucleotides. I. A new and general method for the chemical synthesis of the C<sub>5</sub>'-C<sub>3</sub>' internucleotidic linkage. Syntheses of deoxyribo-dinucleotides. *J. Am. Chem. Soc.* 80:6212-6222.
- Gioeli, C. and Chattopadhyaya, J.B. 1982. The fluoren-9-ylmethoxycarbonyl group for the protection of hydroxy-groups; its application in the synthesis of an octathymidic acid fragment. *J. Chem. Soc. Chem. Commun.* 672-673.
- Gioeli, C., Balgobin, N., Josephson, S., and Chattopadhyaya, J.B. 1981. 2-(Trimethylsilyl)ethyl chloroformate: A convenient reagent for protection of hydroxyl function. *Tetrahedron Lett.* 22:969-972.
- Görtz, H.-H. and Seliger, H. 1981. New hydrophobic protecting groups for the chemical synthesis of oligonucleotides. *Angew. Chem., Int. Ed. Engl.* 20:681-683.
- Grams, G.W. and Letsinger, R.L. 1968. N<sup>6</sup>,3'-O-Disubstituted deoxyadenosine. *J. Org. Chem.* 33:2589-2590.
- Grams, G.W. and Letsinger, R.L. 1970. Synthesis of a diribonucleoside monophosphate by the β-cyanoethyl phosphotriester method. *J. Org. Chem.* 35:868-870.
- Guo, Z., Pfundheller, H.M., and Sanghvi, Y. 1998. A process for the capture and reuse of DMT group during manufacturing of oligonucleotides. Abstracts XIIIth International Round Table: Nucleosides, Nucleotides and their Biological Applications, Montpellier. Poster 194.
- Gupta, K.C., Gaur, R.K., and Sharma, P. 1991. Use of the 4-methoxy-4'-octyloxytrityl group as an affinity handle for the purification of synthetic oligonucleotides. *J. Chromatogr.* 541:341-348.
- Guzaev, A., Salo, H., Azhaye, A., and Lönnberg, H. 1995. A new approach for chemical phosphorylation of oligonucleotides at the 5'-terminus. *Tetrahedron* 51:9375-9384.
- Habus, I. and Agrawal, S. 1994. Improvement in the synthesis of oligonucleotides of extended length by modification of detritylation step. *Nucl. Acids Res.* 22:4350-4351
- Happ, E. and Scalfi-Happ, C. 1988. New trityl-based protecting groups with a mild two-step removal. *Nucleosides Nucleotides* 7:813-816.
- Hasan, A., Stengele, K.-P., Giegrich, H., Cornwell, P., Isham, K.R., Sachleben, R.A., Pfeleiderer, W., and Foote, R.S. 1997. Photolabile protecting groups for nucleosides: Synthesis and photodeprotection rates. *Tetrahedron* 53:4247-4264.
- Hirao, I., Koizumi, M., Ishido, Y., and Andrus, A. 1998. 1,1,3,3-Tetraisopropyl-3-(2-(triphenylmethoxy)ethoxy)disiloxane-1-yl group, a potential 5'-O-protecting group for solid-phase RNA synthesis. *Tetrahedron Lett.* 39:2989-2992.
- Horn, T. and Urdea, M.S. 1985. Enzymatic purification of chemically synthesized oligodeoxyribonucleotides prior to removal from a solid-support. *Nucl. Acids Res. Symp. Ser.* 16:153-156.
- Hosaka, H., Suzuki, Y., Gug-Kim, S., and Takaku, H. 1991. A convenient approach to the synthesis of medium size deoxyribooligonucleotides by improved new phosphite method. *Tetrahedron Lett.* 32:785-788.
- Hotoda, H., Momota, K., Furukawa, H., Nakamura, T., and Kaneko, M. 1994. Biologically active oligodeoxyribonucleotides. II. Structure activity relationships of anti-HIV-1 pentadecadeoxyribonucleotides bearing 5'-end-modifications. *Nucleosides Nucleotides* 13:1375-1395.
- Itakura, K., Rossi, J.J., and Wallace, R.B. 1984. Synthesis and use of synthetic oligonucleotides. *Annu. Rev. Biochem.* 53:323-356.

- Ito, H., Ike, Y., Ikuta, S., and Itakura, K. 1982. Solid phase synthesis of polynucleotides. VI. Further studies on polystyrene copolymers for the solid support. *Nucl. Acids Res.* 10:1755-1769.
- Iwai, S. and Ohtsuka, E. 1988. 5'-Levulinyl and 2'-tetrahydrofuranyl protection for the synthesis of oligoribonucleotides by the phosphoramidite approach. *Nucl. Acids Res.* 16:9443-9456.
- Iwai, S., Sasaki, T., and Ohtsuka, E. 1990. Large scale synthesis of oligoribonucleotides on a solid support: Synthesis of a catalytic RNA duplex. *Tetrahedron* 46:6673-6688.
- Iyer, R.P., Jiang, Z., Yu, D., Tan, W., and Agrawal, S. 1995. Improved procedure for the detritylation of DMT-oligonucleotides: Use of Dowex. *Synth. Commun.* 25:3611-3623.
- Josephson, S. and Chattopadhyaya, J.B. 1981. The application of the 2-phenylsulfonyl-ethyl-, a novel phosphate protecting group, in the synthesis of DNA fragments of defined sequences. *Chem. Scr.* 18:184-188.
- Kamaike, K., Takahashi, M., Utsugi, K., Tomizuka, K., and Ishido, Y. 1995. An efficient method for the synthesis of [4-<sup>15</sup>N]cytidine and [6-<sup>15</sup>N]adenosine derivatives from uridine and inosine. *Tetrahedron Lett.* 36:91-94.
- Kamaike, K., Takahashi, M., Utsugi, K., Tomizuka, K., Okazaki, Y., Tamada, Y., Kinoshita, K., Masuda, H., and Ishido, Y. 1996. An efficient method for the synthesis of [4-<sup>15</sup>N]cytidine, 2'-deoxy[4-<sup>15</sup>N]cytidine, [6-<sup>15</sup>N]adenosine, and 2'-deoxy[6-<sup>15</sup>N]adenosine derivatives. *Nucleosides Nucleotides* 15:749-769.
- Kamaike, K., Takahashi, H., Kakinuma, T., Morohoshi, K., and Ishido, Y. 1997. Oligonucleotide synthesis by the use of a 2-(levulinylloxymethyl)-5-nitrobenzoyl group as the novel base-labile protecting group for the 5'-hydroxyl groups of ribonucleoside and 2'-deoxyribonucleoside 3'-phosphoramidites. *Tetrahedron Lett.* 38:6857-6860.
- Karl, R.M., Klösel, R., König, S., Lehnhoff, S., and Ugi, I. 1995. 1,1-Dianisyl-2,2,2-trichloroethyl ethers—a new protection for the hydroxyl group. *Tetrahedron Lett.* 51:3759-3766.
- Karl, R.M., Richter, W., Klösel, R., Mayer, M., and Ugi, I. 1996. The 1,1-dianisyl-2,2,2-trichloroethyl moiety as a new protective group for the synthesis of dinucleoside trifluoromethylphosphonates. *Nucleosides Nucleotides* 15:379-386.
- Kaufmann, G., Fridkin, M., Zutra, A., and Littauer, U. 1971. Monofunctional substrates of polynucleotide phosphorylase. The monoaddition of 2'(3')-O-isovaleryl-nucleoside diphosphate to an initiator oligonucleotide. *Eur. J. Biochem.* 24:4-11.
- Kawashima, E., Aoyama, Y., Sekine, T., Miyahara, M., Radwan, M.F., Nakamura, E., Kainosho, M., Kyogoku, Y., and Ishido, Y. 1995. Sonochemical and triethylborane-induced tin deuteride reduction for the highly diastereoselective synthesis of (2'R)-2'-deoxy[2'-<sup>2</sup>H]ribonucleoside derivatives. *J. Org. Chem.* 60:6980-6986.
- Kawashima, E., Toyama, K., Ohshima, K., Kainosho, M., Kyogoku, Y., and Ishido, Y. 1997. Novel approach to diastereoselective synthesis of 2'-deoxy[5'-<sup>2</sup>H<sub>1</sub>]ribonucleoside derivatives by reduction of the corresponding 5'-O-acetyl-2'-deoxy-5'-phenylselenoribonucleoside derivatives with a Bu<sub>3</sub>Sn<sup>2</sup>H-Et<sub>3</sub>B system. *Chirality* 9:435-442.
- Kierzek, R., Ito, H., Bhatt, R., and Itakura, K. 1981. Selective N-deacylation of N,O-protected nucleosides by zinc bromide. *Tetrahedron Lett.* 22:3761-3764.
- Klösel, R., König, S., and Lehnhoff, S. 1996. The 1,1-dianisyl-2,2,2-trichloroethyl group as 2'-hydroxyl protection of ribonucleotides. *Tetrahedron* 52:1493-1502.
- Kohli, V., Blöcker, H., and Köster, H. 1980. The triphenylmethyl (trityl) group and its uses in nucleotide chemistry. *Tetrahedron Lett.* 21:2683-2686.
- Kössel, H. and Seliger, H. 1975. Recent advances in polynucleotide synthesis. *Fortschr. Chem. Org. Naturst.* 32:297-508.
- Köster, H. and Sinha, N.D. 1982. Dialkyl aluminium chloride: A reagent for removal of trityl group from trityl ethers of deoxynucleosides, deoxynucleotides, and oligodeoxynucleotides. *Tetrahedron Lett.* 23:2641-2644.
- Köster, H., Stumpe, A., and Wolter, A. 1983. Polymer support oligonucleotide synthesis 13: Rapid and efficient synthesis of oligodeoxynucleotides on porous glass support using triester approach. *Tetrahedron Lett.* 24:747-750.
- Köster, H., Biernat, J., McManus, J., Wolter, A., Stumpe, A., Narang, C.K., and Sinha, N.D. 1984. Polymer support oligonucleotide synthesis. XV. Synthesis of oligodeoxynucleotides on controlled pore glass (CPG) using phosphate and a new phosphite triester approach. *Tetrahedron* 40:103-112.
- Kotschi, U. 1987. Präparative und apparative Beiträge zur Festphasensynthese von Nucleinsäure-Fragmenten. Dissertation, University of Ulm, Germany.
- Krotz, A.H., Cole, D.L., and Ravikumar, V.T. 1999. Dimethoxytrityl removal in organic medium: Efficient oligonucleotide synthesis without chlorinated solvents. *Nucleosides Nucleotides* 18:1207-1209.

- Kwiatkowski, M. and Chattopadhyaya, J. 1984. The 9-(4-octadecyloxyphenyl-xanthen)-9-yl-group. A new acid-labile hydroxyl protective group and its application in the preparative reverse-phase chromatographic separation of oligoribonucleotides. *Acta Chem. Scand. B* 38:657-671.
- Kwiatkowski, M., Heikkilä, S., Björkman, S., and Chattopadhyaya, J.B. 1983. Chemical synthesis of an undecaribonucleoside decaphosphate constituting the 3'-terminal acceptor stem sequence of yeast tRNA<sup>Phe</sup>. *Chem. Scripta* 22:30-48.
- Lakshman, M.K. and Zajc, B. 1996. A rapid, high-yield method for 5'-hydroxyl protection in very reactive and amino group modified nucleosides using dimethoxytrityl tetrafluoroborate. *Nucleosides Nucleotides* 15:1029-1039.
- Lehmann, C., Xu, Y-Z., Christodoulou, C., Tan, Z.-K., and Gait, M.J. 1989. Solid-phase synthesis of oligoribonucleotides using 9-fluorenylmethoxycarbonyl (Fmoc) for 5'-hydroxyl protection. *Nucl. Acids Res.* 17:2379-2390.
- Leonard, N.J. and Neelima 1995. 1,1,1,3,3,3-Hexafluoro-2-propanol for the removal of the 4,4'-dimethoxytrityl protecting group from the 5'-hydroxyl of acid-sensitive nucleosides and nucleotides. *Tetrahedron Lett.* 36:7833-7836.
- Letsinger, R.L. and Finnan, J.L. 1975. Selective deprotection by reductive cleavage with radical anions. *J. Am. Chem. Soc.* 97:7197-7198.
- Letsinger, R.L. and Miller, P.S. 1969. Protecting groups for nucleosides used in synthesizing oligonucleotides. *J. Am. Chem. Soc.* 91:3356-3359.
- Letsinger, R.L. and Ogilvie, K.K. 1967. Use of p-nitrophenyl chloroformate in blocking hydroxyl groups in nucleosides. *J. Org. Chem.* 32:296-300.
- Letsinger, R.L., Fontaine, J., Mahadevan, V., Schexnayder, D.A., and Leone, R.E. 1964. 2,4-Dinitrobenzenesulfonyl as a blocking group for hydroxyl functions in nucleosides. *J. Org. Chem.* 29:2615-2618.
- Letsinger, R.L., Caruthers, M.H., Miller, P.S., and Ogilvie, K.K. 1967. Oligonucleotide syntheses utilizing  $\beta$ -benzoylpropionyl, a blocking group with a trigger for selective cleavage. *J. Am. Chem. Soc.* 89:7146-7147.
- Luzzio, F.A. and Menes, M.E. 1994. A facile route to pyrimidine-based nucleoside olefins: Application to the synthesis of d4T (stavudine). *J. Org. Chem.* 59:7267-7272.
- Ma, Y. and Sonveaux, E. 1987. The 9-fluorenylmethoxycarbonyl (Fmoc) group as a 5'-O base labile protecting group in solid supported oligonucleotide synthesis. *Nucleosides Nucleotides* 6:491-493.
- Ma, Y. and Sonveaux, E. 1989. The 9-fluorenylmethoxycarbonyl group as a 5'-OH protection in oligonucleotide synthesis. *Biopolymers* 28:965-973.
- Mairanovsky, V.G. 1976. Elektro-deblockierung—elektrochemische Abspaltung von Schutzgruppen. *Angew. Chem.* 9:283-294.
- Markiewicz, W.T. 1979. Tetraisopropylidisiloxane-1,3-diyl, a group for simultaneous protection of 3'- and 5'-hydroxy functions of nucleosides. *J. Chem. Res., Miniprint* 0181-0197.
- Markiewicz, W.T. 1980. The reaction of 1,3-dichloro-1,1,3,3-tetraisopropylidisiloxane with some open chain polyhydroxy compounds. *Tetrahedron Lett.* 21:4523-4524.
- Matsuda, A., Inada, M., Nara, H., Ohtsuka, E., and Ono, A. 1993. Nucleosides and nucleotides. 126. Incorporation of a mutagenic nucleoside, 5-formyl-2'-deoxyuridine, into an oligodeoxyribonucleotide. *Bioorg. Med. Chem. Lett.* 3:2751-2754.
- Matteucci, M.D. and Caruthers, M.H. 1980. The use of zinc bromide for removal of dimethoxytrityl ethers from deoxynucleotides. *Tetrahedron Lett.* 21:3243-3246.
- Matteucci, M.D. and Caruthers, M.H. 1981. Synthesis of deoxyoligonucleotides on a polymer support. *J. Am. Chem. Soc.* 103:3185-3191.
- McEldoon, W.L. and Wiemer, D.F. 1995. Synthesis of nucleoside 3'-phosphonates via 3'-keto nucleosides. *Tetrahedron* 51:7131-7148.
- McGall, G.H., Labadie, J., Brock, P., Wallraff, G., Nguyen, T., and Hinsberg, W. 1996. Light-directed synthesis of high-density oligonucleotide arrays using semiconductor photoresists. *Proc. Natl. Acad. Sci. U.S.A.* 93:13555-13560.
- McGall, G.H., Barone, A.D., Diggelmann, M., Fodor, S.P.A., Gentalen, E., and Ngo, N. 1997. The efficiency of light-directed synthesis of DNA arrays on glass substrates. *J. Am. Chem. Soc.* 119:5081-5090.
- Misetic, A. and Boyd, M.K. 1998. The pixyl (Px) group: A novel photocleavable protecting group for primary alcohols. *Tetrahedron Lett.* 39:1653-1656.
- Mitchell, M.J., Hirschowitz, W., Rastinejad, F., and Lu, P. 1990. Boron trifluoride-methanol complex as a non-depurinating detritylating agent in DNA synthesis. *Nucl. Acids. Res.* 18:5321.
- Moris, F. and Gotor, V. 1992a. A novel and convenient route to 3'-carbonates from unprotected 2'-deoxynucleosides through an enzymatic reaction. *J. Org. Chem.* 57:2490-2492.
- Moris, F. and Gotor, V. 1992b. Lipase-mediated alkoxyacylation of nucleosides with oxime carbonates. *Tetrahedron* 48:9869-9876.
- Moris, F. and Gotor, V. 1993. A useful and versatile procedure for the acylation of nucleosides through an enzymatic reaction. *J. Org. Chem.* 58:653-660.
- Mullah, B. and Andrus, A. 1996. Purification of 5'-O-trityl-on oligoribonucleotides. Investigation of phosphate migration during purification and detritylation. *Nucleosides Nucleotides* 15:419-430.
- Natt, F. and Häner, R. 1997. Lipocap: A lipophilic phosphoramidite-based capping reagent. *Tetrahedron* 53:9629-9636.

- Nozaki, K., Uemura, A., Yamashita, J., and Yasumoto, M. 1990. Enzymatic regioselective acylation of the 3'-hydroxyl groups of 2'-deoxy-5-fluorouridine (FUDR) and 2'-deoxy-5-trifluoromethyluridine (CF<sub>3</sub>UdR). *Tetrahedron Lett.* 31:7327-7328.
- Ogilvie, K.K. and Letsinger, R.L. 1967. Use of isobutyloxycarbonyl as a blocking group in preparation of 3'-O-p-monomethoxytritylthymidine. *J. Org. Chem.* 32:2365-2366.
- Ogilvie, K.K., Thompson, E.A., Quilliam, M.A., and Westmore, J.B. 1974. Selective protection of hydroxyl groups in deoxynucleosides using alkylsilyl reagents. *Tetrahedron Lett.* 33:2865-2868.
- Ogilvie, K.K., Usman, N., Nicoghossian, K., and Cedergren, R.J. 1988. Total chemical synthesis of a 77-nucleotide-long RNA sequence having methionine-acceptance. *Proc. Natl. Acad. Sci. U.S.A.* 85:5764-5768.
- Ohtsuka, E. and Iwai, S. 1987. Chemical synthesis of RNA. In *Synthesis and Application of DNA and RNA* (S.A. Narang, ed.) pp. 115-136. Academic Press, Orlando, Fla.
- Ohtsuka, E., Taniyama, Y., Iwai, S., Yoshida, T., and Ikehara, M. 1984. Deoxyribonucleic acids and related compounds. VIII. Solid-phase synthesis of deoxyribooligonucleotides with 3'-modification by elongation in the 3'-direction. *Chem. Pharm. Bull.* 32:85-93.
- Oikawa, Y., Yoshioka, T., and Yonemitsu, O. 1982. Specific removal of o-methoxybenzyl protection by DDQ oxidation. *Tetrahedron Lett.* 23:885-888.
- Ozaki, H., Nakamura, A., Arai, M., Ogawa, Y., and Sawai, H. 1994. Synthesis and property of oligodeoxyribonucleotide bearing 5-aminoalkyl-2'-deoxyuridine derivatives. *Nucl. Acids Res. Symp. Ser.* 31:49-50.
- Ozaki, S., Yamashita, K., Konishi, T., Maekawa, T., Eshima, M., Uemura, A., and Ling, L. 1995. Enzyme aided regioselective acylation of nucleosides. *Nucleosides Nucleotides* 14:401-404.
- Palom, Y., Alazzouzi, E., Gordillo, F., Grandas, A., and Pedroso, E. 1993. An acid-labile linker for solid-phase oligoribonucleotide synthesis using Fmoc group for 5'-hydroxyl protection. *Tetrahedron Lett.* 34:2195-2198.
- Patel, T.P., Millican, T.A., Bose, C.C., Titmas, R.C., Mock, G.A., and Eaton, M.A.W. 1982. Improvements to solid phase phosphotriester synthesis of deoxyoligonucleotides. *Nucl. Acids Res.* 18:5605-5620.
- Patil, S.V., Mane, R.B., and Salunkhe, M.M. 1994. A facile method for detritylation of 5'-O-dimethoxy-trityl-3'-O-tert-butyl dimethylsilyl-2'-deoxynucleosides. *Synth. Commun.* 24:2423-2428.
- Paul, C.H. and Royappa, A.T. 1996. Acid binding and detritylation during oligonucleotide synthesis. *Nucl. Acids Res.* 24:3048-3052.
- Pease, A.C., Solas, D., Sullivan, E.J., Cronin, M.T., Holmes, C.P., and Fodor, S.P.A. 1994. Light-generated oligonucleotide arrays for rapid DNA sequence analysis. *Proc. Natl. Acad. Sci. U.S.A.* 91:5022-5026.
- Pfleiderer, W., Stengele, K.P., Bergmann, F., Resmini, M., and Henke, C. 1995. How to synthesize a tRNA? *Nucleosides Nucleotides* 14:843-846.
- Pieken, W. 1997. Product anchored sequential synthesis: A novel process for the preparation of oligonucleotides. Abstracts, International Symposium on Nucleic Acids and Related Macromolecules, Ulm, Germany.
- Pirrung, M.C. and Bradley, J.-C. 1995a. Dimethoxybenzoin carbonates: Photochemically removable alcohol protecting groups suitable for phosphoramidite-based DNA synthesis. *J. Org. Chem.* 60:1116-1117.
- Pirrung, M.C. and Bradley, J.-C. 1995b. Comparison of methods for photochemical phosphoramidite-based DNA synthesis. *J. Org. Chem.* 60:6270-6276.
- Pirrung, M.C. and Lee, Y.R. 1993. Photochemically removable silyl protecting groups. *J. Org. Chem.* 58:6961-6963.
- Pirrung, M.C., Fallon, L., and McGall, G. 1998. Proofing of photolithographic DNA synthesis with 3',5'-dimethoxybenzoinyloxycarbonyl-protected deoxynucleoside phosphoramidites. *J. Org. Chem.* 63:241-246.
- Prasad, A.K. and Wengel, J. 1996. Enzyme-mediated protecting group chemistry on the hydroxyl groups of nucleosides. *Nucleosides Nucleotides* 15:1347-1359.
- Ramage, R. and Wahl, F.O. 1993. 4-(17-Tetrazabenz[a,c,g,i]fluorenylmethyl)-4',4'-dimethoxytrityl chloride: A hydrophobic 5'-protecting group for the separation of synthetic oligonucleotides. *Tetrahedron Lett.* 34:7133-7136.
- Reddy, M.P., Rampal, J.B., and Beaucage, S.L. 1987. An efficient procedure for the solid phase tritylation of nucleosides and nucleotides. *Tetrahedron Lett.* 28:23-26.
- Reese, C.B. 1978. The chemical synthesis of oligo- and polynucleotides by the phosphotriester approach. *Tetrahedron* 34:3143-3179.
- Reese, C.B. 1985. Some aspects of the chemical synthesis of oligoribonucleotides. *Nucleosides Nucleotides* 4:117-127.
- Reese, C.B. 1989. The chemical synthesis of oligo- and polyribonucleotides. In *Nucleic Acids and Molecular Biology*, Vol. 3 (F. Eckstein and D.M.J. Lilley, eds.) pp. 164-181. Springer-Verlag, Berlin.
- Reese, C.B. and Skone, P.A. 1984. The protection of thymine and guanine residues in oligodeoxyribonucleotide synthesis. *Chem. Soc. Perkin Trans. 1* 1263-1271.
- Reese, C.B. and Stewart, J.C.M. 1968. Methoxyacetyl as a protecting group in ribonucleoside chemistry. *Tetrahedron Lett.* 40:4273-4276.
- Reese, C.B. and Thompson, E.A. 1988. A new synthesis of 1-arylpiperidin-4-ols. *J. Chem. Soc. Perkin Trans. 1* 2881-2885.

- Reese, C.B., Serafinowska, H., and Zappia, G. 1986. An acetal group suitable for the protection of 2'-hydroxy functions in rapid oligoribonucleotide synthesis. *Tetrahedron Lett.* 27:2291-2294.
- Regel, W., Stengele, E., and Seliger, H. 1974. Kinetik der Schutzgruppenabspaltung an Nucleosiden mittels <sup>1</sup>H-NMR-Spektroskopie. *Chem. Ber.* 107:611-615.
- Reiner, T. and Pfeleiderer, W. 1987. β-Substituted ethylsulfonyle chlorides as 5'-OH protecting groups in nucleoside and nucleotide chemistry. *Nucl. Acids Res. Symp. Ser.* 18:161-164.
- Riva, S., Chopineau, J., Kieboom, A.P.G., and Klibanov, A.M. 1988. Protease-catalyzed regioselective esterification of sugars and related compounds in anhydrous dimethylformamide. *J. Am. Chem. Soc.* 110:584-589.
- Robins, M.J., Samano, V., and Johnson, M.D. 1990. Periodinane oxidation, selective primary deprotection, and remarkably stereoselective reduction of *tert*-butyldimethylsilyl-protected ribonucleosides. Synthesis of 9-(β-D-xylofuranosyl)adenine or 3'-deuterioadenosine from adenosine. *J. Org. Chem.* 55:410-412.
- Rosenthal, A., Cech, D., Veiko, V.P., Orezkaja, T.S., Kuprijanova, E.A., and Shabarova, Z.A. 1983. Triester-Festphasensynthese von Oligodesoxyribonucleotiden an Polystyren-Teflon Trägern. *Tetrahedron Lett.* 24:1691-1694.
- Rosowsky, A., Solan, V.C., Sodroski, J.G., and Ruprecht, R.M. 1989. Synthesis of the 2-chloro analogues of 3'-deoxyadenosine, 2',3'-dideoxyadenosine, and 2',3'-dideoxy-2',3'-dideoxyadenosine as potential antiviral agents. *J. Med. Chem.* 32:1135-1140.
- Sachdev, H.S. and Starkovsky, N.A. 1969. Enzymatic removal of acyl protecting groups. The use of dihydrocinnamoyl group in oligonucleotide synthesis and its cleavage by α-chymotrypsin. *Tetrahedron Lett.* 9:733-736.
- Sandström, A., Kwiatkowski, M., and Chattopadhyaya, J. 1985. Chemical synthesis of a pentaribonucleoside tetraphosphate constituting the 3'-acceptor stem sequence of *E. coli* tRNA<sup>Ile</sup> using 2'-O-(3-methoxy-1,5-dicarbomethoxypentan-3-yl)-ribonucleoside building blocks. Application of a new achiral and acid-labile 2'-hydroxyl protecting group in tRNA synthesis. *Acta Chem. Scand. B* 39:273-290.
- Scalfi-Happ, C., Happ, E., and Chládek, S. 1987. New approach to the synthesis of 2'(3')-O-aminoacyl-oligoribonucleotides related to the 3'-terminus of aminoacyl transfer ribonucleic acid. *Nucleosides Nucleotides* 6:345-348.
- Schaller, H., Weimann, G., Lerch, B., and Khorana, H.G. 1963. Studies on polynucleotides. XXIV. The stepwise synthesis of specific deoxyribosepolynucleotides (4). Protected derivatives of deoxyribonucleosides and new syntheses of deoxyribonucleoside-3'-phosphates. *J. Am. Chem. Soc.* 85:3821-3827.
- Schirmeister, H., Himmelsbach, F., and Pfeleiderer, W. 1993. The 2-(4-nitrophenyl)ethoxycarbonyl (npeoc) and 2-(2,4-dinitrophenyl)ethoxycarbonyl (dnpoc) groups for protection of hydroxy functions in ribonucleosides and 2'-deoxyribonucleosides. *Helv. Chim. Acta* 76:385-401.
- Schmidt, G., Schlenk, R., and Seliger, H. 1988. The 4-decyloxytrityl group as an aid in the affinity chromatography of synthetic oligonucleotides. *Nucleosides Nucleotides* 7:795-799.
- Sekine, M. 1993. Selective and rapid O-acylation of hydroxyl groups of nucleosides by means of phase transfer catalysis. *Nat. Prod. Lett.* 1:251-256.
- Sekine, M. 1994. A new method for removal of modified trityl and pixyl groups by use of an acid species generated by reaction of diethyl oxomalonate with methanol. *Nucleosides Nucleotides* 13:1397-1414.
- Sekine, M. and Hata, T. 1983. 4,4',4''-Tris(benzoyloxy)trityl as a new type of base-labile group for protection of primary hydroxyl groups. *J. Org. Chem.* 48:3011-3014.
- Sekine, M. and Hata, T. 1984. 4,4',4''-Tris(4,5-dichlorophthalimido)trityl: A new type of hydrazine-labile group as a protecting group of primary alcohols. *J. Am. Chem. Soc.* 106:5763-5764.
- Sekine, M. and Hata, T. 1985. 4,4',4''-Tris(levulinoyloxy)trityl as a new type of primary hydroxyl protecting group. *Bull. Chem. Soc. Jpn.* 58:336-339.
- Sekine, M. and Hata, T. 1986. Synthesis of short oligoribonucleotides bearing a 3'- or 5'-terminal phosphate by use of 4,4',4''-tris(4,5-dichlorophthalimido)trityl as a new 5'-hydroxyl protecting group. *J. Am. Chem. Soc.* 108:4581-4586.
- Sekine, M. and Hata, T. 1987. 3-(Imidazol-1-ylmethyl)-4',4''-dimethoxytrityl: A new functionalized 5'-hydroxyl protecting group capable of rapid internucleotidic bond formation in the phosphorothio ester approach. *J. Org. Chem.* 52:946-948.
- Sekine, M., Mori, T., and Wada, T. 1993. New 5'-hydroxyl protecting groups for rapid internucleotide bond formation. *Tetrahedron Lett.* 34:8289-8292.
- Seliger, H. 1972. Chlorameisensäureester von Nucleosiden—neue Zwischenprodukte für Synthesen mit Nucleinsäurebausteinen. *Tetrahedron Lett.* 39:4043-4046.
- Seliger, H. 1993. Scale-up of oligonucleotide synthesis. Solution phase. In *Methods in Molecular Biology*, Vol. 20: Protocols for Oligonucleotides and Analogs (S. Agrawal, ed.) pp. 391-435. Humana Press, Totowa, N.J.
- Seliger, H. and Görtz, H.-H. 1981. Specific separation of products in supported oligonucleotide syntheses using the triester method. *Angew. Chem., Int. Ed. Engl.* 20:683-684.
- Seliger, H. and Kotschi, U. 1985. The p-phenylazophenylloxycarbonyl protecting group: Selective deblocking and oligonucleotide synthesis avoiding acid steps. *Nucleosides Nucleotides* 4:153-155.



- Seliger, H. and Schmidt, G. 1987. Derivatization with the 4-decyloxytrityl group as an aid in the affinity chromatography of oligo- and polynucleotides. *J. Chromatogr.* 397:141-151.
- Seliger, H., Holupirek, M., and Görtz, H.-H. 1977a. Oligonucleotide support synthesis with affinity-chromatographic separation of the product. Abstracts, XXVIth IUPAC International Congress of Pure and Applied Chemistry, Tokyo. Abstract 265.
- Seliger, H., Holupirek, M., and Bach, T.C. 1977b. The lipoyl affinity group and its use in oligonucleotide synthesis. Commun. (Posters), Brit. Chem. Soc. Nucl. Group, 10th anniversary meeting, British Chem. Soc., Birmingham, U.K.
- Seliger, H., Holupirek, M., and Görtz, H.-H. 1978. Solid-phase oligonucleotide synthesis with affinity-chromatographic separation of the product. *Tetrahedron Lett.* 24:2115-2118.
- Seliger, H., Klein, S., Narang, C.K., Seemann-Preising, B., Eiband, J. and Hael, N. 1982. Solid-phase synthesis of oligonucleotides using the phosphite method. In *Chemical and Enzymatic Synthesis of Gene Fragments: A Laboratory Manual* (H.G. Gassen and A. Lang, eds.) pp. 81-96. Verlag Chemie, Weinheim, Germany.
- Seliger, H., Zeh, D., Azuru, G., and Chattopadhyaya, J.B. 1983. Two new and efficient routes to the preparation of oligoribonucleotides of defined sequence. *Chem. Scripta* 22:95-101.
- Seliger, H., Gupta, K.C., Kotschi, U., Spaney, T., and Zeh, D. 1986. New preparative methods in oligonucleotide chemistry and their application to gene synthesis. I. Improvements in the chemistry of solid-phase oligonucleotide synthesis. *Chem. Scr.* 26:561-567.
- Seliger, H., Schmidt, G., and Berner, S. 1987. Substituents with long alkyl chains as tools for the purification and immobilization of nucleic acids and their constituents. *Biol. Chem. Hoppe-Seyler* 368:773-774.
- Septak, M. 1996. Kinetic studies on depurination and detriylation of CPG-bound intermediates during oligonucleotide synthesis. *Nucl. Acids Res.* 24:3053-3058.
- Shabarova, Z.A. 1980. The application of solid phase method-synthesized oligodeoxyribonucleotides to molecular biology problems. *Nucl. Acids Res. Symp. Ser.* 7:259-279.
- Shimidzu, T. and Letsinger, R.L. 1968. Synthesis of deoxyguanylyldeoxyguanosine on an insoluble polymer support. *J. Org. Chem.* 33:708-711.
- Singh, H.K., Cote, G.L., and Sikorski, R.S. 1993. Enzymatic regioselective deacylation of 2',3',5'-tri-O-acylribonucleosides: Enzymatic synthesis of 2',3'-di-O-acylribonucleosides. *Tetrahedron Lett.* 34:5201-5204.
- Singh, H.K., Cote, G.L., and Hadfield, T.M. 1994. Manipulation of enzyme regioselectivity by solvent engineering: Enzymatic synthesis of 5'-O-acylribonucleosides. *Tetrahedron Lett.* 35:1353-1356.
- Sinha, N.D., Biernat, J., McManus, J., and Köster, H. 1984. Polymer support oligonucleotide synthesis. XVII. Use of  $\beta$ -cyanoethyl-N,N-dialkylamino/N-morpholino phosphoramidite of deoxynucleosides for the synthesis of DNA fragments simplifying deprotection and isolation of the final product. *Nucl. Acids Res.* 12:4539-4557.
- Smith, M., Rammler, D.H., Goldberg, I.H., and Khorana, H.G. 1962. Studies on polynucleotides. XIV. Specific synthesis of the C3'-C5' interribonucleotide linkage. Syntheses of uridylyl-(3'→5')-uridine and uridylyl-(3'→5')-adenosine. *J. Am. Chem. Soc.* 84:430-440.
- Sonveaux, E. 1986. The organic chemistry underlying DNA synthesis. *Bioorg. Chem.* 14:271-325.
- Sproat, B.S. and Gait, M.J. 1984. Solid-phase synthesis of oligodeoxyribonucleotides by the phosphotriester method. In *Oligonucleotide Synthesis: A Practical Approach* (M.J. Gait, ed.) pp. 83-115. IRL Press, Oxford.
- Takaku, H., Morita, K., and Sumiuchi, T. 1983. Selective removal of terminal dimethoxytrityl groups. *Chem. Lett.* 1661-1664.
- Tanaka, T. and Oishi, T. 1985. Chemical synthesis of deoxyribonucleotides containing deoxyadenosine at the 3'-end on a polystyrene polymer support. *Chem. Pharm. Bull.* 33:5178-5183.
- Tanimura, H. and Imada, T. 1990. Practical chemical synthesis of RNA fragments. Improvements in the preparation of ribonucleoside phosphoramidite units. *Chem. Lett.* 1715-1718.
- Tanimura, H., Fukazawa, T., Sekine, M., Hata, T., Efcavitch, J.W., and Zon, G. 1988. The practical synthesis of RNA fragments in the solid phase approach. *Tetrahedron Lett.* 29:577-578.
- Tanimura, H., Maeda, M., Fukazawa, T., Sekine, M., and Hata, T. 1989. Chemical synthesis of the 24 RNA fragments corresponding to hop stunt viroid. *Nucl. Acids Res.* 17:8135-8147.
- Taunton-Rigby, A. 1973. Oligonucleotide synthesis. III. Enzymatically removable acyl protecting groups. *J. Org. Chem.* 38:977-985.
- Taunton-Rigby, A., Kim, Y.-H., Crosscup, C.J., and Starkovsky, N.A. 1972. Oligonucleotide synthesis. II. The use of substituted trityl groups. *J. Org. Chem.* 37:956-964.
- Temsamani, J., Kubert, M., and Agrawal, S. 1995. Sequence identity of the n-1 product of a synthetic oligonucleotide. *Nucl. Acids Res.* 23:1841-1844.
- Ti, G.S., Gaffney, B.L., and Jones, R.A. 1982. Transient protection: Efficient one-flask syntheses of protected deoxynucleosides. *J. Am. Chem. Soc.* 104:1316-1319.
- Tong, G., Lawlor, J.M., Tregear, G., and Haralambidis, J. 1993. The synthesis of oligonucleotide-polyamide conjugate molecules suitable as PCR primers. *J. Org. Chem.* 58:2223-2231.
- Uemura, A., Nozaki, K., Yamashita, J., and Yasumoto, M. 1989a. Lipase-catalyzed regioselective acylation of sugar moieties of nucleosides. *Tetrahedron Lett.* 30:3817-3818.

- Uemura, A., Nozaki, K., Yamashita, J., and Yasumoto, M. 1989b. Regioselective deprotection of 3',5'-O-acylated pyrimidine nucleosides by lipase and esterase. *Tetrahedron Lett.* 30:3819-3820.
- Usman, N., Ogilvie, K.K., Jiang, M.-Y., and Cedergren, R.J. 1987. Automated chemical synthesis of long oligoribonucleotides using 2'-O-silylated ribonucleoside 3'-O-phosphoramidites on a controlled-pore glass support: Synthesis of a 43-nucleotide sequence similar to the 3'-half molecule of an *Escheria coli* formylmethionine tRNA. *J. Am. Chem. Soc.* 109:7845-7854.
- van Boom, J.H. and Burgers, P.M.J. 1976. Use of levulinic acid in the protection of oligonucleotides via the modified phosphotriester method; synthesis of decaribonucleotide U-A-U-A-U-A-U-A-U-A. *Tetrahedron Lett.* 52:4875-4878.
- van Boom, J.H. and Wreesmann, C.T.J. 1984. Chemical synthesis of small oligoribonucleotides in solution. In *Oligonucleotide Synthesis: A Practical Approach* (M.J. Gait, ed.) pp. 153-183. IRL Press, Oxford.
- Wada, T., Mochizuki, A., Sato, Y., and Sekine, M. 1998a. Functionalisation of solid supports with *N*-unprotected deoxyribonucleotides. *Tetrahedron Lett.* 39:5593-5596.
- Wada, T., Naotake, K., Mori, T., and Sekine M. 1998b. Stereocontrolled synthesis of dithymidine phosphorothioates by use of a functionalized 5'-protecting group bearing an imidazole residue. *Nucleosides Nucleotides* 17:351-364.
- Waldmann, H. and Sebastian, D. 1994. Enzymatic protecting group techniques. *Chem. Rev.* 94:911-937.
- Waldmeier, F., de Bernardini, S., Leach, C.A., and Tamm, C. 1982. 246. Nucleosides and Nucleotides. Part 19. On detritylation with zinc bromide in oligonucleotide synthesis. *Helv. Chim. Acta* 65:2472-2475.
- Wallraff, G., Labadie, J., Brock, P., Di Pietro, R., Nguyen, T., Huynh, T., Hinsberg, W., and McGall, G. 1997. DNA sequencing on a chip. *CHEMTECH* 22-32.
- Weiler, J. and Pfeleiderer, W. 1995. An improved method for large scale synthesis of oligonucleotides applying the NPE/NPEOC-strategy. *Nucleosides Nucleotides* 14:917-920.
- Weiss, R., Birch-Hirschfeld, E., and Witkowski, W. 1984. Comparative study of the synthesis of oligodeoxynucleotides achieved by the phosphotriester method on various supports. *Nucl. Acids Res. Symp. Ser.* 14:313-314.
- Welch, C.J., Zhou, X.-X., and Chattopadhyaya, J. 1986. Synthesis of an mRNA fragment of alanyl-tRNA synthetase gene in *Escherichia coli* using the 6-methyl-3-pyridyl group for protection of the imide functions of uridine and guanosine. *Acta Chem. Scand. B* 40:817-825.
- Winnacker, E.-L. and Dörper, T. 1982. Solid-phase synthesis of oligonucleotides using the phosphoramidite method. In *Chemical and Enzymatic Synthesis of Gene Fragments: A Laboratory Manual* (H.G. Gassen and A. Lang, eds.) pp. 97-102. Verlag Chemie, Weinheim, Germany.
- Wong, C.-H., Chen, S.-T., Hennen, W.J., Bibbs, J.A., Wang, Y.-F., Liu, J.L.-C., Pantoliano, M.W., Whitlow, M., and Bryan, P.N. 1990. Enzymes in organic synthesis: Use of subtilisin and a highly stable mutant derived from multiple site-specific mutations. *J. Am. Chem. Soc.* 112:945-953.
- Yamakage, S., Sakatsume, O., Furuyama, E., and Takaku, H. 1989. 1-(2-Chloroethoxy)ethyl group for the protection of 2'-hydroxyl group in the synthesis of oligoribonucleotides. *Tetrahedron Lett.* 30:6361-6364.
- Yu, D., Tang, J., Iyer, R.P., and Agrawal, S. 1994. Diethoxy-N,N-diisopropyl phosphoramidite as an improved capping reagent in the synthesis of oligonucleotides using phosphoramidite chemistry. *Tetrahedron Lett.* 35:8565-8568.

---

Contributed by H. Seliger  
University of Ulm  
Ulm, Germany