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2-Benzamidoethyl Group − A Novel Type of Phosphate Protecting Group for Oligonucleotide Synthesis

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Abstract: A number of 5′-*O*-(4,4′-dimethoxytrityl)thymidine *N*,*N*-diisopropylamino phosphoramidites protected at P(III) with derivatives of 2-benzamidoethanol were synthesized and incorporated into synthetic oligonucleotides. Depending on substitution patterns at the alkyl chain, amido group, and phenyl ring, the time required for removal of these protecting groups using concentrated ammonium hydroxide varied from 48 h at 55 °C to 25 min at 25 °C. Of the 11 groups studied, 2-[*N*-isopropyl-*N*- (4-methoxybenzoyl)amino]ethyl- (**H**) and *ω*-(thionobenzoylamino)alkyl protections (**I** and **K**) were most easily removed. Derivatives of the 2-[*N*-methyl-*N*-benzoylamino]ethyl group (**E**−**G**) demonstrated moderate stability, but those of the 2-(*N*-benzoylamino)-ethyl group (**A**−**C**) were the most stable. For the most reactive group, **H**, a phosphitylating reagent, bisamidite **60**, was synthesized and used in the preparation of four deoxynucleoside phosphoramidites **28** and **65**−**67**, plus the 2′-*O*-(2-methoxyethyl)-5-methyluridine phosphoramidite **68**. All of these novel building blocks were successfully tested in the preparation of natural, 20-mer oligonucleotides and their phosphorothioate analogues. With the model phosphotriester **37**, the mechanism of deprotection was studied and revealed, in the case of group **H**, a pH-independent formation of the 2-oxazolinium cation **47**. Under aqueous conditions, **47** gave **54**, which in turn was converted in the presence of ammonia to a number of identified products. It is important to note that none of the products formed was reactive toward the oligonucleotide backbone or nucleic bases. Thus, a general strategy for protection of internucleosidic phosphodiester groups is described, which may also find application in synthetic organic chemistry of phosphorus(III) and (V).

Introduction

For the past three decades, oligonucleotide synthesis on solid support has made tremendous progress from the preparation of milligram quantities using a manual, labor-intensive procedure[1] to the manufacturing of the first antisense drug, an anti-*CMV* 21-mer 2′-deoxyoligonucleotide phosphorothioate, on kilogram scale.

Currently, oligonucleotide synthesis on solid support employs a phosphoramidite method[2] and is carried out automatically by stepwise coupling of nucleoside building blocks. This revolutionary approach allows an efficient preparation of DNA and RNA fragments,[3] as well as many types of modified oligonucleotides,[4] on a routine basis. Phosphoramidite building blocks are most often protected at the phosphite moiety by a 2-cyanoethyl group.[5] Final deprotection of the oligonucleotide with ammonia effects *β*-elimination in the 2-cyanoethyl group, thus

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(1) (a) Atkinson, T.; Smith, M. In *Oligonucleotide Synthesis: A Practical Approach*; Gait, M. J., Ed.; Oxford University Press: Oxford, New York, Tokyo, 1984; pp 35−81. (b) Sproat, B. S.; Gait, M. J. In *Oligonucleotide Synthesis: A Practical Approach*; Gait, M. J., Ed.; Oxford University Press: Oxford, New York, Tokyo, 1984; pp 83−115.

(2) (a) Matteucci, M. D.; Caruthers, M. H. *J. Am. Chem. Soc.* **1981**, *103*, 3185−3191. (b) Beaucage, S. L.; Caruthers, M. H. *Tetrahedron Lett.* **1981**, *22*, 1859−1862.

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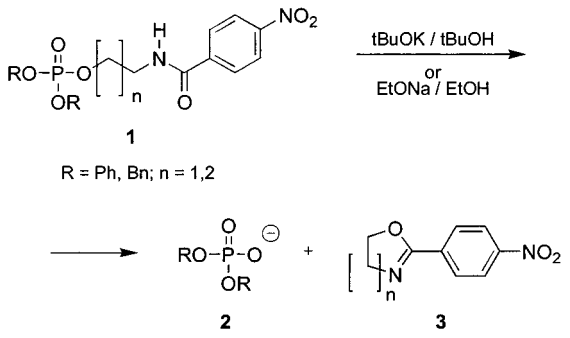
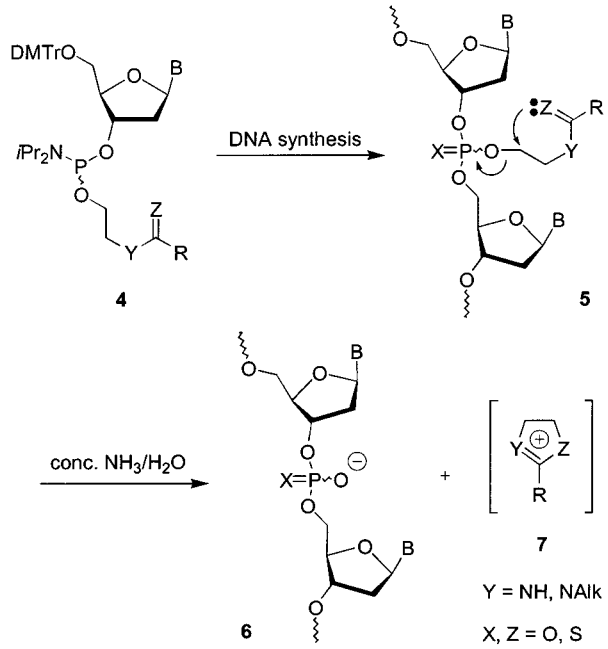
releasing an internucleosidic phosphodiester moiety and acrylonitrile, which is toxic and a potential carcinogen, as a side product. In addition, acrylonitrile has been demonstrated to alkylate nucleic bases under conditions of large-scale oligonucleotide deprotection.[6]

Recent interest in manufacturing oligonucleotides as drugs led to a search for protecting groups without the drawbacks associated with the 2-cyanoethyl and related groups. Of the base labile protections whose removal is governed by mechanisms different from β-elimination, several are worthy of note. Among them, the 2-trialkylsilylethyl protecting group is removed by β-fragmentation, yielding only the harmless products ethylene and trialkylsilanol.[7,8] Recently, ω-(trifluoroacetylamino)alkyl protection has been introduced. It is cleaved by aqueous or gaseous ammonia in two steps. First, the base labile trifluoroacetyl group is removed to yield the ω-aminoalkyl phosphate triester, which in the second step undergoes cyclodeesterification. This releases the final products, the internucleosidic phosphodiester and an azacycloalkane.[6,9,10] Allyl phosphate protection is removed either by aqueous ammonia or, under orthogonal conditions, by treatment with a Pd^0 complex in the presence of a nucleophile.[11] However, there is no known protecting strategy that allows one to control the kinetics of deprotection of internucleosidic phosphates over a wide range. Having such a methodology would allow protecting groups to be chosen in accordance with anticipated deprotection conditions and vice versa.



In our search for a more general methodology for protection of internucleosidic phosphates, we reported recently the use of 2-[(1-naphthyl)carbamoyloxy]ethyl protecting group in DNA synthesis.[12] Now we turned our attention to a reported intramolecular nucleophilic substitution in **1** where 2-oxazoline **3** was formed by releasing phosphodiester **2** as a leaving group.[13] Similarly, solvolysis of thioureido analogues of **1** gave 2-thiazolines and phosphate esters or inorganic phosphate.[14] Similar reactions with more traditional leaving groups, such as halogens, and methyl- or toluenesulfonate, have been studied in detail.[15]

Scheme 1



To the best of our knowledge, use of *N*-acylaminoethyl-protected nucleoside phosphoramidites **4** (Scheme 1) has not been described in the literature. Nevertheless, oligonucleotides similar to **5** have recently been synthesized by different routes via cyclic phosphoramidites.[16,17] When exposed to aqueous ammonium hydroxide, all of them gave deprotected oligonucleotides **6,** although this required a prolonged treatment at elevated temperature. However, structural optimization of the protecting group with respect to deprotection kinetics has not been attempted; nor have the products derived from the protecting groups been identified.

On the basis of these data, we aimed our study at the *N*-acylaminoethyl moieties as protecting groups for internucleosidic phosphates. To this end, we synthesized a number of novel

(3) (a) Caruthers, M. H. *Acc. Chem. Res.* **1991**, *24*, 278−284. (b) Beaucage, S. L.; Iyer, R. P. *Tetrahedron* **1992**, *48*, 2223−2311.

(4) (a) Manoharan, M. In *Antisense Research and Applications*; Crooke, S. T., Lebleu, B. Eds.; CRC Press: Boca Raton, FL, 1993; pp 303−349. (b) Beaucage, S. L.; Iyer, R. P. *Tetrahedron* **1993**, *49*, 1925−1963.

(5) (a) Sinha, N. D.; Biernat, J.; Köster, H. *Tetrahedron Lett.* **1983**, *24*, 5843−5846. (b) Sinha, N. D.; Biernat, J.; McManus, J.; Köster, H. *Nucleic Acids Res.* **1984**, *12*, 4539−4557.

(6) Wilk, A.; Grajkowski, A.; Phillips, L. R.; Beaucage, S. L. *J. Org. Chem.* **1999**, *64*, 7515−7522.

(7) (a) Krotz, A. H.; Wheeler, P.; Ravikumar, V. T. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 2406−2409. (b) Ravikumar, V. T. *Synth. Commun.* **1995**, *25*, 2161−2164. (c) Ravikumar, V. T.; Cole, D. L. *Gene* **1994**, *149*, 157−161. (d) Ravikumar, V. T.; Wyrzykiewicz, T. K.; Cole, D. L. *Tetrahedron* **1994**, *50*, 9255−9266. (e) Ravikumar, V. T.; Sasmor, H.; Cole, D. L. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 2637−2640.

(8) Wada, T.; Sekine, M. *Tetrahedron Lett* **1994**, *35*, 757−760.

(9) Cheruvallath, Z. S.; Capaldi, D. C.; Ravikumar, V. T.; Cole, D. L. U.S. Patent 5,760,209 1998; *Chem. Abstr. 129*: 41382.

(10) Wilk, A.; Srinivasachar, K.; Beaucage, S. L. *J. Org. Chem.* **1997**, *62*, 6712−6713.

(11) (a) Bergmann, F.; Kueng, E.; Iaiza, P.; Bannwarth, W. *Tetrahedron* **1995**, *25*, 6971−6976. (b) Hayakawa, Y.; Hirose, M.; Hayakawa, M.; Noyori, R. *J. Org. Chem.* **1995**, *60*, 925−930. (c) Hayakawa, Y.; Wakabayashi, S.; Kato, H.; Noyori, R. *J. Am. Chem. Soc.* **1990**, *112*, 1691−1696. (d) Hayakawa, Y.; Uchiyama, M.; Kato, H.; Noyori, R. *Tetrahedron Lett.* **1985**, *26*, 6505−6508. (e) Bogdan, F.; Chow, C. S. *Tetrahedron Lett.* **1998**, *39*, 1897−1900. (f) Manoharan, M.; Lu, Y.; Casper, M. D.; Just, G. *Org. Lett.* **2000**, *2*, 243−246.

(12) Guzaev, A. P.; Manoharan, M. *Tetrahedron Lett.* **2000**, *41*, 5623−5626.

(13) Ziodrou, C.; Schmir, G. L. *J. Am. Chem. Soc.* **1963**, *85*, 3258−3264.

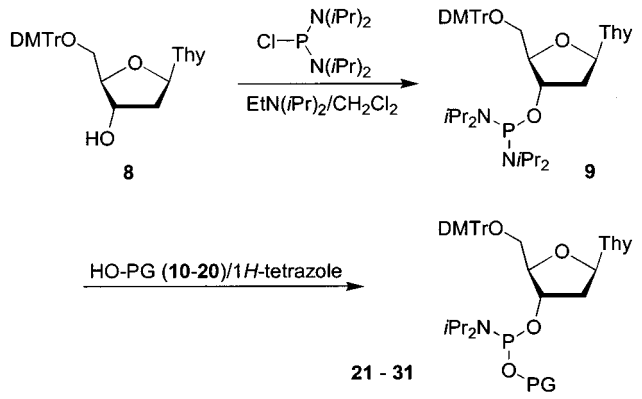
(14) (a) Cherbuliez, E.; Espejo, O.; Willhalm, B.; Rabinowitz, J. *Helv. Chim. Acta* **1968**, *51*, 241−248. (b) Cherbuliez, E.; Willhalm, B.; Espejo, O.; Jaccard, S.; Rabinowitz, J. *Helv. Chim. Acta* **1967**, *50*, 1440−1452. (c) Cherbuliez, E.; Moll, H.; Baehler, B.; Rabinowitz, J. *Helv. Chim. Acta* **1967**, *50*, 1154−1158. (d) Cherbuliez, E.; Baehler, B.; Espejo, O.; Jaccard, S.; Jindra, H.; Rabinowitz, J. *Helv. Chim. Acta* **1966**, *49*, 2408−2415.

(15) For a comprehensive review, see: (a) Lambert, J. B.; Mark, H. W.; Holcomb, A. G.; Magyar, E. S. *Acc. Chem. Res.* **1979**, *12*, 317−324. (b) Capon, B.; McManus, S. P. *Neighboring Group Participation*; Plenum Press: New York, 1976; Vol. 1; p 280. (c) Capon, B. In *Proton-Transfer Reactions*; Caldin, E., Gold, V., Eds.; Chapman & Hall: London, 1975; pp 339−384. (d) Page, M. I. *Chem. Soc. Rev.* **1973**, *2*, 295−323. (e) Goodman, L. *Adv. Carbohydr. Biochem.* **1967**, *22*, 109−175. (f) Capon, B. *Quart. Rev.* **1964**, *18*, 45−111.

(16) (a) Iyer, R. P.; Yu, D.; Devlin, T.; Ho, N.-H.; Agrawal, S. *J. Org. Chem.* **1995**, *60*, 5388−5389. (b) Guo, M. J.; Yu, D.; Iyer, R. P.; Agrawal, S. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2539−2544.

(17) Wilk, A.; Grajkowski, A.; Phillips, L. R.; Beaucage, S. L. *J. Am. Chem. Soc.* **2000**, *122*, 2149−2156.

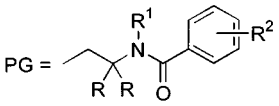
Scheme 2



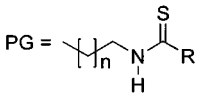
phosphoramidites **4**. These were incorporated into the synthetic oligonucleotides **5** in which Y, Z, and R may vary to form amido, thioamido, or thioureido groups that are capable of efficient intramolecular nucleophilic assistance (Scheme 1). We hypothesized that, once introduced into a synthetic oligonucleotide **5**, these groups might subsequently attack an electrophilic center, either phosphorus or carbon in the P−O−C fragment. If, as with phosphotriester **1**,[13] the nucleophilic attack occurred at the carbon atom, a cyclic intermediate, **7**, would be formed, and the internucleosidic phosphate would serve as a leaving group. In this event, the deprotection of an oligonucleotide backbone would occur and yield **6**. To test this hypothesis, deprotection of **5** with aqueous ammonia was studied. The most promising phosphoramidites, **28** and **65**−**68** (Scheme 7) were successfully employed in standard oligonucleotide synthesis for the preparation of natural oligonucleotides and their antisense phosphorothioate analogues. To illustrate the mechanism of deprotection, the intermediate **47** and stable products formed in the course of the reaction were characterized (Scheme 6). Here we report the results of this study.

Results and Discussion

Synthesis and Evaluation of Thymidine Phosphoramidites Protected by 2-Benzamidoethyl, ω-(Thionobenzoylamino)-alkyl, and 2-(*N*-Phenylthiocarbamoylamino) Ethyl Groups at P(III). Of the many structural motifs capable of an intramolecular nucleophilic cyclization,[15] we chose to study the utility of 2-benzamidoethyl (**A**−**H**), ω− (thionobenzoylamino)alkyl (**I** and **K**), and the 2-(*N*-phenylthiocarbamoylamino)ethyl (**J**) groups (Scheme 2). Several features justify their potential advantage for phosphate protection. First, under basic conditions, the carbonyl function in these systems is known to provide remarkable anchimeric assistance.[15f] On the other hand, in neutral and acidic media, **A**−**K** are chemically inert groups, with no apparent reactivity toward phosphitylating and acylating reagents that are employed in standard oligonucleotide synthesis. Finally, to introduce **A**−**K** at the phosphite moiety of a nucleoside phosphoramidite, the alcohols **10**−**20** required as starting materials are stable compounds which are commercially available or which may be readily synthesized with great structural diversity. For the phosphoramidite synthesis, **8** was first converted to a bisamidite, **9** (Scheme 2).[18] Without isolation, **9** was reacted with **10**−**20** in the presence of 1*H*-tetrazole to give thymidine phosphoramidites **21**−**31**. These were chromatographed on a silica gel column to allow purification and, except for **24**, isolation of pure (*R*)*p*- and (*S*)*p*-isomers of the



PG	HO-PG	Phosphoramidite	R	R^1	R^2	Yield, %
A	**10**	**21**	H	H	H	75.6
B	**11**	**22**	H	H	4-OMe	68.8
C	**12**	**23**	H	H	3-NO_2	61.0
D	**13**	**24**	Me	H	H	80.5
E	**14**	**25**	H	Me	H	78.9
F	**15**	**26**	H	Me	4-OMe	82.1
G	**16**	**27**	H	Me	4-NMe_2	86.0
H	**17**	**28**	H	*i*Pr	4-OMe	88.0



PG	HO-PG	Phosphoramidite	n	R	Yield, %
I	**18**	**29**	1	Ph	73.4
J	**19**	**30**	1	NHPh	70.5
K	**20**	**31**	2	Ph	70.5

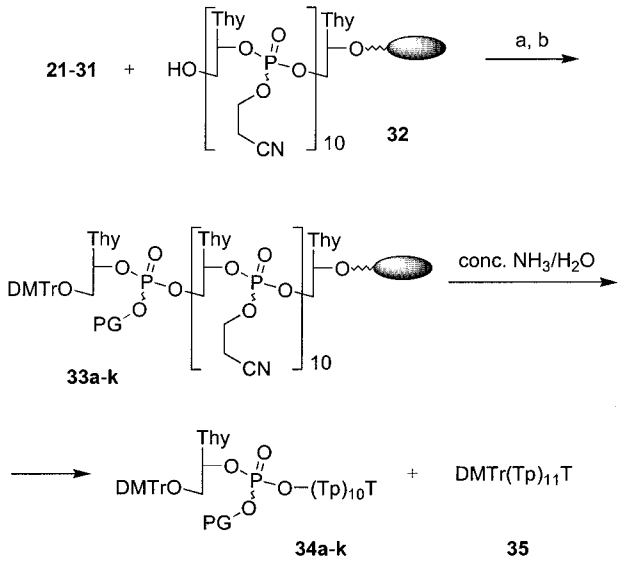
phosphoramidites, along with the isomeric mixture. Because individual (*R*)*p*- and (*S*)*p*-isomers are of limited use in oligonucleotide chemistry,[19] assignment of the absolute configuration of **21**−**31** at the P(III) was not performed. Instead, the individual species were assigned as fast- and slow-eluting isomers in accordance with their chromatographic mobility on silica gel. Nevertheless, the availability of pure (*R*)*p*- and (*S*)*p*-isomers was of paramount importance for spectral characterization of **21**−**31** by NMR. In general, phosphoramidites exhibit rather complex ^{1}H NMR and ^{13}C NMR spectra, due to ^{1}H and ^{13}C long-distance spin−spin couplings to phosphorus and to the overall structural complexity of these molecules. In addition, the amide groups of **21**−**31** apparently formed stable rotamers at the C−N bond, which resulted in a multiplicity of NMR signals. Similar observations have been recently reported for 4-[*N*-methyl-*N*-(trifluoroacetyl)amino]butyl[6] and cyclic *N*-acyl[17] nucleoside phosphoramidites. Furthermore, with the tertiary amides **25**−**28**, hindered rotation around the amide bond resulted in very low signal intensity for the methylene group adjacent to the amide nitrogen. Considering all of these factors, recording the NMR spectra for individual isomers dramatically facilitated assignment of the structures of the phosphoramidites **21**−**31**.

The phosphoramidites **21**−**31** demonstrated excellent shelf life and hydrolytic stability. As revealed by ^{31}P NMR, all of these compounds were > 95% pure after being stored as dry samples for nine months at room temperature under argon. Hydrolytic stability of a representative selection of phosphoramidites was also determined. As measured by ^{31}P NMR, the half-lives of disappearance of compounds **22**, **26**, and **28** (0.1 M in 95% aqueous CD_3CN; 25 °C) were 200, 126, and 73 h,

(18) Marugg, J. E.; Burik, A.; Tromp, M.; van der Marel, G. A.; van Boom, J. H. *Tetrahedron Lett.* **1986**, *27*, 2271−2274.

(19) Stec, W. J.; Zon, G. *Tetrahedron Lett.* **1984**, *25*, 5279−5282.

Scheme 3[a]



[a] (a) 1*H*-Tetrazole/MeCN; (b) for **21**−**28**: I_2/Py/THF/H_2O; for **29**−**31**: *t*-BuOOH/MeCN.

respectively.[20] These data suggest excellent lifetimes for solutions of phosphoramidites while on the DNA synthesizers.

At the beginning of this work, no information was available on the ability of the phosphoramidites **21**−**31** to support oligonucleotide synthesis or the compatibility of the novel protecting groups with the known conditions of oligonucleotide deprotection. Therefore, a proper model system had to be chosen that would allow one to assess the potential of the proposed protecting strategy. In our opinion, this was best achieved by using solid-support-bound oligonucleotides **33a**−**k** as the model compounds (Scheme 3). This model offered several advantages. First, it permitted testing of a variety of phosphoramidites that were prepared on a small scale. Regardless of the structure of the protecting group or the success in removing it, the reaction mixtures that were obtained were likely to be analyzed under standardized conditions by HPLC. These two features allowed a relatively high-throughput screening of a number of protecting groups. Second, this model offered the possibility of testing the phosphoramidites **21**−**31** specifically under conditions of oligonucleotide synthesis on solid support. When the synthesis is carried out on solid phase, the reagents are used in much larger excess, and unwanted side reactions are more pronounced than in solution. In addition, more drastic conditions are often required to remove protecting groups from a negatively charged oligonucleotide backbone than from dinucleoside monophosphates. Thus, with model oligonucleotides **33a**−**k**, the choice for the most suitable protecting groups could be founded on more representative data. The ability of the phosphoramidites **21**−**31** to support oligonucleotide synthesis was tested first (Scheme 3). Using a standard synthetic cycle and ancillary reagents, we attached the phosphoramidites at the 5′-terminus of solid-support-bound undecathymidylate, **32**, to give **33a**−**k**. These were conventionally deprotected with ammonia (6 h/55 °C), and the products were analyzed by HPLC and electrospray MS. The following relevant observations were noted. As revealed by the dimethoxytrityl assay, all phosphoramidites demonstrated high coupling efficiency (>95%). Phosphoramidites **21**−**28** were fully compatible with ancillary reagents that were used in oligonucleotide synthesis, yielding only the expected products, either **35** or, for **21**−**24**, mixtures of **34a**−**d** and **35** (for efficiency of deprotection, see below). For **29**−**31**, use of the standard iodine oxidizer led to formation of unidentified side products. In contrast, oxidation of phosphite triester intermediates derived from **29**−**31** and **32** with *tert*-butyl hydroperoxide[21] led only to **35**. When the oxidation step was replaced with sulfurization using 3*H*-1,2-benzodithiol-3-one 1,1-dioxide,[22] the phosphorothioate analogues of **35** were easily obtained.

(20) Under these conditions, a commercial sample of **77** had a half-life of 200 h.

Table 1. Deprotection of **33a-k** with Concentrated Aqueous Ammonium Hydroxide

protecting group	phosphoramidite	protected oligonucleotide	deprotection conditions	
			time, h	temp, °C
A	**21**	**33a**	48	55
B	**22**	**33b**	48	55
C	**23**	**33c**	48	55
D	**24**	**33d**	8	55
E	**25**	**33e**	6	55
F	**26**	**33f**	5	55
G	**27**	**33g**	1	25
H	**28**	**33h**	<0.5	25
I	**29**	**33i**	<1	25
J	**30**	**33j**	1	25
K	**31**	**33k**	1.5	25

The above data suggested that each of the phosphoramidites **21**−**31** could be used for preparation of synthetic oligonucleotides. They also indicated that, depending on the nature of the protecting group, the deprotection time that is required for complete conversion of **33a**−**k** to **35** differed substantially. To study the removal of protecting groups in more detail, the solid-support-bound compounds **33a**−**k** were treated with concentrated aqueous ammonia at either 25 °C (**33f**−**k**) or 55 °C (**33a**−**g**), and the progress of deprotection was followed by reverse-phase HPLC. In all cases, the final product **35** could be easily separated from the more hydrophobic oligonucleotides **34a**−**k**, and the time required for complete deprotection of **33a**−**k** (>99.5%) was determined (Table 1). The data in Table 1 reveal dramatic differences in reactivity of the protecting groups studied toward concentrated aqueous ammonia, which are discussed below.

Of the protecting groups derived from 2-benzamidoethanol, the tertiary amides **E**−**H** were removed dramatically faster than the secondary ones **A**−**D** (cf. **33e**−**h** and **33a**−**d**). Deprotection was additionally facilitated when bulky substituents were introduced on the aminoethyl skeleton of a protecting group, as predicted by the Thorpe−Ingold *gem*-dialkyl effect.[23] Indeed, the dimethyl-substituted compound **33d** reacted markedly faster than **33a**. Similarly, the *N*-*i*Pr derivative **33h** was more reactive than the *N*-Me analogue **33f**.

Of the electron-donating or -accepting substituents introduced on the phenyl ring, only the 4-dimethylamino group altered the deprotection kinetics to any substantial extent (cf. **33e** and **33g**). In contrast, the influence of NO_2 or MeO groups was insignificant (for secondary amides, cf. **33a**, **b**, and **c**; for tertiary ones, cf. **33e** and **f**).

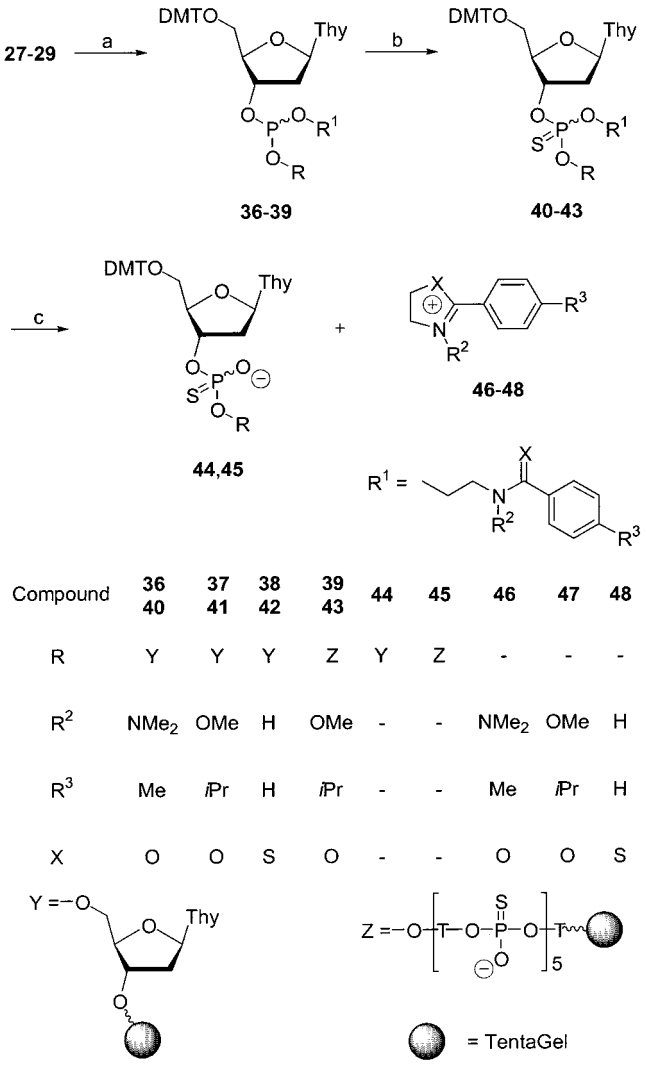
Comparison of the thionocarbonyl derivative **33i** with the corresponding oxygen analogue **33a** indicates dramatically faster deprotection of the former compound, which may reflect an

(21) Alul, R. H.; Singman, C. N.; Zhang, G.; Letsinger, R. L. *Nucleic Acids Res.* **1991**, *19*, 1527−1532.

(22) Iyer, R. P.; Phillips, L. R.; Egan, W.; Regan, J. B.; Beaucage, S. L. *J. Org. Chem.* **1990**, *55*, 4693−4699.

(23) (a) Beesley, R. M.; Ingold, C. K.; Thorpe, J. F. *J. Chem. Soc.* **1915**, *107*, 1080−1106. (b) Ingold, C. K. *J. Chem. Soc.* **1921**, *119*, 305−329.

Scheme 4[a]

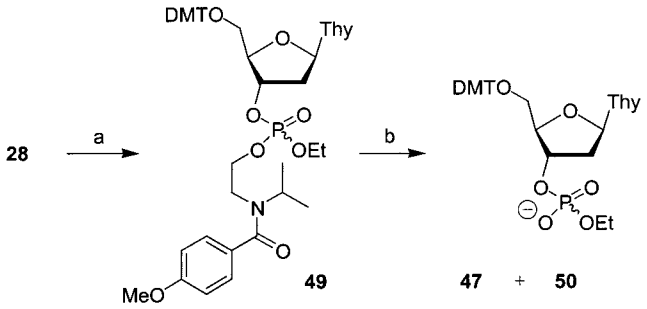


[a] (a) R−OH/1*H*-Tetrazole/MeCN; (b) 3*H*-1,2-benzodithiol-3-one 1,1-dioxide/MeCN; (c) MeCN, 80% aqueous MeCN, MeOH, or THF:2,6-lutidine:H_2O (96:2:2).

enhanced intramolecular participation of the more nucleophilic thiocarbonyl group. In a very similar fashion, groups **J** and **K** were removed under conditions as mild, or almost as mild, as those for **I**.

Removal of the Protecting Groups G−I under Neutral Conditions. It can be seen from Table 1 that the removal of the protecting groups **G**−**K** required ≤1 h at 25 °C. Due to experimental limitations, it is difficult to determine with confidence their stability in concentrated ammonium hydroxide. To provide a more accurate comparison of the deprotection characteristics, removal of the representative groups **G**−**I** was studied under less aggressive conditions. In preliminary experiments, dithymidine monophosphates protected with **G**−**I** proved to be very labile compounds, which prohibited their isolation. Instead, model compounds **40**−**43** were synthesized on a high-loaded Tentagel polymer support as depicted in Scheme 4. Phosphoramidite building blocks **27**−**29** were first converted to solid-support-bound **36**−**38**. The stability of **37** and **38** in MeCN was studied by ^{31}P NMR spectroscopy in gel phase to reveal that both compounds remained unchanged for at least 16 h at room temperature. Compounds **36**−**38** were then sulfurized with 3*H*-benzodithiol-2-one 1,1-dioxide in MeCN[22] to give **40**−**42**. In a similar fashion, **28** was coupled to a solid-support-bound, phosphate-deprotected hexathymidylate pentaphosphorothioate[24] to yield **39**, which was then converted to **43**. Additionally, two simpler model compounds were prepared in solution. Phosphoramidite **28** was reacted with EtOH in the presence of 1*H*-tetrazole, and the product was oxidized to give **49** (Scheme 5). Phosphotriester **52** was synthesized by phosphorylation of **17** with *O*,*O*-diethyl phosphorochloridate (Scheme 6). Both **49** and **52** were isolated by column chromatography on silica gel.

Scheme 5[a]



[a] (a) 1. EtOH/1*H*-Tetrazole/CH_2Cl_2; 2. (3-ClC_6H_4)CO_3H. (b) MeCN, 80% aqueous MeCN, MeOH, or THF:2,6-lutidine:H_2O (96:2:2).

Table 2. Deprotection of **40**−**43**, **49**, and **52** under Neutral Conditions

	half-life: $\tau_{1/2} \times 10^{-2}$, min					
conditions (25 °C)	**40**	**41**	**42**	**43**	**49**	**52**
MeCN	1.0	0.5	0.8	0.8[a]	23.0	171.0
80% aqueous MeCN	1.7	0.5	0.9		3.1	14.7
MeOH	2.0	0.7	0.8		2.6	15.5
THF:2,6-lutidine:H_2O (96:2:2)	1.0	0.7			15.0	

[a] Determined in MeCN:pyridine (95:5).

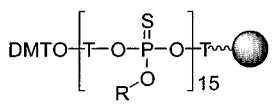
The model compounds **40**−**43**, **49**, and **52** were treated under the mild conditions shown in Table 2. For **40**−**43**, the progress of the deprotection was followed by ^{31}P NMR in gel phase, where their peaks were observed at 68.5−71 ppm. Regardless of the conditions employed, deprotection resulted in the formation of the corresponding thionophosphodiesters. Thus, as seen from Scheme 4, compounds **40**−**42** were converted to **44** (55−61 ppm), and **43** yielded **45** (56.0−56.5 ppm). Simultaneously, the protecting groups **G** and **H** were converted to the corresponding 2-oxazolinium cations **46** and **47**, and group **I** was converted to the 2-thiazolinium cation **48** (for the fate of the protecting groups, see below).

The deprotection of **49** and **52** was studied by conventional ^{31}P NMR in solution. Similarly to the phosphorothioates above, both **49** and **52** were smoothly converted to the phosphodiesters **50** and **53** (Schemes 5 and 6, respectively). In MeCN and THF, the peaks of the starting material were observed at 1.5 to −1.0 ppm and were downfield of the peaks of the respective products **50** and **53** at 1.1 to −1.5 ppm. In 80% aqueous MeCN and MeOH, the peaks of **49** and **52** (−1.2 to −1.4 ppm) were found upfield with respect to the peaks of **50** and **53** (0.5 to −0.2 ppm). Additionally, to reveal the fate of group **H**, the deprotection of **52** was studied by reverse-phase HPLC (see below).

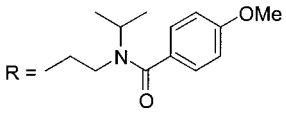
In terms of the half-lives of disappearance of **40**−**43**, **49**, and **52**, several conclusions might be drawn from the results summarized in Table 2. Comparing the half-lives of the dinucleoside phosphorothioates **40**−**42**, the reactivities of the protecting groups **G**−**I** appear to be very similar. Under both aqueous and nonaqueous conditions, the stability of the protecting groups increased in the following order, **H** < **I** < **G**, that

(24) Guzaev, A. P.; Manoharan, M. *J. Org. Chem.*, in press.

is, their relative reactivity remained unchanged in comparison to that in concentrated ammonium hydroxide.



51



or negative charge

The half-lives of **40**−**42** in MeCN demonstrated that the protecting groups **G**−**I** were labile to the extent that they might be removed under the conditions of oligonucleotide synthesis. Furthermore, in comparison with **41**, removal of group **H** from **43** containing the negatively charged backbone was retarded by a factor of < 2. This suggested that a partial deprotection of an oligonucleotide might not inhibit the progress of the further deprotection. For group **H**, this was verified by synthesizing pentadecathymidylate phosphorothioate **51** on a 20-μmol scale using the phosphoramidite **28** as a building block. On completion of the chain assembly, the extent of the protection of the solid-support-bound **51** was immediately determined by ^{31}P NMR. The two major peaks of thionophosphates observed at 68 and 58 ppm were attributed to the triester groups protected by **H** and the deprotected diester groups, respectively. The latter accounted for ca. 90% of the total integrated area, which revealed that the majority of the thionophosphate linkages were deprotected at the end of the synthesis. The deprotection of **51** was completed by a brief treatment of the solid support with 10% pyridine in MeOH (1 h/RT). The ^{31}P NMR spectrum of the product displayed the signal of thionophosphate diester groups and a minor signal of desulfurized phosphodiester groups (−2 ppm, 3.4%). Importantly, no peaks of other side products were detected, which demonstrated that the deprotection was not accompanied by any unwanted reactions of **47** with the internucleosidic thionophosphate residues.

Comparison of the half-lives for compounds **41**, **49**, and **52** that were protected with group **H** demonstrated that nucleosidic residues dramatically facilitated the deprotection. One may tentatively speculate that the bulky nucleosidic residues favor the departure of the protecting group, which reduces steric hindrance around the phosphate moiety.

Our attempts to clarify the influence of protic solvents on the deprotection rates led to conclusive results only for the reactions in homogeneous phase. For compounds **49** and **52,** whose stability was measured in solution, the protic solvents accelerated deprotection by a factor of >10. However, for the solid-support-bound compounds **40**−**42**, either a very minor negative effect (**40**) or no influence of the polar environment on the half-lives of the deprotection was observed. This discrepancy might be tentatively explained by the lack of efficient solvation inside the matrix of the hydrophobic polystyrene solid support rather than by the reactivity of the protecting group **H** in dinucleoside monophosphates **40**−**42**.

The fact that the protecting groups **G**−**I** were removed under the conditions of oligonucleotide synthesis was not considered a disadvantage. We demonstrated recently that, upon conversion of the phosphodiester residues to the 4-dimethylaminopyridinium salt form, the coupling of 2-cyanoethyl phosphoramidite building blocks to phosphate-unprotected oligonucleotides may be carried out as efficiently as the standard coupling.[24] Therefore, the protecting group **H** that provided the best combination of synthetic availability and ease of deprotection was selected for further investigation. Other groups can also be employed as phosphate protecting groups in oligonucleotide synthesis if slower deprotection kinetics is acceptable (**D**−**G**) or if oxidation with iodine solutions can be avoided (**I**−**K**). Finally, **A**, **B**, and **C** are not recommended for routine oligonucleotide synthesis, although they may be suitable for performing reactions in solution. Moreover, the dramatic difference in deprotection time allows keeping **A**, **B**, or **C** intact while the most labile group **H** is completely removed.[25] Thus, a combination of these means of protection may find use in synthesizing selectively modified oligonucleotides.

The Fate of the 2-[*N*-Isopropyl-4-methoxybenzoylamino]-ethyl and 2-(*N*-Thionobenzoylamino)ethyl Groups (H and I) Upon Deprotection. In a recent communication, acrylonitrile, which is released on cleavage of the traditional 2-cyanoethyl phosphate protecting group, has been demonstrated to alkylate nucleic bases.[6] Although this side reaction may occur to a measurable extent only on a manufacturing scale, the study illustrates the importance of analyzing potential consequences before novel protecting groups are introduced in laboratory practice. Although the protecting groups **H** and **I** are labile under the conditions of oligonucleotide synthesis, the extent of the deprotection may depend on the length of the synthesis. For preparations on a small scale, in which faster coupling protocols are used, an oligonucleotide may remain extensively protected at the end of the chain assembly. These protecting groups will have to be removed at the same time as the nucleic base protecting groups. Therefore, to evaluate possible drawbacks of the proposed protecting strategy, the fate of the protecting groups **H** and **I** and the ability of the deprotection products to participate in side reactions were studied.

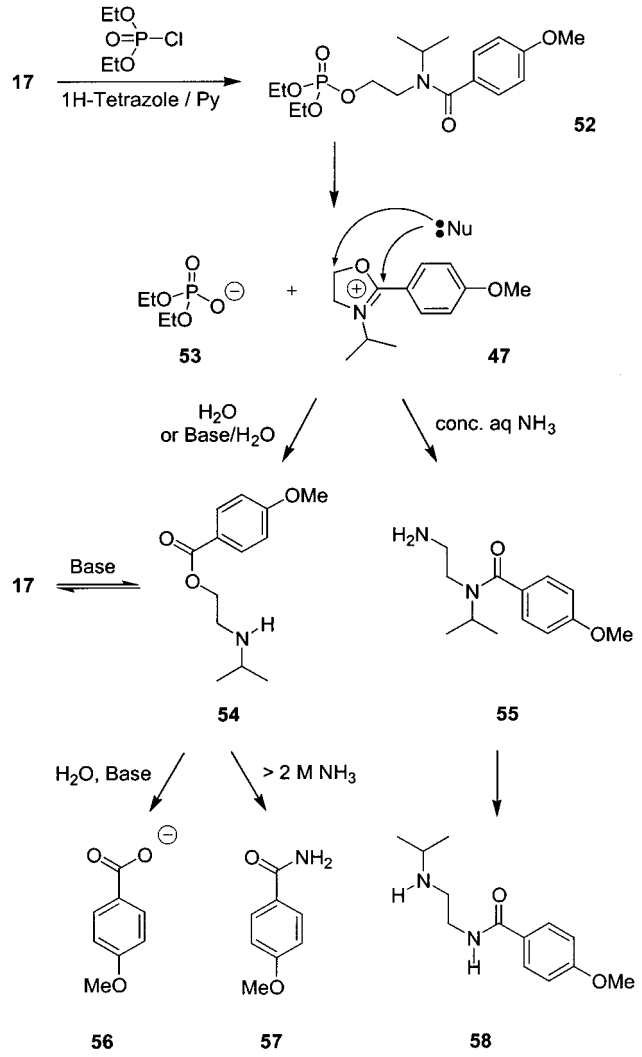
To study the fate of group **H**, a model compound, **52**, was treated with either water or solutions of ammonium hydroxide (0.5 M, 2.0 M, or 28% in water), and the progress of the reactions was followed by HPLC. The products formed were either isolated and characterized (**47** and **54**) or identified by co-injection with authentic samples (**17** and **56**−**58**). The results are summarized in Scheme 6. Regardless of the basicity of the media, **52** rapidly reacted to form diethyl phosphate **53,** which was characterized by ^{1}H, ^{13}C NMR, and ^{31}P NMR and other products. The half-life of disappearance of **52** (380−400 min at 25 °C) was independent of the concentration of ammonium hydroxide. In contrast, the distribution of products other than **53** markedly depended on the concentration of ammonium hydroxide.

Under neutral conditions, **54** was the final product of the reaction, although in early stages, the intermediate **47** accumulated in the reaction mixture. When 70−98% of starting material **52** was consumed, 45−55% of the total integrated area in the HPLC traces accounted for **47**. At this stage, **47** was isolated by HPLC in 90% purity, and its structure was proven by LC/MS, ^{1}H, and ^{13}C NMR spectra. However, the stability of **47** was insufficient to afford its complete characterization. In water, **47** exhibited a half-life of 40 h and underwent hydrolytic ring opening to form **54** in quantitative yield. The latter product was indefinitely stable under neutral and acidic conditions, which permitted its isolation by HPLC as a trifluoroacetate salt and its unambiguous characterization.

In the presence of dilute ammonium hydroxide (0.5 to 2.0 M), the product distribution dramatically changed. Compound **47** was rapidly hydrolyzed so that the extent of its accumulation in the reaction mixture was < 1%. It has been reported that in aqueous K_2CO_3, alkaline hydrolysis of similar *N*-methyl-2-oxazolinium salts gave exclusively *O*-acyl *N*-methylethanolamines as kinetic products.[26] According to very recent findings, this regioselectivity resulted from bifunctional nucleophilic

(25) Guzaev, A.; Manoharan, M. Unpublished results.

Scheme 6

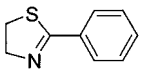


catalysis provided by the buffer that was used. More generally, predominant formation of *N*-acylated *N*-methylethanolamines as kinetic products along with *O*-acylated isomers has been observed under basic conditions.[27] In contrast to these findings, **47** was hydrolyzed to give mainly **54** as the kinetic product.

In turn, **54** was consumed by three concurrent reactions. First, it underwent reversible *O*→*N* acyl migration to give **17**. This was proven by subjecting **17** to the same conditions. On equilibration, a mixture of **17** and **54** (3:1) was obtained. Second, basic hydrolysis of the *O*-anisoyl group led to **56**. Finally, at a concentration for ammonium hydroxide of 2.0 M and higher, ammonolysis of **54** gave **57**. It is reasonable to expect that *N*-isopropylaminoethanol was also formed in the latter two reactions, although no attempt was made to identify it.

In the presence of 28% aqueous ammonium hydroxide, the product distribution was somewhat more complex. Along with the products mentioned above, a new compound tentatively assigned as **55** was formed in approximately 10% yield. On longer exposure to the reaction conditions, **55** was converted to **58**, which was confirmed by co-injection with an authentic sample synthesized by an independent route.

To study the fate of group **I**, the solid support **42** (40 μmol) was treated with 80% aqueous MeCN as described above until the deprotection was complete (Scheme 4). The solid support was then washed with 5% aqueous $NaHCO_3$ and ethyl acetate, which converted **48** to the 2-phenyl-2-thiazoline-free base (**59**) and eluted it from the solid support. On evaporation of the organic phase, **59** was obtained in 85% yield. The structure of **59** was consistent with its ^{1}H NMR and IR spectra, which were in good agreement with those reported in the literature,[28] and with its ^{13}C NMR spectrum. The treatment of **59** with concentrated aqueous ammonium hydroxide for 5 days revealed no appreciable chemical transformations, which demonstrated its high stability under the conditions of oligonucleotide deprotection.



59

Formation of **47**, **54**, and **59** demonstrated above confirms the proposed intramolecular mechanism of deprotection where the phosphodiester anion is substituted with the oxygen atom of the amido group or the sulfur of the thionoamido group (Scheme 1). Moreover, it is important to note that no products that indicate reactivity of **52** by any other route were detected in the reaction mixtures.

The occurrence of minor side reactions of the products derived from group **H** with nucleic bases was studied. Each of the 5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxynucleosides (dA, dC, dG, and T) was mixed with **52** (10-fold excess) and treated with concentrated ammonium hydroxide for 24 h at 55 °C. When the reaction mixtures were analyzed by reverse-phase HPLC, no new nucleosidic products were observed. Of the compounds presented in Scheme 7, only compound **47** may in principle be considered as an alkylating agent. As seen from the reactivity of **47** toward ammonia, the products of alkylation, that is, **55** and **58**, were formed only in concentrated ammonium hydroxide in low yield (<10%). Thus, **47** is a very weak alkylating agent that is, however, highly labile toward hydrolysis. Rapid consumption of **47** by hydrolysis under basic conditions further reduces its chance to react with nucleosides. In view of these considerations, the observed lack of modifications of nucleic bases is not surprising.

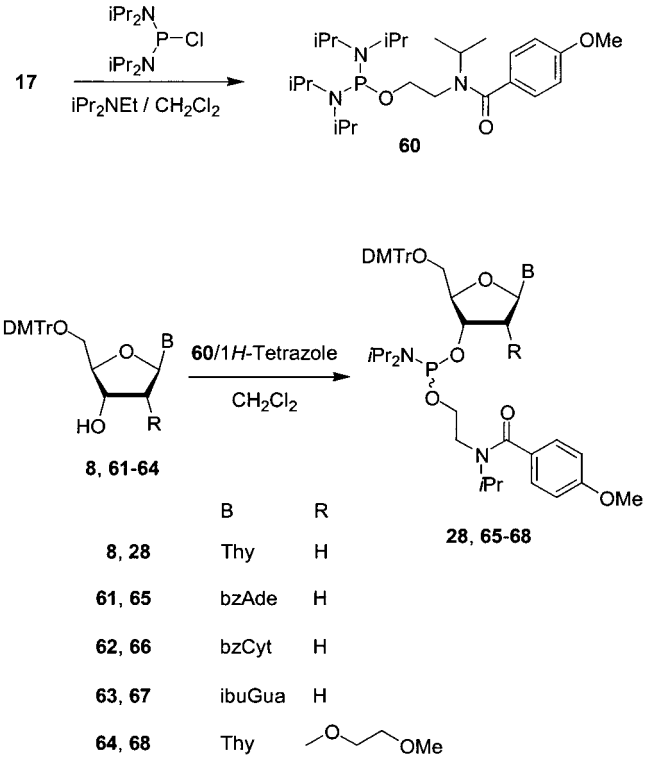
Utility of the 2-[*N*-Isopropyl-4-methoxybenzoylamino]-ethyl-Protected Nucleoside Phosphoramidites in Oligonucleotide Synthesis. The usefulness of the proposed protection strategy for routine preparation of synthetic oligonucleotides and their phosphorothioate analogues was rigorously tested as described below. First, a novel phosphitylating reagent, bisamidite **60**, was synthesized from **17**, as depicted in Scheme 7. After column purification, **60** was obtained in 84.5% yield as a stable crystalline material. Under dry conditions, it could be stored at −18 °C for >2 months without any detectable decomposition. When the protected 2′-deoxynucleosides **8** and **61**−**63** were reacted with a slight excess of **60**, the corresponding 2′-deoxynucleoside phosphoramidites **28** and **65**−**67** were synthesized in 91−98% yield. Similarly, the 2′-*O*-(2-methoxyethyl)-5-methyluridine phosphoramidite **68** was prepared from **64** in 96% yield. The phosphoramidites **28** and **65**−**68** were employed in the synthesis of the natural 20-mer oligonucleotides **69**−**71**, the oligonucleotide phosphorothioates with proven antisense activity, **72**−**75**, and the 2′-*O*-(2-methoxyethyl)-modified oligonucleotide phosphorothioate **76** (Table 3). For comparison, the same sequences were likewise prepared using commercial phosphoramidites **77**−**80** protected with a 2-cyanoethyl group at the phosphite moiety.

(26) Deslongchamps, P.; Dube, S.; Lebreux, C.; Patterson, D. R.; Taillefer, R. J. *Can. J. Chem.* **1975**, *53*, 2791−2807.

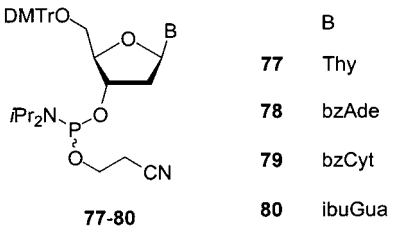
(27) Perrin, C. L.; Engler, R. I.; Young, D. B. *J. Am. Chem. Soc*. **2000**, *122*, 4877−4881.

(28) (a) Ino, A.; Murabayashi, A. *Tetrahedron* **1999**, *55*, 10271−10282. (b) Vorbrüggen, H.; Krolikiewicz, K. *Tetrahedron* **1993**, *49*, 9353−9372.

Scheme 7



On a small scale, the coupling cycle was first optimized using the oligonucleotide **69** as an example. The coupling of phosphoramidite **28** was slower than that of **77**. To obtain the high stepwise yields, the extended 5-min coupling time for **28** was used. However, the selectivity of the reaction of **28** with the 5′-hydroxy functions in the presence of unprotected phosphate moieties was higher than that of **77**. We demonstrated recently



that under standard conditions, the coupling of **77** to phosphate-unprotected oligonucleotides resulted in a stepwise yield of 93.8−96.3%.[24] As demonstrated above for **51**, the protecting group **H** was almost completely removed in the course of the oligonucleotide synthesis. Nevertheless, using **28** as a building block, the oligonucleotide **69** was synthesized in 97.4% stepwise yield. When optimized conditions for the phosphoramidite coupling to phosphate-unprotected oligonucleotides were used,[24] the coupling yields could be further improved. In a modified synthetic cycle, the detritylation of solid-support-bound oligonucleotides was followed by a brief wash with a solution of 0.1 M 4-dimethylaminopyridinium 1*H*-tetrazolide (DMAP−Tet) in MeCN. This neutralized the acidic phosphodiester groups. Furthermore, the protonated DMAP that was retained on the solid support as a counterion of phosphodiester residues served as a nucleophilic catalyst in the following coupling reaction. Under these conditions, **69** was synthesized in 98+% stepwise yield. In a similar fashion, the mixed-base oligonucleotides **70**−**74** were synthesized in yields consistent with a high coupling efficiency. When the phosphoramidite **68** was used, a coupling for 10 min was required to ensure 97+% coupling efficiency.

Solid-support-bound oligonucleotides were deprotected with concentrated aqueous ammonium hydroxide for 2 h at room temperature (**69** and **76**) or for 6 h at 55 °C (**70**−**74**). Considering the rates of deprotection of compounds **41** and **43** (Table 2) and the fact that the release of diethyl phosphate from **52** occurred in a pH-independent manner, the use of a base might be unnecessary for the removal of the remaining phosphate protecting groups. However, these conditions were required for the removal of the protecting groups from nucleic bases and for the release of the oligonucleotides from the solid support. After deprotection, all of the oligonucleotides were routinely isolated by DMTr-On reverse-phase HPLC. When the 5′-terminal DMTr group was removed, compounds **69**−**76** were desalted and characterized by electrospray MS. Their purity was confirmed by HPLC and capillary gel electrophoresis.

The utility of protecting group **H** for synthesis of oligonucleotides on a large scale was next tested. On a preparative scale, an extensive optimization of the coupling protocol is required for the successful preparation of 20-mer oligonucleotides. Therefore, a lower yield of the product might be initially expected for a set of novel phosphoramidite building blocks that are used under unoptimized conditions. The oligonucleotide **75** was synthesized on a 150 μmol scale using 2.5−3 equiv. of **28** and **65**−**67** and a 20-min coupling time. Indeed, the yield of **75** using **28** and **65**−**67** was lower than that obtained with the standard building blocks **77**−**80** (43.9 and 55.0%, respectively). When it was deprotected with concentrated ammonium hydroxide, the crude product was analyzed by reverse-phase HPLC and LC/MS. It is important to note that no products indicating modification of **75** at the nucleic bases were detected in the reaction mixture. This agreed with the observation presented above that **52** was inert toward nucleosides in concentrated ammonium hydroxide.

Conclusion

The data presented suggest that 2-benzamidoethyl, ω-(thionobenzoylamino)alkyl, and 2-(*N*-phenylthiocarbamoylamino)ethyl groups are a novel, versatile class of protecting groups for phosphodiester functions. The utility of these groups was demonstrated by preparing building blocks for oligonucleotide synthesis, the nucleoside phosphoramidites **21**−**31** and **65**−**68**. All of these compounds were synthesized in high yields using well-established methods and demonstrated excellent solubility in MeCN, stability in both solid state and in solution, and coupling efficiency.

Studying the structure−activity relationship revealed that, depending on substitution, removal of the 2-benzamidoethyl, ω-(thionobenzoylamino)alkyl and 2-(*N*-phenylthiocarbamoylamino)ethyl protection occurs under a wide range of conditions. We demonstrated here the successful use of the most labile protecting group for oligonucleotide synthesis on solid support. Other more stable groups allow one to synthesize chimeric oligonucleotides by deprotecting certain internucleosidic phosphate linkages while other selected groups are kept protected. In addition, this may make the protecting strategy attractive for synthetic applications whose requirements for deblocking conditions differ from those of oligonucleotide chemistry.

The fate of the most convenient protecting groups, **H** and **I,** upon deprotection was studied. The mechanism of deprotection was confirmed by isolation of the intermediate **47** or the stable product **59**. The products formed from **47** were identified, and the dependence of their distribution on the concentration of ammonium hydroxide in solution was determined. The lack of reactivity of these compounds toward nucleic bases or the oligonucleotide backbone was unambiguously demonstrated.

Table 3. Oligonucleotides Synthesized with the Aid of **28** and **65**−**68**

compound	sequence (5′ → 3′)	backbone	scale, μmol	yield, %	target
69	T_{20}	P═O	1	69.2	
70	$GC_3A_2GCTG_2CATC_2GTCA$	P═O	1	63.1	
71	$TC_3GC_2(TG)_2ACATGCAT_2$	P═O	20	61.5	
72	$GC_3A_2GCTG_2CATC_2GTCA$	P═S	1	52.3	human ICAM-1
73	$TC_3GC_2(TG)_2ACATGCAT_2$	P═S	1	50.6	human C-*raf*
74	$ATGCAT_2CTGC_5A_2G_2A$	P═S	20	56.5	mouse C-*raf*
75	$AGCT_2CT_3G(CA)_2TGTA_3$	P═S	150	43.9	human MDM-2
76	T^*_{20} [a]	P═S	1	49.2	

[a] T* stands for 2′-*O*-(2-methoxyethyl)-5-methyluridine residue.

One may finally conclude that the proposed protecting strategy is a valuable addition to the portfolio of methods for oligonucleotide synthesis and that it may eventually attain general applicability in the organic chemistry of phosphorus. Recently, synthesis of oligonucleotides without nucleoside-base protection has been reported.[29] Combining this methodology with the use of the protecting group **H** that is removed under neutral conditions may result in a novel synthetic strategy that involves no deprotection procedures except for the release from a solid support.

Experimental Section

Oligonucleotide synthesis. The oligonucleotide synthesis was performed on an ABI 380B DNA Synthesizer on a 1-μmol scale according to the manufacturer's recommendations. The phosphoramidites **21**−**31** were used as 0.1 M solutions in dry MeCN. When **29**−**31** were employed, the oxidation step was carried out with *tert*-butyl hydroperoxide[21] (10% in MeCN). For preparation of the phosphorothioate oligonucleotides, 3*H*-1,2-benzodithiol-3-one 1,1-dioxide[22] (0.05 M in MeCN) was used as a sulfur-transfer reagent. Solid-support-bound **33a**−**k** were deprotected as depicted in Scheme 3 under conditions specified in Table 1. The 5′-DMT-protected oligonucleotides were analyzed by reverse-phase HPLC and characterized by ES/MS.

For preparation of **69**−**74** and **76** (Table 3) by the modified cycle, a solution of 0.1 M DMAP and 0.1 M 1*H*-tetrazole in MeCN was placed in positions 15 or 17 of the synthesizer (PS and PO cycles, respectively). The standard detritylation subroutine was followed by a brief washing with MeCN and flushing with argon. Next, the solution of DMAP-TET was delivered to the columns for 15 or 45 s (1- and 20-μmol cycles, respectively), followed by washing with MeCN and flushing with argon. For the coupling step, phosphoramidites **28** and **65**−**68** (0.2 M in MeCN) were delivered in 15- and 5-fold excess over the solid support (1- and 20-μmol cycles, respectively), and the phosphoramidite condensation was carried out for 5 (**28** and **65**−**67**) or 10 min (**68**). The solid-support-bound material was deprotected with concentrated aqueous ammonium hydroxide under standard conditions (8 h at 55 °C for **70**−**75** or 2 h at RT for **69** and **76**). The 5′-DMT-protected oligonucleotides were isolated by reverse-phase HPLC. The 5′-DMT group was cleaved using 10% aq AcOH (30 min), and the oligonucleotides were desalted by reverse-phase HPLC and characterized by ES/MS. The purity of the isolated oligonucleotides was confirmed by HPLC and capillary gel electrophoresis.

The synthesis of **75** was carried out on a MilliGene/Biosearch 8800 Reagent Delivery Module on a 150-μmol scale using 2.5−3 equivs of **28** and **65**−**67** (0.3 M in MeCN) and a 20-min coupling time. The solid support was treated with concentrated aqueous ammonium hydroxide overnight at room temperature. The solution obtained was heated at 55 °C for 6 h and evaporated. The crude product was analyzed by reverse-phase HPLC and LC/MS. Compound **75** was isolated and characterized as described above for **70**−**75**.

General Procedure for the Preparation of 10−**15 and 17.** Acyl chloride (0.1 mol) in THF (100 mL) was added dropwise to a solution of an aminoethanol (0.4 mol) in THF (200 mL) under magnetic stirring at 4 to 10 °C. The reaction mixture was stirred for 2 h at room temperature, and the solvent was evaporated in vacuo. The residue was dissolved in ice-cold water (200 mL). The solution was brought to pH 6 with concentrated hydrochloric acid and extracted with ethyl acetate (5 × 100 mL). Extracts were washed with brine (100 mL), dried over Na_2SO_4, and evaporated. Crude 2-(*N*-benzoylamino)ethanols (85−95%) were used without further purification. For characterization, **10**−**13** and **17** were recrystallized from water or a mixture of toluene and hexane. **14** and **15** were purified by column chromatography on silica gel using a gradient from 5 to 20% MeOH in CH_2Cl_2 to give colorless oils. Compounds **10**, **11**, **13**,[30] **14,** and **15**,[26] were identical to those reported previously.

***N*-(2-hydroxyethyl)-*N*-isopropyl-4-methoxybenzamide (15).** 88% yield: white solid, mp 71.5−72 °C (toluene−hexane). ^{1}H NMR ($CDCl_3$): δ 7.32 (2H, d, J = 8.6 Hz), 6.89 (2H, d, J = 8.6 Hz), 4.40 (1H, br s), 4.09 (1H, m), 3.80 (3H, s), 3.90−3.70 (1H, m), 3.53 (2H, t, J = 4.6 Hz), 1.13 (6H, d, J = 6.8 Hz). ^{13}C NMR ($CDCl_3$): δ 173.56, 160.60, 128.71, 128.16, 113.89, 63.69, 55.39, 50.60, 44.45, 21.19. FAB−HRMS: calcd for $C_{13}H_{19}NO_3$, 238.1443 (MH^+); found, 238.1444.

***N,N,N′,N′*-Tetraisopropyl-*O*-[2-[*N*-isopropyl-*N*-(4-methoxybenzoyl)-amino] ethyl] phosphordiamidite (60).** Chloro bis[(*N*,*N*,-diisopropyl)-amino]phosphite (1.73 g, 6.5 mmol) in CH_2Cl_2 (20 mL) was added to a stirred solution of **17** (1.19 g (5.0 mmol) and *N*,*N*-diisopropylethylamine (1.03 g, 8.0 mmol) in CH_2Cl_2 (5 mL) dropwise under argon atmosphere at −78 °C. The mixture was stirred at −78 °C for 10 min and was allowed to warm to room temperature. The solution was treated with triethylamine (2.5 mL) and hexane (50 mL). The mixture was evaporated to dryness and coevaporated twice with triethylamine−hexane (5:95; 25 mL). The residue was dissolved in triethylamine−hexane (5:95; 25 mL), filtered, and applied on a short silica gel column. The column was eluted with triethylamine−ethyl acetate−hexane (5:5:90). Fractions were evaporated to give 60 (1.98 g, 84.5%) as a white solid: mp 58.5−59.5 °C. ^{1}H NMR ($CDCl_3$): δ 7.35−7.29 (2H, m), 6.95−6.85 (2H, m), 3.82 (3H, s), 3.80−3.34 (9H, m), 1.40−1.05 (30H, m). ^{13}C NMR ($CDCl_3$): δ 171.6, 160.4, 129.8, 128.2, 127.7, 113.8, 62.8, 62.4, 55.3, 45.2, 44.5, 44.2, 24.8, 24.6, 23.8, 23.7, 21.0. ^{31}P NMR ($CDCl_3$): δ 124.6; (CD_3CN): δ 130.9. FAB−HRMS: calcd for $C_{25}H_{46}N_3O_3P$ ($M + Na^+$), 490.3174; found, 490.3166.

General Procedure for the Preparation of 28 and 65−**68.** ***N*²-Isobutyryl-5′-*O*-(4,4′-dimethoxytrityl)-3′-*O*-(*N*,*N*-diisopropylamino)-[2-[*N*-isopropyl-*N*-(4-methoxybenzoyl)amino]ethoxy]phosphinyl-2′-deoxyguanosine (67).** 1*H*-Tetrazole (0.45 M in MeCN, 1.29 mL, 0.58 mmol) was added to a mixture of **60** (766 mg, 1.64 mmol), **63** (928 mg, 1.45 mmol), and CH_2Cl_2 (15 mL), and the resulting solution was stirred for 2 h at room temperature. Aqueous $NaHCO_3$ (5%, 10 mL) was added, the emulsion was diluted with brine (50 mL), and the product was extracted with ethyl acetate (3 × 75 mL). Extracts were washed with brine (3 × 50 mL), dried over Na_2SO_4, and evaporated to dryness. The residue was dissolved in toluene (25 mL), applied on a silica gel column; and separated, eluting with a gradient from 40:55:5 ethyl acetate:hexane:triethylamine to 5:90:5 ethanol:ethyl acetate:triethylamine. Collected fractions were evaporated, coevaporated with dry MeCN (2 × 50 mL), and dried on an oil pump to give **67**, fast diastereomer (170 mg); **67**, slow diastereomer (161 mg); and their mixture (1006 mg) totaled in 1330 mg (91.2%) of **67**. **67**, fast diastereomer, ^{1}H NMR ($CDCl_3$): δ 7.78 (1H, s), 7.44−7.15 (13H, m), 6.94−6.74 (6H, m), 6.28 (1H, m), 4.56 (1H, m), 4.23 (1H, m), 3.78 (3H, s), 3.76 (6H, s), 4.15−3.20 (10H, m), 2.80−2.65 (1H, m), 2.43−2.25 (1H, m), 1.3−0.8 (24H, m). ^{13}C NMR ($CDCl_3$): δ 179.93, 172.53,

(29) Hayakawa, Y.; Kataoka, M. *J. Am. Chem. Soc.* **1998**, *120*, 12395−12401.

160.44, 158.62, 155.82, 148.19, 144.53, 135.58, 129.98, 129.03, 128.02, 127.94, 127.63, 126.96, 121.31, 113.89, 113.24, 86.52, 85.83, 84.53, 74.15, 73.75, 63.67, 62.57, 62.40, 55.34, 55.22, 43.26, 43.02, 40.85, 35.45, 24.75, 24.60, 24.45, 21.25, 18.90. ^{31}P NMR ($CDCl_3$): δ 146.72. FAB−HRMS: calcd for $C_{54}H_{68}N_7O_{10}P$ ($M + Na^+$), 1028.4663; found, 1028.4630. **67**, slow diastereomer, ^{1}H NMR ($CDCl_3$): δ 7.77 (1H, s), 7.48−7.12 (13H, m), 6.90−6.70 (6H, m), 6.30 (1H, dd, J = 7.7, 5.5 Hz), 4.67 (1H, m), 4.38 (1H, m), 3.76 (3H, s), 3.74 (6H, s), 4.15−3.18 (10H, m), 2.84−2.60 (1H, m), 2.53−2.35 (1H, m), 1.3−0.8 (24H, m). ^{13}C NMR ($CDCl_3$): δ 179.44, 172.32, 160.46, 158.63, 155.82, 148.52, 148.15, 144.66, 135.71, 131.62, 130.04, 129.18, 128.12, 128.02, 127.95, 127.00, 121.54, 113.85, 113.21, 86.44, 85.65, 85.53, 84.12, 73.78, 73.51, 63.87, 61−62 (br m), 55.33, 55.24, 43.22, 43.98, 39.67, 35.74, 24.75, 24.61, 21.16, 18.86. ^{31}P NMR ($CDCl_3$): δ 144.07. FAB−HRMS: calcd for $C_{54}H_{68}N_7O_{10}P$ ($M + Na^+$), 1028.4663; found, 1028.4628.

5′-*O*-(4,4′-dimethoxytrityl)-3′-*O*-(*N*,*N*-diisopropylamino)[2-[*N*-isopropyl-*N*-(4-methoxybenzoyl)amino]ethoxy]phosphinyl-2′-deoxythymidine (28) was synthesized analogously from **60** (468 mg, 1.1 mmol) and **8** (545 mg, 1.0 mmol) and isolated by column chromatography to afford **28**, fast diastereomer (96 mg); **28**, slow diastereomer (125 mg); and their mixture (668 mg) totaled in 889 mg (97.6%) of **28** as a white amorphous solid. **28**, fast diastereomer, ^{1}H NMR ($CDCl_3$): δ 7.61 (1H, s), 7.40−7.16 (12H, m), 6.90−6.74 (6H, m), 6.39 (1H, dd, J = 8.0, 5.7 Hz), 4.63 (1H, m), 4.19 (1H, m), 3.77 (3H, s), 3.74 (6H, s), 3.95−3.26 (9H, m), 2.45 (1H, ddd, J = 13.0, 5.4, 1.5 Hz), 2.28 (1H, ddd, J = 13.0, 7.2, 6.18 Hz), 1.35 (3H, s), 1.20−1.10 (18H, m). ^{13}C NMR ($CDCl_3$): δ 171.88, 164.00, 150.43, 135.77, 111.16, 86.96, 85.98, 84.88, 73.71, 73.42, 63.45, 61.34, 61.04, 55.27, 43.15, 42.91, 40.19, 24.74, 24.62, 21.15, 11.74. ^{31}P NMR: δ 147.64. FAB−HRMS: calcd for $C_{50}H_{63}N_4O_{10}P$ ($M + Na^+$), 933.4180; found 933.4166. **28**, slow diastereomer, ^{1}H NMR ($CDCl_3$): δ 7.60 (1H, s), 7.41−7.18 (12H, m), 6.90−6.74 (6H, m), 6.43 (1H, br t), 4.66 (1H, m), 4.14 (1H, m), 3.79 (3H, s), 3.76 (6H, s), 3.96−3.25 (9H, m), 2.64−2.48 (1H, m), 2.42−2.20 (1H, m), 1.40 (3H, s), 1.28−1.0 (18H, m). ^{13}C NMR ($CDCl_3$): δ 171.98, 163.98, 150.41, 135.79, 111.21, 86.93, 85.61, 85.52, 84.76, 73.96, 73.59, 63.42, 61.30, 60.99, 55.27, 43.09, 42.85, 40.19, 24.66, 24.54, 21.20, 11.73. ^{31}P NMR: δ 147.96. FAB−HRMS: calcd for $C_{50}H_{63}N_4O_{10}P$ ($M + Na^+$), 933.4180; found, 933.4166.

***N*^{6}−Benzoyl-5′-*O*-(4,4′-dimethoxytrityl)-3′-*O*-(*N*,*N*-diisopropylamino)[2-[*N*-isopropyl-*N*-(4-methoxybenzoyl)amino]ethoxy]phosphinyl-2′-deoxyadenosine (65)** was synthesized analogously from 60 (5.14 g, 11.0 mmol) and 61 (6.58 g, 10.0 mmol). Column separation using a gradient from 25:70:5 ethyl acetate:hexane:triethylamine to 96:5 ethyl acetate:triethylamine gave **65**, fast diastereomer (1.88 g); **65**, slow diastereomer (1.01 g); and their mixture (6.74 g) totaled in 9.63 g (94.0%) of **65**. **65**, fast diastereomer, ^{1}H NMR ($CDCl_3$): δ 8.99 (1H, br s), 8.73 (1H, s), 8.20 (1H, s), 8.06−7.96 (2H, m), 7.65−7.45 (3H, m), 7.45−7.15 (11H, m), 6.9−6.7 (6H, m), 6.53 (1H, dd, J = 6.2, 6.4 Hz), 4.77 (1H, m), 4.41 (1H, m), 3.80 (3H, s), 3.76 (6H, s), 4.2−3.3 (9H, m), 3.04−2.86 (1H, m), 2.72−2.57 (1H, m), 1.3−1.1 (18H, m). ^{13}C NMR ($CDCl_3$): δ 171.91, 164.67, 160.44, 158.55, 152.56, 151.53, 149.50, 144.58, 142.25, 135.73, 133.81, 132.75, 130.08, 129.46, 128.87, 128.23, 127.90, 126.92, 123.59, 113.79, 113.17, 86.52, 86.38, 85.09, 74.65, 73.35, 63.56, 61.45, 61.12, 55.33, 55.24, 43.36, 42.93, 39.64, 24.78, 24.68, 21.20. ^{31}P NMR ($CDCl_3$): δ 147.30. FAB−HRMS: calcd for $C_{57}H_{66}N_7O_9P$ ($M + Na^+$), 1046.4557,; found, 1046.4515. **65**, slow diastereomer, ^{1}H NMR ($CDCl_3$): δ 9.01 (1H, br s), 8.72 (1H, s), 8.18 (1H, s), 8.06−7.94 (2H, m), 7.65−7.45 (3H, m), 7.45−7.15 (11H, m), 6.9−6.7 (6H, m), 6.54 (1H, dd, J = 7.3, 6.4 Hz), 4.76 (1H, m), 4.32 (1H, m), 3.80 (3H, s), 3.76 (6H, s), 4.2−3.3 (9H, m), 3.04−2.84 (1H, m), 2.80−2.62 (1H, m), 1.3−1.05 (18H, m). ^{13}C NMR ($CDCl_3$): δ 171.99, 164.68, 160.44, 158.55, 152.51, 151.53, 149.49, 144.55, 141.77, 135.70, 133.81, 132.73, 130.07, 129.39, 128.21, 127.88, 127.74, 126.92, 123.59, 128.85, 113.80, 113.16, 86.50, 86.11, 86.01, 85.02, 74.14, 73.76, 63.60, 61.34, 61.04, 55.33, 55.24, 43.19, 42.95, 39.43, 24.73, 24.61, 21.23. ^{31}P NMR ($CDCl_3$): δ 147.94. FAB−HRMS: calcd for $C_{57}H_{66}N_7O_9P$ ($M + Na^+$), 1046.4557; found, 1046.4515.

***N*^{4}−Benzoyl-5′-*O*-(4,4′-dimethoxytrityl)-3′-*O*-(*N*,*N*-diisopropylamino)[2-[*N*-isopropyl-*N*-(4-methoxybenzoyl)amino]ethoxy]phosphinyl-2′-deoxycytidine (66)** was synthesized analogously from **60** (5.14 g, 11.0 mmol) and **62** (6.18 g, 10.0 mmol). Column separation using a gradient from 25:70:5 ethyl acetate:hexane:triethylamine to 70:25:5 ethyl acetate:hexane:triethylamine gave 66, fast diastereomer (1.46 g); **66**, slow diastereomer (1.63 g); and their mixture (6.51 g) totaled in 9.60 g (96.0%) of **66**. **66**, fast diastereomer, ^{1}H NMR ($CDCl_3$): δ 8.56 (1H, br s), 8.33 (1H, d, J = 7.5 Hz), 7.88 (2H, m), 7.66−7.15 (15H, m), 6.90−6.74 (6H, m), 6.28 (1H, dd, J = 5.9, 5.7 Hz), 4.67 (1H, m), 4.27 (1H, m), 3.79 (9H, s), 4.25−3.35 (9H, m), 2.79 (1H, m), 2.33 (1H, m), 1.30−1.10 (18H, m). ^{13}C NMR ($CDCl_3$): δ 171.87, 166.50, 162.08, 160.41, 158.71, 154.77, 144.82, 144.21, 135.58, 135.29, 133.29, 133.10, 130.20, 130.07, 129.46, 128.23, 128.05, 127.57, 127.14, 113.78, 113.34, 96.37, 87.28, 86.97, 86.20, 71.85, 71.51, 62.38, 61.41, 61.15, 55.51, 43.15, 42.92, 41.21, 24.76, 24.68, 21.30. ^{31}P NMR ($CDCl_3$): δ 148.00. FAB−HRMS: calcd for $C_{56}H_{66}N_5O_{10}P$ ($M + Na^+$), 1022.4445; found, 1022.4487. **66**, slow diastereomer, ^{1}H NMR ($CDCl_3$): δ 8.64 (1H, br s), 8.29 (1H, d, J = 7.3 Hz), 7.88 (2H, m), 7.66−7.10 (15H, m), 6.92−6.80 (6H, m), 6.32 (1H, t, J = 5.8 Hz), 4.62 (1H, m), 4.23 (1H, m), 3.80 (9H, s), 4.18−3.35 (9H, m), 2.81 (1H, m), 2.31 (1H, m), 1.30−1.0 (18H, m). ^{13}C NMR ($CDCl_3$): δ 171.96, 166.65, 162.05, 160.4, 158.73, 154.70, 144.78, 144.14, 135.47, 135.26, 133.29, 133.11, 130.19, 129.47, 129.03, 128.05, 127.57, 127.17, 113.80, 113.33, 96.42, 87.21, 86.98, 86.01, 85.80, 72.52, 72.16, 62.51, 61.18, 60.91, 55.26, 43.14, 42.89, 41.43, 25.01, 24.87, 24.77, 24.51, 21.20. ^{31}P NMR ($CDCl_3$): δ 148.15. FAB−HRMS: calcd for $C_{56}H_{66}N_5O_{10}P$ ($M + Na^+$),1022.4445; found, 1022.4488.

5-Methyl-5′-*O*-(4,4′-dimethoxytrityl) −2′-*O*-(2-methoxyethyl)- 3′-*O*-(*N*,*N*-diisopropylamino)[2-[*N*-isopropyl-*N*-(4-methoxybenzoyl)-amino]ethoxy]phosphinyluridine (68) was synthesized analogously from **60** (0.98 g, 2.1 mmol) and **64** (1.21 g, 2.0 mmol). Column separation using a gradient from 15:80:5 to 80:15:5 ethyl acetate:hexane:triethylamine gave **68**, fast diastereomer (0.15 g); **68**, slow diastereomer (0.41 g); and their mixture (1.33 g) totaled in 1.89 g (96.2%) of **68**. **68**, fast diastereomer, ^{1}H NMR ($CDCl_3$): δ 8.53 (1H, br s), 7.68 (1H, s), 7.50−7.20 (11H, m), 6.93−6.78 (6H, m), 6.05 (1H, d, J = 4.8 Hz), 4.48 (1H, ddd, J = 10.0, 4.5, 4.5 Hz), 4.31 (1H, m), 4.25 (2H, t, J = 4.5 Hz), 3.81 (3H, s), 3.78 (6H, s), 3.33 (3H, s), 3.93−3.27 (12H, m), 1.33 (3H, s), 1.22−1.13 (12H, m), 1.08 (3H, d, J = 7.3 Hz), 1.05 (3H, d, J = 7.3 Hz).^{13}C NMR ($CDCl_3$): δ 171.81, 163.99, 160.42, 158.74, 150.45, 144.39, 135.87, 135.51, 135.36, 129.41, 128.02, 127.70, 127.15, 113.78, 113.29, 110.78, 87.33, 86.96, 83.28, 81.61, 81.54, 72.32, 71.05, 70.79, 70.01, 62.31, 60.98, 60.68, 59.11, 55.27, 43.23, 42.99, 24.79, 24.68, 21.12, 11.70. ^{31}P NMR ($CDCl_3$): δ 149.35. FAB−HRMS: calcd for $C_{53}H_{69}N_4O_{12}P$ ($M + Na^+$), 1007.4547; found, 1007.4529. **68**, slow diastereomer, ^{1}H NMR ($CDCl_3$): δ 8.15 (1H, br s), 7.66 (1H, s), 7.45−7.20 (11H, m), 6.93−6.78 (6H, m), 6.08 (1H, d, J = 5.0 Hz), 4.48 (1H, ddd, J = 10.0, 4.5, 4.5 Hz), 4.27 (2H, t, J = 4.9 Hz), 4.22 (1H, m), 3.82 (3H, s), 3.78 (6H, s), 3.32 (3H, s), 3.92−3.26 (12H, m), 1.31 (3H, s), 1.20−1.15 (12H, m), 1.01 (6H, d, J = 6.7 Hz).^{13}C NMR ($CDCl_3$): δ 171.94, 164.02, 160.40, 156.76, 150.45, 144.27, 135.95, 135.42, 130.25, 129.56, 128.27, 127.18, 113.78, 113.49, 110.80, 87.44, 87.01, 83.16, 83.08, 82.37, 72.53, 70.60, 70.43, 70.26, 62.68, 61.80, 61.50, 59.11, 55.26, 43.02, 42.77, 24.76, 24.66, 21.19, 11.62. ^{31}P NMR ($CDCl_3$): δ 149.56. FAB−HRMS: calcd for $C_{53}H_{69}N_4O_{12}P$ ($M + Na^+$), 1007.4547,; found, 1007.4529.

***O*,*O*-Diethyl-*O*-[2-[*N*-isopropyl-*N*-(4-methoxybenzoyl)amino] ethyl] phosphate (52).** *O*,*O*-Diethyl phosphorochloridate (777 mg, 4.5 mmol) in CH_2Cl_2 (3 mL) was added to a stirred solution of **17** (712 mg, 3.0 mmol) and 1*H*-tetrazole (210 mg, 3.0 mmol) in pyridine (2 mL) and CH_2Cl_2 (3 mL) under argon atmosphere at ambient temperature. The mixture was stirred for 30 min, evaporated, and treated with Na_2CO_3 (5% aq, 25 mL). The product was extracted with ethyl acetate (3 × 50 mL). Extracts were washed with brine (2 × 50 mL), dried over Na_2SO_4, and evaporated. Purification on a silica gel column eluting with a gradient of ethanol in CH_2Cl_2 (0−3%) afforded **52** (894 mg, 79.8%) as a colorless oil. ^{1}H NMR ($CDCl_3$): δ 7.35−7.20 (2H, m), 6.95−6.85 (2H, m), 4.3−4.05 (7H, m), 3.80 (3H, s), 3.57 (2H, t, J = 6.3 Hz), 1.31 (6H, t, J = 7.1 Hz), 1.15 (6H, d, J = 6.6 Hz). ^{13}C NMR ($CDCl_3$): δ 172.1, 160.6, 129.1, 128.3, 113.8, 64.7, 64.6, 63.9, 63.8, 55.3, 50.0, 41.3, 41.2, 21.1, 16.2, 16.1. ^{31}P NMR ($CDCl_3$): δ −1.32. Anal. Calcd for $C_{17}H_{28}NO_6P$: C, 54.68; H, 7.56; N, 3.75. Found: C, 54.52; H, 7.51; N, 3.68.

Hydrolysis of 52 under Neutral Conditions and Preparation of

2-[(4-Methoxybenzoyl)oxy]-*N*-isopropylethanaminium trifluoroacetate (40·CF_3COOH). A solution of **52** (224 mg, 0.6 mmol) in water (50 mL) was kept at 25 °C, with progress of the hydrolysis followed by HPLC. After 50 h, **52** disappeared (>98% conversion), and the reaction mixture contained an intermediate, **47**, and product, **54**, in a 46:54 ratio. At this moment, part of the reaction mixture (10 mL) was withdrawn, acidified with TFA to pH 2, and separated by HPLC. After evaporation of the collected fractions, **47** trifluoroacetate was obtained as a 90:10 mixture with **54** trifluoroacetate. LC/MS: calcd for $(C_{13}H_{18}NO_2)^+$, 220.29; found M^+, 220.30. ^{1}H NMR (1% TFA in D_2O): δ 7.82 (2H, m), 7.20 (2H, m), 5.03 (2H, t, J = 9.6 Hz), 4.69 (1H, sept, J = 6.6 Hz), 4.35 (2H, t, J = 9.8 Hz), 3.93 (3H, s), 1.43 (6H, d, J = 6.6 Hz). ^{13}C NMR (1% TFA in D_2O): δ 171.3, 163.3 (q), 132.8, 119.8, 115.6, 113.0, 70.9, 56.4, 51.8, 46.0, 19.7.

After the disappearance of **47** (7 days) the mixture was evaporated, coevaporated with MeCN, and dried to give >98% HPLC-pure, crystalline **54** diethyl phosphate (186 mg, 99%), mp 102−103 °C. ^{1}H NMR (DMSO-d_6): δ 9.35 (2H, br s); 8.03−7.99 (2H, m), 7.06−7.02 (2H, m), 4.45 (2H, t, J = 5.2 Hz), 3.82 (3H, s), 3.65(4H, pent, J = 7.2 Hz), 3.30−3.20 (3H, m), 1.23 (6H, d, J = 6.4 Hz), 1.07 (6H, t, J = 7.2 Hz). ^{1}H NMR (D_2O): δ 7.93−7.89 (2H, m), 6.98−6.93 (2H, m), 4.48−4.44 (2H, m), 3.79 (4H, dt, J = 7.2 Hz), 3.77 (3H, s), 3.40 (1H, sept, J = 6.4 Hz), 3.37 (2H, t, J = 5.2 Hz), 1.22 (6H, d, J = 6.4 Hz), 1.12 (6H, J = 7.2 Hz). ^{13}C NMR (D_2O): δ 168.4, 164.3, 132.6, 121.8, 114.7, 62.9, 62.8, 61.3, 56.3, 51.8, 44.1, 18.7, 16.3. ^{31}P NMR (DMSO-d_6): δ 3.21. ^{31}P NMR (D_2O): δ 1.82.

Crude **54** was dissolved in water (10 mL) and acidified with TFA (1% aq; 1 mL). The product was isolated by HPLC to afford crystalline **54** trifluoroacetate (147 mg, 88%), mp 123−125 °C. ^{1}H NMR (DMSO-d_6): δ 9.18 (2H, br s), 8.02−7.98 (2H, m), 7.08−7.03 (2H, m), 4.44 (2H, t, J = 5.2 Hz), 3.83 (3H, s), 3.44−3.31 (3H, m), 1.24 (6H, d, J = 6.4 Hz). ^{1}H NMR (D_2O): δ 7.93−7.89 (2H, m), 6.98−6.93 (2H, m), 4.48−4.44 (2H, m), 3.77 (3H, s), 3.40 (1H, sept, J = 6.4 Hz), 3.38 (2H, t, J = 5.0 Hz), 1.24 (6H, d, J = 6.4 Hz). ^{13}C NMR (D_2O): δ 168.4, 164.3 (q), 132.5, 121.7, 114.7, 61.3, 56.2, 51.8, 44.1, 18.7. Anal. Calcd for $C_{15}H_{20}F_3NO_5$: C, 51.28; H, 5.74; N, 3.99. Found: C, 51.09; H, 5.71; N, 3.88.

2-Phenyl-2-thiazoline (59). Compound **29** (171 mg, 0.2 mmol) and 1*H*-tetrazole (63 mg, 0.9 mmol) in MeCN (2 mL) were reacted with a detritylated commercial DMT-T derivatized Tentagel N (45 μmol; 0.208 mmol g^{-1}) for 10 min. The solid support was washed with MeCN (3 mL), treated with 3*H*-1,2-benzodithiol-3-one 1,1-dioxide (60.0 mg, 0.3 mmol)[22] in MeCN (3 mL) for 1 min, and detritylated with a solution of dichloroacetic acid (3% in CH_2Cl_2). After washing with MeCN (3 mL), the solid support was suspended in 80% aq MeCN (5 mL) and stirred overnight. The liquid phase was withdrawn, and the solid support was alternately washed with ethyl acetate and 5% aq $NaHCO_3$ (3 × 10 mL each). The solution in 80% aq MeCN and aqueous washings were combined and extracted with ethyl acetate (3 × 10 mL). The ethyl acetate washings and extracts were dried over Na_2SO_4 and evaporated to give **59** (ca. 90% pure, 6.3 mg, 85.7%). ^{1}H NMR ($CDCl_3$): δ 7.89−7.80 (2H, m), 7.52−7.34 (3H, m), 4.46 (2H, t, J = 8.4 Hz), 3.42 (2H, t, J = 8.4 Hz). ^{13}C NMR ($CDCl_3$): δ 163.7, 131. 4, 128.7, 128.6, 65.5, 33.9. IR (Nujol): ν 1610 cm^{-1}.

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Supporting Information Available: Materials and methods, HPLC techniques. Synthetic procedures and spectral data for **12**, **16**, **21**−**31**, **49**, and **58**. ^{31}P NMR spectra for solid-support-bound compounds **37**, **39**, **41**, **43**, **44**, **45**, and **51**. ^{1}H and ^{13}C NMR spectra for **47** and **59**. ESMS Data and reverse-phase HPLC profiles for oligonucleotides **69**−**76**. This material is available free of charge via the Internet at http://pubs.acs.org.

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