Protective Groups in Organic Synthesis, Third Edition. Theodora W. Greene, Peter G.M. Wuts

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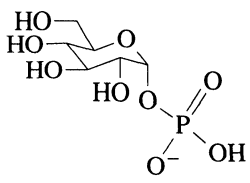
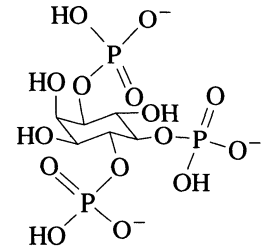
PROTECTION FOR THE PHOSPHATE GROUP

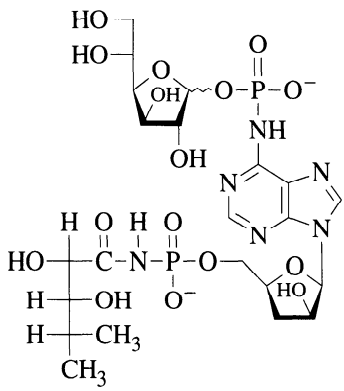
"Phosphate esters and anhydrides dominate the living world."[1] Major areas of synthetic interest include oligonucleotides[2] (polymeric phosphate diesters), phosphorylated peptides, phospholipids, glycosyl phosphates, and inositol phosphates.[2b,3]



a glycosyl phosphate



D-*myo*-inositol 1,4,5-triphosphate



Agrocin 84[4]

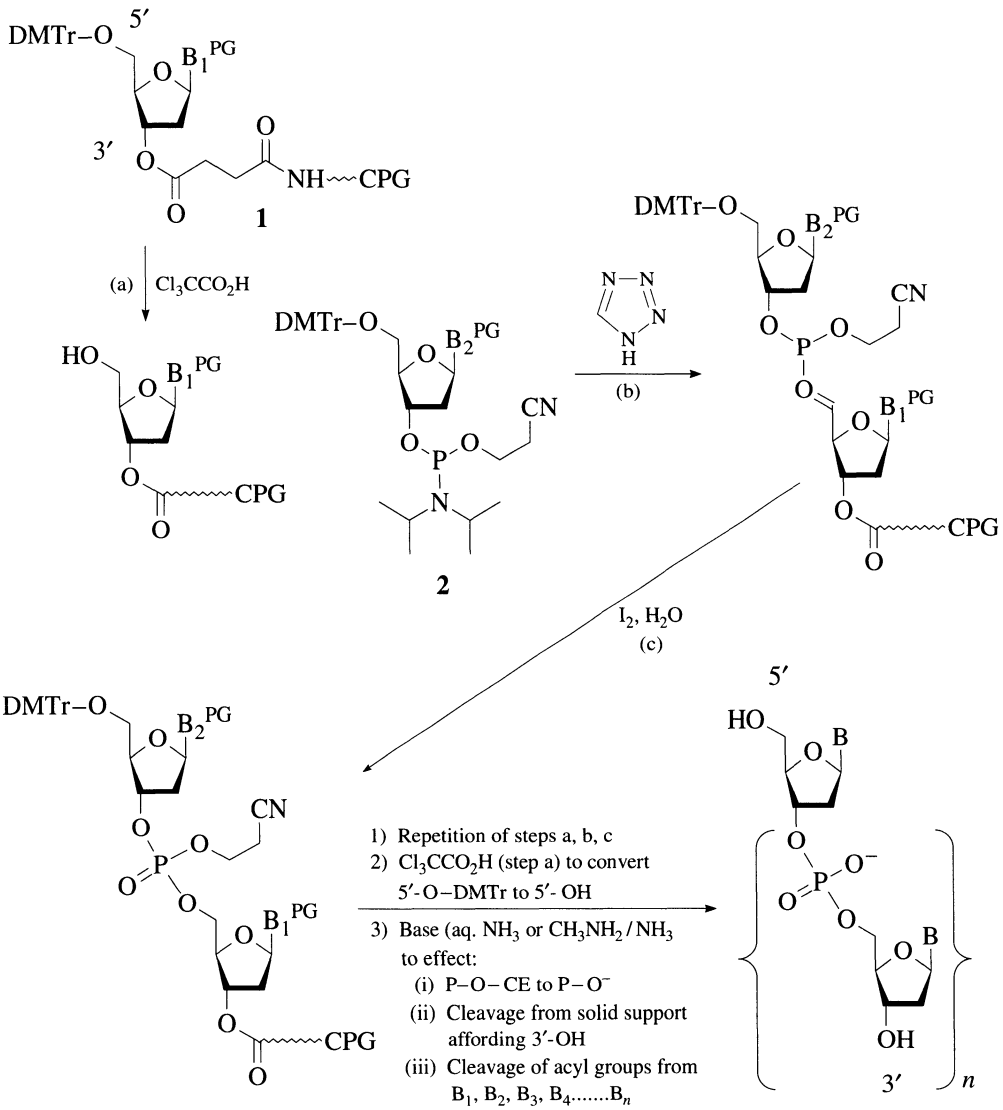
The steps involved in automated oligonucleotide synthesis illustrate the current use of protective groups in phosphate chemistry (Scheme 1). Oligonucleotide synthesis involves the protection and deprotection of the 5′-OH, the amino groups on adenine, guanine, and cytosine, and –OH groups on phosphorus.

A difference in the problems associated with the protection and deprotection of phosphoric acid species, compared with the other functionalities in this book (alcohols, phenols, aldehydes and ketones, carboxylic acids, amines, and thiols), lies in the fact that phosphoric acid is tribasic (pK_1 = 2.12, pK_2 = 7.21, pK_3 = 12.66). These large differences in pKa's are reflected in large differences in rates of alkaline hydrolysis of the corresponding esters [e.g., $t_{1/2}$ at 1 *M* NaOH in water, 35°: $(CH_3O)_3PO$, 30 minutes; $(CH_3O)_2PO_2^-$, 11 years].[5] Large differences are often found in the rates of successive removal of blocking groups from phosphate derivatives, especially under nonacidic conditions. Phosphate esters are also hydrolyzed by acid,[5] but here the relative rates are closer together.

A consequence of the tribasic nature of phosphoric acid (three –OH groups attached to phosphorus) is the increased number of options available in the overall process of conversion of alcohol to protected phosphate. The conversion might be carried out by the sequence

$$R\text{–}O\text{–}H \rightarrow R\text{–}O\text{–}PO_3H_2 \rightarrow R\text{–}O\text{–}P(O)(OH)\text{–}O\text{–}PG$$

or by the formation of the R–O–P attachment *after* the formation of P–O–PG, i.e., introduction of the phosphate moiety in a form that is already protected. Another major difference in protection (and deprotection) in the phosphorus area lies in the availability of two major valence states, P(III) and P(V), of this second-row element. Both of these aspects [the order of formation of the bonds to P and the use of P(III) as well as P(V)] are important in current phosphate protection practice.



DMTr = 4,4′-dimethoxytrityl

B^{PG} = acetyl, benzoyl, isobutyryl

CPG = "Controlled Pore Glass" (solid support)

B_1, B_2, B_3, B_4 = adenyl, cytidyl, guanyl, thymidyl

CE = 2-cyanoethyl

1 and **2** (B_1, B_2, B_3, B_4), commercially available

Scheme 1. Automated Synthesis of Oligonucleotides. Synthetic Cycle for the Phosphoroamidite Method.

Phosphate protection may begin at the stage of phosphoryl chloride (phosphorus oxychloride). A protective group may be introduced by reaction of this

acid chloride with an alcohol[6] to afford an ester with the desired combination of stability to certain conditions and lability to others:

$$POCl_3 + ROH \rightarrow \underset{\text{a phosphorodichloridate}}{RO\text{-}P(O)Cl_2} \xrightarrow{\text{slower}} \underset{\text{a phosphorochloridate}}{(RO)_2P(O)Cl} \xrightarrow{\text{slower}} (RO)_3PO$$

A disadvantage of phosphoryl chloride reagents is that they are not very reactive. In the mid-1970s, Letsinger and co-workers introduced a new paradigm that makes use of the more reactive phosphorus(III) reagents.[7] In this approach a monoprotected phosphorodichloridite ($ROPCl_2$)[8,9] is coupled with an alcohol, followed by a second condensation with another alcohol, to produce a triester. Oxidation with aqueous iodine affords a phosphate:[2,10]

$$ROPCl_2 + R'OH \rightarrow ROP(OR')Cl \xrightarrow{R''OH} ROP(OR')(OR'')$$
$$\xrightarrow{I_2,\ H_2O} ROP(O)(OR')(OR'')$$

The disadvantage of this method is that the dichloridites and monochloridites are sensitive to water and thus could not be used readily in automated oligonucleotide synthesis. This problem was overcome by Beaucage and Caruthers, who developed the phosphoramidite approach. In this method, derivatives of the form $R'OP(NR_2)_2$ react with one equivalent of an alcohol (catalyzed by species such as 1*H*-tetrazole) to form diesters, $R'OP(OR'')NR_2$, which usually are stable, easily handled solids. These phosphoroamidites are easily converted to phosphite triesters by reaction with a second alcohol (catalyzed by 1*H*-tetrazole). Here, again, oxidation of the phosphite triester with aqueous iodine affords the phosphate triester. Over the years, numerous protective groups and amines have been examined for use in this approach. Much of the work has been reviewed.[2,10]

SOME GENERAL METHODS FOR PHOSPHATE ESTER FORMATION

1. Phosphoric acids may be esterified using an alcohol and an activating agent:
   (a) carbodiimides, e.g., DCC.[11,12]
   (b) arylsulfonyl chloride and a base (TPS, Pyr).[13]
   (c) Various sulfonamido derivatives ($ArSO_2$–Z, Z = 1-imidazolyl, 1-triazolyl, 1-tetrazolyl).[2j,14,15]
   (d) CCl_3CN.[16–18]
   (e) $SOCl_2$, DMF, –20°, 70–90% yield:[19] $RP(O)(OH)_2 \rightarrow RP(O)(OH)OR$.
   (f) $[(Me_2N)_3PBr]^+PF_6^-$, DIEPA, CH_2Cl_2.[20]
2. Nucleophilic (S_N2) reactions for the formation of benzyl, allyl, and certain alkyl phosphates [e.g., Me_4N^+ $(RO)_2P(O)O^-$ and an alkyl halide in refluxing DME].[21,22]

3. Reaction of a phosphoric acid with a diazoalkane [CH_2N_2,[23,17] $ArCHN_2$, (*N*-oxido-α-pyridyl)CHN_2, Ar_2CN_2].[24]
4. Primary alcohols may be phosphorylated by use of the Mitsunobu reaction (Ph_3P, DEAD, HBF_4, Pyr). Of several salts examined, the potassium salt of the phosphate was the best.
5. One of the most widely used methods for the formation of phosphate esters involves the conversion of a P–N bond of a phosphorus(III) compound to a P–O bond by ROH, catalyzed by 1*H*-tetrazole, followed by oxidation to the phosphorus(V) derivative:[2]

(a) $R'OH + (R''O)P(NR_2)_2 \xrightarrow{1H\text{-tetrazole}} (R'O)P(NR_2)OR''$

phosphorodiamidite → phosphoroamidite

(b) $(R'O)P(NR_2)OR'' \xrightarrow{I_2,\ H_2O} (R'O)P(O)(NR_2)OR''$

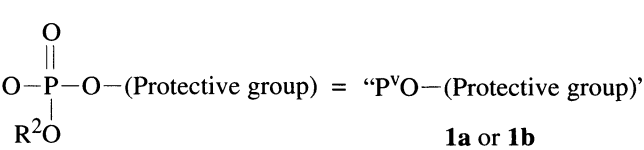
phosphoroamidite → phosphoroamidate

6. Preparation of $(MeO)_2P$–O–R: ROH, $(MeO)_3P$, CBr_4, Pyr, 70–98% yield.[25] The alkyl dimethyl phosphite may then be oxidized to the corresponding phosphate by aq. iodine, *t*-butyl hydroperoxide, or peracid.

REMOVAL OF PROTECTIVE GROUPS FROM PHOSPHORUS

All the approaches for deblocking protective groups described earlier in this book have found application in the removal of protective groups from phosphorus derivatives. Because phosphate protection and deprotection are commonly associated with compounds that contain acid-sensitive sites (e.g., glycosidic linkages and DMTr–O– groups of nucleotides), the most widely used protective groups on phosphorus are those that are deblocked by base.

In the following list, "P^v–O–" stands for phosphorus(V) derivatives — usually $(R^1O)P(O)(OR^2)$–O–, in which R^1 and R^2 are not specified:



1a $R^1 = R^2$ = alkyl or aryl

1b R^1 = H, R^2 = alkyl or aryl

1. Groups removed by base (in one step or in the second of two steps):
   (a) One-step removal via β-elimination of various β-substituted ethyl derivatives:

(i) P^v–O–CH_2CH_2CN + TEA → P^v–O^- + CH_2=CHCN Ref. 26

(ii) P^v–O–CH_2CH_2–$SiMe_3$ + $Bu_4N^+F^-$, THF → P^v–O^- Ref. 4

(b) Two-step removal:

(i) oxidation–elimination

$$P^v\text{–O–}CH_2CH_2\text{–S–R} \xrightarrow{\text{Oxid'n}} P^v\text{–}OCH_2CH_2\text{–}SO_2R \xrightarrow{\text{base}} P^v\text{–}O^- \quad \text{Ref. 14}$$

(ii) reduction-elimination

P^v–O–CH_2–(2-anthryl-9,10-quinone) $\xrightarrow{\text{Red'n}}$ corresponding hydroquinone $\xrightarrow{\text{base}}$ P^v–O^- Ref. 27

(c) Aryl phosphates and strong base. As stated earlier, dialkyl phosphates are quite stable to base. The P^v–O–aryl moiety is more labile to base than the P^v–O–alkyl moiety (hydroxide attack at P and ejection of Ar–O^-):

$$P^v\text{–O–Aryl} + OH^- \rightarrow P^v\text{–}O^- + ArO^- \quad \text{Ref. 28}$$

2. Hydrogenolysis: P^v–O–CH_2Ph, H_2, Pd.[29]
3. Reduction: P^v–O–CH_2CCl_3, Zn/Cu, DMF.[30]
4. S_N2 displacement:
   (a) P^v–O–CH_2Ph + NaI, CH_3CN → P^v–OH (or P^v–O^-) Ref. 31
   (b) P^v–O–CH_3 + PhS^-, DMF → P^v–O^- + PhSMe Ref. 32
5. Acid: P^v–O–*t*-Bu + H^+ → P^v–OH Ref. 33
6. Photolysis: P^v–O–R $\xrightarrow{h\nu}$ P^v–OH (or P^v–O^-) Ref. 34
   R = 3,5-dinitrophenyl, 2-nitrobenzyl, 3,5-dimethoxybenzyl, pyrenylmethyl, desyl, 4-methoxybenzoylmethyl
7. Oxidation: P^v–O–C_6H_4–*p*-NHTr, I_2, acetone, NH_4OAc.[35]
8. Metal ion catalysis: P^v–O–8-quinolinyl, $CuCl_2$, DMSO, H_2O → P^v–O^- Ref. 36
9. TMSCl, TMSBr or TMSI: P^v–O–CH_3, TMSI, CH_3CN.[37]
10. Cleavage of P^v–NHR to P^v–OH: P^v–NH–Ph, isoamyl nitrite, HOAc.[38]
11. Cleavage of P^v–S–R:
    (a) P^v–S–Et, I_2, Pyr → P^v–O^- Ref. 39
    (b) P^v–S–Ph, Zn → P^v–O^- Ref. 40
12. Transesterification: conversion of P^v–O–R to P^v–O–R′.
    (a) Transesterification–hydrogenolysis:

P^v–O–Ph + Bn–O^- → P^v–O–Bn $\xrightarrow{H_2, Pd}$ P^v–OH (or P^v–O^-) Ref. 41

(b) Transesterification–elimination:

$$P^{v}\text{–O–R}+R'\text{–CH=N–O}^{-} \rightarrow P^{v}\text{–O–N=CHR} \xrightarrow{\text{base}} P^{v}\text{–O}^{-}+R'CN \quad \text{Ref. 42}$$

13. Electrolysis (has seen little use):

$$P^{v}\text{–O–CH}_2CCl_3 \xrightarrow{\text{electrolytic reduction}} P^{v}\text{–O}^{-} \quad \text{Ref. 43}$$

The following sections primarily describe many of the methods used for the cleavage of some of the more common phosphate protective groups. Since most of these groups are introduced by either the phosphate or phosphite method, little information is included here about their formation. The cited references generally describe the means that were used to introduce the protective group. In some cases, methods of formation are described, but this is done only when alternative methods to the phosphate or phosphite procedure were used.

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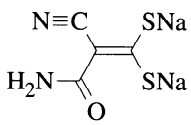
ALKYL PHOSPHATES

Methyl: CH_3-

Formation

1. A phosphonic acid can be esterified with CH_2N_2 in 88–100% yield.[1,2]

Cleavage

1. 2-Mercaptobenzothiazole, *N*-methylpyrrolidone, DIPEA. The reagent has the advantage that it is odorless and does not lead to internucleotide cleavage, but the cleavage rate is 10 times slower than when thiophenol is used.[3]
2. Thiophenol, TEA, DMF or dioxane.[4] In the case of dimethyl phosphonates, this method can be used to remove selectively only one methyl group.[5] Lithium thiophenoxide is also effective.[6]
3.  DMF. This odorless and easily prepared reagent is relatively nonbasic (pK_B = 8.4) and cleaves the methyl group about four times faster than thiophenol. It is also used to remove the 2,4-dichlorobenzyl group from phosphates and dithiophosphates.[4]
4. Ammonia. Cleavage is not as clean as with thiophenol.[7]
5. 10% Me_3SiBr, CH_3CN, 1–2 h, 25°, >97% yield.[8,9] This reagent is also useful for the cleavage of ethyl phosphates[10] and phosphonates.[11]
6. 1 *M* Me_3SiBr, thioanisole, TFA.[8,12]
7. 45% HBr, AcOH.[13,14,15] This method and the use of TMSI were not suitable for the deprotection of phosphorylated serines.[16] Diethyl phosphates are cleaved very slowly.[17]
8. TMSI, CH_3CN.[14,15,18] *In situ*–generated TMSI is also effective.[19]
9. Aqueous pyridine.[20]
10. NaI, acetone.[21,22]
11. The use of TMSOTf and thioanisole results in rapid ($t_{1/2}$ = 7 min) cleavage of one methyl in a dimethyl phosphate, whereas the second methyl is cleaved only slowly ($t_{1/2}$ = 12 h).[23] The method has been further refined for peptide synthesis.[24]
12. Fmoc chemistry is compatible with methyl phosphates when methanolic

K_2CO_3 is used to remove the Fmoc group instead of the usual amines.[25]

13. Dimethyl sulfide, CH_3SO_3H. Methyl phosphates are selectively cleaved in the presence of other alkyl phosphates.[26]
14. *t*-Butylamine, 46°, 15 h.[27]

Ethyl: C_2H_5-

Cleavage

1. Ethyl phosphates are usually cleaved by acid hydrolysis.[28]
2. TMSBr, CH_3CN.[29]
3. NH_4OH, MeOH.[29] These conditions result in cleavage of only one ethyl group of a diethyl phosphonate. Selective monodeprotection of a number of alkyl-protected phosphates is fairly general for cases where cleavage occurs by the release of phosphate or phosphonate anions.
4. LiBr has been used to cleave the ethyl group.[30]

4-(*N*-Trifluoroacetylamino)butyl: $CF_3C(O)NH(CH_2)_4-$

Ammonia treatment removes the TFA group, which then releases the phosphate and pyrrolidine through intramolecular cyclization. The analogous pentyl derivative was also prepared.[31]

Isopropyl: $(CH_3)_2CH-$

A diisopropyl phosphonate is cleaved with TMSBr, TEA, CH_2Cl_2, rt.[32] Dioxane can also be used as solvent.[33]

Cyclohexyl (cHex): $C_6H_{11}-$

Cleavage

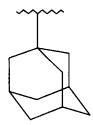
1. The cyclohexyl phosphate, used in the protection of phosphorylated serine derivatives, is introduced by the phosphoramidite method and cleaved with TFMSA/MTB/*m*-cresol/1,2-ethanedithiol/TFA, 4 h, 0° to rt.[34]
2. Monocyclohexyl phosphates and phosphonates can be cleaved by a two-step process in which the ester is treated with an epoxide such as propylene oxide to form another ester, which, upon treatment with base, releases the cyclohexyl alcohol.[35]

***t*-Butyl:** $(CH_3)_3C-$

t-Butyl phosphates are acid sensitive.[36] They are not stable to Zn/AcOH.[37]

Cleavage

1. 1 *M* HCl, dioxane, 4 h.[16,38]
2. TFA, thiophenol[12] or thioanisole.[39]
3. TMSCl, TEA, CH_3CN, 75°, 2 h.[40]

1-Adamantyl: 

An adamantyl phosphonate, prepared from adamantyl bromide and Ag_2O, is easily cleaved with TFA in CH_2Cl_2.[41]

Allyl: $CH_2{=}CHCH_2{-}$

Cleavage

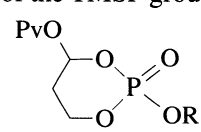
1. $Rh(Ph_3P)_3Cl$, acetone, H_2O, reflux, 2 h, 86% yield.[42]
2. $Pd(Ph_3P)_4$, Ph_3P, RCO_2K, EtOAc, 25°, 83% yield.[42,43] Diethylammonium formate,[44] NH_3,[45] and $BuNH_2$[46,47] have also been used as allyl scavengers in this process. In a diallyl phosphate, deprotection results in the cleavage of only a single allyl group.[48]
3. $Pd_2(dba)_3$–$CHCl_3$, Ph_3P, butylamine, formic acid, THF, 50°, 0.5–1 h.[49]
4. Concd. ammonia, 70°.[50]
5. $PdCl_2(Ph_3P)_2$, Bu_3SnH; $ClB(OR)_2$, then aqueous hydrolysis.[51]
6. NaI.[52]

2-Trimethylsilylprop-2-enyl (TMSP): $CH_2{=}C(TMS)CH_2{-}$

This derivative is stable to AcOH and methanolic ammonia, but not to 0.5 *N* aq. NaOH.

Cleavage

1. H_2, Pd–C, EtOH. [53]
2. $Et_4N^+F^-$, CH_3CN, 48 h, reflux. TMSF and allene are formed in the cleavage reaction. These conditions are not compatible with phenyl phosphates, which are cleaved preferentially with fluoride.[53] Cleavage of a bis TMSP phosphate results in the cleavage of only one of the TMSP groups.

3-Pivaloyloxy-1,3-dihydroxypropyl Derivative: 

This group was designed as an enzymatically cleavable protective group. Cleavage is achieved using an esterase present in mouse plasma or hog liver carboxylate esterase.[54]

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2-Substituted Ethyl Phosphates

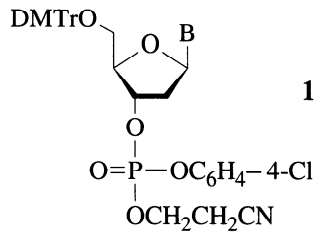
2-Cyanoethyl: $NCCH_2CH_2-$

Formation

1. $NCCH_2CH_2OH$, triisopropylbenzenesulfonyl chloride, Pyr, rt, 15 h.[1]
2. $NCCH_2CH_2OH$, DCC, Pyr.[2]
3. $NCCH_2CH_2OH$, 8-quinolinesulfonyl chloride, 1-methylimidazole, Pyr, rt.[3]
4. For monoprotection of a phosphonic acid: $NCCH_2CH_2OH$, Cl_3CCN, 74–93% yield.[4]

Cleavage

1. Aqueous ammonia, dioxane.[5]
2. Alkaline hydrolysis.[2]
3. TMSCl, DBU, CH_2Cl_2, 25°. The presence of TMSCl allows for complete deprotection of a biscyanoethyl phosphate. Without TMSCl, only one cyanoethyl group was cleaved.[6]
4. $Bu_4N^+F^-$, THF, 30 min.[7]
5. In a study of the use of various amines for the deprotection of the cyanoethyl group, it was found that primary amines are the most effective in achieving rapid cleavage. The following times for complete cleavage of the cyanoethyl group in phosphate **1** were obtained: TEA, 180 min; DIPA, 60 min; Et_2NH, 30 min; *s*-$BuNH_2$, 20 min; *t*-$BuNH_2$, 10 min; *n*-$PrNH_2$, 2 min.[8] Further study showed that *t*-$BuNH_2$ was most suitable because it did not react with protected nucleobases. Methylamine/ammonia was also a fast (5 min), effective reagent for deprotection.[9]



1

2-Cyano-1,1-dimethylethyl (CDM): $CNCH_2C(CH_3)_2-$

Cleavage

1. Ammonia.[10]
2. DBU, *N,O*-bis(trimethylsilyl)acetamide.[11] Thiophosphorylated derivatives are cleaved more rapidly than the phosphorylated counterpart.
3. 0.2 *N* NaOH, dioxane, CH_3OH.[10]
4. Guanidine, tetramethylguanidine, or $Bu_4N^+OH^-$.[12]

4-Cyano-2-butenyl

This is a vinylogous analogue of the cyanoethyl group that is removed by δ-elimination with ammonium hydroxide.[13]

***N*-(4-Methoxyphenyl)hydracrylamide, *N*-Phenylhydracrylamide, and *N*-Benzylhydracrylamide Derivatives:** $ArNHC(O)CH_2CH_2-$

These derivatives, used for 5′-phosphate protection, are prepared by using the DCC coupling protocol and are cleaved with 2 *N* NaOH at rt.[14] The protected phosphates can be purified using benzoylated DEAE–Cellulose.

2-(Methyldiphenylsilyl)ethyl (DPSE): $(C_6H_5)_2CH_3SiCH_2CH_2-$

2-(Trimethylsilyl)ethyl (TSE): $(CH_3)_3SiCH_2CH_2-$

These groups, along with a number of other trialkylsilylethyl derivatives, were examined for protection of phosphorothioates. Only the phenyl-substituted silyl derivative was useful, because simple trialkylsilyl derivatives were prone to acid-catalyzed thiono–thiolo rearrangement.[15] Other trialkylsilylethyl derivatives also suffer from inherent instability upon storage,[16] but the trimethylsilylethyl group has been used successfully in the synthesis of the very sensitive agrocin 84[17] and for internucleotide phosphate protection with the phosphoramidite approach.[18]

Formation

1. The ester is introduced by means of the phosphoramidite method.[15,19]

Cleavage

1. Ammonium hydroxide, rt, 1 h.[15,19,20]
2. $Bu_4N^+F^-$ THF, Pyr, H_2O.[15,21,22]
3. Methylamine, H_2O.[15]
4. SiF_4, CH_3CN, H_2O, 20 min.[23]
5. NH_4F, methanol, 60°. One of two DPSE groups is cleaved.[24]
6. HF, CH_3CN, H_2O. In this case, both DPSE groups are removed.[24] This method effectively removes the trimethylsilylethyl group.[25]
7. TFA, CH_2Cl_2 or TFA, phenol, 30 min.[16]

2-(Triphenylsilyl)ethyl: $(C_6H_5)_3SiCH_2CH_2-$

This group, used for 5′-phosphate protection, has hydrophobicity similar to that of the dimethoxytrityl group and thus was expected to assist in reverse-phase HPLC purification of product from failure sequences in oligonucleotide synthesis. The group is cleaved with $Bu_4N^+F^-$ in DMSO at 70°.[26]

2-(*S*-Acetylthio)ethyl (SATE): $CH_3C(O)SCH_2CH_2-$

Formation

1. The SATE ester is formed from a phosphite using PvCl activation followed by oxidation to the phosphate with I_2/H_2O.[27]

Cleavage

1. Enzymatic hydrolysis exposes the sulfide, which undergoes episulfide formation releasing the phosphate.[27] This method was developed for

intracellular delivery of a monophosphate. This concept was also extended to the use of an *S*-glucoside that could be activated by a glucosidase to release the thiol.[28]

2. Treatment of $(EtO)_2P(S)SCH_2CH_2SC(O)R$ (R = Bz was preferred) with ammonia gives $(EtO)_2P(S)S^-$.[29]

2-(4-Nitrophenyl)ethyl (NPE): $4\text{-}NO_2C_6H_4CH_2CH_2-$

The use of this group in nucleotide and nucleoside synthesis has been reviewed.[30,31]

Cleavage

1. 0.5 *M* DBU in Pyr or CH_3CN. In this study,[32] the cleavage of a series of **2-(pyrazin-2-yl)ethyl phosphates** was compared with that of the NPE group. The former group was found to be cleaved with DBU in CH_3CN.[32–34] The related **2-(2-chloro-4-nitrophenyl)ethyl** ester is cleaved with the weaker base TEA in CH_3CN.[35] The addition of thymine during DBU deprotection improves the yield, because thymine scavenges the released 4-nitrostyrene.[36] The **2-(2-nitrophenyl)ethyl** group is cleaved about six times more slowly with DBU as the base.[37] Upon DBU treatment, a **bis-2-(4-nitrophenyl)ethyl** phosphate releases only a single Npe group.[38]

2-(2′-Pyridyl)ethyl (Pyet)

Cleavage

1. NaOMe, MeOH, Pyr or *t*-BuOK, Pyr, *t*-BuOH.[39] This group is reasonably stable to aqueous NaOH, ammonia, and 80% acetic acid.
2. MeI, CH_3CN.[40]
3. PhOCOCl, CH_3CN, 20°, 6 h; ammonia, Pyr.[41]

2-(4′-Pyridyl)ethyl

The 4′-pyridylethyl group was found to be more effective for internucleotide phosphate protection than the 2′-pyridylethyl group, because its cleavage proceeded with greater efficiency. It is cleaved in a two-step process: acylation with PhOCOCl increases the acidity of the benzylic protons, facilitating E-2 elimination by ammonia.[42]

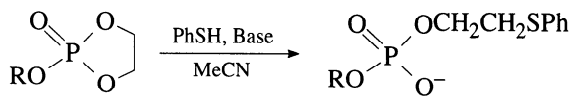
2-(3-Arylpyrimidin-2-yl)ethyl

Cleavage of this ester with DBU is faster than cleavage of the Npes group; it can also be cleaved with the weaker base TEA/Pyr.[43]

2-(Phenylthio)ethyl: $C_6H_5SCH_2CH_2-$

Formation

1. From $ROP(O)(OH)_2$: $PhSCH_2CH_2OH$, DCC.[44]

2. 

Ref. 45

3. $PhSCH_2CH_2OH$, triisopropylbenzenesulfonyl chloride, DMF, HMPA, rt, 8 h, 65–70% yield.[46]

Cleavage

1. $NaIO_4$, 1 h, rt; 2 *N* NaOH, 30 min, rt.[44,45]
2. *N*-Chlorosuccinimide; 1 *N* NaOH.[47] With this method, the sulfide is oxidized completely to the sulfone, which is cleaved with hydroxide more readily than the sulfoxide formed by periodate oxidation. It has been reported that oxidation of the sulfide leads to oxidation of adenine and guanine.[48] However, see the discussion of the TPTE group below.

2-(4′-Nitrophenyl)thioethyl (PTE)

This group is stable to TEA and morpholine in pyridine at 20°. It is cleaved by oxidation with MCPBA followed by elimination with TEA in Pyr, 10 min, 20°.[49] The rate of cleavage is proportional to the strength of the electron-withdrawing group on the phenyl ring.[50]

2-(4′-Triphenylmethylphenylthio)ethyl (TPTE): 2-[4-$(C_6H_5)_3CC_6H_4S$]CH_2CH_2-

The TPTE group, an analogue of the 2-(phenylthio)ethyl group, was developed to impart lipophilicity to protected oligonucleotides so that they could be isolated by solvent extraction. It is formed from the phosphoric acid and the alco-hol using either DCC or TPS as coupling agents. Cleavage is effected by base treatment after oxidation with $NaIO_4$ or NCS.[51]

2-[2′-(Monomethoxytrityloxy)ethylthio]ethyl

This easily prepared lipophilic 5′-phosphate protective group is cleaved by NCS oxidation (dioxane, triethylammonium hydrogen carbonate, 2 h, rt) followed by ammonia-induced β-elimination.[3]

Dithiodiethanol Derivative (DTE): $HOCH_2CH_2SSCH_2CH_2-$

Cleavage

1. Reduction of the disulfide by a reductase exposes the thiol, which then closes to give an episulfide, releasing the phosphate.[27]

2-(*t*-Butylsulfonyl)ethyl (B′SE): $(CH_3)_3CSO_2CH_2CH_2-$

The B′SE group was used for internucleotide protection and is removed with ammonia, also used to remove *N*-acyl protective groups. Compared with the methylsulfonylethyl group,[52] the B′SE group has better solubility properties for solution phase synthesis.[53]

2-(Phenylsulfonyl)ethyl (PSE): $C_6H_5SO_2CH_2CH_2-$

The use of this group avoids the problems associated with the oxidation of the phenylthioethyl group.

Cleavage

1. TEA, Pyr, 20°, <3 h.[48,54]

2-(Benzylsulfonyl)ethyl

This group is cleaved with 2 eq. of TEA in Pyr at a rate somewhat slower than that of the phenylsulfonylethyl group.[55]

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Haloethyl Phosphates

2,2,2-Trichloroethyl: Cl_3CCH_2O-

Myoinositol bis(trichloroethyl)phosphates were not as stable to pyridine at 20°, as were the related benzyl analogs.[1]

Formation

1. Trichloroethanol, DCC, Pyr, rt, 15 h.[2]
2. A phosphonic acid was monoesterified with trichloroethanol, CCl_3CN in Pyr at 100°.[3]

Cleavage

1. Electrolysis at a Hg cathode, −1.2 V (Ag wire), CH_3CN, DMF, $Bu_4N^+BF_4^-$, 2,6-lutidine.[4] LiCl or $LiClO_4$ have been used as electrolytes in the electrochemical removal of haloethyl phosphates.[5]
2. Zn, acetylacetone, DMF, Pyr.[6,7] Chelex resin can be used to remove the zinc from these deprotections.[8]
3. Na, ammonia.[9] These conditions also remove cyanoethyl- and benzyl-protective groups. Phosphorothioates are similarly deprotected.

4. Zn(Cu), DMF.[10,11]
5. NaOH, aqueous dioxane.[12]
6. The trichloroethyl group is stable to Pd-catalyzed hydrogenolysis in AcOH/TFA, but when hydrogenolysis was attempted using EtOAc/MeOH as solvent, partial removal of the trichloroethyl group occurred along with Fmoc cleavage. Clean cleavage was observed in aqueous ethanol as solvent.[13,14]
7. Hydrogenolysis: Pd, Pyr.[15]
8. $Bu_4N^+F^-$, THF.[16]
9. Zn, anthranilic acid. Anthranilic acid was used to prevent complexation of the zinc with the oligonucleotides.[17]

2,2,2-Trichloro-1,1-dimethylethyl(TCB): $Cl_3CC(CH_3)_2O-$

Formation

1. The ester is introduced as the bis-TCB monochlorophosphate.[18]

Cleavage

1. Cobalt(I)-phthalocyanine, CH_3CN, 48 h. In a phosphate with two TCB groups, the first is cleaved considerably faster than the second.[18,19]
2. Bu_3P, DMF, TEA, 80°, quant.[20,21] Trichloroethyl phosphates are also cleaved.
3. Zn, acac, TEA, CH_3CN.[22]

2,2,2-Tribromoethyl: Br_3CCH_2-

Formation

1. $(RO)(Cl_3CCH_2O)P(O)Cl$, Br_3CCH_2OH.

Cleavage

1. Electrolysis at a Hg cathode, −0.5 to −0.6 V, $LiClO_4$, CH_3CN, Pyr. The trichloroethyl ester, which requires a greater reduction potential for cleavage, is retained under these conditions.[4]
2. Zn(Cu), DMF, 20°.[23]
3. Zn(Cu), Bu_3N, H_3PO_4, Pyr, rt.[24]

2,3-Dibromopropyl: $BrCH_2CHBrCH_2-$

Treatment of this protective group with KI/DMF for 24 h results in complete cleavage. The group is stable to Pyr/TEA/H_2O, but not to 7 *M* NH_4OH/MeOH.[25]

2,2,2-Trifluoroethyl: CF_3CH_2-

The trifluoroethyl group was used as an activating group in the phosphotriester approach to oligonucleotide synthesis, as well as a protective group that could be removed with 4-nitrobenzaldoxime (tetramethylguanidine, dioxane, H_2O).[26]

1,1,1,3,3,3-Hexafluoro-2-propyl: $(CF_3)_2CH-$

Cleavage of this group is achieved with tetramethylguanidinium *syn*-2-pyridinecarboxaldoxime.[27,28] Tris(hexafluoro-2-propyl) phosphites are sufficiently reactive to undergo transesterification with alcohols in a stepwise fashion.[29]

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BENZYL PHOSPHATES

Benzyl (Bn): $C_6H_5CH_2-$

Formation

1. From a tributylstannyl phosphate: BnBr, $Et_4N^+Br^-$, CH_3CN, reflux. Phenacyl, 4-nitrobenzyl, and simple alkyl derivatives were similarly prepared. Yields are substrate and alkylating-agent dependent.[1]
2. Diphenyl phosphates are converted by transesterification to dibenzyl phosphates upon treatment with BnONa in THF at 25° in 83% yield.[2]

Cleavage

1. Pd–C, H_2, formic acid.[3]
2. Pd–C, EtOH, $NaHCO_3$, H_2.[4] Hydrogenolysis in the presence of NH_4OAc cleaves only one benzyl group of a dibenzyl phosphate.[5]
3. Na, ammonia.[6,7] Cyanoethyl and trichloroethyl phosphates are also deprotected.
4. 1 *M* TFMSA in TFA, thioanisole.[8] Dibenzyl phosphates are only partially labile to TFA alone.
5. TFA, thiophenol.[9]
6. A dibenzyl phosphate is monodeprotected with TFA–CH_2Cl_2.[10]
7. LiSPh, THF, HMPA, 30 min, >95% yield.[11]
8. NaI, CH_3CN.[12]
9. TMSBr, Pyr, CH_2Cl_2, rt, 1.5 h.[13] Phenolic phosphates were stable to this reagent.[14]
10. With dibenzyl phosphates or phosphonates, treatment with refluxing *N*-methylmorpholine results in monodebenzylation (60–100% yield).[15]
11. Quinuclidine, toluene, reflux.[16] In dibenzyl phosphates, only one benzyl group is removed.

o-Nitrobenzyl: $2\text{-}NO_2\text{-}C_6H_4CH_2-$

Formation

1. *o*-Nitrobenzyl alcohol, DCC, rt, 2 days. Pyridine slowly reacts to displace the nitrobenzyl ester, forming a 2-nitrobenzylpyridinium salt.[17]

Cleavage

1. Photolysis.[18–20]
2. Cleavage of an *S*-2-nitrobenzyl phosphorothioate is achieved with thiophenoxide in 5 min.[21]

4-Nitrobenzyl: 4-$NO_2C_6H_4CH_2$–

The 4-nitrobenzyl group, used in the synthesis of phosphorylated serine, is introduced by the phosphoramidite method and can be cleaved with TFMSA/MTB/*m*-cresol/1,2-ethanedithiol/TFA, 4 h, 0° to rt.[22] *N*-Methylmorpholine at 80° also cleaves a 4-nitrobenzyl phosphate triester.[23]

2,4-Dinitrobenzyl: 2,4-$(NO_2)_2$–$C_6H_3CH_2$–

Formation

This group has been used for the protection of a phosphorodithioate and is cleaved with 4-methylthiophenol and TEA.[24]

4-Chlorobenzyl: 4-$ClC_6H_4CH_2$–

Cleavage

1. Hydrogenolysis: Pd–C, *t*-BuOH, NaOAc, H_2O.[25–27]
2. From a phosphorothioate: TFMSA, *m*-cresol, thiophenol, TFA. These conditions minimized the migration of the benzyl group to the thione.[28]

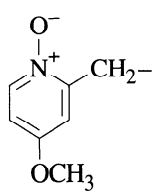
4-Chloro-2-nitrobenzyl: 4-Cl–2-$NO_2C_6H_3CH_2$–

The 4-chloro-2-nitrobenzyl group was useful in the synthesis of dithymidine phosphorothioates. It could be cleaved with a minimun of side reactions with PhSH, TEA, Pyr.[29]

4-Acyloxybenzyl: 4-$RCO_2C_6H_4CH_2$–

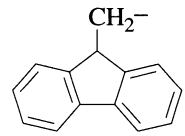
4-Acyloxybenzyl esters were designed to be released under physiological conditions. Porcine liver carboxyesterase efficiently releases the phosphate by acetate hydrolysis and quinonemethide formation. In a diester, the first ester is cleaved faster than the second.[30]

1-Oxido-4-methoxy-2-picolyl:



The oxidopicolyl group increases the rate and efficiency of internucleotide phosphodiester synthesis.[31] It is cleaved with piperidine.[32]

Fluorenyl-9-methyl (Fm):



Formation

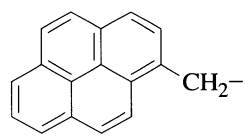
1. 5′-Nucleoside phosphates are protected using triisopropylbenzenesulfonyl chloride in Pyr.[33]

Cleavage

1. TEA, Pyr, 20°, 2 h.[34] These conditions were developed for use with 2-chlorophenyl protection at the internucleotide junctions.
2. TEA, CH_3CN, 14 h, rt.[35]
3. 0.1 *M* NaOH, 0°, 10 min.[33]
4. Concd. NH_4OH, 50°, 2 h.[33]

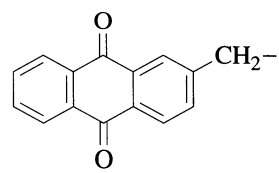
The fluorenyl-9-methyl group has been shown to be of particular value in studies of deoxynucleoside dithiophosphates.[36]

Pyrenylmethyl:



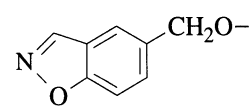
This derivative, synthesized by a silver oxide–promoted condensation of pyrenylmethyl chloride and a dialkyl phosphate (92% yield), is quantitatively cleaved by photolysis at >300 nm in 60 min.[37]

2-(9,10-Anthraquinonyl)methyl or 2-Methyleneanthraquinone (MAQ):



This group is stable to TEA/Pyr and to 80% acetic acid. It is cleaved by reduction with sodium dithionite at pH 7.3.[38]

5-Benzisoxazolylmethylene (Bim):



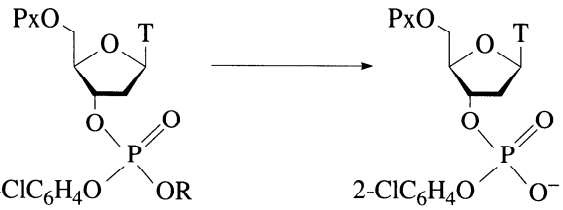
This group was effective in the synthesis of oligonucleotides using the phosphotriester approach.

Cleavage

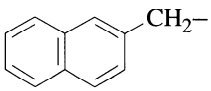
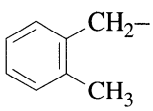
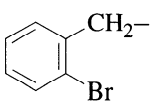
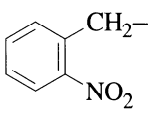
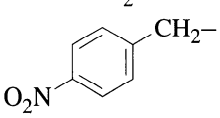
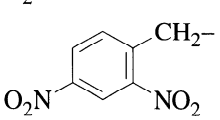
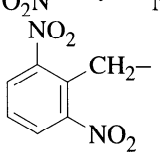
1. TEA, Pyr, < 2 h.[39]

Cleavage Rates of Various Arylmethyl Phosphates

The following table compares the cleavage rates for a variety of benzyl phosphates using thiols or pyridine for the reaction[40,41]



Px = 9-phenylxanthen-9-yl (pixyl)

Substrate R =	*p*-Thiocresol/TEA/ACN		Pyridine	Ratio of half-lives
	$t_{½}$ (min)	(min)	$t_{½}$ (h)	(Pyr/RSH)
CH_3–	45	—	12	16
Bn–	30	—	12	24
	5	60	5	60
	7	90	3	26
	4	45	10	150
	5	60	68	820
	2	20	40	1200
	~10 sec	~1	120	~43,000
	~10 sec	~1	45	~16,000

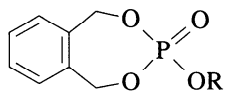
Diphenylmethyl (Dpm): $(C_6H_5)_2CH-$

The reaction of phosphoric acid with diphenyldiazomethane in dioxane gives the triphosphate.[42,43]

Cleavage

1. $(DpmO)_3PO$, upon reaction with NaI, Pyr at 100°, gives $(DpmO)_2P(O)ONa$ quantitatively. $Bu_3N^+HI^-$ can also be used to remove a single Dpm group.[42]
2. H_2, Pd-C, aqueous methanol.[42]
3. Trifluoroacetic acid.[43]

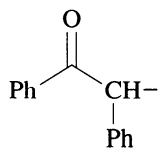
***o*-Xylene Derivative:**



Cleavage

1. Hydrogenolysis: H_2, Pd–C, rt, 17 h.[44–46]

Benzoin Derivative:



Formation

1. From $(EtO)_2P(O)Cl$: benzoin, Ag_2O.[37]
2. Bu_3NH–cAMP, desyl bromide.[47]

Cleavage

1. Photolysis, >300 nm.[37,48]

3′,5′-Dimethoxybenzoin Derivative (3′,5′-DMB)

The phosphate ester, prepared either through phosphoramidite or phosphoryl chloride protocols, is cleavable by photolysis (350 nm, benzene, 83–87% yield).[49–51]

4-Hydroxyphenacyl: $4\text{-}HOC_6H_4C(O)CH_2^-$

The 4-hydroxyphenacyl group is removed by photolysis (300 nm, CH_3CN, tris buffer).[52,53]

4-Methoxyphenacyl: $4\text{-}CH_3OC_6H_4C(O)CH_2-$

Introduced with α-diazo-4-methoxyacetophenone, the phenacyl group is cleaved by photolysis with Pyrex-filtered mercury light in 74–86% yield.[54]

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PHENYL PHOSPHATES

Phenyl: C_6H_5-

Cleavage

1. PtO_2 (stoichiometric), TFA, AcOH, H_2, 91% yield.[1,2] This method cannot be used in substrates that contain a tyrosine, because tyrosine is easily reduced in the acidic medium. Neutral conditions fail to cleave phenyl phosphates.[3]
2. Aqueous HCl, reflux.[4]

3. $Bu_4N^+F^-$, THF, Pyr, H_2O, rt, 30 min.[5] These conditions result in the formation of a mixture of fluorophosphate and phosphate. In the case of oligonucleotides, some internucleotide bond cleavage is observed with this reagent.
4. NaOH, THF[6] or LiOH, dioxane.[7]
5. See the discussion of the cleavage of 2-chlorophenyl (below) for oximate rate comparisons.

2-Methylphenyl and 2,6-Dimethylphenyl

These groups were more effective than the phenyl group for protection of phosphoserine during peptide synthesis. They are cleaved by hydrogenolysis with stoichiometric PtO_2 in AcOH.[8]

2-Chlorophenyl: 2-Cl-C_6H_4–

Cleavage

1. Tetramethylguanidinium 4-nitrobenzaldoxime, dioxane, H_2O, 20°, 22 h.[9] This reagent cleaves the 2-chlorophenyl ester 2.5 times faster than the 4-chlorophenyl ester and 25 times faster than the phenyl ester. The use of *syn*-2-nitrobenzaldoxime increases the rate an additional 2.5 to 4 times.[10] Oximate cleavage proceeds by nucleophilic addition–elimination to give an oxime ester that, with base, undergoes another elimination to give a nitrile and phosphate anion.[11]
2. NaOH, Pyr, H_2O, 0°.[12]
3. *syn*-Pyridine-2-aldoxime, tetramethylguanidine, dioxane, Pyr, H_2O.[13]

4-Chlorophenyl: 4-Cl-C_6H_4–

Halogen-substituted phenols were originally introduced for phosphate protection to minimize internucleotide bond cleavage during deprotection.[14]

1. NH_4OH, 55°, 3 h.[15]
2. Treatment of an internucleotide 4-chlorophenyl ester with CsF and an alcohol (MeOH, EtOH, neopentylOH) results in transesterification.[16]

2,4-Dichlorophenyl: 2,4-$Cl_2C_6H_3$–

1. 4-Nitrobenzaldoxime, tetramethylguanidine, THF.[17]
2. Aqueous ammonia, dioxane, 12 h, 60°.[18]

2,5-Dichlorophenyl: 2,5-$Cl_2C_6H_3$–

Cleavage

1. 4-Nitrobenzaldoxime, TEA, dioxane, H_2O.[19] Cleavage occurs in the presence of 4-nitrophenylethyl phosphate.

2. Pyridine-2-carbaldoxime, TEA, H_2O, dioxane. The 2-(1-methyl-2-imidazolyl)phenyl group is not removed under these conditions.[20]

2,6-Dichlorophenyl: 2,6-$Cl_2C_6H_3$–

Cleavage

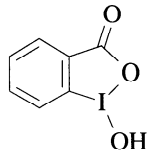
1. 4-Nitrobenzaldoxime, TEA, dioxane, H_2O.[21]

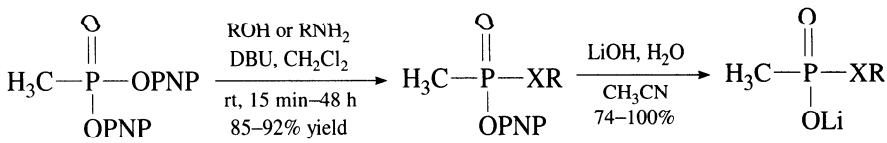
2-Bromophenyl: 2-BrC_6H_4–

Cleavage of the bromophenyl group is achieved with $Cu(OAc)_2$ in Pyr, H_2O. The 2-chlorophenyl group is stable to these conditions.[22]

4-Nitrophenyl (PNP): 4-$NO_2C_6H_4$–

Cleavage

1. *p*-Thiocresol, TEA, CH_3CN.[9] The 4-nitrophenyl group is removed in the presence of a 2-chlorophenyl group.
2.  (Organoindinane), aqueous micellar cetyltrimethylammonium chloride, pH 8.[23]
3. Tetrabutylammonium acetate, 20 h, 20°. For comparison, the 2,4-dichlorophenyl group was removed in 100 h.[24]
4. *syn*-4-Nitrobenzaldoxime, tetramethylguanidine, dioxane, CH_3CN, 16 h.[24]
5. 0.125 *N* NaOH, dioxane.[24]
6. 4-Nitrophenyl phosphonates are transesterified in the presence of DBU and an alcohol.[25]



X = NH or O

7. Zr^{+4}, H_2O, pH 3.5, 37°.[26]

3,5-Dinitrophenyl: 3,5-$(NO_2)_2C_6H_3$–

Photolysis through a Pyrex filter in Pyr, EtOH, H_2O cleaves this phosphate ester.[27] The rate increases with increasing pH.

4-Chloro-2-nitrophenyl: 4-Cl-2-$NO_2C_6H_3$–

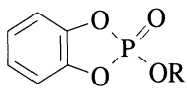
Cleavage is achieved with refluxing NaOH (15 min), but some deamination occurs with deoxyriboadenosine-5′-phosphate.[28] The ester is formed using the DCC protocol for phosphate ester formation.

2-Chloro-4-triphenylmethylphenyl

The lipophilicity of this phosphate protective group helps in the chromatographic purification of oligonucleotides. It is removed by the oximate method.[29]

2-Methoxy-5-nitrophenyl

This ester is cleaved by photolysis at >300 nm in basic aqueous acetonitrile.[30]

1,2-Phenylene: 

The phenylene group is removed oxidatively with $Pb(OAc)_4$ in dioxane.[31]

4-Triphenylmethylaminophenyl: 4-[$(C_6H_5)_3CNH$]C_6H_4–

Formation

1. $TrNHC_6H_4OH$, DCC, Pyr.

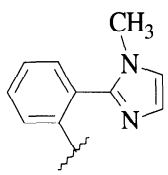
Cleavage

1. Iodine, acetone or DMF, ammonium acetate, rt, 2 h. The tritylaminophenyl group is stable to isoamyl nitrite/acetic acid.[32]

4-Benzylaminophenyl: 4-[$C_6H_5CH_2NH$]C_6H_4–

Cleavage

1. Electrolysis: 0.6–1.0 V, 3 h, DMF, H_2O, $NaClO_4$.[33] The related 4-tritylaminophenyl and 4-methoxyphenyl groups were not cleanly cleaved.

1-Methyl-2-(2-hydroxyphenyl)imidazole Derivative: 

The rate of oligonucleotide synthesis by the triester method using mesitylenesulfonyl chloride was increased five- to tenfold when this group was used as a protective group during internucleotide bond formation. It was removed with concd. NH_4OH at 60° for 12 h[18] or by the oximate method.[20]

8-Quinolyl

This group is stable to acid and alkali. It has been used as a copper-activated leaving group for triphosphate protection.[34]

Formation

1. Ph_3P, 2,2′-dipyridyl disulfide, Pyr, rt, 6 h.[35]
2. $(PhO)_3P$, 2,2′-dipyridyl diselenide, Pyr, rt, 12 h.[36]

Cleavage

1. $CuCl_2$, DMSO, H_2O, 40–45°, 5 h.[35]

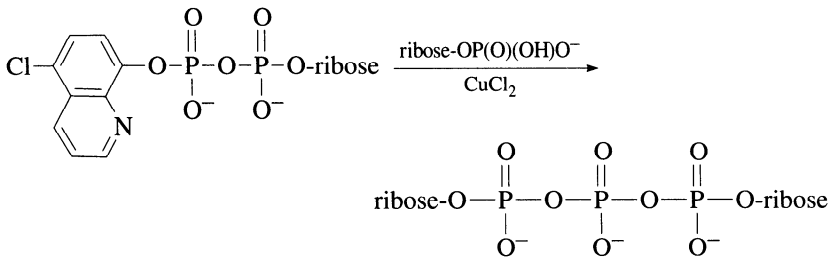
5-Chloro-8-quinolyl

Formation

1. 5-Chloro-8-hydroxyquinoline, $POCl_3$, Pyr, 92% yield.[37]
2. 2,2′-Dipyridyl diselenide, $(PhO)_3P$, Pyr, rt, 12 h, 80–85% yield.[38]

Cleavage

1. Aqueous ammonia, 2 days, 27°.[39]
2. $Zn(OAc)_2$, Pyr, H_2O, 28 h, 98% yield.[12]
3. 2-Pyridinecarboxaldoxime, tetramethylguanidine, dioxane, H_2O, 90% yield.[12]
4. $ZnCl_2$, aq. Pyr, rt, 12 h.[38,40]
5. Pyr, *t*-$BuNH_2$, H_2O. Cleavage occurs in the presence of the 2,6-dichlorophenyl phosphate.[41]
6. The 5-chloro-8-quinolyl group can also be activated with $CuCl_2$ under anhydrous conditions and used in triphosphate formation.[42,43]



Thiophenyl: C_6H_5S-

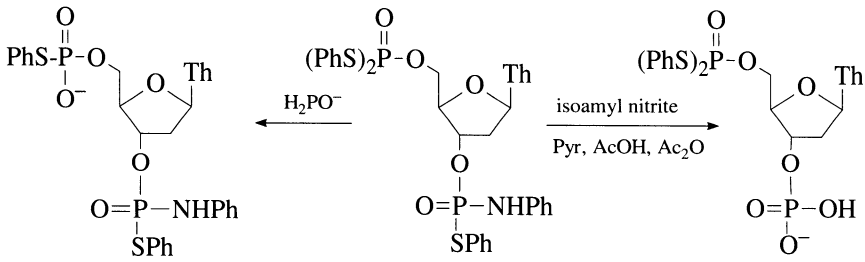
The phosphorodithioate is stable to heating at 100°, 80% acetic acid (1 h), dry or aqueous pyridine (days) and refluxing methanol, ethanol or isopropyl alcohol for 1 h.

Formation

1. $(ArS)_2P(O)O^-$ $C_6H_{11}NH_3^+$ is prepared from the phosphinic acid with TMSCl, TEA, PhSSPh in THF at rt, 20 h, in 83% yield.[44]

Cleavage

1. Treatment of $ROP(O)(SPh)_2$ (**1**) with 0.2 *N* NaOH (dioxane, rt, 15 min)[44] or pyridinium phosphinate (Pyr, TEA)[45] quantitatively gives $ROP(O)(SPh)O^-$ (**2**).



2 **1** **3**

Ref. 45

2. AgOAc (Pyr, H_2O) cleaves both thioates of **1** to give a phosphate.[44]
3. Treatment of **2** with I_2 or AgOAc also gives the phosphate.[44]
4. Treatment of **1** with Zn (acetylacetone, Pyr, DMF) gives the phosphate.[44]
5. Treatment of **1** with phosphinic acid and triazole gives **2**.[44]
6. Treatment of $(RO)_2P(O)SPh$ with Bu_3SnOMe converts it to $(RO)_2P(O)$-OMe.[46,47]
7. $(Bu_3Sn)_2O$; TMSCl; H_2O.[48,49]
8. Treatment of $ROP(O)(SPh)_2$ with H_3PO_3/Pyr gives $ROP(O)(SPh)OH$.[50]
9. Phosphorothioates, when activated with $AgNO_3$ under anhydrous conditions in the presence of monophosphates, are converted into diphosphates.[51]
10. Tributylstannyl 2-pyridine-*syn*-carboxaldoxime, Pyr.[48]

Salicylic Acid Derivative

Salicylic acid was used for phosphite protection in the synthesis of glycosyl phosphites and phosphates. This derivative is very reactive and readily forms a phosphite upon treatment with an alcohol or a phosphonic acid upon aqueous hydrolysis.[52]

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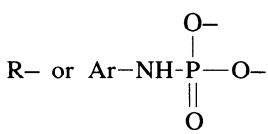
AMIDATES



Anilidate: C_6H_5NH-

A polymeric version of this group has been developed for terminal phosphate protection in ribooligonucleotide synthesis.[1]

Formation

1. Ph_3P, 2,2′-dipyridyl disulfide, aniline, 60% yield.[2]

Cleavage

1. Isoamyl nitrite, Pyr, acetic acid.[3,4]

4-Triphenylmethylanilidate: 4-$(C_6H_5)_3CC_6H_4NH$–

This highly lipophilic group is cleaved with isoamyl nitrite in Pyr/AcOH.[5] The use of a lipophilic 5′-phosphate protective group aids in reverse-phase HPLC purification of oligonucleotides.

[*N*-(2-Trityloxy)ethyl]anilidate: $(C_6H_5)_3COCH_2CH_2$-C_6H_4-N–

This lipophilic group, developed for 5′-phosphate protection in oligonucleotide synthesis, is removed with 80% AcOH in 1 h.[6,7] The related trityloxyethylamino group has been used in a similar capacity for phosphate protection and is also cleaved with 80% AcOH.[8]

***p*-(*N*,*N*-Dimethylamino)anilidate:** *p*-$(CH_3)_2NC_6H_4NH$–

This group was developed to aid in the purification of polynucleotides by adsorbing the phosphoroanilidates on an acidic ion-exchange resin.[9] Derivatives containing this group as a terminal phosphate protective group could be adsorbed on an acid ion-exchange resin for purification. The group is removed with 80% acetic acid at 80° for 3 h.[10]

Formation

1. DCC, *N*, *N*-dimethyl-*p*-phenylenediamine.[10]

Cleavage

1. 80% acetic acid, 80°, 3 h.[10]
2. Isoamyl nitrite, Pyr, AcOH.[11]

3-(*N*, *N*-Diethylaminomethyl)anilidate: 3-$[(C_2H_5)_2NCH_2]C_6H_4NH$–

Cleavage is effected with isoamyl nitrite in Pyr/AcOH.[12,13]

***p*-Anisidate:** *p*-$CH_3OC_6H_4NH$–

Cleavage

1. Pyr, AcOH, isoamyl nitrite.[14,15]
2. $Bu_4N^+NO_2^-$, Ac_2O, Pyr, rt, 10 min.[16]

2,2′-Diaminobiphenyl Derivative

Formation

1. 2,2′-Diaminobiphenyl, Ph_3P, $(PyS)_2$.[17]

Cleavage

1. Isoamyl nitrite, Pyr, AcOH, AgOAc, benzoic anhydride.[17]

n-Propylamine and *i*-Propylamine Derivatives

These derivatives provide effective protection for phosphotyrosine in Fmoc-based peptide synthesis. They are cleaved with 95% TFA.[18]

N,N'-Dimethyl-(*R,R*)-1,2-diaminocyclohexyl

This group was used as a protective group and chiral directing group for the asymmetric synthesis of α-aminophosphonic acids. It is cleaved by acid hydrolysis.[19]

Morpholino Derivative

Morpholine has been used for 5'-phosphate protection in oligonucleotide synthesis and can be cleaved with 0.01 *N* HCl without significant depurination of bases having free exocyclic amino functions.[20,21]

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MISCELLANEOUS DERIVATIVES

Ethoxycarbonyl: EtO_2C-

The ethoxycarbonyl group was developed for the protection of phosphonates. The derivative is prepared by reaction of tris(trimethylsilyl) phosphite with ethyl chloroformate and can be cleaved by hydrolysis of the ester followed by silylation with bistrimethylsilylacetamide.[1]

(Dimethylthiocarbamoyl)thio: $(CH_3)_2NC(S)S-$

This group, used for internucleotide protection, is introduced with 8-quinolinesulfonyl chloride, $[(CH_3)_2NC(S)S]_2$, and Ph_3P and is cleaved with BF_3 (dioxane, H_2O, rt).[2]

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2. H. Takaku, M. Kato, and S. Ishikawa, *J. Org. Chem.*, **46**, 4062 (1981).