Oligo Modifications

Spacers Introduction

Spacer modifcations C3, 9, C12 and 18 are used to insert a spacer arm in an oligonucleotide. These modifications can be added in multiple additions when a longer spacer is required. 3'-Spacer C3 CPG may also act as a blocker of exonuclease and polymerase activity at the 3'-terminus. dSpacer is used to introduce a stable abasic site within an oligonucleotide. See "Photo-Cleavable" modification category for photo-cleavable Spacer.

Spacers Design Protocols

Hydrophobic vs Hydrophilic Spacers—Design Considerations

Spacer 9 and Spacer 18 are polyethylene glycol (PEG)-based, and thus hydrophilic, while Spacers C3, C12, PC Linker, and PC Spacer are aliphatic, and thus hydrophobic. Thus, when designing oligos that require one or more Spacer modifications, it is important to consider possible effects of Spacer hydrophobicity/hydrophilicity on the properties of the oligo. For example, post-synthetic labeling of an amino or thiol-modified oligo at the 5'-end with a dye, hapten or enzyme is commonly performed in aqueous solution. The active amino or thiol group typically is attached to the oligo via a hydrophobic linker, and when incorporated into long oligos or those with significant secondary structure, poor coupling of the label to the oligos is often observed. Consequently, optimization of the coupling reaction may require incorporation of a hydrophilic spacer (such as Spacer 18 or Spacer 9) next to the amino/thiol linker.

Spacers Applications

Some common usages of Spacer phosphoramidites are to position various tags/labels at a desired length from an oligonucleotide, to immobilize oligonucleotides to a solid phase (like a microsphere or microarray slide), or to form non-nucleoside folds and hairpin loops within an oligonucleotide (1-3). Spacers can be placed anywhere within an oligo, and multiple incorporations of a Spacer can be performed to effectively generate spacers of virtually any desired length. For example, six successive incorporations of dSpacer were used to create the optimal separation for FRET between the donor and acceptor fluorophores in energy transfer fluorescent sequencing primers (4).

In addition to being used as a spacer arm, the Spacer C3 modification also can be placed at the 3'-end of an oligonucleotide to effectively block that end from enzymatic reactions (e.g., extension by PCR) (5). dSpacer and rSpacer also can be used to mimic abasic sites in oligonucleotides slated for use in DNA damage/repair studies (6). Photocleavable linkers and spacers can be used to introduce photocleavable tags/labels onto the ends of an oligo, or link two separate oligonucleotides through a short, photocleavable spacer arm for use in photo-triggered hybridization applications (7).

References

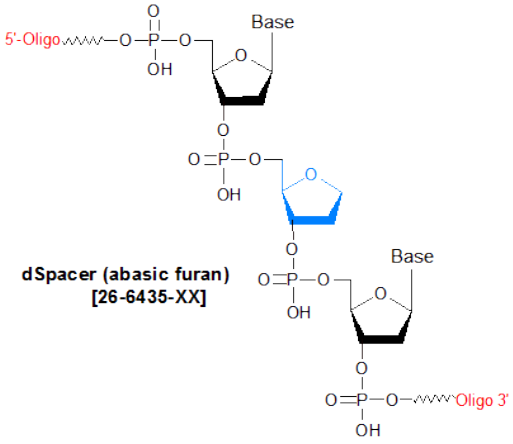
(1) Li, H., McGall, G. Photoactivatable Silanes: Synthesis and Uses in Biopolymer Array Fabrication on Glass Substrates. In Frontiers in Biochip Technology. X, W-L., Cheng, J. (Ed.) Springer Science+Business Media, Inc. (2006), pp. 176-190.
(2) Salunkhe, M., Wu, T.F., Letsinger, R.L. Control of folding and binding of oligonucleotides by use of non-nucleotide linker. J. Am. Chem. Soc. (1992), 114: 8768-8772.
(3) Durand, M., Chevrie, K., Chassignol, M., Thuong, N.T., Maurizot, J. Circular dichroism studies of an oligodeoxyribonucleotide containing a hairpin loop made of a hexaethylene glycol chain : conformation and stability. Nucleic Acids Res. (1990), 18: 6353-6359.
(4) Ju, J., Glazer, A.N., Mathies, R.A. Cassette labeling for facile construction of energy transfer fluorescent primers. Nucleic Acids Res. (1996), 24: 1144-1148.
(5) Zhou, L., Myers, A.N., Vandersteen, J.G., Wang, L., Wittwer, C.T. Closed-Tube Genotyping with Unlabeled Oligonucleotide Probes and a Saturating DNA Dye. Clin. Chem. (2004), 50: 1328-1335.
(6) Takeshita, M., Chang, C.N., Johnson, F., Will, S., Grollman, A.P. Oligodeoxynucleotides containing synthetic abasic sites. Model substrates for DNA polymerases and apurinic/apyrimidinic endonucleases. J. Biol. Chem. (1987), 262: 10171-10179.
(7) Ordoukhanian, P., Taylor, J-S. Design and synthesis of a versatile photocleavable DNA building block. Application to phototriggered hybridization. J. Am. Chem. Soc. (1995), 117: 9570-9571.

Modificaton Code List

Modification	Code	Catalog Number
Abasic Site (dSpacer abasic furan-THF)	[dABS]	26-6435
PC Linker (photocleavable)	[PCL]	26-6888
PC Spacer C3 (photocleavable)	[PC-Sp-C3]	26-6889
rAbasic Site (rSpacer abasic furan)	[rABS]	26-6442
Spacer 18 (hexaethyleneglycol)	[Sp18]	26-6447
Spacer 9	[Sp9]	26-6440
Spacer C12	[SpC12]	26-6441
Spacer C3	[SpC3]	26-6439
Spacer C6	[SpC6]	26-6945

Abasic Site (dSpacer tetrahydrofuran-THF)

Category	Spacers
Modification Code	dABS
Reference Catalog Number	26-6435
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	180.1



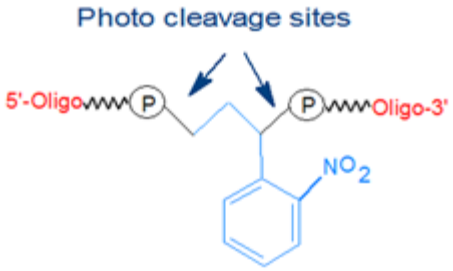
dSpacer (abasic furan) is a tetrahydrofuran derivative, in which a methylene group occupies the 1 position of 2'-deoxyribose. dSpacer is commonly used to mimic an abasic site in an oligonucleotide. In DNA, abasic sites are generated by hydrolysis of the glycosidic linkage to the nucleotide base, leaving just the sugar-phosphate backbone at that position. In the cell, abasic site formation occurs after a spontaneous depurination/depyrmidination event, by UV ionizing radiation, or as a Base Excision Repair (BER) intermediate (1, 2). Because such sites are fragile, they are easily susceptible to single-stranded/double-stranded breakage, and if not repaired by the BER mechanism, abasic lesions often lead to mutation by translesion synthesis during replication. The particular base incorporated opposite the lesion varies depending on organism and environmental conditions (3).

dSpacer is used as an abasic site mimic in synthetic oligonucleotides because it not only is structurally very similiar to the natural site, but it is considerably more stable, and thus can tolerate the chemical conditions used in oligo synthesis and purification (4).One or more consecutive dSpacer modifications can also be used simply to provide varying amounts of separation between different parts of an oligo sequence. **References**

1. Lindahl, T. Instability and decay of the primary structure of DNA. *Nature.* (1993), **362**: 709-715.
2. Nilsen, H., Krokan, H.E. Base excision repair in a network of defence and tolerance.*Carcinogenesis* (2001), **22**: 987-998.
3. Lehman, A. Replication of damaged DNA by translesion synthesis in human cells. *FEBS Letters.* (2005), **579**: 873-876.
4. Takeshita, M., Chang, C.N., Johnson, F., Will, S., Grollman, A.P. Oligodeoxynucleotides containing synthetic abasic sites. Model substrates for DNA polymerases and apurinic/apyrimidinic endonucleases. *J. Biol. Chem.* (1987), **262**: 10171-10179.

PC Linker (photocleavable)

Category	Photo Cleavable
Modification Code	PCL
Reference Catalog Number	26-6888
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	259.15



PC Linker (photocleavable)
[26-6888-XX]

PC Linker (photocleavable) is a non-nucleosidic molety that can be used to link two nucleotide sequences through a short, UV photo-cleavable C3 spacer arm, this can be added at any position of the sequence. Photo-cleavage of PC Linker by UV light yields one 5'-phosphorylated oligo and one 3'-phosphorylated oligo.

The utility of PC Linker for photo-triggered hybridization applications was first demonstrated by Ordoukhanian and Taylor in 1995 (1). They incorporated PC Linker into the sugar-phosphate backbone of a DNA hairpin. Upon irradiation by UV light, photo-cleavage released a 5-phosphorylated 18-mer oligonucleotide having 9X greater hybridization affinity for a complementary DNA strand.

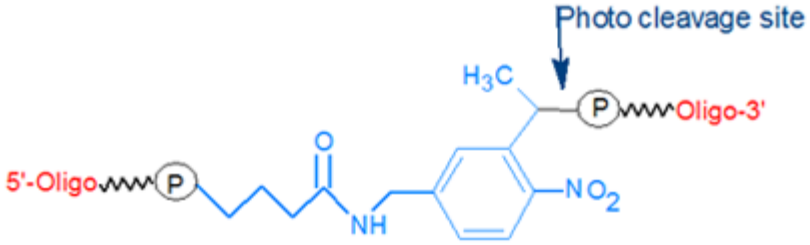
The use of PC Linker has also been explored in designing multi-functional single-stranded nucleotide conjugates for use in in vitro selection of novel DNA or RNA-based catalysts for bio-molecular or organic reactions (for example Diels-Alder) (2,3). PC Linker-modified oligonucleotides are the centerpiece of Bruker Daltonik's genoSNIP, a MALDI-TOF MS based assay system for SNP detection (4).

Cleavage Protocol
Cleavage occurs by irradiation with near-UV light (300-350 nm, complete cleavage occurs within 5 minutes. Try using a Black Ray XX-15 UV lamp (Ultraviolet Products Inc., San Gabriel, CA) at a distance of 15 cm (emission peak 365 nm, 300 nm cut-off, 1.1 mW intensity at~31 cm).

References
1. Olejnik, J., Krzymanska-Olejnik, E., Rothschild, K.J. Photocleavable aminotag phosphoramidites for 5'-termini DNA/RNA labeling. *Nucleic Acids Res.* (1998), **26**: 3572-3576.
2. Olejnik, J., Ludemann, H-C., Olejnik, E.K, Berkenkamp, S., Hillenkamp, F., Rothschild, K.J. Photocleavable peptide-DNA conjugates: synthesis and applications to DNA analysis using MALDI-MS. *Nucleic Acids Res.* (1999), **27**: 4626-4631.
3. Tang, X., Su, M., Yu, LiLi, Lv, C., Wang, J., Li, Z. Photomodulating RNA cleavage using photolabile circular antisense oligodeoxynucleotides.*Nucleic Acids Res.* (2002), **38**: 3848-3855.

PC Spacer (photocleavable)

Category	Photo Cleavable
Modification Code	PC-Sp-C3
Reference Catalog Number	26-6889
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	344.26



PC Spacer (photocleavable)
[26-6889-XX]

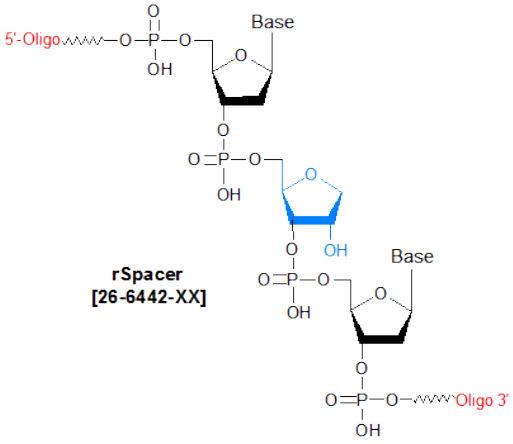
PC Spacer (photocleavable) is a non-nucleosidic molety that can be used as an intermediary to attach any available phosphoramidite modification at either end of an oligonucleotide through a UV photo-cleavable C3 spacer, as well as insert such a spacer internally. An example is the use of PC Spacer to incorporate a photo-cleavable 6-FAM tag onto the 5'-end of oligonucleotides immobilized on glass slides. These fluorescently-labeled oligo arrays were then UV irradiated in order to test the efficacy of photo-cleavage in removing the 6-FAM tag from these oligos, as part of developing sequencing-by-synthesis applications (1).

Cleavage Protocol
Cleavage occurs by irradiation with near-UV light (300-350 nm, complete cleavage occurs within 5 minutes. Try using a Black Ray XX-15 UV lamp (Ultraviolet Products Inc., San Gabriel, CA) at a distance of 15 cm (emission peak 365 nm, 300 nm cut-off, 1.1 mW intensity at~31 cm).

References
1. Olejnik, J., Krzymanska-Olejnik, E., Rothschild, K.J. Photocleavable aminotag phosphoramidites for 5'-termini DNA/RNA labeling. *Nucleic Acids Res.* (1998), **26**: 3572-3576.
2. Olejnik, J., Ludemann, H-C., Olejnik, E.K, Berkenkamp, S., Hillenkamp, F., Rothschild, K.J. Photocleavable peptide-DNA conjugates: synthesis and applications to DNA analysis using MALDI-MS. *Nucleic Acids Res.* (1999), **27**: 4626-4631.
3. Tang, X., Su, M., Yu, LiLi, Lv, C., Wang, J., Li, Z. Photomodulating RNA cleavage using photolabile circular antisense oligodeoxynucleotides.*Nucleic Acids Res.* (2002), **38**: 3848-3855.

rSpacer

Category	Spacers
Modification Code	rABS
Reference Catalog Number	26-6442
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	196.09

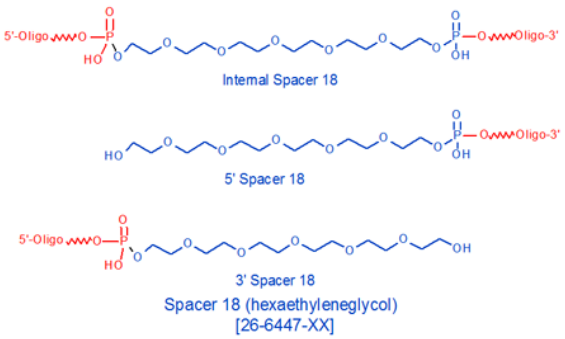


Ribo rAbasic Site (rSpacer abasic furan) RiboSpacer (rSpacer) is a tetrahydrofuran derivative, in which a methylene group occupies the 1 position of 2'-ribose. rSpacer is commonly used to mimic an abasic site in an RNA oligonucleotide. Naturally-occurring abasic sites in RNA are less common than in DNA, due to RNA being less susceptible to depurination (1). However, once generated, either spontaneously or via an enzymatic pathway, RNA abasic sites are about 15-fold more stable than DNA abasic sites; this fairly high level of stability could have important biological consequences for long-lived RNAs (for example, tRNAs or rRNA) (2). While such biological consequences have been largely unexplored thus far, abasic site effects on RNA structure and activity has been observed for the case of the hammerhead ribozyme, which catalyzes phosphodiester bond cleavage (3). Introduction of abasic sites at different positions of this ribozyme's core significantly reduced ribozyme activity. Interestingly, the activity was partially rescued for some abasic positions by exogenous addition of the missing base. rSpacer-modified oligonucleotides could serve as important research tools for elucidating the effects of abasic sites on the structure and function of long-lived RNAs and ribozymes. **References**

1. Kochetkov, N.K., Budovskii, E.I. Hydrolysis of N-glycosidic bonds in nucleosides, nucleotides and their derivatives. In *Organic Chemistry of Nucleic Acids* New York: Plenum; (1993). pp. 425-448.
2. Kupfer, P.A., Leumann, C.J. The chemical stability of abasic RNA compared to abasic DNA. *Nucleic Acids Res.* (2007), **35**: 58-68.
3. Peracchi, A., Beigelman, L., Usman, N., Herschlag, D. Rescue of abasic hammerhead ribozymes by exogenous addition of specific bases. *Proc. Natl. Acad. Sci. USA.* (1996), **93**: 11522-11527.

Spacer 18

Category	Spacers
Modification Code	Sp18
Reference Catalog Number	26-6447
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	344.3

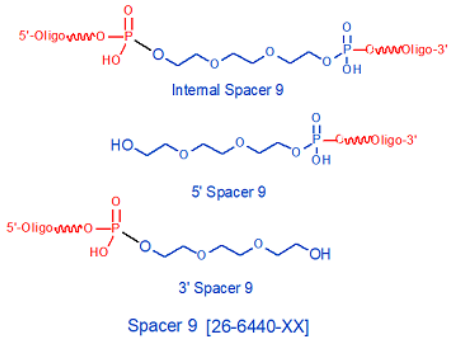


Spacer 18 is a hexaethylene glycol chain that is 18 atoms long (12 carbons + 6 oxygens), and is used to incorporate a long spacer arm into an oligonucleotide. Spacer 18 can be incorporated in consecutive additions whenever a longer spacer is required. Spacer 18 had been used to form bold folds and hairpin loops in oligonucleotides (1,2), and for solid-phase immobilization of hybridization probes (3). Spacer 18 has also been used to modify random primers used in whole genome amplification (WGA)-based applications, as a way to eliminate self-priming events that form spurious DNA products (that is, false-positive amplification) in the PCR reactions (4). **References**

1. Salunkhe, M., Wu, T.F., Letsinger, R.L. Control of folding and binding of oligonucleotides by use of non-nucleotide linker. *J. Am. Chem. Soc.* (1992), **114**: 8768-8772.
2. Durand, M., Chevrie, K., Chassignol, M., Thuong, N.T., Maurizot, J. Circular dichroism studies of an oligodeoxyribonucleotide containing a hairpin loop made of a hexaethylene glycol chain: conformation and stability.*Nucleic Acids Res.* (1990), **18**: 6353-6359.
3. Zhang, Y., Coyne, M.Y., Will, S.G., Levenson, C.H., Kawasaki, E.S. Single-base mutational analysis of cancer and genetic diseases using membrane bound modified oligonucleotides. *Nucleic Acids Res.* (1991), **19**: 3929-3933.
4. Brukner, I., Paquin, B., Belouchi, M., Labuda, D., Krajinovic, M. Self-priming arrest by modified random oligonucleotides facilitates the quality control of whole genome amplification.*Anal. Biochem.* (2005), **339**: 345-347.

Spacer 9

Category	Spacers
Modification Code	Sp9
Reference Catalog Number	26-6440
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	212.14



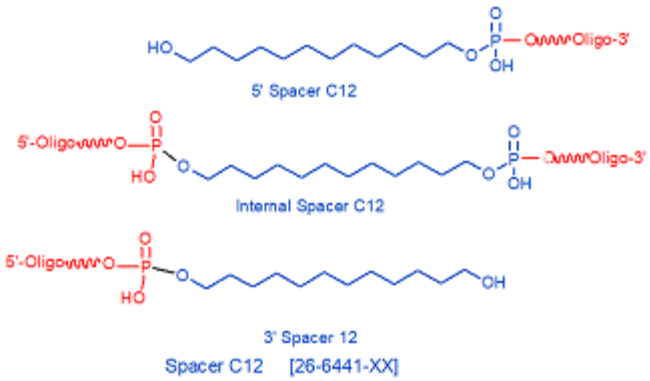
Spacer 9 [26-6440-XX]

Spacer 9 is a triethylene glycol chain that is 9 atoms long (6 carbons + 3 oxygens), and is used to incorporate a spacer arm into an oligonucleotide. Spacer 9 can be incorporated in consecutive additions whenever a longer spacer is required. Spacer 9 has been used to form non-nucleotide bridges in hairpin loops in oligonucleotides (1), for linking oligonucleotides to epitopes for drug development (2), and for solid-phase immobilization of hybridization probes (3). Multiple incorporation of Spacer 9 has been used to form long, flexible linker arms between the two domains (double-helix forming and triple-helix forming, respectively) of a bifunctional DNA oligonucleotide, in order to maximize the binding flexibility of the two domains for their respective targets (4). This oligo was used to form a peptide nucleic acid (PNA)-DNA conjugate for use in site-directed recombination applications. **References**

1. Nelson, J.S., Giver, L., Ellington, A.D., Letsinger, R.L. Incorporation of Non-Nucleotide Bridge into Hairpin Oligonucleotides Capable of High-Affinity Binding to the Rev Protein of HIV-1. *Biochemistry.* (1996), **35**: 5339-5344.
2. Palma, E., Klapper, D.G., Cho, M.J. Antibodies as Drug Carriers III: Design of Oligonucleotides with Enhanced Binding Affinity for Immunoglobulin G. *Pharm. Res.* (2005), **22**: 122-127.
3. Beattie, W.G., Meng, L., Turner, S.L., Varma, R.S., Dao, D.D., Beattie, K.L. Hybridization of DNA targets to glass-tethered oligonucleotide probes. *Mol. Biotechnol.* (1995), **4**: 213-225.
4. Rogers, F.A., Vasquez, K.M., Egholm, M., Glazer, P.M. Site-directed recombination via bifunctional PNA-DNA conjugates. *Proc. Natl. Acad. Sci. USA* (2002), **99**: 16695-16700.

Spacer C12

Category	Spacers
Modification Code	SpC12
Reference Catalog Number	26-6441
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	264.3



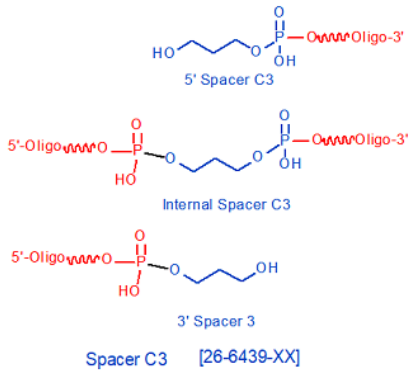
Spacer modifications C3, C6, C9, C12 and triethylene glycol Spacer 9 and 18 are used to insert a spacer arm in an oligonucleotide. These modifications can be added in multiple additions when a longer spacer is required. 3'-Spacer C3 CPG may also act as a blocker of exonuclease and polymerase activity at the 3'-terminus. dSpacer is used to introduce a stable abasic site within an oligonucleotide. See "Photo-Cleavable" modification category for photo-cleavable Spacer.

Spacer C12 is a 12-carbon spacer that is used to incorporate a long spacer arm into an oligonucleotide. Spacer C12 can be incorporated in consecutive additions if a longer spacer is required. Spacer C12 is frequently used for solid-phase immobilization of DNA probes or aptamers for microarray applications (1,2), but can be used for any oligonucleotide-based application requiring a long spacer arm. **References**

1. Reese, M.O., van Dam, R.M., Scherer, A., Quake, S.R. Microfabricated Fountain Pens for High-Density DNA Arrays. *Genome Res.* (2003), **13**: 2348-2352.
2. Lao, Y-H., Peck, K., Chen, L-C. Enhancement of Aptamer Microarray Sensitivity through Spacer Optimization and Avidity Effect. *Anal. Chem.* (2006), **81**: 1747-1754.

Spacer C3

Category	Spacers
Modification Code	SpC3
Reference Catalog Number	26-6439
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	138.06

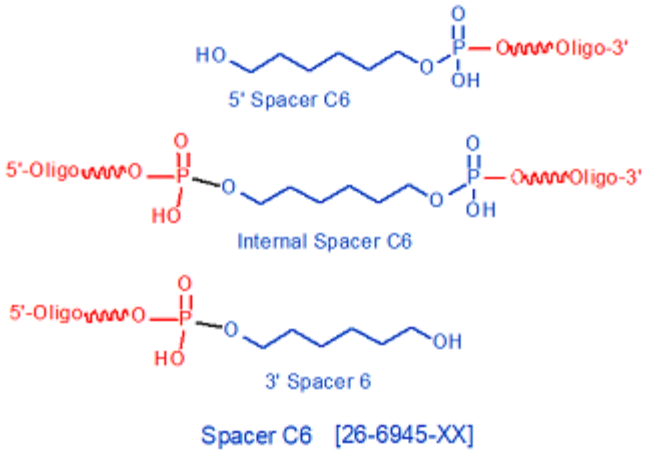


Spacer C3 is a three-carbon spacer that is used to incorporate a short spacer arm into an oligonucleotide. Spacer C3 can be incorporated in consecutive additions if a longer spacer is required. Spacer C3-modified oligos have been used in a number of different applications, including protein-RNA functional studies (1), as a DNA abasic site mimic to study the utility of small synthetic ligands (such as pteridines) for nucleotide recognition in SNP typing applications (2), and for solid-phase immobilization of hybridization probes (3). Spacer C3 incorporated at the 3'-end of an oligo functions as an effective blocking agent against polymerase extension at that end in PCR reactions (4). **References**

1. Pritchard, C.E., Grasby, J.A., Harny, F., Zacharek, A.M., Singh, M., Karn, J., Gait, M.J. Methylphosphonate mapping of phosphate contacts critical for RNA recognition by the human immunodeficiency virus tat and rev proteins. *Nucleic Acids Res.* (1994), **22**: 2592-2600.
2. Dai, Q., Xu, C-Y., Sato, Y., Yoshimoto, K., Nishizawa, S., Teramae, N. Enhancement of the Binding Ability of a Ligand for Nucleobase Recognition by Introducing a Methyl Group. *Anal. Sci. (Japan)* (2006), **22**: 201-203.
3. Li, H., McGall, G. Photoactivatable Silanes: Synthesis and Uses in Biopolymer Array Fabrication on Glass Substrates. In *Frontiers in Biochip Technology*, X, W-L., Cheng, J. (Ed.). Springer Science+Business Media, Inc. (2006). pp. 176-190.
4. Zhou, L., Myers, A.N., Vandersteen, J.G., Wang, L, Wittwer, C.T. Closed-Tube Genotyping with Unlabeled Oligonucleotide Probes and a Saturating DNA Dye. *Clin. Chem.* (2004), **50**: 1328-1335.

Spacer C6

Category	Spacers
Modification Code	SpC6
Reference Catalog Number	26-6945
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	180.06



Spacer C6 phosphoramidite is used to incorporate a six carbon spacer arm into oligonucleotides. Spacer phosphoramidites can be added sequentially to create longer spacer arms.