LINK

Product Update

February 2016

Structural Studies

Modifications for Epigenetics

The study of DNA damage resulting from metabolic processes and environmental factors, along with their associated repair mechanisms has led to a better understanding of the occurrence of genetic mutations, neurodegenerative diseases, cancers and the aging process.

At LINK we are expanding our range of modifiers for this purpose. In particular we are introducing the amidites of 5-hydroxy-dC (**2543**), 5-hydroxy-dU (**2541**), 5-hydroxymethyl-dU (**2542**), 5-hydroxymethyl-dC (**2544**), 5-carboxy-dC (**2545**), 5-formyl-dC (**2546**), 5-hydroxymethyl-dC II (**2547**) and 5-formyl-dC III (**2548**) for use in the study of oxidative damage and repair, methylation and epigenetics.

Oxidised pyrimidines such as 5-hydroxy dU and 5-hydroxy dC are derived from dC *via* oxidative metabolic processes, UV or ionising radiation to form 5-HO-dC which spontaneously undergoes deamination to form 5-HO-dU (see Figure 1).

Although there are repair mechanisms to convert 5-HO-pyrimidines back to dC,[1] the fact that they are observed in cellular DNA at consistent levels suggests that these repair mechanisms are inefficient,[2] at least in certain cell types. Oligonucleotides modified with **2541** or **2543** are useful in understanding such processes.

The presence of either 5-HO-dU or 5-HO-dC can both lead to mutations resulting from their ability to mismatch with A and A/C respectively hence where the repair mechanism fails, such mutations can be permanently incorporated into the resulting gene.

5-Hydroxymethyl-dU (5-hmdU, **2542**) is also a result of oxidative process or ionizing radiation but

1 **Base excision repair in a network of defence and tolerance**, H. Nilsen and H.E. Krokan, *Carcinogenesis*, **22**, 987-998, 2001.

2 **Endogenous oxidative damage of deoxycytidine in DNA**, J.R. Wagner, H. Chia-Chieh and B.N. Ames, *Proc. Nat. Acad. Sci.*, **89**, 3380-3384, 1992.

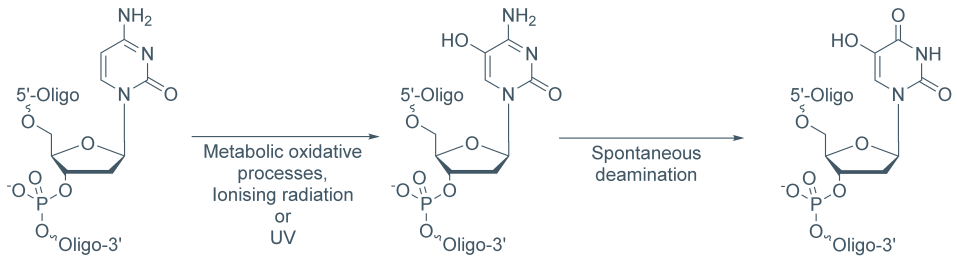
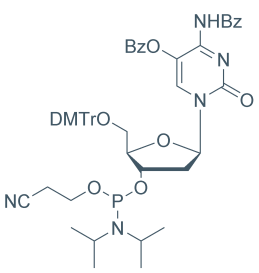
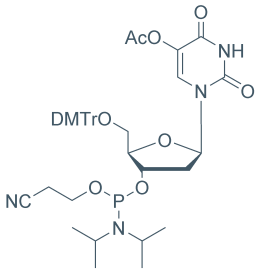


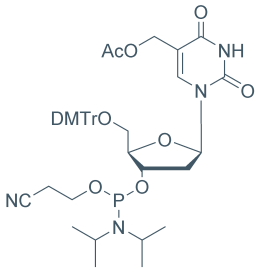
Figure 1. Formation of 5-HO-dC and 5-HO-dU from dC.



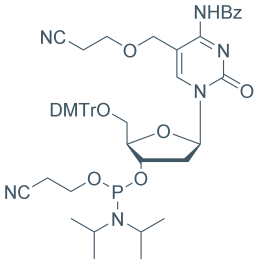
2543



2541



2542



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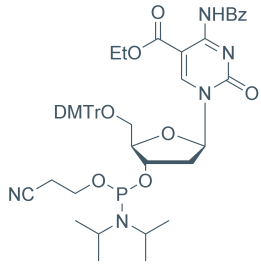
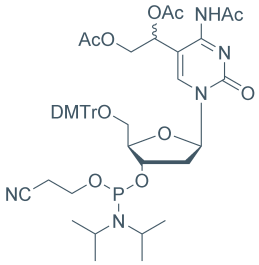
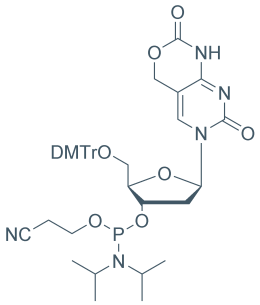


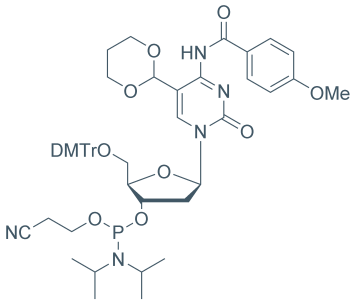
2545



2546



2547



2548

in this case dT is modified.[3] It is also possible that 5-hmdU is formed by deamination of 5-hmdC but Müller and Carell recently showed that this does not contribute to the steady state levels of hmdU in mouse embryonic stem cells, but that dT is a substrate for ten eleven translocation enzymes (Tet) leading to the formation of 5-hmdU.[4] Hence, **2542** is an important reagent for the study of both oxidative processes and epigenetics.

Epigenetics is the study of heritable silencing of genes where there is no change to the coding sequence. Interest in this area has grown significantly over the past few years particularly looking at changes induced and sustained by non-coding RNA gene silencing, histone modification and DNA methylation of cytidine in CpG islands.[5] Phosphoramidites **2544** - **2548** are applicable to the latter.

Once incorporated into an oligonucleotide, these modifiers represent the various products in the biochemical pathway of the modification of dC (see Figure 2).

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3 **Oxidative damage to DNA: formation, measurement, and biological significance**, J. Cadet, M. Berger, T. Douki and J.-L. Ravanat, *Rev. Physiol. Biochem. Pharmacol.*, **131**, 1-87, 1997.

4 **Tet oxidizes thymine to 5-hydroxymethyluracil in mouse embryonic stem cell DNA**, T. Pfaffeneder, F. Spada, M. Wagner, C. Brandmayr, S.K. Laube, D. Eisen, M. Truss, J. Steinbacher, B. Hackner, O. Kotljarova, D. Schuermann, S. Michalakis, O. Kosmatchev, S. Schiesser, B. Steigenberger, N. Raddaoui, G. Kashiwazaki, U. Müller, C.G. Spruijt, M. Vermeulen, H. Leonhardt, P. Schär, M. Müller and T. Carell, *Nat. Chem. Biol.*, **10 (7)**, 574-81, 2014.

5 **Epigenetics in human disease and prospects for epigenetic therapy**, G. Egger, G. Liang, A. Aparicio and P.A. Jones. *Nature*, **429**, 457-463, 2004.

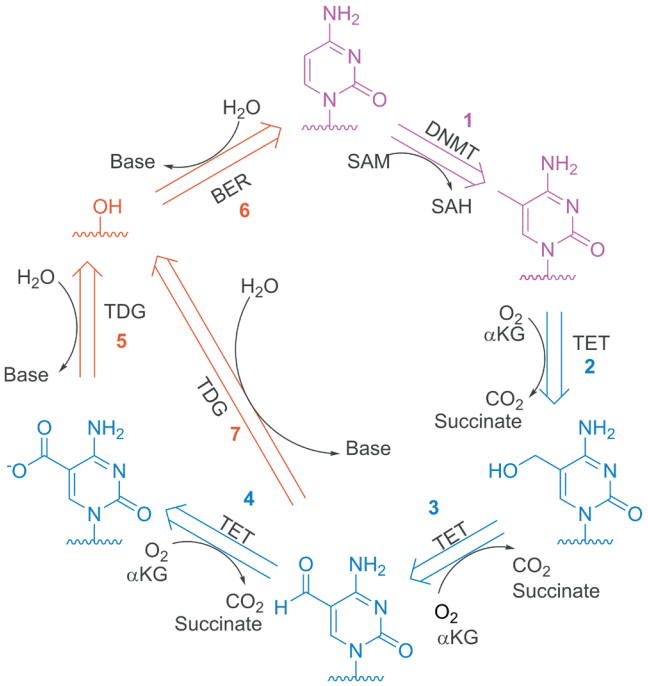


Figure 2. A complete pathway for dynamic modifications of C (adapted from reference 6).

In DNA, cytidine is methylated by a DNA methyl transferase catalysed reaction with S-adenosylmethionine to form 5-mdC. This is oxidised by Tet enzymes to 5-hydroxymethyl-dC which is further oxidised to 5-formyl-dC, which in turn is further oxidised to 5-carboxy-dC. Both 5-carboxy-dC and 5-formyl-dC can be converted back to dC *via* thymidine DNA glycosylase mediated base excision repair.[6]

Until now our range of products in this area of research has been limited to 5-methyl-dC (**2017** [N-Bz] and **2529** [N-Ac]) therefore the addition of these modifiers to our catalogue provides our customers working in this area the tools required to progress our understanding of these important pathways.

Other Structural Studies: Use of Me-U

5-Me-U residues occur naturally in RNA *via* the catalytic reaction of an RNA-methyltransferase on U residues[7] and as such 5-Me-U CE Phosphoramidite (rT-CE Phosphoramidite; **2091**) is useful in the study of methylation of RNA.

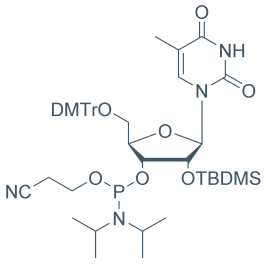
This modifier is also useful in controlling the Tm of DNA:RNA duplexes since methylated U residues have also been shown to have a slight destabilising effect on DNA:RNA duplexes[8] but have very little influence on structure.[9] Similarly, this is utilised in structural and activity relationship studies.

6 **Tet enzymes, TDG and the dynamics of DNA methylation**, R.M. Kholi and Y. Zhang, *Nature*, **502**, 472-479, 2013.

7 **RNA nucleotide methylation**, M. Helm, *Wiley Interdisciplinary Reviews. RNA 2011*, **2(5)**, 611-31.

8 **The solution structure of a DNA·RNA duplex containing 5-propynyl U and C; comparison with 5-Me modifications**, J.I. Gyi, D. Gao, G.L. Conn, J.O. Trent, T. Brown and A.N. Lane, *Nucleic Acids Research*, **31**, 2683–2693 (2003).

9 **Impact of CpG methylation on structure, dynamics and solvation of cAMP DNA responsive element**, S. Derreumaux, M. Chaoui, G. Tevanian and S. Fermandjian, *Nucleic Acids Research*, **29**, 2314-2326 (2001).



2091

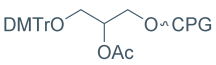
Glyceryl Modification

3'-Glyceryl CPG (**2326**) is used to introduce a glycerol moiety at the 3'-end of an oligonucleotide (see (**1**) in Figure 3).[10] There are several options as to how to utilise this modification; it can easily be converted to introduce an aldehyde functionality (**2**) or a carboxylate (**3**) functionality by oxidation. Reduction of the aldehyde will introduce an ethylene glycol (**4**). The methods for each of these reactions are described by Urata and Akagi in reference 10.

These conversions provide a means of preparing authentic samples for the identification of degradation products arising from the H-abstraction of d-ribose within DNA.

The aldehyde and carboxylate can also be utilised as a means of attaching oligonucleotides to an amino functionalised solid surface or biomolecule.

10 **A convenient synthesis of oligonucleotides with a 3'-phosphoglycolate and 3'-phosphoglycaldehyde terminus**, H. Urata and M. Akagi, *Tetrahedron Lett.*, **34**, 4015-4018 (1993).



2326

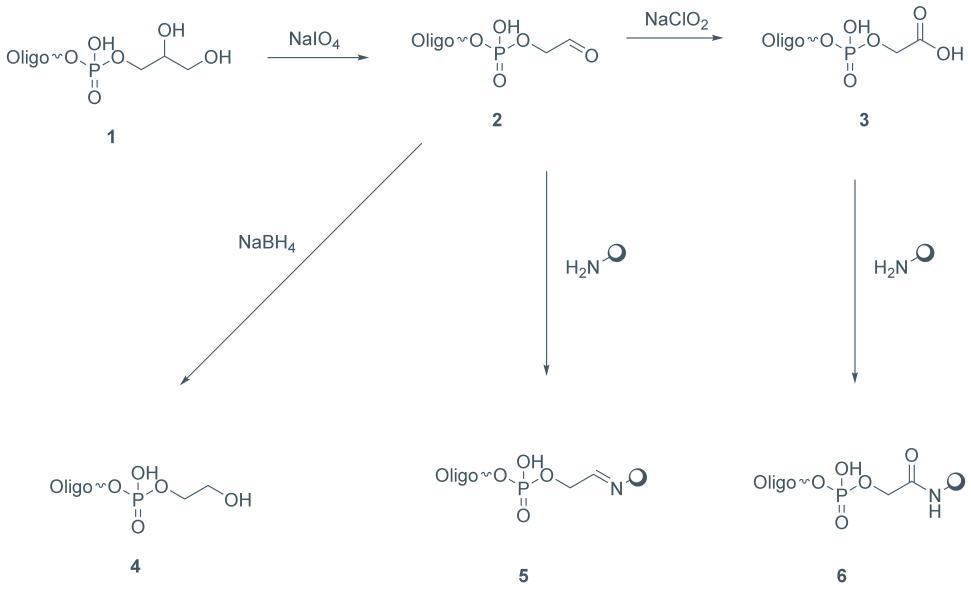


Figure 3. Oxidation of glycerol modified oligos for attachment to amino surface.

High load CPG

We have expanded our range of SynBase™ CPG supports to include high loaded 500Å version (loading of 80-130µmol/g) for use in large scale oligo synthesis. This product is particularly suitable for oligos up to 30mers. For an updated comparison of all our SynBase™ CPG supports, see our Help Centre article **Choosing a SynBase™ Solid Support** (https://linktechsupport.zendesk.com/hc/en-us/articles/202426156-Choosing-a-SynBase-Solid-Support).

Data and Protocols

Physical Data

			Dilution (0.1M)/ml			Dilution (0.067M)/ml		
Item No.	Mol. Wt.	Unit Wt.	250mg	500mg	1g	250mg	500mg	1g
2091	875.09	320.19	2.86	5.71	11.43	4.26	8.53	17.06
2255	-	304.20	-	-	-	-	-	-
2256	-	329.21	-	-	-	-	-	-
2257	-	313.21	-	-	-	-	-	-
2258	-	289.18	-	-	-	-	-	-
2326	-	154.06	-	-	-	-	-	-
2541	788.83	306.17	3.17	6.34	12.68	4.73	9.46	18.92
2542	802.86	320.19	3.11	6.23	12.46	4.65	9.30	18.59
2543	954.03	305.18	2.62	5.24	10.48	3.91	7.82	15.64
2544	917.00	319.21	2.73	5.45	10.91	4.07	8.14	16.28
2545	905.97	333.19	2.76	5.52	11.04	4.12	8.24	16.47
2546	915.96	317.19 (Formyl), 349.23 (Diol)	2.73	5.46	10.92	4.07	8.15	16.29
2547	785.82	319.21	3.18	6.36	12.73	4.75	9.50	18.99
2548	950.02	317.19 (Formyl), 375.27 (Acetal)	2.63	5.26	10.53	3.93	7.86	15.71

Protocols

Dissolution

Prepare the amidite solutions in anhydrous acetonitrile (**4050**) to the desired concentration as above.

Epigenetics Phosphoramidites

2541	5-Hydroxy-dU-CE Phosphoramidite
2542	5-Hydroxymethyl-dU-CE Phosphoramidite (hmdU)
2543	5-Hydroxy-dC-CE Phosphoramidite
2544	5-Hydroxymethyl-dC-CE Phosphoramidite (hmdC)
2545	5-Carboxy-dC-CE Phosphoramidite
2546	5-Formyl-dC-CE Phosphoramidite
2547	5-Hydroxymethyl-dC II-CE Phosphoramidite
2548	5-Formyl-dC III-CE Phosphoramidite

Coupling

A coupling time of 25-60s is recommended for all amidites, except for **2548** which should be coupled for 180s.

Cleavage & Deprotection

Recommendations are specific to each product, but will also need to take into account the requirements of other bases and modifications in the oligo.

2541 - Use Fast or UltraMILD protection on the nucleobases (plus UltraMILD Cap A, **4210**). Cleave and deprotect with ammonium hydroxide at RT for 24h.

2542 - Use Fast or UltraMILD protection on the nucleobases (plus UltraMILD Cap A, **4210**). Cleave and deprotect with AMA at 65ºC for 10min (although this is not compatible with Bz-dC), or use ammonium hydroxide at RT for 2h, then deprotect the nucleobases as required.

2543 - Use UltraMILD protection on the nucleobases (plus UltraMILD Cap A, **4210**). Cleave and deprotect with 0.05M Potassium carbonate in methanol at RT for 4h, or ammonium hydroxide at RT for 2h.

2544 - Any nucleobase protection can be used, however the product is not compatible with AMA or UltraMILD deprotection methods. Cleavage and deprotection is with 30% ammonium hydroxide at 75°C for 17h.

2545 - Use a combination of iBu-dG and Ac-dC nucleobase protection, or UltraMILD (plus UltraMILD Cap A, **4210**). Cleave and deprotect with 0.4M NaOH in MeOH/water 4:1 (v/v) at RT for 17h.

2546 - Any nucleobase protection can be used, dependent on the deprotection strategy required. Typically, use ammonium hydroxide at 55ºC for 17h, AMA at 65ºC for 10 min, or 0.4 M NaOH in MeOH/water 4:1 (v/v) at RT for 17h. Then oxidise with 50mM sodium periodate at 4ºC for 30min, and desalt (G25).

2547 - Either utilise iBu-dG/Ac-dC base protection and deprotect with 0.4M NaOH in MeOH/water 4:1 at RT for 17h, or use UltraMILD deprotection (plus UltraMILD Cap A, **4210**) and deprotect with 0.05M potassium carbonate in methanol at RT for 4h.

2548 - Either utilise dmf-dG/Ac-dC base protection and deprotect with 30% ammonium hydroxide at RT for 17h and immediately evaporate, or employ iBu-dG/Ac-dC or UltraMILD (plus UltraMILD Cap A, **4210**) and deprotect with 0.4 M NaOH in MeOH/water 4:1 (v/v) at RT for 17h, desalt (G25) and evaporate. Subsequently, remove the acetal protecting group using 80% acetic acid in water at 20ºC for 6h.

Storage & Stability

All products are stored dry in a freezer at -10 to -30°C, or short term at 2-8°C (except **2548** where -10 to -30°C is required). Stability in solution is 2-3 days, except for **2546** and **2548** which must be used with 1-2 days.

Use of 5-Me-U

2091	5-Me-U-CE Phosphoramidite

Coupling

As with other 2'-OTBDMS products, a 12min coupling time is recommended.

Cleavage & Deprotection

As this has no nucleobase protection, complete the cleavage and deprotection with conditions suitable for the other nucleobases in the oligo.

Desilylation

The desilylation procedure is the same for DMT ON and DMT OFF oligos. Suspend the residue in dry N-methyl pyrrolidone/Et_3N/$Et_3N.3HF$ (6:3:4 v/v/v) and deprotect silyl groups for 2.5h at 65°C in a sealed sterile tube. Alternatively, DMSO (or DMF)/$Et_3N.3HF$ (3:1 v/v) can be used.

Storage & Stability

Store dry in a freezer at –10 to –30°C. The phosphoramidite is stable in anhydrous acetonitrile solution for 2-3 days.

Glyceryl Modification

2326	3'-Glyceryl SynBase™ CPG 1000

For oxidation and reduction methods for converting the glycerol group, see reference 10.

Coupling

As with all non-nucleosidic supports, an additional deblock step is required.

Cleavage and Deprotection

This is dependent on the nucleobase protection and other modifications but is typically ammonium hydroxide at 55ºC for 4h, or AMA at 65ºC for 10min.

Storage & Stability

Store dry in a freezer at –10 to –30°C.

High Load CPG

Synthesis protocols are exactly the same as for other SynBase™ CPG supports; see page 166 of the **Guidebook for the Synthesis of Oligonucleotides** (*Product Guide 2015/16*). Download from http://discover.linktech.co.uk/download-link-guidebook-web/.

Ordering Information

Product	Pack Size	Cat. No.
Epigenetics		
5-Hydroxy-dU-CE Phosphoramidite	100µmol	2541-F100
	250mg	2541-B250
5-Hydroxymethyl-dU-CE Phosphoramidite (hmdU)	100µmol	2542-F100
	250mg	2542-B250
5-Hydroxy-dC-CE Phosphoramidite	100µmol	2543-F100
	250mg	2543-B250
5-Hydroxymethyl-dC-CE Phosphoramidite (hmdC)	100mg	2544-F100
	250mg	2544-B250
5-Carboxy-dC-CE Phosphoramidite	100µmol	2545-F100
	250mg	2545-B250
5-Formyl-dC-CE Phosphoramidite	100µmol	2546-F100
	250mg	2546-B250
5-Hydroxymethyl-dC II-CE Phosphoramidite	100µmol	2547-F100
	250mg	2547-B250
5-Formyl-dC III-CE Phosphoramidite	100µmol	2548-F100
	250mg	2548-B250

Product	Pack Size	Cat. No.
Glyceryl Modification		
3'-Glyceryl-SynBase™ CPG 1000	100mg	2326-B100
	1g	2326-C001
ALL-FIT Columns*	10 x 0.2µmol	2326-P002
	10 x 1µmol	2326-P008
5-Me-U		
5-Me-U-CE Phosphoramidite (ribo-T)	100µmol	2091-F100
	250mg	2091-B250

* Please enquire about alternative column types and scales.

High Load CPG

Product	Pack Size	Cat. No.			
		Bz-dA	Bz-dC	iBu-dG	dT
SynBase™ 500/50 H	1g	2257-C001	2258-C001	2256-C001	2255-C001
	5g	2257-C005	2258-C005	2256-C005	2255-C005
	25g	2257-C025	2258-C025	2256-C025	2255-C025

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