

The GLEN REPORT

Newsletter

Volume 31.2 | December 2019

Ethynylpyridone C-Nucleoside Phosphoramidite (dW): A High Affinity Replacement for Thymidine

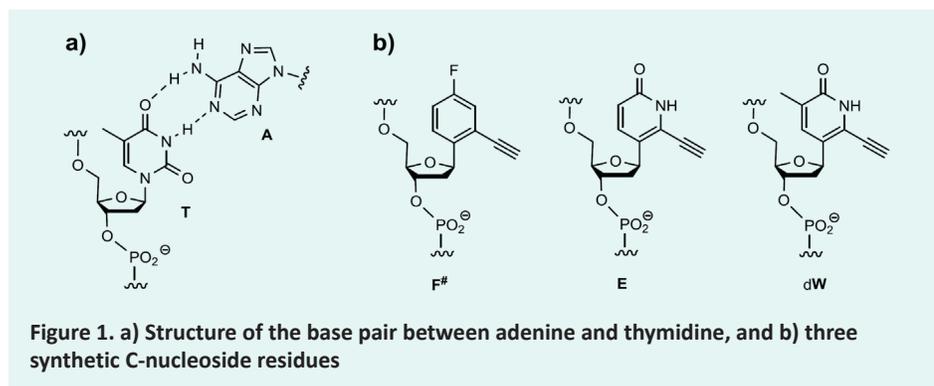
Authors: Juri Eyberg and Clemens Richert

Institute of Organic Chemistry, University of Stuttgart, D-70569 Stuttgart, Germany

Email: lehrstuhl-2@oc.uni-stuttgart.de

DNA base pairs are known to differ in strength. The T:A pair (Figure 1a) is weaker and more prone to mispairing than the C:G pair.¹ Likewise, U:A base pairs in RNA:RNA and RNA:DNA duplexes are weaker than the corresponding C:G base pairs, and often similar in stability to U:G wobble base pairs.² Weak base pairing makes it difficult to bind sequences with low G/C content, which not only complicates the detection of A/T-rich sequences in a genomic context, but also presents a challenge for many other hybridization probes or primers that depend on reliable pairing between complementary sequences.

Previously, we published an approach describing how to obtain isostable duplexes using 'decorated probes'.³ However, this method failed to account for the physicochemical property changes that resulted from adding multiple lipophilic side chains to bases. In order to address this limitation, we developed an approach that relies on nucleobases similar in size to natural bases.



Through improved shape complementarity, these nucleobases achieve high affinity and high-fidelity base pairing for adenine in target strands. We opted for C-nucleosides as replacements for thymidine because unlike canonical nucleosides, which have a glycosidic C-N bond between the sugar and the nucleobase, C-nucleosides feature a C-C bond at the anomeric position. Figure 1b shows three C-nucleosides developed in recent years.

The first of the C-nucleosides synthesized was fluorobenzene derivative F#, inspired in part by the elegant work of Kool and co-workers on isosteres of natural bases.⁴ The fluorobenzene pairs with A, but the base pair is destabilizing compared to T:A base pairs,⁵ making the base analog unsuitable for the reinforcement of A/T-rich duplexes. The destabilizing effect is much

Continued on Page 2

Ethynylpyridone
C-Nucleoside
Phosphoramidite (dW):
A High Affinity
Replacement
for Thymidine 1

New Product – dW CE
Phosphoramidite 3

New Product – beta
L-DNA Phosphoramidites 4

Cross-linkers and
Mechanism of RNA
Induced Silencing
Complex (Part 2) 7

Technical Brief – Universal
Support III PS Cleavage
and Dephosphorylation 11

Glen Research
22825 Davis Drive
Sterling, VA 20164
Phone: 703-437-6191
support@glenresearch.com



Continued from Front Page

smaller than that of the 2,4 difluorotoluene C-nucleoside,⁶ indicating that an ethynyl group at what is the 2-position in thymidine can have a positive influence on duplex stability in base pairs with adenine. In light of this, we began developing a synthetic route for the preparation of ethynylpyridone C-nucleoside E, retaining the ethynyl group, while also featuring the NH and carbonyl groups that engage in hydrogen bonding with adenine in the T:A base pair. When E was incorporated into oligodeoxynucleotides, it was found to stabilize duplexes with strands containing an adenine opposite the C-nucleoside in their sequence.⁷ A single E residue increased the UV-melting point of duplexes by approx. 2.1–2.8 °C, and overall base pairing strength was close to that of C:G base pairs.

The latest development in the evolution of strong pairing analogs of thymidine is represented by ethynylmethylpyridone dW (Figure 1b), the phosphoramidite of which is shown in Figure 2. This phosphoramidite was reported last year⁸ and is now available from Glen Research. While E had to be incorporated into DNA by a manual coupling after strand phosphitylation on solid support,⁷ the phosphoramidite allows for introduction of dW into oligonucleotides by automated DNA synthesis using conventional chain extension cycles. The pivaloyl (Piv) group is easily removed with the ammonia solution used to deprotect the natural nucleobases. Removal of the triisopropylsilyl (TIPS) protecting group within the ethynyl group is induced under conditions similar to those used for 2'-TBDMS protected RNA

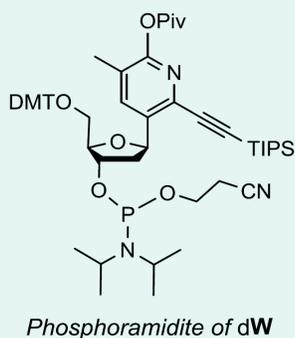


Figure 2. Structure of the phosphoramidite of dW

strands, with tetrabutylammonium fluoride (TBAF) as deprotection agent.

We studied the base pairing of dW-containing oligonucleotides in duplexes with DNA and RNA strands, and found that dW pairs slightly better with adenine than E, resulting in a base pairing strength nearly identical to that of C:G. In order for a dodecamer to hybridize to the fully complementary RNA strand, a melting point increase of 2.9°C was measured.⁸ Table 1 lists UV-melting points of fully complementary DNA duplexes containing dW residues.⁸ The ΔT_m values given are compared to duplexes that contain T residues instead of dW residues in the oligodeoxynucleotide chain. From this data, we can discern that although the melting point increase per dW residue depends on the sequence context and the number of modified residues, substantial stabilization is observed in all cases studied. Furthermore, dW possesses excellent base pairing fidelity, all but suppressing wobble base pairing with G, as evidenced by a ΔT_m of -20.5 °C for a single mismatched G in the sequence context.⁸ These results demonstrate just how strongly and selectively the new C-nucleoside pairs with A.

Sequence	T_m [°C]	ΔT_m [°C]
5'-CTTTTCWTTCTT-3'	44.5	+2.9
5'-CCWCCTT-3'	32.3	+5.8
5'-TGGWWGAC-3'	45.3	+9.0
5'-GTCWWWWWWGC-3'	64.0	+17.5

Table 1. UV-melting points of duplexes with fully complementary strands, as measured for the oligodeoxynucleotides containing dW residues listed above.

What are the reasons for the high stability of dW:A base pairs that contain one hydrogen bond less than C:G base pairs? Figure 3 shows the base pair with adenine. The ethynyl group reaches into the minor groove and likely gets into van der Waals contact with the CH group at position 2 of the adenine ring. This additional interaction is a probable contributor to pairing strength, as evidenced by the results of theoretical studies.⁸ Stacking interactions with neighboring bases is another, as well as hydrophobic/solvation effects. The effect of the ethynyl group is thus reminiscent of what has been reported for 5-propynylpyrimidines.⁹

In conclusion, the ethynylmethylpyridone C-deoxynucleoside dW represents an appealing replacement for thymidine in oligonucleotides designed to pair with target strands containing adenines. The phosphoramidite makes incorporation into oligonucleotides a facile process easily performed by laboratories that are well versed in the art of oligonucleotide synthesis. Given that the new nucleobase shows a significantly stronger binding and higher base pairing fidelity than T, dW will be an interesting option for applications that rely on hybridization between complementary strands. We hope that by placing the phosphoramidite of dW into the hands of interested researchers, Glen Research will foster new research by providing a solution to a classical problem in nucleic acid chemistry, opening the door to applications ranging from hybridization probes to building blocks for nanostructures.

References

1. J. SantaLucia, Jr.; H.T. Allawi; P. A. Seneviratne, *Biochemistry*, 1996, **35**, 3555–3562.
2. R. Kierzek; M.E. Burkard; D.H. Turner, *Biochemistry*, 1999, **38**, 14214–14223.
3. C. Ahlborn, K. Siegmund, C. Richert, J. Am. Chem. Soc., 2007, **129**, 15218–15232.
4. E.T. Kool; J.C. Morales; K.M. Guckian,

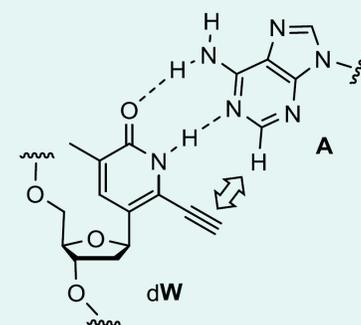


Figure 3. Proposed molecular interactions in the base pairing of a dW residue with adenine in a double helix. Besides the hydrogen bonds known from T:A base pairs, there is potential for van der Waals interactions between the ethynyl group and the lower rim of adenine facing the minor groove, as indicated by the double-headed arrow.



- Angew. Chem. Int. Ed. Engl., 2000, **39**, 990-1009.
- N. Griesang; C. Richert, *Tetrahedron Lett.*, 2002, **43**, 8755-8758.
 - S. Moran; R.X. Ren; S. Rumney; E.T. Kool, *J. Am. Chem. Soc.*, 1997, **119**, 2056-2057.
 - M. Minuth; C. Richert, *Angew. Chem. Int. Ed.*, 2013, **52**, 10874-10877.
 - T.J. Walter; C. Richert, *Nucleic Acids Res.*, 2018, **46**, 8069-8078.
 - R.W. Wagner; M. D. Matteucci; J.G. Lewis; A.J. Gutierrez; C. Moulds, *B.C. Froehler, Science*, 1993, **260**, 1510-1513.

New Product – dW CE Phosphoramidite

Over the years, Glen Research has introduced many products to modulate hybridization interactions, some of which continue to be quite popular. Those that stabilize the A – T base pair have been of particular interest because of how much weaker the interaction is compared to the G – C base pair. Products in this family include N6-Me-dA, pdU, and 2-amino-dA. Due to a third hydrogen bond interaction with T, 2-amino-dA provides the strongest stabilization of duplexes (Glen Report 11.1). However, the 2-amino-dA – T interaction was still weaker than that of a G – C base pair. When we first learned about Professor Richert's research on a new A base pairing analog called W,¹ we were intrigued, and saw it as an excellent new product candidate. Their team showed that W paired with A almost as strongly as G paired with C. With Dr. Richert's assistance, we are now able to provide customers with another option for strengthening A – T base pair interactions. We called this product dW, to clarify that it is for DNA.

In our hands, we have found that a 15 min. coupling time was sufficient for good coupling efficiency. Also, the use of 3 % dichloroacetic acid (DCA) in dichloromethane is highly recommended as the deblock, for reasons that will be elaborated on below. For deprotection, typical conditions would be ammonium hydroxide at room temperature for 17 hr (sufficient for dmf-dG) or ammonium hydroxide/aqueous methylamine 1:1 at room temperature

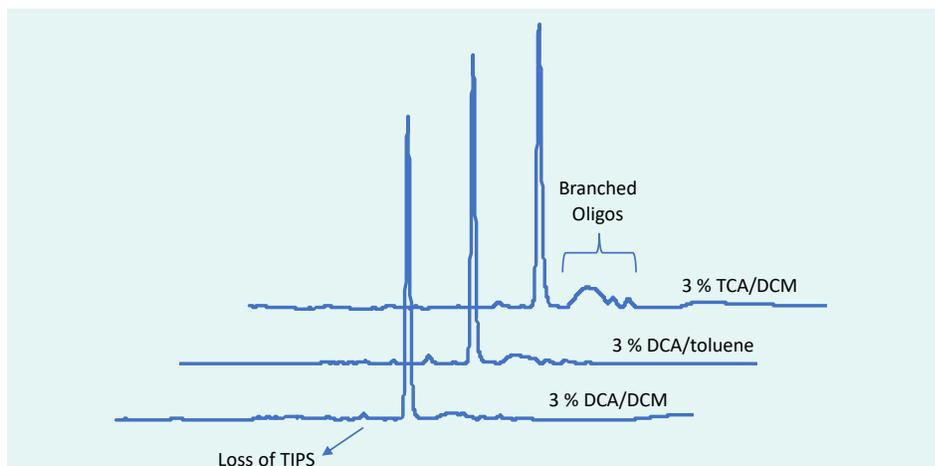


Figure 1. Comparison of different deblock formulations. The sequence TTT TTT TTW TTT was synthesized DMT-on, deprotected with NH₄OH for 2 hr at room temperature, and analyzed by RP-HPLC (A254). TCA, trichloroacetic acid; DCA, dichloroacetic acid; DCM, dichloromethane; TIPS, triisopropylsilyl.

for 2 hr, but all common deprotection solutions are compatible, as long as no elevated temperature is used. Otherwise, the triisopropylsilyl (TIPS) group can be removed prematurely, and the resulting unprotected alkyne will be susceptible to addition of water across the triple bond to give the ketone.² For example, in one overnight deprotection of an oligo with ammonium hydroxide at 55 °C, more than 95 % of the TIPS groups were removed, and about a fifth of the alkynes were converted to the ketone. After deprotection, the oligo is dried down, resuspended in 0.5 mL of DMF and 0.1 mL of TBAF/THF, mixed thoroughly, and incubated at room temperature for 30 min. The mixture is then treated with 0.5 mL 2 M TEAA and desalted on a Glen Gel-Pak™ column or equivalent.

During the development of this product, we noticed that typical oligo syntheses resulted in several later-eluting peaks in addition to the desired product on RP-HPLC. Investigation into this revealed that the peaks were a result of branching, and the culprit was the deblock. If we capped a dW-containing oligo, treated it with deblock for an extended period of time, and performed one extra cycle of coupling, we were able to observe significant coupling relative to a control. It turns out the pivaloyl (Piv) group is susceptible to removal during the deblocking step, and once the Piv group

is lost, an oligo branch can grow from there. The DCA in dichloromethane minimizes this issue. As shown in Figure 1, 3 % DCA in dichloromethane is superior to both 3 % DCA in toluene and 3 % trichloroacetic acid (TCA) in dichloromethane. For purification, we found that branched species containing multiple DMT groups were typically well resolved from the desired single DMT-on product by RP-HPLC. It should be noted that phenyl phosphate linkages are less stable than standard ones. Because of this, deprotection with 0.4 M sodium hydroxide in methanol/water (4:1) overnight at room temperature will cleave off a portion of the undesirable branches, effectively increasing the yield of desired product. Purification of such mixtures will require a size- and/or length-based purification method.

References

- T.J. Walter, and C. Richert, *Nucleic Acids Res.*, 2018, **46**, 8069-8078.
- S.A. Ingale, H. Mei, P. Leonard, and F. Seela, *J. Org. Chem.*, 2013, **78**, 11271-11282.

Item	Pack	Catalog No.
dW-CE Phosphoramidite	50 μmole	10-1527-95
	100 μmole	10-1527-90
	0.25 g	10-1527-02

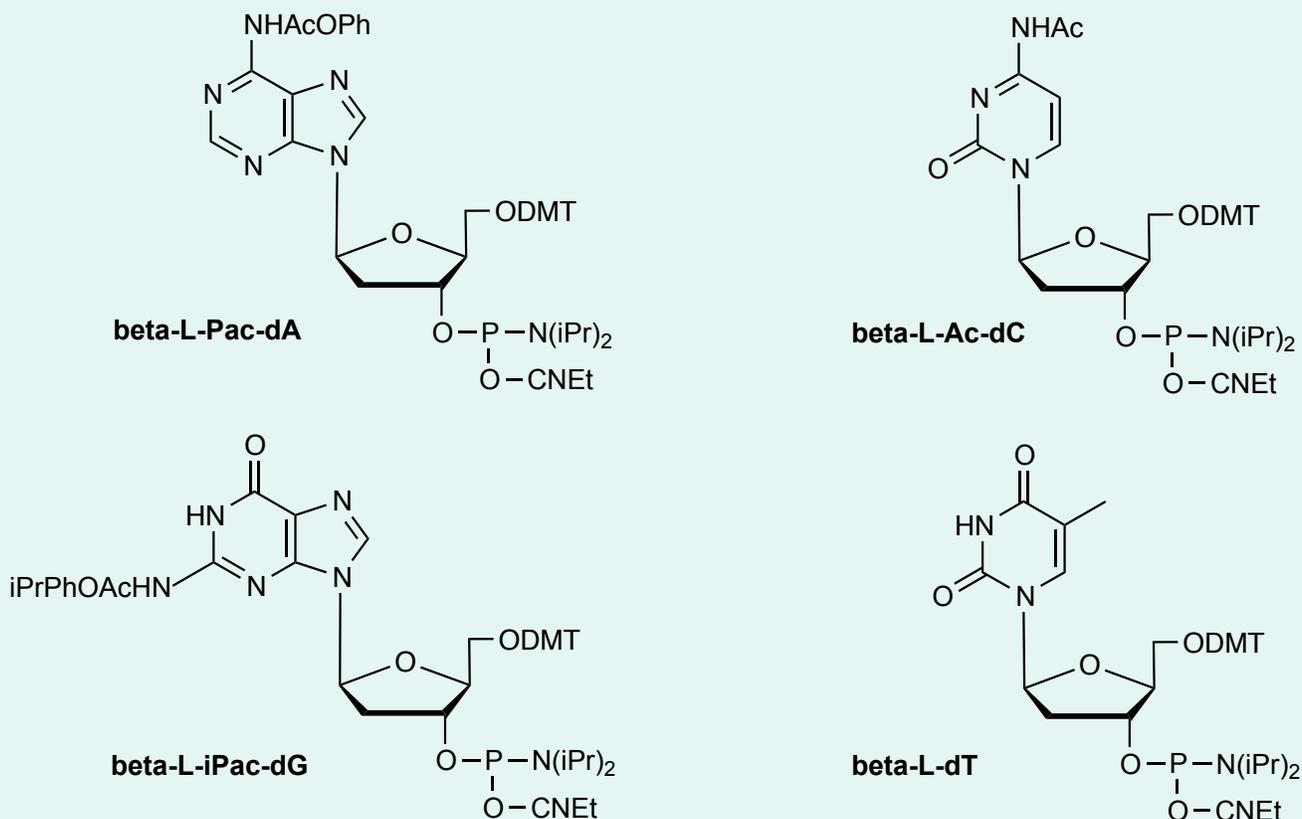


Figure 3: Structures of L-DNA Phosphoramidites

Aptamers

The unique properties of L-DNAs have made them attractive for many biological applications, including use in the field of aptamer therapeutics. Aptamers are functional nucleic acids that, through three-dimensional structure, bind tightly and specifically to molecular targets in the same way that antibodies do. Over the years, many D-DNA aptamers have been developed for targets that range in size from small molecules all the way to whole cells, and one aptamer is currently approved for medical use. D-DNA aptamers, at least native ones, generally have short lifetimes in biological environments due to degradation by nucleases, while L-DNA aptamers do not suffer from this issue. Although the development of L-DNA aptamers is more laborious,¹ many of these affinity agents have been developed. Several have been or

are currently being investigated in clinical trials involving cancer, diabetes, and other conditions as well.²

Molecular Beacons (MBs)

L-DNA has also been used to enhance molecular beacons (MBs). Since the stem domain plays a crucial role in the stability of the MB probes, efforts were devoted into enhancing stem stability and minimizing off-target effects. In a series of experiments, Kim et al. showed that MB chimeras containing an L-DNA stem and a D-DNA loop gave a signal enhancement ratio that was double that of the all D-DNA MB.³ This observation was primarily due to a reduction of background signal, as no intramolecular stem-to-loop interaction was possible for the chimeric MB. Additionally, the overall stability of the MB chimera was higher than that of the all D-DNA version, with a melting temperature elevation of 5-10 °C.

Molecular Tagging

In addition to aptamers and MBs, L-DNA has been used as a handle for surface immobilization. In one example, primers containing a section of L-DNA at the 5'-end were used for PCR.⁴ Since the L-DNA overhang was non-amplifiable, PCR gave double-stranded D-DNA product with a single-stranded L-DNA molecular tag. The resulting L-DNA-tagged PCR products were successfully immobilized by hybridization with complementary L-DNA on a Surface Plasmon Resonance (SPR) gold surface.⁴

Drug Nanocarriers

Finally, L-DNAs have been investigated as nanocarriers of drugs. DNA can easily be designed to form three-dimensional structures, and due to a number of desirable properties, including being non-toxic and biodegradable, DNA has been investigated as

Continued on Page 6



Continued from Page 5

a polymer for nanocarriers to facilitate the delivery of a whole host of drug candidates. In such an application, L-DNA would offer significant advantages to D-DNA. As discussed earlier, L-DNA is going to be much more stable, and for the delivery of D-DNA/D-RNA drugs, there is the added advantage that there will not be any drug to carrier base-pairing interactions. Several investigations have been published regarding this, and in one of them, the L-DNA tetrahedron nanocarriers were not degraded in 10 % mouse serum after 10 hours of exposure.⁵ The authors were also able to construct aptamer-tethered L-DNA carriers to successfully deliver aptamers to cells.

Glen Research is introducing L-DNA Phosphoramidites with UltraMild protecting groups (Figure 3). They are unique products in the market, providing access to L-DNA oligos that require UltraMild deprotection. Of course, these monomers are also compatible with standard deprotection processes for oligos that do not require UltraMild deprotection.

Use of L-DNA Phosphoramidites

The synthesis of L-DNA oligonucleotides is very similar to that of D-DNA oligonucleotides. The L-DNA Phosphoramidites can be dissolved in anhydrous acetonitrile to standard concentrations. Regarding the coupling of L-DNA, no changes are needed from standard methods recommended by the synthesizer manufacturer. To avoid any exchange of the iPr-Pac group on the dG with acetyl, please use the UltraMild Cap Mix A (40-4210-xx/40-4212-xx). For deprotection, UltraMild deprotection will be carried out with either 0.05 M potassium carbonate in methanol, 4 hr at room temperature OR 2 hr at room temperature in 30 % ammonium hydroxide. As previously mentioned, UltraMild monomers will work well in standard syntheses with some conversion of the iPr-Pac-dG to Ac-dG. Ac-dG is deprotected using normal deprotection conditions.

References

1. K.P. Williams, et al., Proc Natl Acad Sci U S A, 1997, **94**, 11285-11290.
2. A. Vater, and S. Klussmann, Drug Discov Today, 2015, **20**, 147-155.
3. Y. Kim, C.J. Yang, and W. Tan, Nucleic Acids Res., 2007, **35**, 7279-7287.
4. G. Hayashi, M. Hagihara, and K. Nakatani, Nucleic Acids Symp Ser (Oxf), 2005, 261-262.
5. K.-R. Kim, T. Lee, B.-S. Kim, and D.-R. Ahn, Chemical Science, 2014, **5**, 1533-1537.

Item	Pack	Catalog No.
beta-L-Pac-dA-CE Phosphoramidite	0.25 g	10-2101-02
	0.5 g	10-2101-05
	1.0 g	10-2101-10
beta-L-Ac-dC-CE Phosphoramidite	0.25 g	10-2115-02
	0.5 g	10-2115-05
	1.0 g	10-2115-10
beta-L-iPr-Pac-dG-CE Phosphoramidite	0.25 g	10-2121-02
	0.5 g	10-2121-05
	1.0 g	10-2121-10
beta-L-dT-CE Phosphoramidite	0.25 g	10-2130-02
	0.5 g	10-2130-05
	1.0 g	10-2130-10

Cross-linkers and Mechanism of RNA Induced Silencing Complex (Part 2)

Author: Dick Keys, Ph.D.

See Part 1 in Glen Report 31.1

Introduction

Previously, we reviewed the use of 5-Bromo- and 5-Iodo-pyrimidine nucleosides within synthetic oligonucleotides for probing nucleic acid-protein interactions at their binding interface.¹ Recently, a much broader role for RNA in regulating gene expression has been established, through the characterization of previously poorly understood or unknown activities among the many noncoding RNAs, including lncRNA, miRNA², and others. Here in Part 2, we describe studies of RNA interference that use 5-Iodo-Uracil (5IU) in siRNA oligonucleotides to discern biological mechanism details whereby siRNA is processed in the RNA-induced silencing complex (RISC).

Regulation of Gene Expression and RNA Interference: the RISC

Tomari et al. used homologous siRNA molecules labeled with 5IU and applied photo-cross-linking to discern the processing details of siRNA interaction with several protein subcomponents of the RISC of *Drosophila*.³

Since the discovery of RNA interference by Fire and Mello⁴, the RNAi pathway has been elucidated in many eukaryotes. We now know that an important mode of regulation of gene expression utilizes ribonucleases and RNA binding proteins (RBP), which in turn oversee the production and action of siRNA and miRNA via the following pathways (Figure 1).⁵

After nuclear processing by Drosha, pre-miRNAs are transported to the cytoplasm, where processing pathways for miRNA and exogenous and endogenous siRNA converge. Dicer cleaves these molecules, yielding miRNA or short ~21 nucleotide double-

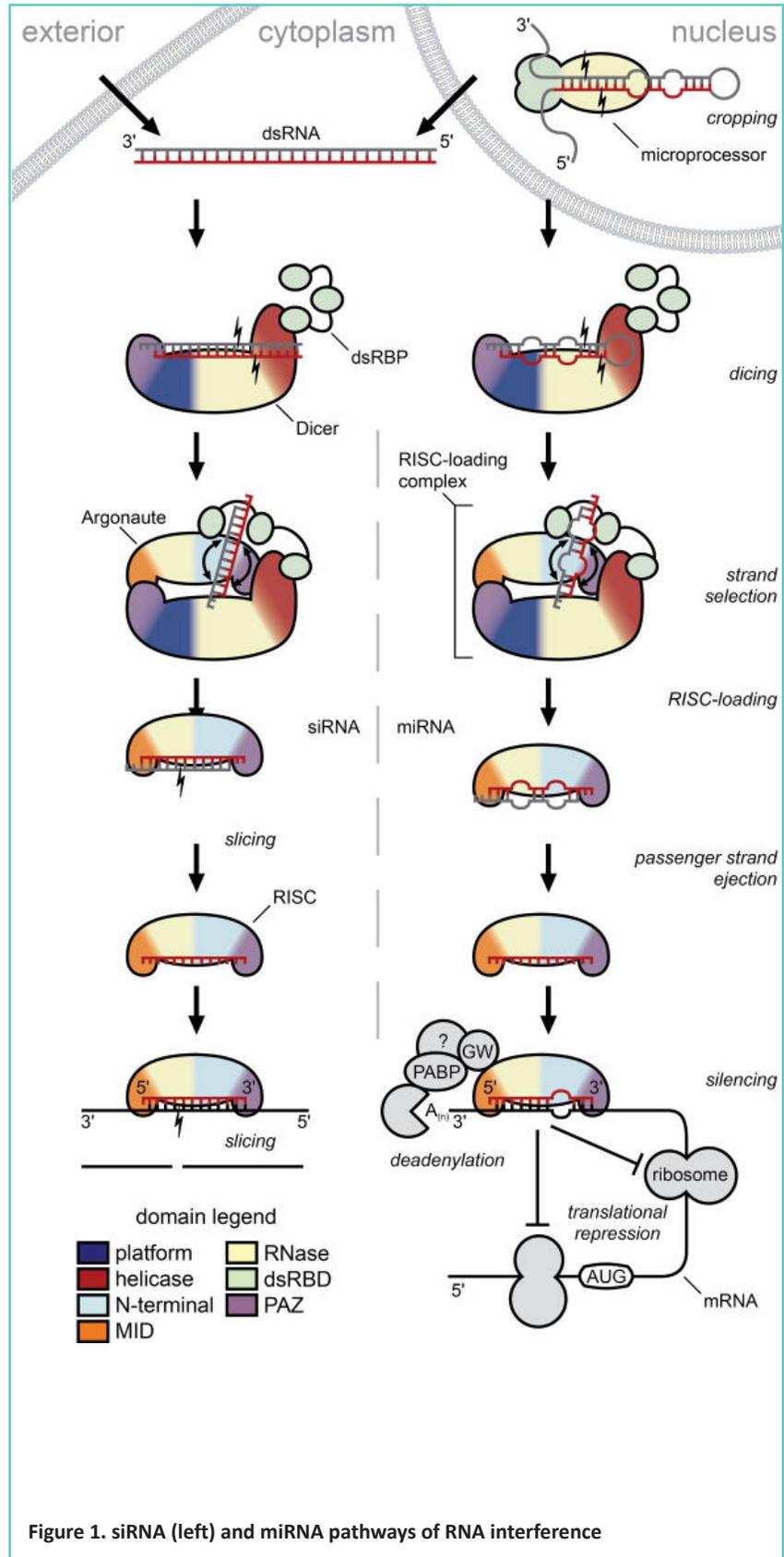


Figure 1. siRNA (left) and miRNA pathways of RNA interference

Continued on Page 8



Continued from Page 7

stranded siRNA, with a two-base overhang on each 5'-end. Each siRNA interacts with a protein complex, including Dicer, RNA binding protein, and an Argonaute family endoribonuclease. These 3 key protein components and others form a gene-silencing ribonucleoprotein complex, the RNA-induced silencing complex. This RNP complex unwinds siRNA into a passenger strand and a guide strand. The passenger strand is degraded into inactive fragments, while the Argonaute-bound guide strand remains bound to the RISC and base-pairs with a target mRNA molecule. This complex affects gene silencing through either intrinsic endonucleolytic cleavage activity on mRNA or by translational repression of the corresponding mRNA.

Determination of siRNA Processing Mechanisms in the RISC

The Tomari team investigated RISC assembly, in which the formation of RISC loading complex (RLC) containing double stranded siRNA, RNA binding protein (known as R2D2), and Dicer (Dcr-2) acts to identify siRNA asymmetry, recognize guide versus passenger strand, initiate unwinding of siRNA, and associate the guide strand with Argonaute. Previous studies *in vitro* have shown that recombinant Dcr-2/R2D2 heterodimer or Dcr-2 alone cannot catalyze siRNA unwinding as it binds to the RLC. Studies were carried out using *Drosophila* embryo lysates, and by photo-crosslinking a series of 5IU labeled siRNAs, it was shown that fully assembled RLC initiates unwinding of bound siRNA through an ordered stepwise process.

Previous researchers found that photo-cross-linking of siRNA to RISC was highly dependent on the position of 5IU in the sequence.⁶ In this work, siRNAs 1 and 2 were used to target the Luciferase gene (Table 1), where red U indicates the presence of 5IU and asterisks show the position of ³²P. Labeled siRNA was pre-incubated with lysate in microwells and then photo-cross-linked by irradiation for 10–15 min at 302 nm using an inverted transilluminator.

Table 1. siRNAs targeting firefly luciferase

siRNA 1	CGUACGCGGAAUACUUCGAUU GUGCAUGCGCCUUAUGAAGCU - p*
siRNA 2	p* - CGUACGCGGAAUACUUCGAUU GUGCAUGCGCCUUAUGAAGCU

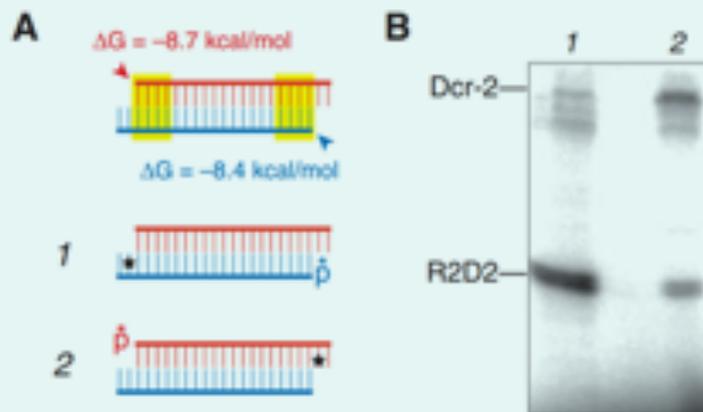


Figure 2. Cross-linking to RBP or Dicer-2

PAGE autoradiography showed that R2D2 was more efficiently cross-linked to oligo when 5IU was on the siRNA antisense strand. Alternately, when 5IU was on the sense strand, it more efficiently cross-linked to Dicer-2 (Figure 2). Thus, Dicer-2 is adjacent to this siRNA sense strand's 3'-end on entering the RISC.

Additional siRNAs *a* through *g* were prepared to target human superoxide dismutase gene (*sod1*), where the first listed, siRNA *a*, is nearly symmetric, while the remainder, *b* through *g*, are highly asymmetric due to a 5'-base mismatch (Table 2). Note that when there is a 5'-base mismatch, that strand is loaded into the RISC nearly exclusively. Thus *b-d* have a single mismatch at the 5'-end of the upper strand, while *e-g* have a single mismatch at the 5'-end of the lower strand (Figure 3, C).

In duplexes *b* and *c*, siRNA *b* contains 5IU near the 3'-end of the antisense strand (one base in from the terminus), while in duplex *c*, 5IU is positioned next to the 3'-end of the sense strand, respectively. The siRNA *d* contains both phosphorylated strands of *b* and *c*. The gel reveals that siRNA *b* crosslinks predominantly to Dcr-2 and contains only double-stranded RNA (Figure 3, D, lane b). In contrast, siRNA *c* crosslinks to RBP and to Argonaute protein, and not at all to Dcr-2. It also contains significant single-stranded RNA, due to siRNA unwinding. siRNA *d* crosslinks to proteins with a combination of the results in *b* and *c*, which is expected since 5IU is present in both termini of corresponding *b* and *c* strands.

The reader can follow the gel data in the similar series of siRNAs *e*, *f*, and *g*, in which asymmetry of the single 5'-mismatch is positioned on the opposite strand to siRNAs



Table 2. siRNAs Targeting Human sod1

siRNA a	GUCACAUUGCCCAAGUCUCTT TUCAGUGUAACGGGUUCAGAG - p*
siRNA b	UUCACAUUGCCCAAGUCUCTT TUCAGUGUAACGGGUUCAGAG - p*
siRNA c	p* -UUCACAUUGCCCAAGUCUCUT TTCAGUGUAACGGGUUCAGAG
siRNA d	p* -UUCACAUUGCCCAAGUCUCUT TUCAGUGUAACGGGUUCAGAG - p*
siRNA e	GUCACAUUGCCCAAGUCUATT TUCAGUGUAACGGGUUCAGAG - p*
siRNA f	p* -GUCACAUUGCCCAAGUCUAUT TTCAGUGUAACGGGUUCAGAG
siRNA g	p* -GUCACAUUGCCCAAGUCUAUT TUCAGUGUAACGGGUUCAGAG - p*

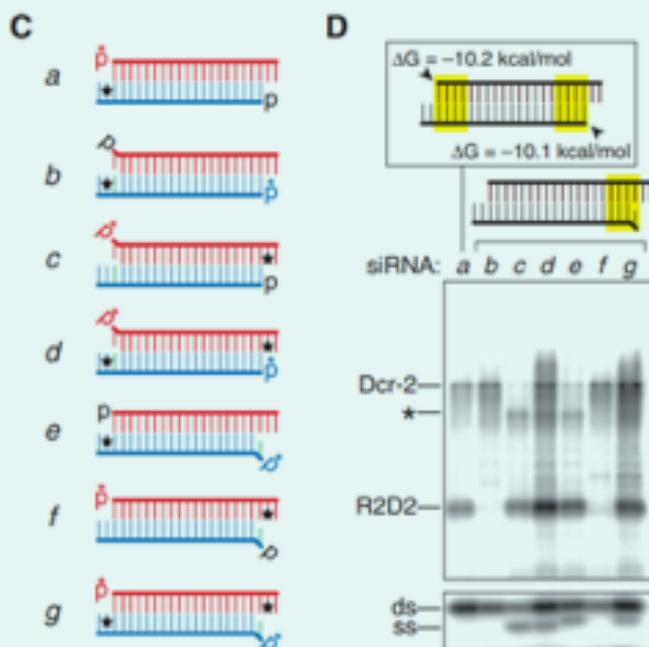


Figure 3. Highly Ordered Crosslinking of siRNA to RISC. C) siRNA designs, D) Autoradiograms of PAGE siRNA/Lysate Crosslink mixtures. The asterisk position indicates a crosslink to Argonaute (Ago2).

b, *c*, and *d*, while keeping the 5IU at the corresponding location, respectively, adjacent to the terminus of each corresponding oligo. The data are consistent with analogous positioning of the duplex and the less-stable mismatched siRNA end preferentially loading into the RISC.

The authors propose that “R2D2 orients the Dcr-2/R2D2 heterodimer on the siRNA within the RLC. As siRNA unwinding proceeds, the heterodimer is exchanged for Ago2, the core component of the RISC,” and siRNA unwinding only occurs when the RISC, containing Argonaute, is assembled. Further studies support and expand these conclusions and readers are directed to recent reviews.⁷

Conclusion

Photo-crosslinking synthetic DNA and RNA to protein using 5-bromo and 5-iodopyrimidine nucleosides continues to facilitate research. The crosslink specificity to adjacent protein residues can reveal details of dynamic processes occurring in a large multifunctional ribonucleoprotein complex, as discussed for the RISC. These efficient crosslinkers are well-suited to additional research, among the growing collection of non-coding RNAs and RNP complexes regulating gene expression. Studies may be readily undertaken using the 5-halogen substituted amidites, judicious design of modified oligonucleotides, and readily available photo-cross linking methods.

The Glen Research line of molecules includes halogenated-deoxycytidine and-deoxyuridine phosphoramidites and bromodeoxyuridine CPG support. For RNA constructs, bromo- and iodo-uridine phosphoramidites are available TBDMS protected, and as the bromo-uridine, 2'-O-methyl phosphoramidite. In general, mild deprotection is required. See use instructions for dissolution, coupling, and deprotection conditions for monomers.

Continued on Page 10



Continued from Page 9

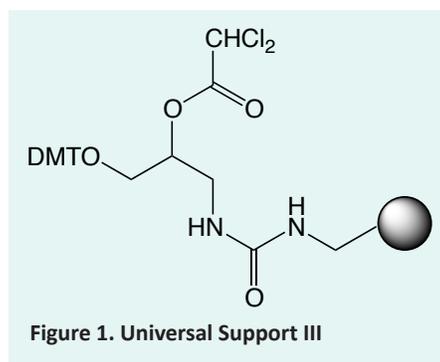
References

1. The Glen Report, 2019, 31.1, 3-10. <https://www.glenresearch.com/reports/gr31-12>
2. Abbreviations used: dsRBP, double stranded RNA binding protein; lncRNA, long non-coding RNA; mRNA, messenger RNA; miRNA, microRNA; RBP, RNA binding protein-also called dsRBP in Figure 1; RNAi, RNA Interference; RISC, RNA-induced silencing complex; RNP, ribonucleoprotein; siRNA, small interfering RNA; sod1, superoxide dismutase 1; ssRNA, single-stranded RNA.
3. Y. Tomari, C. Matranga, B. Haley, N. Martinez, P.D. Zamore, *Science*, 2004, **306** (5700), 1377-1380.
4. A. Fire, S. Xu, M.K. Montgomery, S.A. Kostas, S.E. Driver, C.C. Mello, *Nature*, 1998, **391** (6669): 806-811.
5. R. Wilson and J.A. Doudna, *Annu. Rev. Biophys.*, 2013, **42**: 217–239.
6. Y. Tomari, T. Du, B. Haley, D.S. Schwarz, R. Bennett, H.A. Cook, B.S. Koppetsch, W.E. Theurkauf, P.D. Zamore, *Cell*, 2004, **116** (6): 831-841.
7. A.V. Olina, A.V. Kulbachinskiy, A.A. Aravin, D.M. Esyunina, *Biochemistry (Moscow)*, 2018, **83**: 483-497.

Item	Pack	Catalog No.
5-Br-dC-CE Phosphoramidite	100 μ mole	10-1080-90
	0.25 g	10-1080-02
5-I-dC-CE Phosphoramidite	100 μ mole	10-1081-90
	0.25 g	10-1081-02
5-Br-dU-CE Phosphoramidite	100 μ mole	10-1090-90
	0.25 g	10-1090-02
5-Br-dU-CPG	0.1 g	20-2090-01
1 μ mole columns	Pack of 4	20-2090-41
0.2 μ mole columns	Pack of 4	20-2090-42
Br-U-CE Phosphoramidite	50 μ mole	10-3090-95
	100 μ mole	10-3090-90
	0.25 g	10-3090-02
5-I-U-CE Phosphoramidite	100 μ mole	10-3091-90
	0.25 g	10-3091-02
2'-OMe-5-Br-U-CE Phosphoramidite	100 μ mole	10-3190-90
	0.25 g	10-3190-02

Technical Brief – Universal Support III PS Cleavage and Dephosphorylation

Since 2001, Glen Research has been offering a version of Universal Support III PS (USIII) as a matrix option for oligonucleotide synthesis. Due to the mild and anhydrous conditions that may be used for oligonucleotide cleavage and phosphate elimination, this product has proven to be a truly universal support and an excellent complement to UnySupport products. In particular, USIII is well-suited for the synthesis of RNA.



Oligos synthesized on USIII are typically cleaved and deprotected in two separate steps. First, oligos are released from the support in 2M anhydrous ammonia in methanol at room temperature for 20-60 mins. Subsequently, deprotection is completed based on the protecting groups of the nucleobases and the sensitivity of any modifications. A detailed protocol can be found at: https://www.glenresearch.com/media/productattach/import/tbn/TB_Universal_Support_III.pdf.

Over the years, a number of customers have inquired whether the first step could be skipped or whether alternative procedures were possible. The reason a separate cleavage condition is necessary is because the cleavage reaction for the universal linker requires 1) an amide to form a weak intramolecular hydrogen bond and 2) the presence of the cyanoethyl group of the first

phosphoramidite addition. Any environment that interferes with the former or accelerates the removal of the latter will affect the yield. **As such, preliminary elimination of cyanoethyl deprotecting groups using hindered bases such as diethylamine or DBU will prevent cleavage and result in no yield.**

To confirm how critical our recommended two-step procedure is, we evaluated four alternative methods of cleavage/dephosphorylation. The results are summarized in Table 1. In short, anhydrous conditions will give better yields than aqueous conditions. The choice is yours: a faster, single-step cleavage and deprotection reaction, or higher final yields with a two-step cleavage and deprotection reaction. For those performing UltraMild syntheses, a third option would be to cleave and deprotect using 50 mM potassium carbonate in methanol at room temperature for 4 hr (UltraMild Cap Mix A) or overnight (regular capping).

Reagent	Time (min)	Temp (°C)	Relative yield (%)
AMA*	15	RT	51
AMA	10	65	57
EMAM	10	65	58
8 M MeNH ₂ /EtOH*	15	RT	85
2M NH ₃ /MeOH*	60	RT	100

Table 1. Cleavage and dephosphorylation of USIII. Five 20 nt oligos were synthesized, and yield was quantified by OD measurement at A260 following deprotection. *Suspensions were filtered to remove support matrix, and deprotection was continued by adding AMA and heat (65 °C, 10 min). AMA, ammonium hydroxide/aqueous methylamine 1:1; EMAM, ethanolic methylamine/aqueous methylamine 1:1; RT, room temperature.

Item	Pack	Catalog No.
Universal Support III PS	0.1 g	26-5010-01
	0.25 g	26-5010-02
	1.0 g	26-5010-10
1 μmole columns (ABI)	Pack of 4	26-5110-41
0.2 μmole columns (ABI)	Pack of 4	26-5110-42
40 nmole columns (ABI)	Pack of 4	26-5110-45
10 μmole column (TWIST format) (ABI)	Pack of 1	26-5110-13
1 μmole columns (Expedite)	Pack of 4	26-5210-41
0.2 μmole columns (Expedite)	Pack of 4	26-5210-42
40 nmole columns (Expedite)	Pack of 4	26-5210-45
15 μmole column (TWIST format) (Expedite)	Pack of 1	26-5210-14
1 μmole columns (MerMade, etc.)	Pack of 96	26-5110-91
200 nmole columns (MerMade, etc.)	Pack of 96	26-5110-92
40 nmole columns (MerMade, etc.)	Pack of 96	26-5110-95
200 nmole columns (AB 3900)	Pack of 10	26-5110-52
40 nmole columns (AB 3900)	Pack of 10	26-5110-55



Literature Highlights

Epigenetics of Modified DNA

A general strategy exploiting m5C duplex-remodelling effect for selective detection of RNA and DNA m5C methyltransferase activity in cells

T. Yang; J. J A Low; E. C Y Woon, *Nucleic Acids Res.*, gkz1047, <https://doi.org/10.1093/nar/gkz1047>

5-Formylcytosine-induced DNA–peptide cross-links reduce transcription efficiency, but do not do cause transcription errors in human cells

S. Ji; D. Park; K. Kropachev; M. Kolbanovskiy; I. Fu; S. Broyde; M. Essawy; N. E. Geacintov; N. Y. Tretyakova, *J. Biol. Chem.*, <http://www.jbc.org/cgi/doi/10.1074/jbc.RA119.009834>

