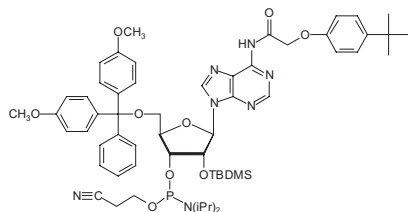


User Instructions

RNA Phosphoramidites



rA(tac) Phosphoramidite

Product Description

Chemical Formula: C₅₈H₇₆N₇O₉PSi

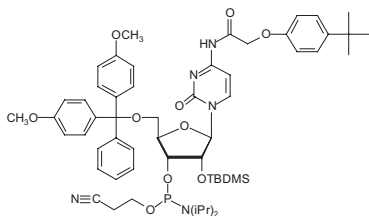
Formula Weight: 1074.4

Storage: -20°C

DMT-rAdenosine(N6-tac)(O2'-TBDMS)-β-Cyanoethylphosphoramidite

Product List

A212081-01	0.5g PE™ 8900 and Polygen™ compatible
A212031-01	0.5g ABI™ compatible
A212061-01	1g ABI™ compatible
A212010-01	10g bulk



rC(tac) Phosphoramidite

Product Description

Chemical Formula: C₅₇H₇₆N₅O₁₀PSi

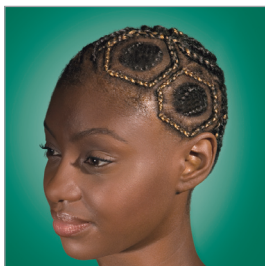
Formula Weight: 1050.3

Storage: -20°C

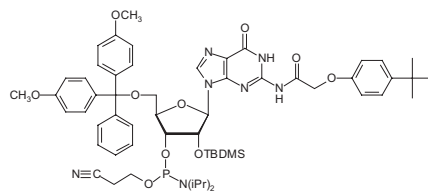
DMT-2'-O-Methyl-rCytidine(N4-tac)-β-Cyanoethylphosphoramidite

Product List

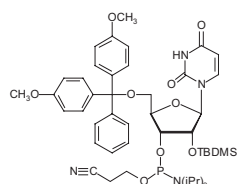
C212081-01	0.5g PE 8900 and Polygen™ compatible
C212031-01	0.5g ABI™ compatible
C212061-01	1g ABI™ compatible
C212010-01	10g bulk



RNA Phosphoramidites



rG(tac) Phosphoramidite



rU Phosphoramidite

RNA Synthesis

The synthesis cycle for RNA oligonucleotides consists of the same series of reactions as the cycle that is employed for Fast Deprotection DNA monomers.

However, the rate of coupling for RNA monomers is slower, compared to that of DNA monomers (a coupling time of 10 minutes for RNA monomers is recommended compared to 90 seconds for DNA monomers). With the

exception of the monomers and supports, RNA synthesis is accomplished with the same reagents as DNA synthesis.

All RNA phosphoramidites from Proligo® Reagents are diluted with dry acetonitrile. Fast Deprotection CAP A is employed to prevent the transacylation of guanosine bases, similar to synthesis of tert-butylphenoxyacetyl (TAC) DNA monomers.

Product Description

Chemical Formula: $C_{58}H_{76}N_7O_{10}PSi$

Formula Weight: 1090.3

Storage: $-20^{\circ}C$

DMT-rGuanosine(N2-tac)(O2'-TBDMS)- β -Cyanoethylphosphoramidite

Product List

G212081-01	0.5g PE™ 8900 and Polygen™ compatible
G212031-01	0.5g ABI™ compatible
G212061-01	1g ABI™ compatible
G212010-01	10g bulk

Product Description

Chemical Formula: $C_{45}H_{61}N_4O_9PSi$

Formula Weight: 861.1

Storage: $-20^{\circ}C$

DMT-2'-O-Methyl-rUridine- β -Cyanoethylphosphoramidite

Product List

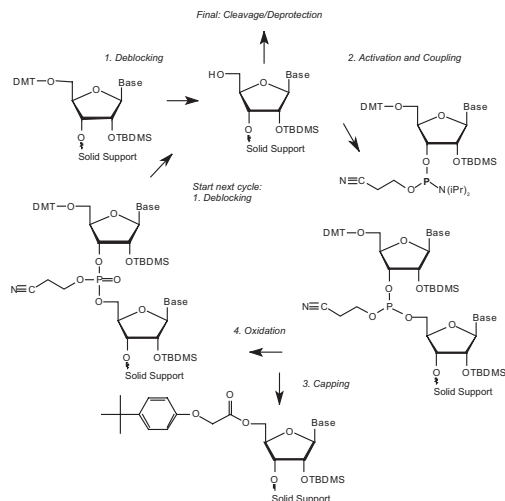
U211181-01	0.5g PE™ 8900 and Polygen™ compatible
U211131-01	0.5g ABI™ compatible
U211061-01	1g ABI™ compatible
U211110-01	10g Bulk



RNA Phosphoramidites



The Synthesis Cycle



RNA Monomers

RNA monomers feature hydroxyl groups at the 2'-position. In order to prevent the formation of unnatural 2'-5' phosphodiester bonds during chain elongation, the 2'OH group is protected with a trialkyl-silyl group, tert-butyldimethylsilyl (TBDMS). The TBDMS group is stable under the acidic conditions used to remove the DMT group during the synthesis cycle, but can be removed by a variety of methods after cleavage and deprotection of the RNA oligomer, e.g. with a solution of tetrabutylammonium fluoride (TBAF) in tetrahydrofuran (THF) or with triethylamine hydrofluoride.

TAC RNA Monomers

RNA oligonucleotides prepared from TAC protected RNA monomers can be base-deprotected under very mild conditions. Recommended cleavage and deprotection conditions for TAC base-protected oligonucleotides are 15 minutes at 55°C or 2 hours at room temperature, in a mixture of concentrated ammonia solution and

ethanol (3/1, v/v). Alternatively, AMA reagent (concentrated ammonia/40% aqueous methylamine 1/1, v/v) can be employed for 30 minutes at room temperature. The shorter exposure time of the oligonucleotide to the alkaline deprotecting agent, compared to conventionally protected RNA oligonucleotides, reduces chain degradation and provides a higher yield of full length RNA product.

Although Proligo Reagents' TAC RNA monomers are stable for several days in solution, we recommend reconstitution of fresh amidites after 6 days on the instrument, to achieve optimal results.

RNA Supports

The supports for RNA synthesis consist of an RNA nucleoside covalently attached through either the 2'- or the 3'-position to controlled pore glass (CPG).

The remaining free hydroxyl group is protected with a base-labile acyl group. The pore size of Proligo Reagents' CPG for RNA synthesis is 500Å. Proligo Reagents offers ready-to-use synthesis columns for RNA synthesis at 1mmol scale.

Methods

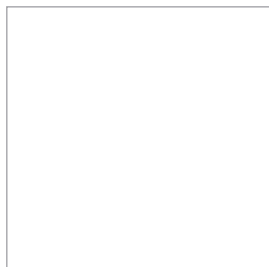
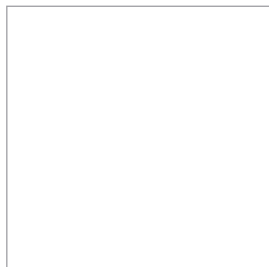
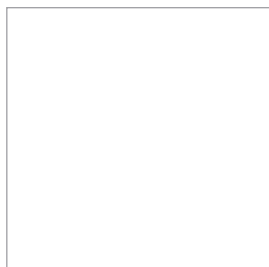
1. Use anhydrous acetonitrile (water content < 30ppm) as diluent. It is important to maintain anhydrous conditions while dissolving RNA amidites in acetonitrile.
2. For use on PE 8900 instruments, add 10ml of acetonitrile to 0.5g RNA monomer, to obtain a concentration of 50mg/ml. For use on PE 390 series instruments, add 5ml acetonitrile to 0.5g RNA monomer to obtain a concentration of 100mg/ml.



RNA Phosphoramidites



3. Gently swirl the vial until the powder is completely dissolved.
4. Attach the dissolved phosphoramidite to the appropriate position on the synthesizer. Ensure that the delivery line to the synthesis chamber is sufficiently primed.
5. Once TAC RNA phosphoramidite has been dissolved and placed on your instrument, the phosphoramidite should be used within 6 days.
6. Enter the sequence of the RNA oligonucleotide you wish to synthesize. A minimum coupling time of 10 minutes is recommended for 2'-TBDMSprotected RNA amidites.
7. Fast deprotection CAP A must be employed in all RNA synthesis with the TAC-protected rG RNA phosphoramidite.
8. Proceed as you would with a standard DNA oligonucleotide synthesis. Depending on your intended further use of the oligomer, you can choose either DMT-On or DMT-Off procedures. The coupling efficiency of RNA monomers may be determined by standard dimethoxytrityl cation assays.
9. Cleave from the support and deprotect the RNA oligonucleotide with a mixture of concentrated ammonia and ethanol 3/1, v/v, at 55°C for 15 minutes, or at room temperature for 120 minutes. Alternatively, AMA reagent (concentrated ammonia/40% aqueous methylamine 1/1, v/v) can be employed for 10 minutes at 65°C.
10. **It is essential to employ sterile conditions from this step forward.** Always use sterilized water: preferably water recently treated with DEPC (diethyl pyrocarbonate, stir 1L of HPLC grade water with 100µl DEPC overnight and autoclave twice) and use baked glassware (250°C+ for more than 4 hours).
11. Transfer the supernatant solution of the RNA oligonucleotide into a separate vial.



The yield of the RNA oligonucleotide can be improved by rinsing the support with ethanol/acetonitrile/water 3/1/1, v/v, and combining the oligonucleotide solution with the washing solution. Evaporate to dryness.

12. Add a 1M solution of tetrabutylammonium fluoride (TBAF) in THF and incubate for 24 hours at room temperature. The deprotection time can be shortened to 6 hours if a TBAF-solution, with water content less than 5%, w/w, is employed. Following deprotection, add an equal volume of 1M TEAA buffer pH 7, followed by another volume of water. Alternatively, deprotection can be accomplished using a mixture of neat triethylamine trihydrofluoride, triethylamine and N-methylpyrrolidone, 4/3/6, v/v, which can be employed for 90 minutes at 65°C. The deprotection reaction is quenched by the addition of an equal volume of water in this case. Note that the application of triethylamine trihydrofluoride in the DMT-On mode will lead to detritylation, due to the acidity of the reagent.
13. Desalt the RNA oligonucleotide by using a desalting matrix such as Sephadex™ G25, an ion exchange cartridge, or a reversed phase purification cartridge. Optimal conditions for desalting vary greatly with the employed matrix/product. Conditions recommended by the manufacturer should generally be applied. The lyophilized crude RNA oligonucleotide product can be purified by AX-HPLC or by preparative gel electrophoresis.

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