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Inspiring Science

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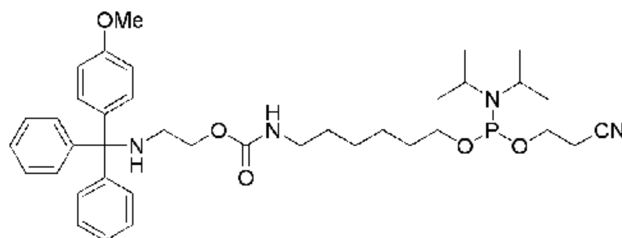
User instructions

ssH-Linker

Product Description

Chemical Formula : $C_{38}H_{53}N_4O_5P$
Formula Weight : 676,83
Storage : < -20°C



Method

1. Use anhydrous acetonitrile (water content ≤ 30ppm) to dissolve the ssH-Linker*. It is important to maintain anhydrous conditions during liquid transfer and dissolution.

2. For use on Expedite™ instruments, add 5ml acetonitrile to 0.25g ssH-Linker (M010982-01) to obtain a concentration of 50mg/ml. For use on ABI® instruments, add 3.7ml acetonitrile to 0.25g ssH-Linker (M010932-01) to prepare a 0.1M solution.

3. The ssH-Linker is a viscous oil that requires more time to dissolve than powdered phosphoramidites. Gently swirl the vial until the linker 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. Enter the sequence of the oligonucleotide you wish to synthesize with ssH-Linker. The coupling time for ssH-Linker is the same as that recommended by the instrument manufacturer for the four standard DNA phosphoramidites A, C, G and T. Note that the ssH-Linker will terminate the synthesis and can only be employed in the last coupling step on the 5' terminus.

* ssH-Linker is covered by the patents / patent applications JP 2005-217026 and PCT/JP2005/021135.

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6. Proceed as you would with a standard DNA oligonucleotide synthesis. Depending on your intended further usage of the oligomer, you can either choose Trityl-On or Trityl-Off procedures. The coupling efficiency of the ssH-Linker may be determined by a monomethoxytrityl cation assay in Trityl-Off mode. Standard deblock steps as used for the removal of DMT-groups during oligonucleotide chain assembly can be applied for the removal of the MMT*-group in Trityl-Off mode.

7. After synthesis in Trityl-Off mode the oligonucleotide is ready for on-support labelling. Perform the labelling reaction by incubating the support in the respective reaction mixture and wash the support appropriately**.

8. Cleave and deprotect the oligonucleotide with ammonia at 40°C for 24 hours with standard protected nucleobases, or, if TAC-protected phosphoramidites are used, at 55°C for 15 minutes.

9. The oligonucleotide is now ready for further processing, such as desalting or purification with RP-HPLC, AX-HPLC or gel-based methods. MMT-protected ssH-Linker oligonucleosides are particularly suitable for cartridge-based reverse phase purification.

10. Oligonucleotides prepared in Trityl-On mode are further deprotected by a treatment with 10% aqueous acetic acid for 20 minutes at room temperature. Acetic acid is removed by evaporation under vacuum. Free MMT residues can be removed, if desired, by extraction of an aqueous solution of the oligonucleotide with diethyl ether.

* monomethoxytrityl

** During oligonucleotide deprotection in ammonia the amino group should either be MMT-protected or conjugated to a reporter group. Unprotected amino groups will react with the internal carbamate linkage under deprotection conditions resulting in a derivative which is unreactive to common labelling reagents.

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