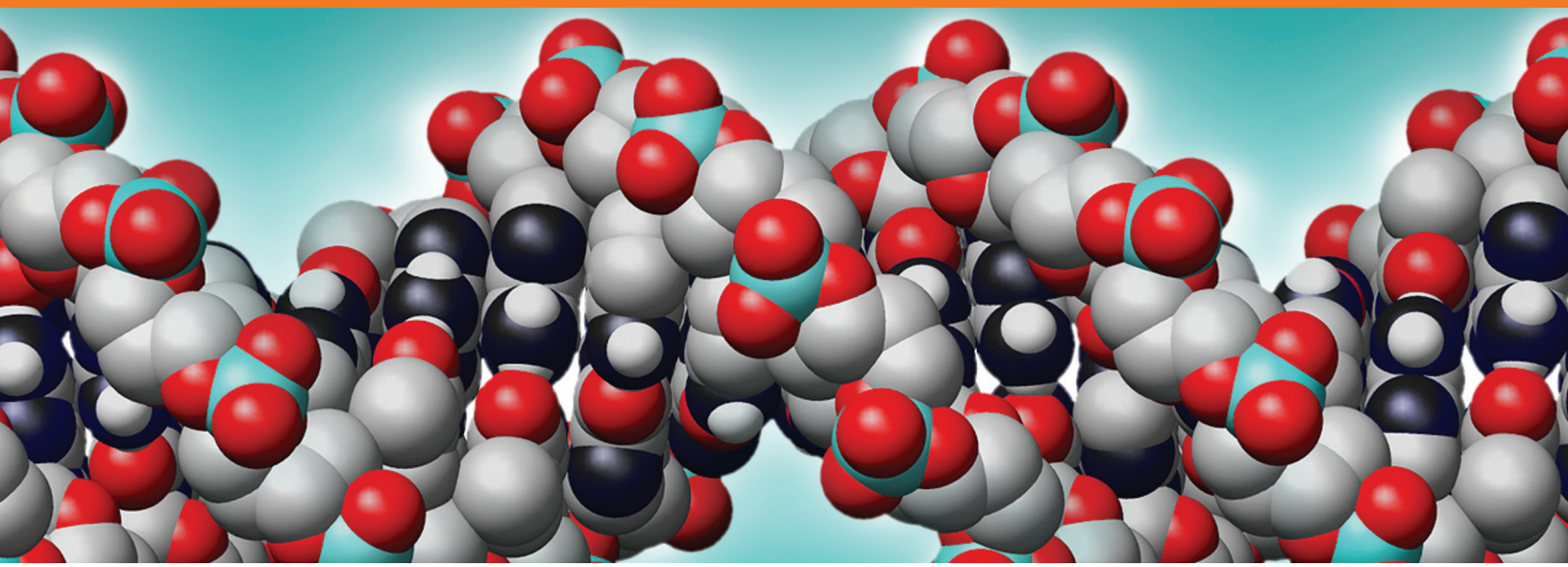
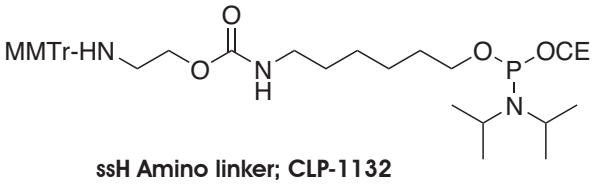
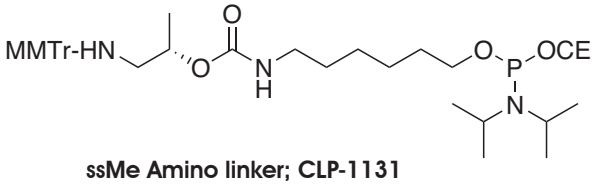
ssR Amino Linkers



ssR Amino Linkers: ssH & ssMe Amidites



ssH Amino linker; CLP-1132



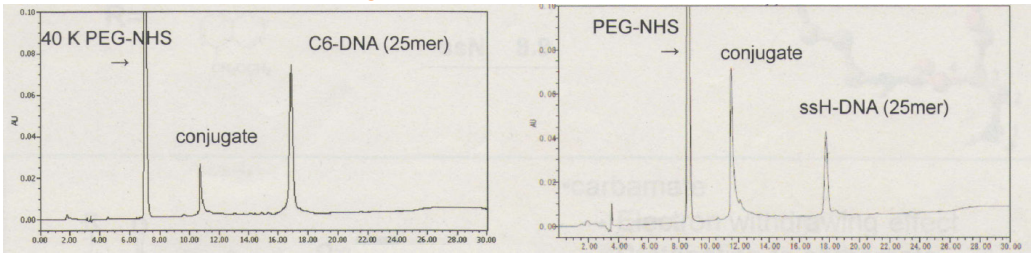
ssMe Amino linker; CLP-1131

Advantages and Key Features:

ssR amino linkers with an aminoethyl carbamate main linkage and a side chain residue have several following advantages over conventional amino linkers.

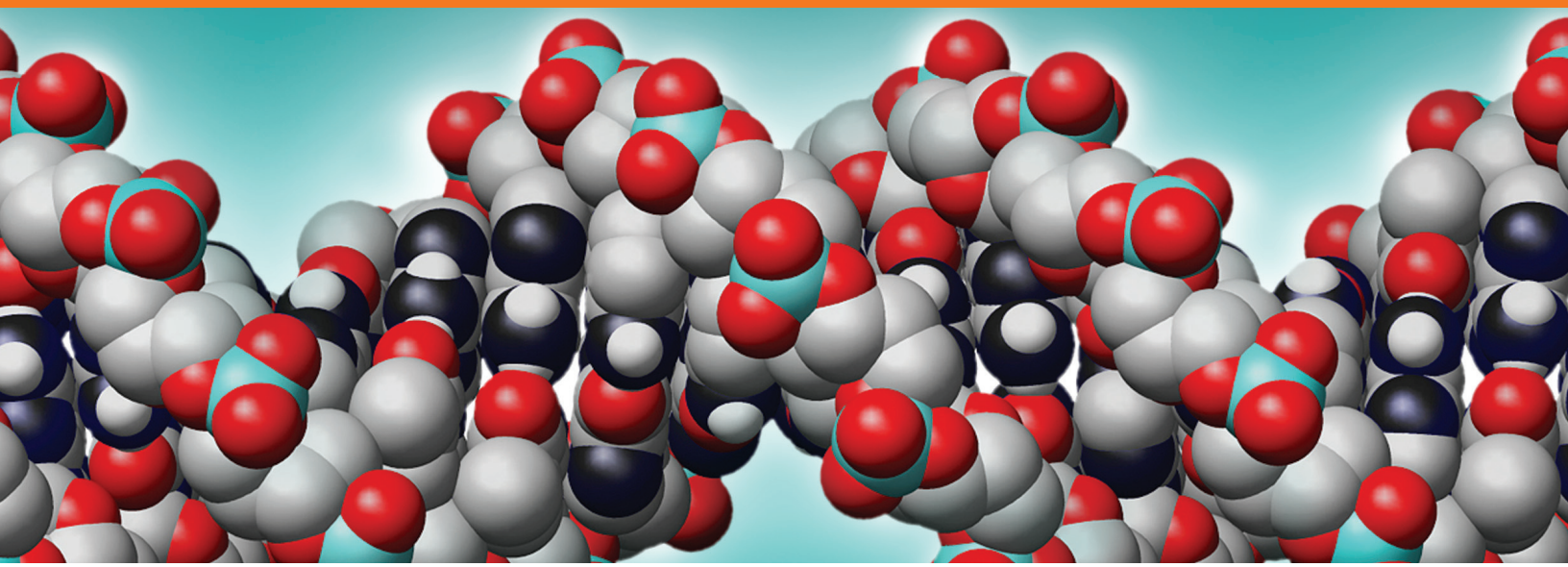
- Strong hydrophobicity of MMTr group assists in easy purification of the amino modified oligonucleotides.
- High solubility in acetonitrile and standard oligonucleotides synthesis protocols can be applied and it allows measurement of the coupling efficiency through colorimetric MMT-assays.
- The aminoethyl carbamarte structure facilitates deprotection of MMTr group under very mild acidic conditions compared to the standard aliphatic amine and this feature avoids the depurination side reaction.
- ssR amino modifications have displayed very high labeling efficiency with an active esters, isothiocyanate compared. (1) with Biotin-NHS/phosphate buffer (pH8), 77-79% yield for ssH and 82-84% yield for ssMe, with FITC/phosphate buffer (pH8), 57-59% yield for ssH and 63-65% yield for ssMe reporter group at the 5′ end of oligonucleotides.
- **Pegylation** with polyethylene glycol: 40K PEG-NHS* with ssH-linker yield is 70-75%.
- **Pegylation** 60% for ssH and 65.6% for ssMe amino linkers.
- **Cholesterol** attachment coupling efficiency is 70-80%.
- High-throughput purification due to increased conjugation efficiency.

Comparative illustration of pegylation with Conventinal amino linker and ssH amino linker



application contd ..... see back cover

ssR Amino Linkers



Applications of ssR linkers; ..... Contd.....

- ssH and ssMe amino linkers are stable under alkaline condition. ssH-linker is stable even in carbonate buffer.
- For solid phase labeling, transacetylation reactions were suppressed by a) keeping the terminal amino groups protonated and b) by activating the exogeneous molecules before the coupling reaction.
- pKa of the aminoethyl carbamate structure is lower than the aliphatic amine, this might be responsible for the **rapid MMTr removal and efficient labeling reaction**.
- Attachment of oligonucleotides to **chips** (**microarray**: high quality amino-ODNs). Immobilization of the modified oligonucleotides to various surfaces for oligonucleotide labeling with corresponding reporter molecules.

Reaction conditions of the PEGYLATION:

Conjugation condition with Branched 40 K PEG*:

Amino-modified oligos of 25-bases (5 μmole) were incubated with activated PEG-NHS* (500 μmole) in 250 mM phosphate buffer (pH8) at 40 ^{o}C. After 20 min, aliquots (30 μL) removed from the solvent, was combined with distilled water, followed by HPLC analysis using anion-exchange DEAE column.

*PEG-NHS: Shown in the example in figure is symmetrical branched PEG-NHS Ester-MW 40,000

ssMe-linker:

1. ssMe is superior to ssH is both conjugation efficiencies and MMTr-removal.
2. The cost of ssMe-linker is higher due to single isomer (S) being used in the synthesis.
3. Degradation of ssMe-linker under alkaline conditions is slightly more as compared to the ssH linker oligos.

Important Note: It is preferable not to deprotect MMTr during oligo synthesis, as some carbamate cleavage during aq. ammonia deprotection can occur.

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References:

1. Komatsu, Y. et. al. *Bioorg Med. Chem.* **2008**, *16*, 941.
2. Kojima, N. et. al. *Bioorg. Med. Chem. Lett.* **2009**, 19, 2144.
3. Komatsu, Y. et. al. US 2008/0227968 A1.