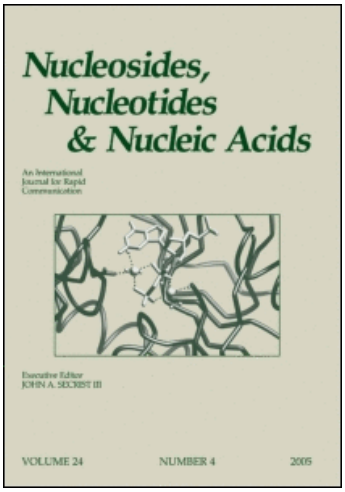

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Solid Phase Conjugation Chemistry: Use of Alloc as a Protecting Group for 2'-Aminolinker Containing Oligonucleotides

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To cite this Article Barber-Peoc'h, Isabelle , Manoharan, Muthiah and Cook, P. Dan(1997) 'Solid Phase Conjugation Chemistry: Use of Alloc as a Protecting Group for 2'-Aminolinker Containing Oligonucleotides', Nucleosides, Nucleotides and Nucleic Acids, 16: 7, 1407 — 1410
To link to this Article: DOI: 10.1080/07328319708006193
URL: http://dx.doi.org/10.1080/07328319708006193

NUCLEOSIDES & NUCLEOTIDES, 16(7-9), 1407-1410 (1997)

SOLID PHASE CONJUGATION CHEMISTRY: USE OF ALLOC AS A PROTECTING GROUP FOR 2'-AMINOLINKER CONTAINING OLIGONUCLEOTIDES

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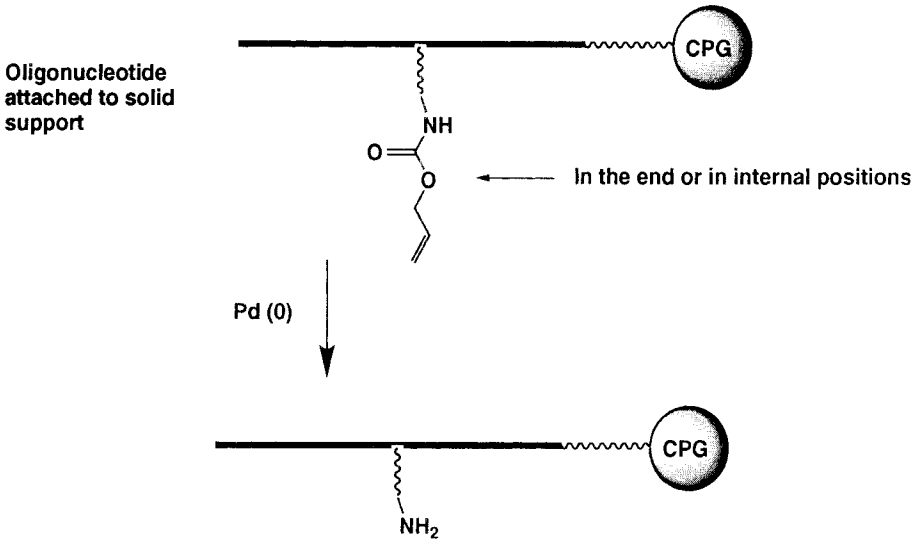
The drug properties of antisense and antigene oligonucleotides can be enhanced by strategic positioning of ligands capable of ameliorating these properties.[1,2] Certain ligands may improve the cellular delivery of oligomers and increase their affinity for the target gene and resistance to nucleases. The 2'-*O*-position is an attractive modification site.[3] Oligonucleotides possessing the 2'-*O*-alkyl modifications exhibit higher chemical stability under depurination conditions, higher stability to enzymatic cleavage by both endo- and exonucleases, and increased affinity for target mRNA. In addition, they form highly stable triple helices. Thus they promise to be versatile compounds in controlling gene expression by antisense and antigene technologies.

Conjugation of different ligands to modulate the function of oligonucleotides via suitable reactive groups (-NH_2 or -SH) is of significant value in antisense technology. Often this chemistry requires introducing a suitable tether containing the reactive group (e.g., -NH_2) to the oligonucleotide and post-synthetically adding the ligand of interest (intercalators, chemical nucleases, cellular permeation and targeting agents) as an active ester and carrying out the conjugation reaction in solution. Although this method is widely used, it suffers from the disadvantage of requiring several steps for purification of the conjugate.

Conjugations at the 2'-position of the carbohydrate moiety may interfere less with base pairing and/or stacking interactions than do conjugations at the internucleotide backbone sites or nucleobase sites. Secondly, this site provides a way to perform multiple conjugations in the minor groove. Thirdly, one can augment the desired properties of the 2'-*O*-alkyls by 2'-*O*-alkyl-ligand conjugates.

Recently, we described[4] a new approach to functionalize oligonucleotides at the 2'-*O*-carbohydrate site via an alkyl aminolinker and an alkyl thiol tether placed at the adenosine nucleoside. In this report we describe the synthesis and incorporation into oligonucleotides of nucleosides containing an *O*-aminohexyl moiety either at the 2' or 3' position of uridine. The *O*-aminohexyl moiety can be used as such to produce amphipathic oligonucleotides.[5] The resulting aminotether[4] has also been used as a site of conjugation.[6]



- Deprotection either in solid support or in solution
- Alkylamine oligos for further studies
- Amino group for conjugation

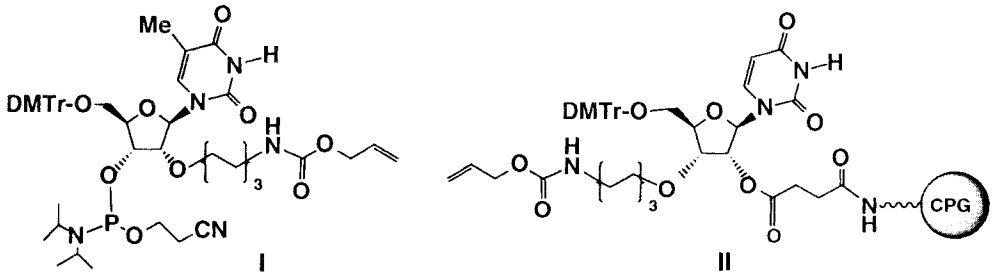


Figure 1

An alternative approach is to generate the free amino group while the oligonucleotide is still bound to the solid support and then carry out conjugation chemistry at the solid support, while all other protecting groups are intact. To use this approach, The base labile trifluoroacetyl group is not compatible with this approach. The FMOC appeared as another group to evaluate for for this purpose, but we had limited success with this group with the commercial solid supports. The acid labile MMT (monomethoxytrityl) group for amino group protection is a possibility but it limits the use of the aminolinker only at the 5'-end of the oligonucleotide.

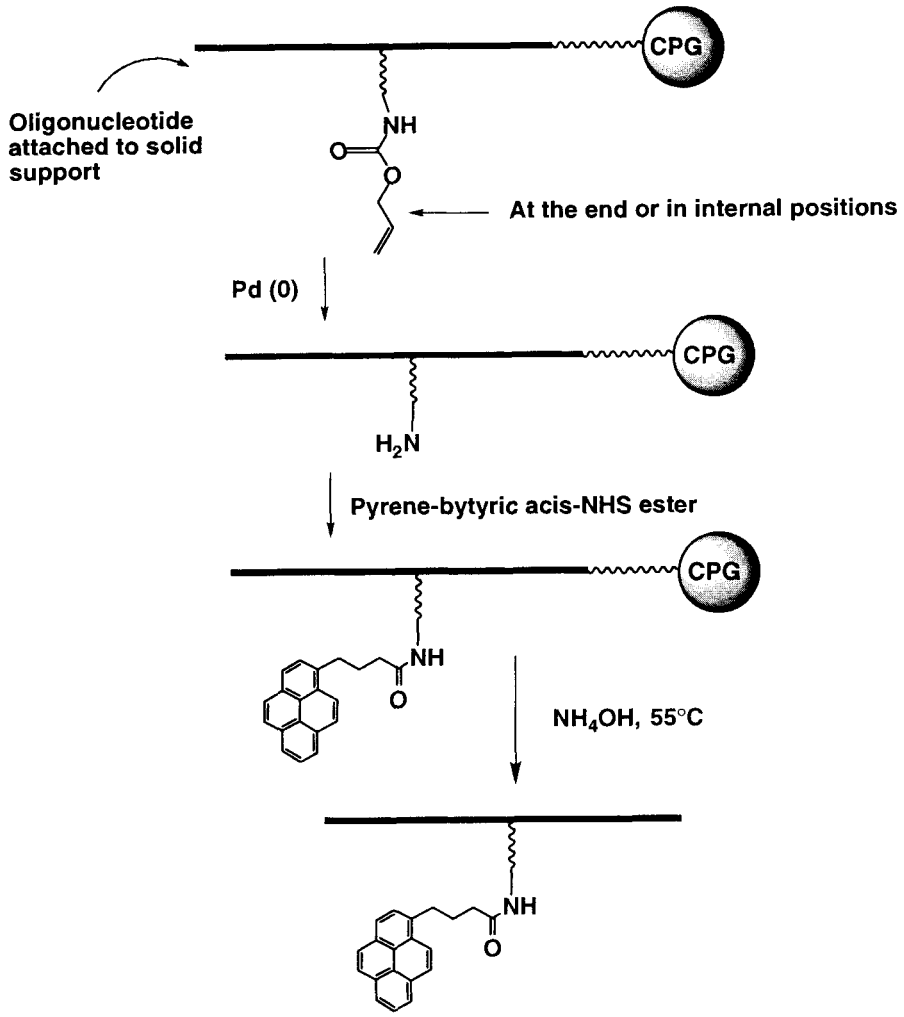


Figure 2

In order to place aminolinker-containing nucleosides at any position of oligonucleotides (internal and terminal positions), we synthesized alloc-protected compounds **I** and **II** and used them to make oligonucleotide phosphorothioates. Before cleavage of the oligo from CPG, the alloc group was conveniently removed with Pd(0). The free amine was then further conjugated with pyrene butyric acid to demonstrate its utility as a linker. While the alloc group is convenient for use on solid support, it may also be used for solution-phase conjugation chemistry.

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