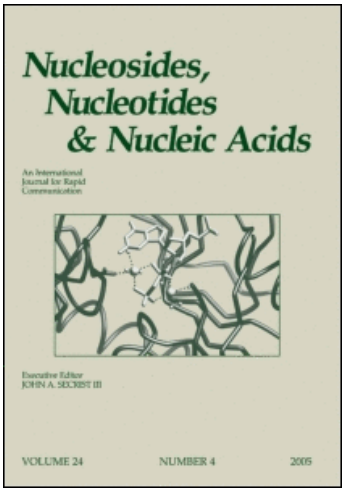

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Oligodeoxyribonucleotides Covalently Linked via Nucleic Bases with 3'-5' and 5'-5' Polarities

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To cite this Article Asseline, Ulysse and Thuong, N. T.(1995) 'Oligodeoxyribonucleotides Covalently Linked via Nucleic Bases with 3'-5' and 5'-5' Polarities', Nucleosides, Nucleotides and Nucleic Acids, 14: 3, 889 — 893
To link to this Article: DOI: 10.1080/15257779508012496
URL: http://dx.doi.org/10.1080/15257779508012496

NUCLEOSIDES & NUCLEOTIDES, 14(3-5), 889-893 (1995)

OLIGODEOXYRIBONUCLEOTIDES COVALENTLY LINKED *VIA* NUCLEIC BASES WITH 3'-5' AND 5'-5' POLARITIES.

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Abstract: The solid-phase preparation of oligodeoxyribonucleotides covalently linked via nucleic bases with normal (3'-5') or inverted (5'-5') polarities is reported. The key-step of these syntheses is the preparation of the tethered dimers.

The discovery of the triple-helix structure in synthetic polyribonucleic acids[1], has provided the basis for the development of a new strategy to selectively control gene expression[2]. However, at the present time one significant limitation to the practical application of triple-helix is its requirement for oligopurine tracts in the target double helix . To overcome this limitation several approaches[3-8] have shown that the repertoire of the triplex forming sequences can potentially be expanded to adjacent blocks of pyrimidines and purines by allowing the third strand to pair with purines on alternate strands by crossing the major groove . Among them[5-8] the use pyrimidine third strands with (5'-5')-ends or (3'-3')-ends junction in order to meet the relative polarity requirement between the third strand and the purine containing strand of the duplex has been investigated [5-8] (FIG 1a). More recently, it has been demonstrated that 3'-5' linked oligopyrimidine sequences can form a triple helix on a single-stranded DNA sequence[9] (FIG. 1b). In this structure one strand folds back on the double-helix to form an intramolecular triplex. The ligation of these tethered oligomers was achieved through the hydroxyl groups of the sugar residues via a long linker. In order to obtain a good cooperative binding between the oligonucleotide segments what requires a short linker, we chose to use interbase linkages for the ligation of the two oligopyrimidine sequences. In a previous paper[10] we have described the preparation of symmetrical 3'-3' linked oligonucleotides starting from a tethered dimer immobilized on a support which allows

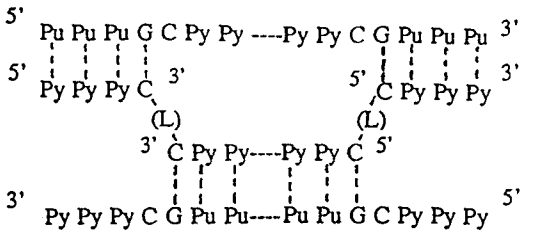


FIGURE 1a: Model for triple helix formation on alternated target sequence.

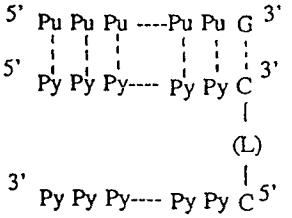
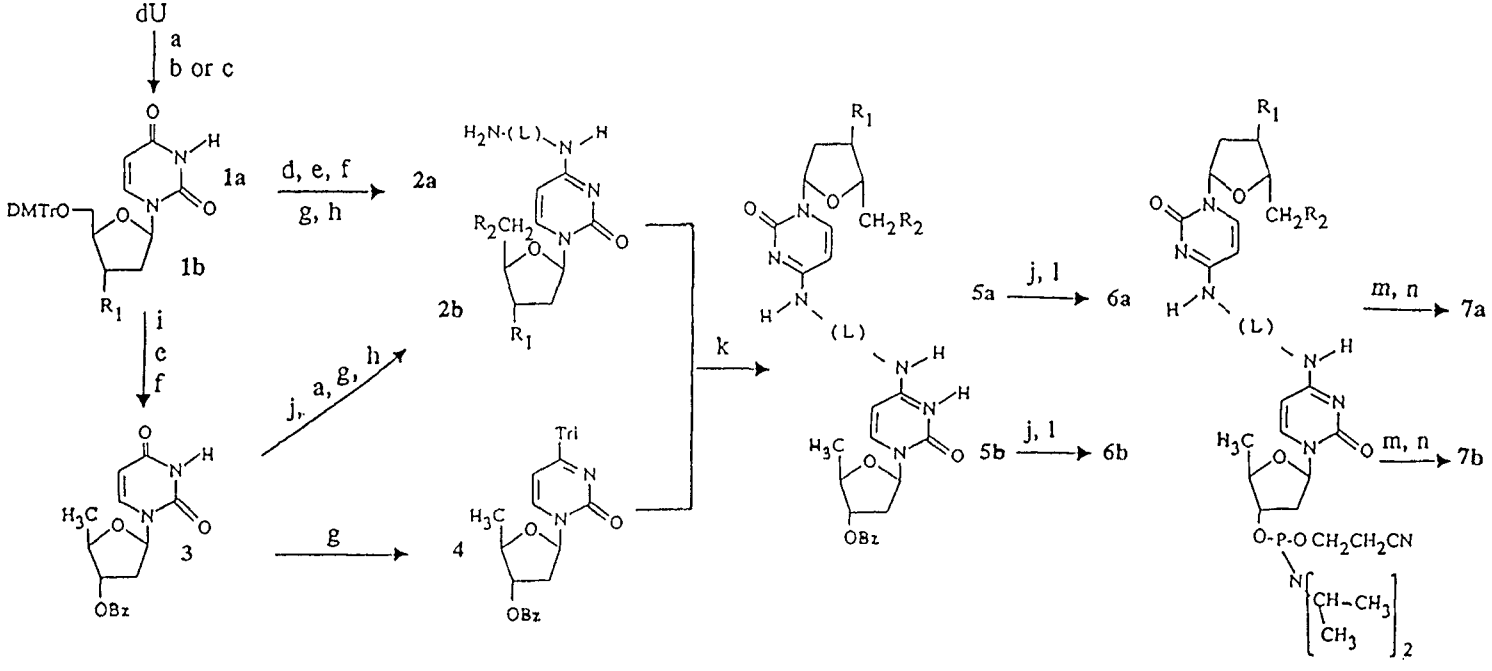


FIGURE 1b: Model for triple helix formation on a single stranded target.

the synthesis of both sequences simultaneously. We report here the synthesis of asymmetrical sequences tethered via nucleic bases with natural (3'-5') and inverted (5-5') polarities. The key step of these syntheses is the preparation of the modified bridged dinucleosides **5a** and **5b** described on the FIG. 2. The chosen strategy consists in using dimethoxytrityl group on the hydroxyl function (5' or 3' position) of one nucleoside and a phosphoramide group on an hydroxyl function (5' or 3' position) of the second nucleoside. In order to avoid the problem of protective group selectivity we chose to remove[11,12] the two remaining hydroxyl groups not involved in the synthesis (one on each nucleoside). The dinucleoside **5a** (tritylated at the 5'-position of one nucleoside and benzoylated at the 3'-position of the second nucleoside) has been obtained by reaction of the 2',3'-dideoxyuridine tritylated at the 5'-position and substituted at the 4-position by the 2,2'-(ethylenedioxy)diethylamine **2a** with the triazolo derivative of the 2',5'-dideoxyuridine substituted at the 3'-position by a benzoyl group **4**. The dinucleoside **5b** has been obtained by reaction of the 2',5'-dideoxyuridine tritylated at the 3'-position and substituted at the 4-position by 1,3-diaminopropane **2b** with **4**. After debenzoylation of **5a** and **5b** and phosphytilation of free hydroxyl groups the dinucleoside



1a R_1 = -O-Si(t-Bu)Me$_2$ 2a R_1 = H 2b R_1 = DMTrO-
1b R_1 = -OBz R_2 = DMTrO- R_2 = H

5a, 6a R_1 = H R_2 = DMTrO- L= -$(CH_2)_3$-
5b, 6b R_1 = DMTrO- R_2 = H L = -$(CH_2CH_2O)_2CH_2CH_2$-

7b1 = d 3' TTTTCTTTTCCC-5'(L)5'-CCCTTTTCTTTT 3'
7b2 = d 3' TTCTTCTTCTTC-5'(L)5'-CCCTTTTCTTTT 3'
7b3 = d 3' TTCTCTCTCTCTTC-5'(L)5'-CCCTTTTCTTTT 3'

7a = d 5' TCTTCCTCTCTC-3'(L)-5'CTCTCTCCTTCTAGCCTCC 3'

FIGURE 2: DMTr= dimethoxytrityl; Si(t-Bu)Me$_2$ = tert-butyldimethylsilyl; Bz= benzoyl; Tri= triazolyl; a = DMTrCl,Pyridine; b = Si(t-Bu)Me$_2$-Cl, imidazole, pyridine; c = BzCl, pyridine; d = $F^-N^+Bu_4$; e = PhO(S) CCl, pyridine; f = n Bu_3SnH, AIBN, toluene; g = phosphorus oxychloride,1,3,4-triazole, NEt_3, CH_3CN; h = $H_2N(L)NH_2$; i = H^+; j = OH^-; k = CH_3CN; l = 2-cyanoethyl-N,N-diisopropylamido-chlorophosphite, diisopropylethylamine, $ClCH_2CH_2Cl$; m = oligonucleotide chain elongation; n = deprotection.

phosphoramidites **6a** and **6b** have been obtained, respectively. 3'-5' asymmetrical linked oligonucleotides have been obtained by starting the synthesis of the first oligonucleotide in the usual 3'-5' direction with conventional 3'-phosphoramidites, then adding the dimer **6a** and continuing the synthesis with assembly of the second oligonucleotide in the same direction by using nucleoside 3'-phosphoramidites. 5'-5'-asymmetrical oligonucleotides have been prepared by assembly of the first oligonucleotide with 3'-phosphoramidites, then addition of the dimer **6b** and then by assembly of the second oligonucleotide in the opposite direction with nucleoside 5'-phosphoramidites. After the chain elongations have been achieved, the removal of the protective groups from the modified oligomers as well as their releasing from the supports have been carried out following the standard procedure (concentrated ammonia and acetic acid treatments). After purification and analysis by ion exchange and reversed-phase chromatographies the full deprotection and the nucleic base composition of the interbase linked oligonucleotides **7a**, **7b1**, **7b2** and **7b3** have been confirmed by nuclease degradation followed by reversed-phase analysis[13] of the hydrolysates through comparison with standard monomers and modified dimers samples obtained after full deprotection of compounds **5a** and **5b**, respectively. The described method which involves the removal of the hydroxyl groups not used in the synthesis allows the preparation of asymmetrical linked oligonucleotides with any polarities. We reported here only the preparation of linked oligonucleotides with 3'-5' and 5'-5' polarities but 3'-3' tethered oligonucleotides could be obtained by using the same strategy.

Acknowledgements

This work was supported by the *Agence Nationale de Recherche sur le SIDA*, Rhône-Poulenc and bio-Mérieux.

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13. Base composition analysis were performed on a lichrospher 100RP18(5μm) column (125 x4mm.) using a linear gradient of CH_3CN in 0,1M aqueous triethylammonium acetate buffer, pH7, with a flow rate of 1ml/min., 0% CH_3CN for 5min. then 0 to 20% CH_3CN in 20 min. and then 20 to 50% CH_3CN in 15 min.. $Rt_{5'a}$= 23 min.; $Rt_{5'b}$= 36 min.11 sec.