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Synthesis of Triple Helix Forming Oligonucleotides with a Stretched Phosphodiester Backbone

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ABSTRACT: Total syntheses of novel DMT-phosphoramidites of thymidine (**11** and **15**) and 2'-deoxyguanosine (**8** and **20**), and their utility in the preparation of triple helix forming oligodeoxyribonucleotides with a stretched phosphodiester backbone are described.

Recently it has been demonstrated that in the presence of divalent cations, certain short guanine rich oligonucleotides can bind to specific sites in duplex DNA to form triple helices[1-3] at physiological pH. It has also been shown that the formation of such sequence-specific triple helices can inhibit DNA replication[4,5] and block transcription initiation, thus resulting in the specific inhibition of the synthesis of disease associated proteins. Therefore, the potential therapeutic significance of these triple helix forming oligonucleotides (TFOs) is obvious.

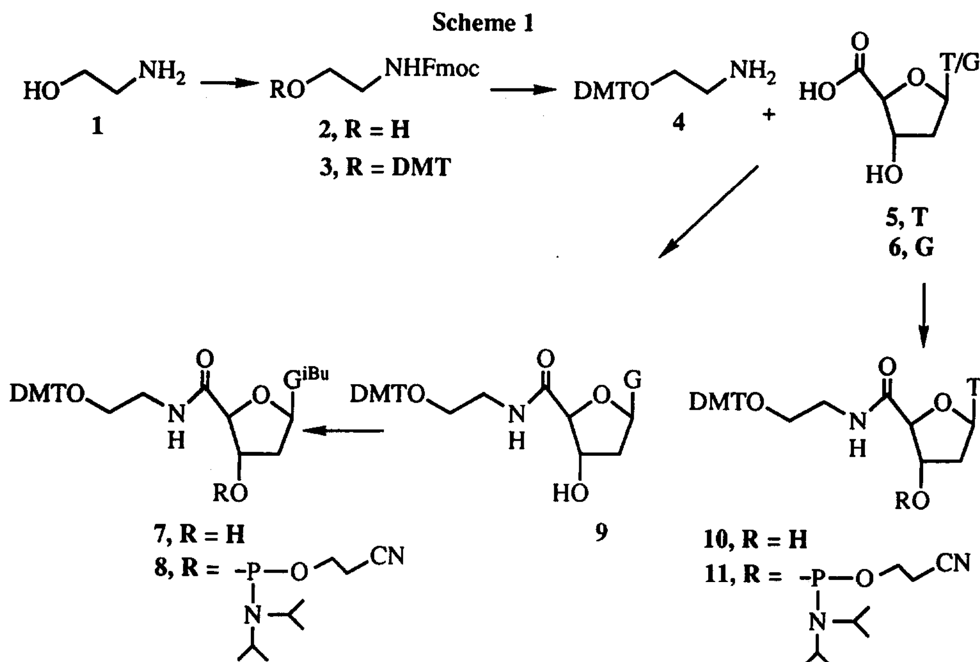
The major goal of TFO design is to develop molecules which can bind to any duplex DNA sequence, without regard for purine content or other symmetry consideration. H-bonding of the Hoogsteen or reverse Hoogsteen type normally occurs with purine bases in the major groove of an underlying duplex. Consequently, a polypurine/polypyrimidine duplex target presents an orderly array of bond formers positioned upon one side of the major helix groove. However, at sites of CG or TA inversion, the corresponding purine target base is placed upon the opposing half of the major groove and can be reached for the purposes of hydrogen bonding only a) upon extension of the TFO backbone, b) by distension of the duplex, or c) both. If triple helix formation could be made stable at sites of TA and CG inversion, TFOs could be designed against any duplex site, rather than just at purine rich targets. One solution to the binding problem at CG inversion sites would be to have an extended backbone. We now report the synthesis of triple helix forming oligonucleotides with a novel, extended phosphodiester backbone, employing solid-support, phosphoramidite chemistry.

In a typical synthesis of the monomeric units **8** and **11** (*Scheme 1*) ethanolamine (**1**) was reacted with 9-fluorenylmethyl chloroformate in the presence of *N,N*-diisopropylethylamine in anhydrous *N,N*-dimethylformamide to give 9-fluorenylmethoxycarbonylethanolamine (**2**) in essentially quantitative yield. Dimethoxytritylation of **2** with 4,4'-dimethoxytrityl chloride (DMT-Cl) in anhydrous pyridine afforded the corresponding DMT derivative (**3**) in a 86% yield. Removal of the Fmoc protecting group from **3** was readily achieved by treating with excess piperidine and the requisite DMT-ethanolamine (**4**)[6] was isolated in a 64.5% yield after silica gel column chromatography.

Condensation of **4** with 1,2-dideoxy-1-(thymin-1-yl)-β-D-ribofuranuronic acid (**5**)[7] in dry DMF in the presence of 1-hydroxybenzotriazole and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide

hydrochloride (EDC) and triethylamine, followed by work up of the reaction mixture and silica gel column chromatography afforded analytically pure 4-[1,2-dideoxy-1-(thymin-1-yl)-β-D-ribofuranuronamido]-O-(4,4'-dimethoxytrityl) ethanol (**10**) in a 86% yield. Compound **10** on phosphitylation with 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite in the presence of *N,N*-diisopropylethylamine gave the corresponding phosphoramidite (**11**, 85.5%)[8] as a colorless powder.

Scheme 1

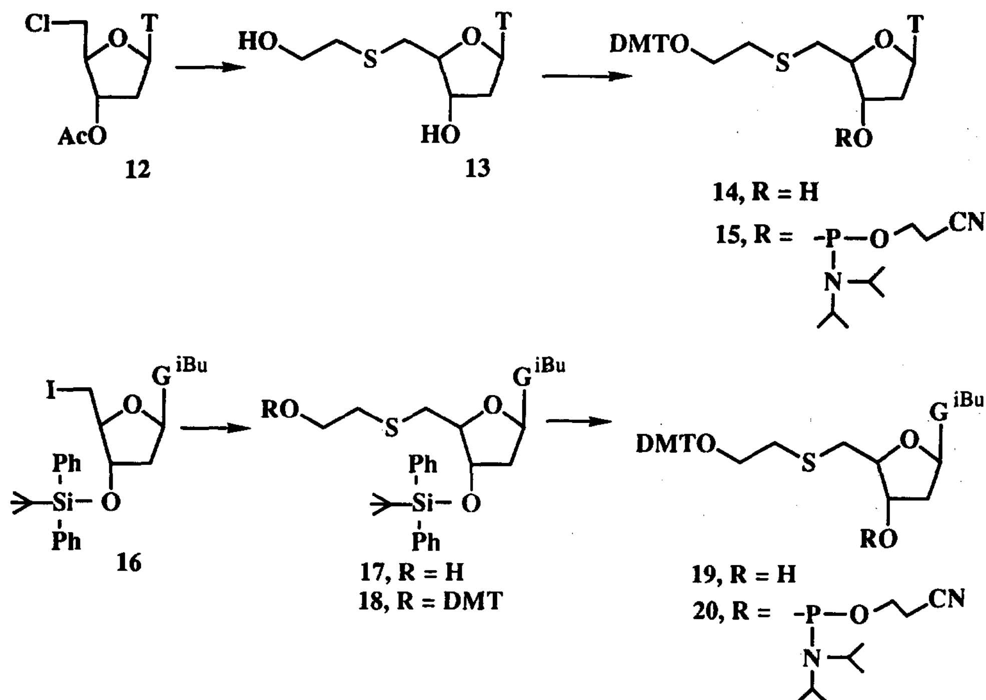


Similarly, condensation of **4** with 1,2-dideoxy-1-(guanin-9-yl)-β-D-ribofuranuronic acid triethylammonium salt[9] gave 4-[1,2-dideoxy-1-(guanin-9-yl)-β-D-ribofuranuronamido]-O-(4,4'-dimethoxytrityl) ethanol (**9**) in a 74% yield. However, in the case of 2'-deoxyguanosine derivative (**8**), it is essential to protect the exocyclic amino function prior to phosphitylation. Thus, the protection of the amino functionality was achieved by employing transient protection methodology[10]. Compound **9** on treatment with chlorotrimethyl-silane, followed by isobutyryl chloride and subsequent removal of the silyl protecting groups gave suitably protected monomer **7** (82%), which on phosphitylation furnished the phosphoramidite (**8**) [11] in a 82% yield.

Synthesis of the other two building blocks **15** and **20** (*Scheme* 2) was successfully achieved starting from 3'-protected-5'-halo nucleosides. Thus, 3'-O-acetyl-5'-chloro-5'-deoxythymidine (**12**)[12] on reaction with the sodium salt of 2-mercaptoethanol[13] in 2 N NaOH solution gave 5'-deoxy-5'-(2-hydroxyethylthio)thymidine (**13**, 85%). Compound **13** was converted to the corresponding DMT derivative **14** (75%) *via* conventional procedure, which on treatment with 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite afforded the target building block (**15**)[14] in a 78% yield. Similarly, reaction of N^2-isobutyryl-3'-O-*t*-butyldiphenylsilyl-5'-iodo-2',5'-dideoxyguanosine (**16**)[15] with the

sodium salt of 2-mercaptoethanol in anhydrous dioxane afforded **17** in a 86.5% yield, which on treatment with 4,4'-dimethoxytrityl chloride gave **18** in a 85% yield. Removal of the silyl group from **18** was accomplished by using *n*-tetrabutylammonium fluoride to give **19** (79%). Phosphitylation of **19** gave the target building block (**20**)[16] and the homogeneous product was isolated in a 90% yield.

Scheme 2



All these novel building blocks (**8**, **11**, **15** and **20**) were found to be stable under solid-support, oligonucleotide synthesis and deblocking conditions[17]. A series of TFOs were prepared based on the following model sequence, with a stepwise coupling efficiency ranging from 95.6% to 98.5%.

GGGGTTGGGGGGTTGGGGGTTGGGGG

All base labile protecting groups on the oligodeoxyribonucleotides were removed by the treatment with concentrated NH_4OH and the TFO was purified by HPLC using an ion exchange Q-sepharose (Pharmacia) column. The purified product was desalted by passing through C_{18} sep-pack (Waters) cartridge and analyzed on a 20% denaturing polyacrylamide gel after labeling with ^{32}P-ATP using polynucleotide kinase. Unmodified oligonucleotide was used as the standard for comparison of mobility and purity. The modified TFOs were found to be $\geq$ 95% pure and with the expected length. Complete synthetic details of the monomeric building blocks, the oligonucleotides and their binding analyses will be presented in a full paper.

In summary, this communication describes, for the first time, a convenient synthesis of suitably protected novel monomeric phosphoramidite building blocks and their utility in the preparation of triple helix forming oligonucleotides with a stretched phosphodiester backbone, using solid-support, phosphoramidite chemistry.

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14. ^{31}P NMR (CD_3CN): δ 149.33; ^{1}H NMR (CD_3CN): δ 1.0 (m, 12 H, 2 CH(CH_3)$_2$), 1.77 (s, 3 H, C_5CH_3), 2.15 - 2.90 (m, 8 H, H_2C-S-, $C_{5'}H_2$, NC-CH_2, $C_{2'}H$ and $C_{2''}H$), 3.20 (m, 2 H, 2 NCH(CH_3)$_2$), 3.40- 3.60 (m, 4 H, OCH_2CH_2), 3.75 (s, 6 H, 2 OCH_3), 4.05 (m, 1 H, $C_{4'}H$), 4.45 (m, 1 H, $C_{3'}H$), 6.18 (t, 1 H, J = 6.8 Hz, $C_{1'}H$), 6.70 - 7.45 (m, 13 H, *DMT*) and 7.46 (s, 1 H, C_6H).
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16. ^{31}P NMR (CD_3CN): δ 149.21 and 149.38. 1H NMR (CD_3CN): δ 1.10 (m, 12 H, 2 CH(CH3)2), 2.40 - 3.30 (m, 9 H, SCH2CH2-, C5'H2, CH2CN and CH(CH3)2), 3.50 - 3.80 (m, 4 H, 2-CH2O-), 3.75 (s, 6 H,2 OCH_3), 4.15 (m, 1 H, $C_{4'}H$), 4.60 (m, 1 H, $C_{3'}H$), 6.18 (t, 1 H, J = 6.8 Hz, $C_{1'}H$), 6.80 - 7.40 (m, 13 H, *DMT*) and 7.85 (s, 1 H, C_8H).
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