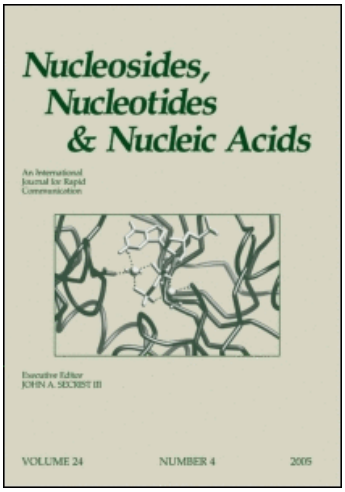

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SYNTHESIS OF 3′-*S*-PHOSPHOROTHIOLATE OLIGONUCLEOTIDES FOR THEIR POTENTIAL USE IN RNA INTERFERENCE

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□ *The potency of RNA interference (RNAi) undoubtedly can be improved through chemical modifications to the small interfering RNAs (siRNA). By incorporation of the 3′-S-phosphorothiolate modification into strands of RNA, it is hoped that specific regions of a siRNA duplex can be stabilised to enhance the target binding affinity of a selected antisense strand into the activated RNA-induced silencing complex (RISC*). Oligonucleotides composed entirely of this modification are desirable so unconventional 5′ → 3′ synthesis is investigated, with initial solution-phase testing proving successful. The phosphoroamidite monomer required for solid-phase synthesis has also been produced.*

Keywords RNA Interference; siRNA; phosphorothiolate; reverse synthesis; 5′-3′

INTRODUCTION

RNA interference (RNAi) was discovered in 1998 by Fire and Mello et al.,[1] and is a potent method for gene knockout with potential use in therapeutics. During the RNAi process, the double-stranded small interfering RNAs (siRNAs) are incorporated into an RNA-induced silencing complex (RISC), which is activated (RISC*) by ATP-driven unwinding of the siRNA. It is established that the strand selected for assembly into RISC* is the one whose 5′-end is less tightly paired to its complement. Various chemical modifications have been investigated in the siRNA duplex to improve the potency of RNAi.[2]

The sugar conformations of DNA and RNA differ, with RNA preferring the C3′-*endo* (north) conformer and DNA the C2′-*endo* (south) conformer. ^{1}H NMR studies of the 3′-*S*-phosphorothiolate linkage (Figure 1) by Beevers et al. has shown there is a shift to the north pucker of the sugar directly attached to the sulphur atom.[3] Hence, the 3′-*S*-phosphorothiolate modification is an excellent mimic of natural RNA. It also has been observed that the deoxyribose pucker of the 3′-*S*-phosphorothiolate actually populates the north conformation to a greater extent than natural RNA.[4] For these

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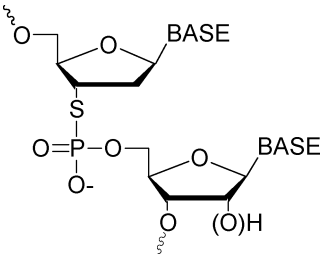
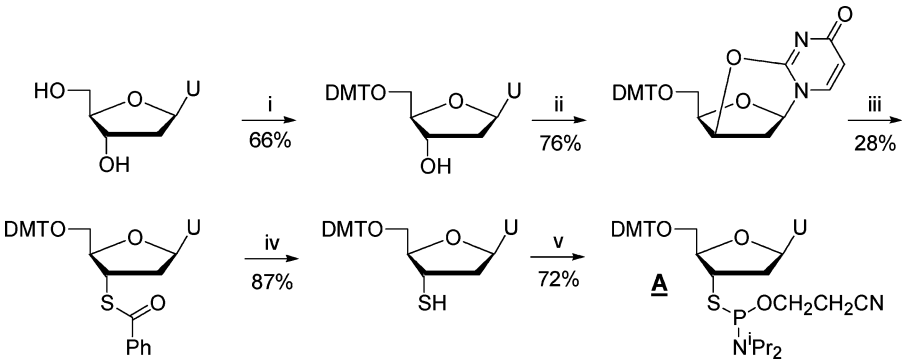


FIGURE 1 3′-*S*-phosphorothiolate linkage.

reasons, it is expected that integration of the 3′-*S*-phosphorothiolate linkage into RNA will enhance duplex stability of a modified RNA strand to its complement.

RESULTS AND DISCUSSION

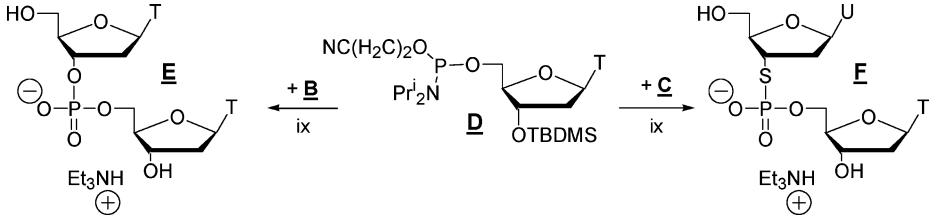
We are currently investigating the incorporation of 3′-*S*-phosphorothiolate analogues into the phosphodiester backbone of RNA to improve efficacy of RNAi. For the incorporation of the 3′-*S*-phosphorothiolate modification, the phosphorothioamidite of 2′-deoxyuridine has been synthesised (A, Scheme 1). This currently is being integrated into the RNA sequence 5′-GCGU$_{10}$GCG-3′ (U10). Once purified, UV thermal melting (T_m) studies of the RNA:RNA duplexes containing the modified U10 sequence and its complement will be measured, with an increase in Tm expected. Stabilisation of specific regions of a siRNA duplex using the 3′-*S*-phosphorothiolate could then be used to direct the required antisense strand into the RISC* complex of a specific gene target.



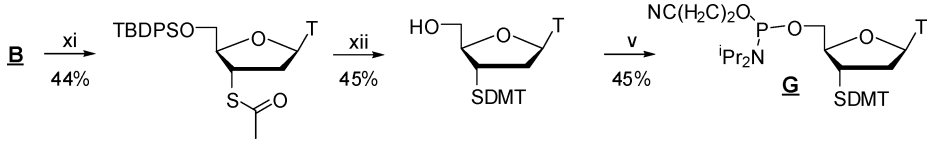
SCHEME 1 i) DMT-Cl (1.1eq), pyridine, RT, 3h; ii) DIAD (1.5eq), PPh_3 (1.5eq), EtOAc, RT, overnight; iii) NaSCOPh (10eq), DMF, 110°C, 3h; iv) 10M NaOH in EtOH, then KH_2PO_4; v) $P(NPr_2)_2(OCH_2CH_2CN)$, Tetrazole, CH_2Cl_2, RT, overnight.

Standard solid-phase oligonucleotide synthesis generally occurs in a 3′→5′ manner. However, there is a limitation to the number of 3′-*S*-phosphorothiolate modifications which can be incorporated into oligonucleotides using this method due to the reduced reactivity of the phosphorothioamidite. Conversely, oligonucleotides can be very efficiently synthesised in the reverse direction (5′→3′) by using monomers functionalised with a 5′-amidite and a 3′-DMT protected hydroxyl group.[5] Application of this approach to the 3′-*S*-phosphorothiolate linkage involves nucleophilic attack of a 3′-thiol on a reactive 5′-*O*-phosphoroamidite.

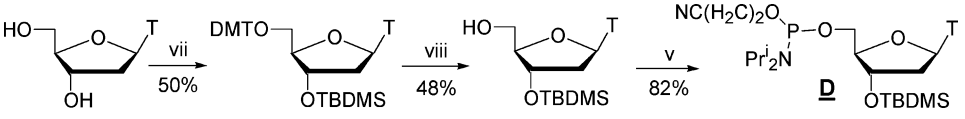
Initial tests were successfully undertaken using solution-phase conditions by synthesising an unmodified TT dimer (E, Scheme 2,). For the synthesis of a 3′-*S*-phosphorothiolate modification, the UsT dimer (F, Scheme 2) was successfully prepared from the 3′-thiol of uridine (C, Scheme 3) and 5′-amidite of thymidine (D, Scheme 4) using the same conditions for the TT dimer. For application for solid-phase synthesis, the appropriate 5′-phosphoroamidite monomer with a 3′-DMT-protected thiol was synthesised successfully in a reasonable yield (G, Scheme 5). Initial solid phase synthesis is currently ongoing and it is hoped that coupling yields will be enhanced so that oligonucleotides composed entirely of the phosphorothiolates can be synthesised.



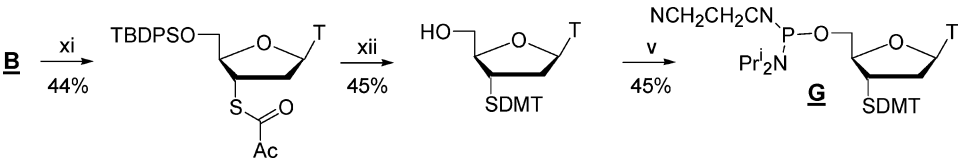
SCHEME 2 ix) a. Tetrazole, MeCN, Under Argon; b. 4% (w/v) solution of I_2 in pyridine/water/THF (1:1:8); c. NH_3, 50°C, Overnight; d. NEt_3.3HF, RT, 24 h.



SCHEME 3 vi) TBDPS-Cl or TBDMS-Cl (1.1eq), Imidazole, DMF, RT, overnight; ii-iv) See Scheme 1.



SCHEME 4 vii) a. DMT-Cl (1.1eq), pyridine, RT, 3h; b. TBDMS-Cl, Imidazole, DMF, RT, 2h; viii) 80% AcOH, RT, overnight; v) See Scheme 1.



SCHEME 5 ix) a. Tetrazole, MeCN, Under Argon; b. 4% (w/v) solution of I2 in pyride/water/THF (1:1:8); c, NH3, 50°C, Overnight; d. NHt_3.3HF, RT, 24H.

CONCLUSIONS

The phosphorothioamidite of 2′-deoxyuridine has been synthesised in moderate yield and is being incorporated into strands of RNA. It is expected to result in increased binding affinity in an RNA/RNA duplex. Stabilisation of specific regions of a siRNA duplex using the 3′-*S*-phosphorothiolate could then be used to direct the required antisense strand into the RISC* of a specific gene target. Incorporation of the 3′-*S*-phosphorothiolate modification into TT and UsT dimers via solution-phase 5′→3′ reverse synthesis was successfully achieved together with the phosphoroamidite monomer required for solid-phase synthesis.

REFERENCES

1. Fire, A.; Xu, S.Q.; Montgomery, M.K.; Kostas, S.A.; Driver, S.E.; Mello, C.C. Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. *Nature* **1998**, 391, 806–811.
2. Manoharan, M. RNA interference and chemically modified small interfering RNAs. *Curr. Opin. Chem. Biol*, **2004**, 8, 570–579.
3. Beevers, A.P.G.; Fettes, K.J.; O'Neil, I.A.; Roberts, S.M.; Arnold, J.R.P.; Cosstick, R.; Fisher, J. Probing the effect of a 3′-S-phosphorothiolate link on the conformation of a DNA: RNA hybrid; implications for antisense drug design. *Chem. Comm.* **2002**, 1458–1459.
4. Beevers, A.P.G.; Witch, E.M.; Jones, B.; Cosstick, R.; Arnold, J.R.P.; Fisher, J. Conformational analysis of 3′-S-PO3-linked ribo- and deoxyribodinucleoside monophosphates. *Magn. Res. Chem.* **1999**, 37, 814–820.
5. Wagner, T.; Pfleiderer, W. Synthesis of 2′-deoxyribonucleoside 5′-phosphoramidites: New building blocks for the inverse (5′-3′)-oligonucleotide approach. *Helv. Chimica Acta* **2000**, 83, 2023–2035.