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SYNTHESIS AND HYBRIDIZATION PROPERTY OF AN OLIGONUCLEOTIDE CONTAINING A 3′-THIOFORMACETAL LINKED PENTATHYMIDYLATE

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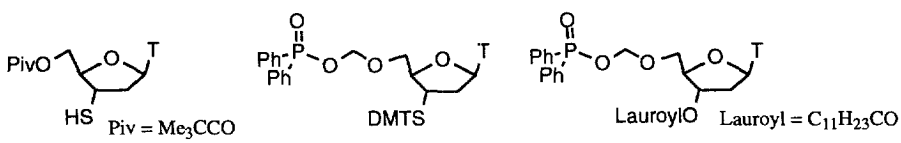
Abstract: A pentathymidylate fully substituted with 3′-thioformacetal internucleotidic linkages was synthesized and subsequently incorporated into an oligonucleotide (ON) 15mer. Tm analysis was performed on the resulting ON hybridized with its complementary RNA. This duplex demonstrated slightly improved binding affinity relative to the control phosphate diester ON/RNA hybrid.

The modulation of gene expression through the antisense strategy offers potential as a novel drug design paradigm.[1] Extensive studies have been made toward the development of oligonucleotide (ON) analogs.[2] Elimination of the anionic charges of the native phosphate diesters may enhance binding affinity towards target RNA molecules due to the reduction of interstrand charge repulsion. A variety of neutral phosphate diester surrogates have been made;[3] however, few of these modifications actually exhibit improved binding affinity. One improved modification is the 3′-thioformacetal internucleotidic linkage. Incorporation of the 3′-thioformacetal linked dinucleotides into ONs resulted in higher binding affinity toward both RNAs and DNAs.[4] We now describe the synthesis of a pentanucleotide fully substituted with 3′-thioformacetal linkages through a sequential elongation approach and the hybridization property of an ON containing this pentathymidylate with its complementary RNA.

The synthesis of a 3′-thioformacetal pentathymidylate by sequential addition of nucleobases required three key intermediates **1**, **2**, and **3** (Figure 1). Compound **1** and **3** were prepared as previously reported[5] and the synthesis of compound **2** is described below (Scheme 1). The readily available thymidine analog **4**[6] was detritylated with methanesulfonic acid in 5% MeOH/CH_2Cl_2 to give **5** in 89% yield. Subsequent treatment of **5** with benzoyl peroxide and methyl sulfide gave the methylthiomethyl ether **6**, which was converted to the desired phosphinate **7** with diphenylphosphic acid and *N*-iodosuccinimide in excellent yield. Phosphinate **7** was carefully deprotected with methanolic NH_3 at 0 °C and then the crude thiol product was subjected to DMTCl and pyridine to give phosphinate **2** in 78% yield. Compound **2** can be stored for a long period when refrigerated.



1 **2** **3**

Figure 1

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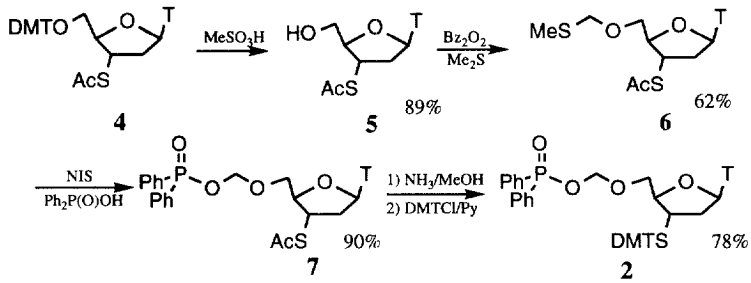


Scheme 1

The displacement of a diphenylphosphinyloxy group by a sulfhydryl group occurs under mild conditions to give the thioformacetal moiety.[5] Initially, compound **1** was condensed with **7**; however, no desired dinucleotide was isolated. It was believed that an S-acyl migration occurred[7] and the more stable DMT protected compound **2** was prepared. Condensation of **1** with **2** proceeded smoothly to give dinucleotide **8** in 90% yield (Scheme 2). Deprotection of **8** with methanesulfonic acid in the presence of an excess of triethylsilane gave dimer sulfhydryl **9** in 95% yield. Coupling of **9** with **2** afforded trimer **10** in 90% yield and deprotection gave trimer sulfhydryl **11**. Tetranucleotides **12** and **13** were obtained in 72% and 90% yields, respectively. The final addition was performed with **13** and phosphinyl methyl ether **3** to give pentamidylate **14** in 62% yield. The use of lauroyl group in **3** was to increase the solubility and extractibility of the pentamer **14**. Lower yields were observed as the chain elongated. This was mainly due to the increased extractive and chromatographic loss for larger ONs. Double saponifications of **14** gave pentamer **15**, which was monotritylated with DMTCl and pyridine at 0 °C to yield pentamer **16**. All the key intermediates gave satisfactory mass spectroscopy data.[8] Pentamer **16** was subsequently attached to succinylated controlled pore glass support to give **17**,[9] which was then subject to ON automated synthesis using standard H-phosphonate chemistry. The phosphodiester and 3′-thioformacetal chimeric ON **18** was synthesized and purified by polyacrylamide gel electrophoresis (PAGE). Maldi mass spectroscopy analysis of **18** confirmed its structure.[10] The hybridization property of **18** and its complementary ssRNA **19** was determined by thermal denaturation (Tm) analysis and compared to that of the phosphate diester control duplex (**19+20**). The following buffer was used in the Tm analyses: 140 mM KCl; 5 mM Na_2HPO_4 (10 mM Na+); 1 mM $MgCl_2$; and pH 7.2.

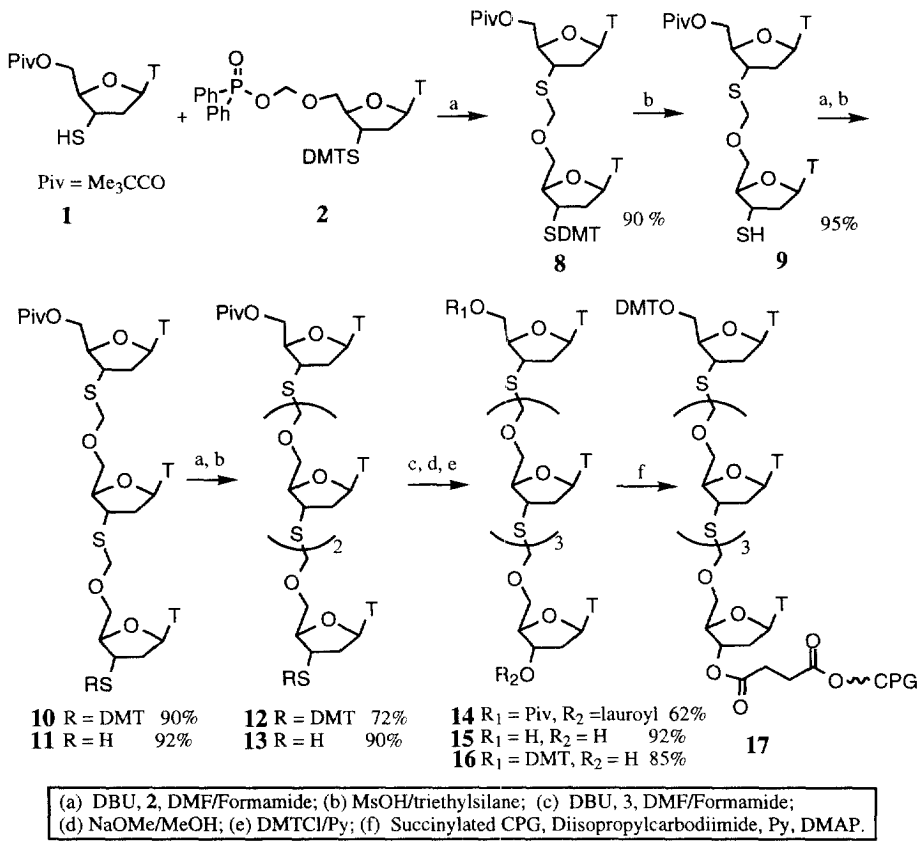
18 5′-dTpC^{m}TpC^{m}pTpC^{m}pTpC^{m}pTpC^{m}pTpC^{m}pTpC^{m}pT*T*T*T*T

19 ssRNA sequence: 3'-rAGAGAGAGAGAAAAA

20 5′-dTpC^{m}TpC^{m}pTpC^{m}pTpC^{m}pTpC^{m}pTpC^{m}pTpC^{m}pTpTpTpTpT

p = phosphate diester; * = 3′-thioformacetal

T = Thymidine, C^m = 5-Methyl Cytidine



Scheme 2

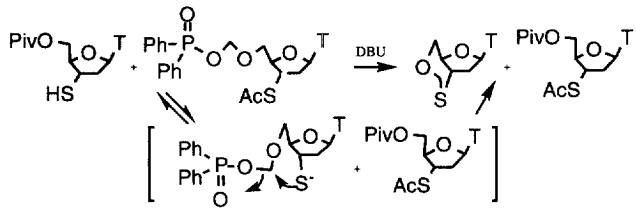
The 3′-thioformacetal containing ON **18** showed a slightly improved binding affinity towards RNA **19** (Tm = 63 °C) relative to the diester control ON **20** (Tm = 62 °C). This is consistent with the previous report.[4] The slight increase in binding affinity is likely due to the preferred C-3 endo conformation that 3′-thioformacetal adopts[11] and the reduced interstrand charge repulsion.

In summary, the neutral, achiral 3′-thioformacetal backbone modification continues to be an attractive choice for the phosphodiester replacement. Using a phosphinate intermediate, ONs with consecutive 3′-thioformacetal linkages can be synthesized. This efficient coupling and deprotecting cycle would be well suited for solid phase syntheses of ONs bearing 3′-thioformacetal linkages.

Acknowledgement: We thank Terry Terhorst for the oligonucleotide synthesis.

References and Notes

1. (a) Milligan, J. F.; Matteucci, M. D.; Martin, J. C. *J. Med. Chem.* **1993**, *36*, 1923. (b) Ulman, E.; Peyman, A. *Chem Rev.* **1990**, *90* , 543. (c) Wagner, R. W.; Flanagan, W. M. *Molecular Medicine Today* **1997**, 31. d) Matteucci, M. D.; Wagner, R. W. *Nature* **1996**, *Supp. 20*, 384.
2. (a) Matteucci, M. D. In *Perspective in Drug Discovery and Design*; Trainer, G., Ed.; ESCOM: Leiden, 1996, Vol. 4, pp 1–16. (b) Cook, P. D. In *Antisense Research and Applications*; Crooke, S., Lebleu, B., Ed.; CRC: Boca Raton, 1995; pp 149–187. (c) De Mesmaker, A.; Haner, R.; Martin, P.; Moser, H. E. *Acc. Chem. Res.* **1995**, *28*, 366.
3. Varma, R. S. *Synlett.* **1993**, 621.
4. Jones, R. J.; Lin, K.-Y., Milligan; J. F., Wadwani; S.; Matteucci, M. D. *J. Org. Chem.* **1993**, *58*, 2983.
5. Zhang, J.; Matteucci, M. D. *Tetrahedron Lett.* **1995**, *36*, 8375.
6. (a) Cao, X.; Matteucci, M. D. *Tetrahedron Lett.* **1994**, *35*, 2325. (b) Li, X., Andrews, D. M.; Cosstick, R. *Tetrahedron* **1992**, 2729.
7. It was postulated that an S-acetyl migration occurred initially and followed by a concomitant intramolecular nucleophilic cyclization reaction. Replacements of the S-acetyl group with sterically more hindered acyl groups (Pivoloyl, adamantoyl) did not prevent this unwanted process from occuring.



8. Mass spectroscopic analyses of coupling products. Dimer **13**: HRMS for $C_{47}H_{54}N_4O_{11}S_2$ + Na, calcd, 937.6281; obsd, 937.3116. Trimer **15**: HRMS for $C_{58}H_{68}N_6O_{15}S_3$+Na, calcd, 1207.9193; obsd, 1207.3794. Tetramer **17**: Fab-MS for $C_{69}H_{82}N_8O_{19}S_4$+Na, calcd, 1478.1, obsd, 1478.6. Pentamer **19**: FABMS for $C_{71}H_{100}N_{10}O_{23}S_4$, calcd, 1589.2; obsd, 1589.6.
9. Dahma, M. J.; Giannaris, P. A.; Zabarylo, S. V. *Nucleic Acid Res.* **1990**, *18*, 3813.
10. Maldi MS analysis for ON **19:** $C_{154}H_{205}N_{35}O_{86}P_{10}S_4$+$H^+$, calcd, 4361.5; obsd, 4361.6.
11. Veal, J. M.; Brown, F. K. *J. Am. Chem. Soc.* **1995**, *117*, 1873.