Tetrahedron Letters, Vol.31, No.17, pp 2385-2388, 1990
Printed in Great Britain

0040-4039/90 $3.00 + .00
Pergamon Press plc

Deoxyoligonucleotide Analogs Based on Formacetal Linkages

Mark Matteucci
Gilead Sciences, Inc.
344/346 Lakeside Drive, Foster City, CA 94404

Abstract: This report describes the synthesis of a trimer of thymidine bearing formacetal linkages, its incorporation into a longer deoxyoligonucleotide and characterization of the hybridization properties with a complementary RNA sequence.

The use of deoxyoligonucleotide analogs for the inhibition of gene expression is a growing field[1]. Phosphate linkage analogs have been investigated as a means of increasing cell permeation and nuclease stability of oligonucleotides[1]. Neutral phosphate analogs, namely methylphosphonates[2], have been extensively studied in pursuit of these properties. Other neutral analogs such as phosphotriesters[3], phosphoramidates[4] and carbamates[5] have been characterized to the point of measuring the oligonucleotide's ability to hybridize to complementary sequences. All of the above linkages, with the exception of carbamates suffer from the creation of a chiral center and consequently 2^n diasteriomers, where n is the number of linkages in the oligonucleotide. Carbamates suffer from no detectable hybridization as oligo thymidine analogs[5a], possibly due to restricted rotation about the trigonal carbamate linkage.

A formacetal is the simplest and smallest achiral isostere for a phosphate linkage. This linkage is reminiscent of the acetal linkage in another class of biopolymers, the polysaccharides. This report describes the synthesis of a trimer of thymidine bearing formacetal linkages, its incorporation into a longer deoxyoligonucleotide and characterization of the hybridization properties with a complementary RNA sequence.

The synthetic scheme is outlined in Figure 1. The coupling chemistry is a modified form of a method used to synthesize disaccharides[6]. A dimer of thymidine is generated from a 5'dimethoxytrityl 3'methylthioacetal thymidine (**2**) and a 5'OH 3'thexyldimethylsilyl thymidine. **2** is activated with N-bromo succinimide in the presence of 2,6,di-t-butylpyridine in methylene chloride. Isolation of the dimer (**3**), detritylation with 20% H_2O/acetic acid and a second round of coupling with activated **2** yields the protected trimer (**4**). **4** is desilylated with tetrabutyl-ammonium fluoride (TBAF) in THF[7] and succinylated. The resulting carboxylic acid is coupled to a controlled pore glass support via standard methods[8]. This support (**5**) serves as the starting material for standard deoxyoligonucleotide synthesis using H-phosphonate chemistry[9].

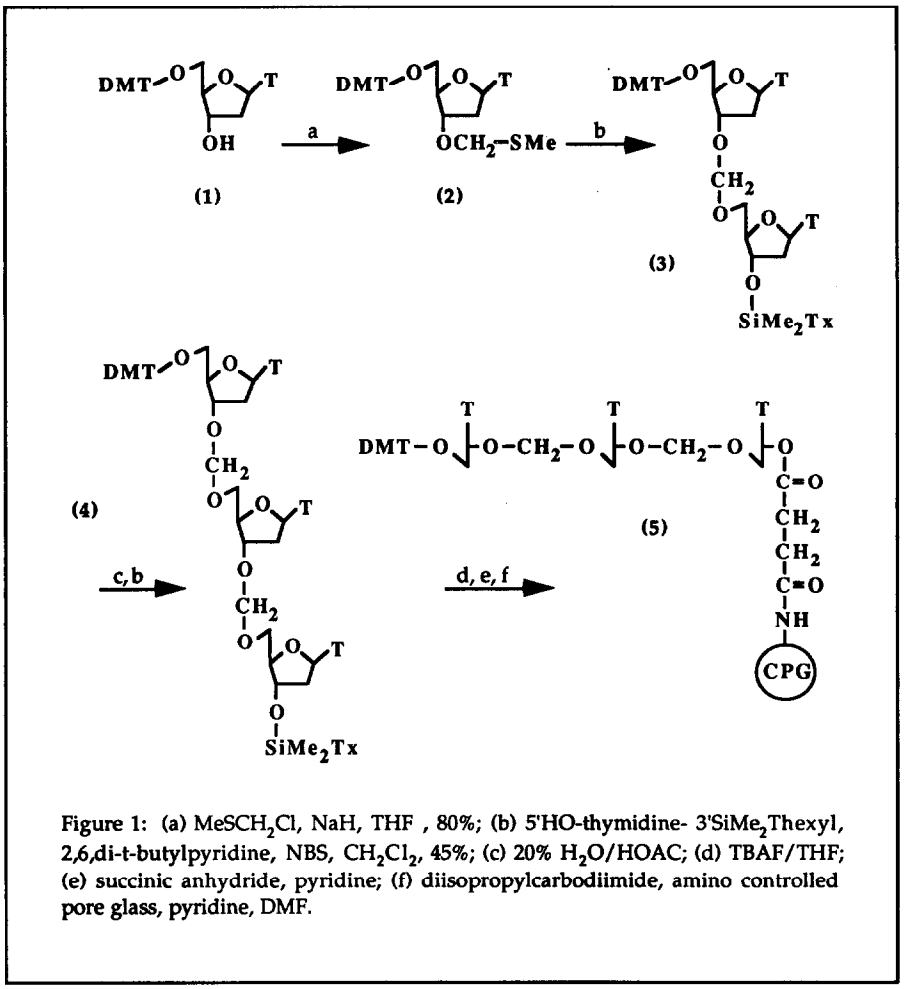

Figure 1: (a) $MeSCH_2Cl$, NaH, THF , 80%; (b) 5'HO-thymidine- 3'$SiMe_2$Thexyl, 2,6,di-t-butylpyridine, NBS, CH_2Cl_2, 45%; (c) 20% H_2O/HOAC; (d) TBAF/THF; (e) succinic anhydride, pyridine; (f) diisopropylcarbodiimide, amino controlled pore glass, pyridine, DMF.

The sequence 5' TTCCCTCTCTTT-OCH_2O-T-OCH_2O-T (6) (see Table 1) was synthesized, the N benzoyl amides on cytidine removed with aqueous ammonia and the oligonucleotide purified by reverse phase HPLC. The purified product was characterized by treatment with 20% H_2O/formic acid at 80°C for 1 hour. The T_3 formacetal is stable to moderate acid treatment such as 1M HCl (20°C, 3 hr) or 20% H_2O/HOAc (45°C, 3 hr) but formic acid at 80°C results in complete decomposition. This is useful for characterization by electrophoretic mobility in polyacrylamide gels as shown in Figure 2. Additionally, 6 is stable to snake venom phosphodiesterase under conditions sufficient for the complete destruction of the phosphodiester parent (data not shown).

The T_m of 6 with its RNA complement (generated via T_7 transcription[10]) was determined[11]. For comparative purposes, three additional oligonucleotides were prepared and their respective T_m's determined. The T_m data obtained is listed in Table 1. This data demonstrates that the T_3 formacetal 6

	Sequence	T_m (°C)
(6)	$^{5'}$ TCTCCCTCTCTTT—O—CH_2—O—T—O—CH_2—O—T—OH (Acetal)	59.0
(7)	$^{5'}$ TCTCCCTCTCTTT—O—P(=O)(O^-)—O—T—O—P(=O)(O^-)—O—T—OH (Diester)	59.5
(8)	$^{5'}$ TCTCCCTCTCTTT—O—P(=O)(NH⟨⟩OMe)—O—T—O—P(=O)(NH⟨⟩OMe)—O—T—OH (Amidate)	58.5
(9)	$^{5'}$ TCTCCCTCTCTTT—OH (OH)	56.5

Table 1: Melting temperatures (T_m) measured in 10 mM NaPhos pH 7.4, 150 mM NaCl. T_m measurements are +/- 0.2° C.

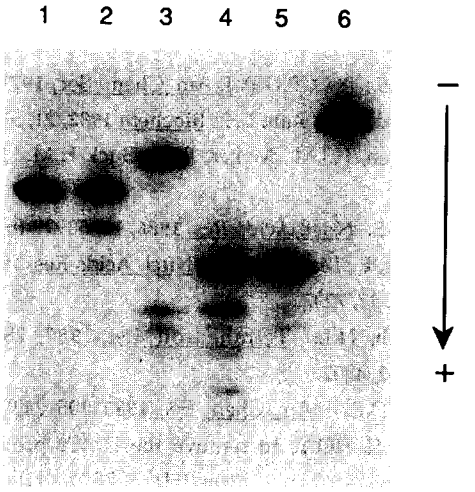

Figure 2: Autoradiogram resulting from electrophoresis of oligomers 6-9 in a 20% polyacrylamide 7M urea gel. All oligomers were radiolabeled using T_4 kinase and gamma ^{32}P ATP. **Lane 1:** 7; **lane 2:** 7 treated with 20% H_2O/formic acid, 1 hr, 80°C; **lane 3:** 6; **lane 4:** 6 treated with 20% H_2O/formic acid; **lane 5:** 9; **lane 6:** 8.

hybridizes with a complementary sequence of RNA. While the T_m is not as high as T_3 diester (7) it is higher than that of the methoxyethylphosphoramidates (8), which in turn are higher than deletion of the terminal two thymidines (9).

In summary, a formacetal analog of a deoxyoligonucleotide has been synthesized, shown to be chemically and enzymatically stable, and demonstrated to hybridize to complementary RNA. This achiral and neutral tetrahedral linkage has potential applications in the control of gene expression by the *in vivo* blocking of the translation of specific messenger RNA.

Acknowledgements: The author thanks John Helms, Sarah McCurdy and Matt Stephens for technical assistance; Dr. John Latham for the T_7 transcript; Brian Froehler for helpful discussions; Dr. Mike Riordan for moral support and B.F., J.L. and M.R. for critical reading of this manuscript.

References and Notes

1. For recent review, see: van der Krol, A.R.; Mol, J.N.M.; Stuitje, A.R. BioTechniques **1988**, 6, 958.
2. (a) Miller, P.S. ; Ts'O P.O.P. Anti Cancer Drug Design **1987**, 2,117. (b) Smith, C.C.; Aurelian, L.; Reddy, M.; Miller, P.S.; Ts'O, P.O.P. Proc. Natl. Acad. Sci. **1986**, 83, 2787. (c) Miller, P.S.; McParland, K.B.; Jayaraman, K.; Ts'O, P.O.P. Biochem. **1981**, 20, 1874. (d) Blake, K.R.; Murakami, A.; Miller, P.S. Biochem. **1985**, 24, 6132.
3. (a) Miller, P.S.; Fang, K.N.; Kondo, N.S.; Ts'O, P.O.P. J. Am. Chem. Soc. **1971**, 93, 6657. (b) Miller, P.S.; Chandrasegaran, S.; Dow, D.L.; Pulford, S.M.; Kan, L.S. Biochem **1982**, 21, 5468. (c) Moody, H.M.; van Genderen, M.H.P.; Koole, L.H.; Kocken, H.J.M.; Meijer, E.M.; Buck H.M. Nucl. Acids Res. **1989**, 17, 4769.
4. (a) Letsinger, R.L.; Bach, S.A.; Eadie, J.S. Nucl. Acids Res. **1986**, 14, 5399. (b) Froehler, B. Tetrahedron Let. **1986**, 27, 5575. (c) Froehler, B.; Ng, P.; Matteucci, M. Nucl. Acids Res. **1988**, 16, 4831. (d) Jager, A.; Levy, M.J.; Hecht, S.M. Biochem 1988, 27, 7237.
5. (a) Coull, J.M.; Carlson, D.V.; Weith, H.L. Tetrahedron Let. **1987**, 28, 745. (b) Stirchak, E.P.; Summerton, J.E. J. Org. Chem. **1987**, 52, 4202.
6. Nicolaou, K.C.; Seitz, S.P.; Papahatjis, D.P. J. Am. Chem. Soc. **1983**, 105, 2430.
7. A small aliguot was treated with 80% HOAc to remove the DMTr protecting group. 1H NMR, D6DMSO, 7.65(s,1H), 7.49(s,1H), 7.47(s,1H), 6.13(m,3H), 4.78(m,4H), 4.30(m,2H), 4.20(m,1H), 4.05(m,1H), 3.92(m,1H), 3.87(m,1H), 3.70(d,2H), 3.60(d,2H), 3.42(q,2H), 2.10-2.30(m,6H), 1.86(s,6H). MS m/z 1036 (MH+) as di(Me_2ThexylSilyl) ether derivative.
8. Matteucci, M.; Caruthers, M.H. J. Am. Chem. Soc. **1981**,103, 3185.
9. Froehler, B.; Ng, P.; Matteucci, M. Nucl. Acids Res. **1986**, 14, 5399.
10. Mulligan, J.F.; Groebe, D.R.; Witherell,G.W.; Uhlenbeck, O.C. Nucl. Acids Res. **1987**, 15, 8783.
11. For the T_m experimental procedure, see: Summers, M. F.; Powell, C.; Egan, W.; Byrd, R.A.; Wilson, W.D.; Zon G.Q. Nucl. Acids Res. **1986**, 14, 7421.

(Received in USA 23 January 1990)