Pergamon

Tetrahedron Letters, Vol. 35, No. 15, pp. 2325-2328, 1994
Elsevier Science Ltd
Printed in Great Britain
0040-4039/94 $6.00+0.00

0040-4039(94)E0294-8

Oligodeoxynucleotides Containing 3'-Allylether, 3'-Allylsulfide and Their Saturated Derivatives as Phosphate Mimics

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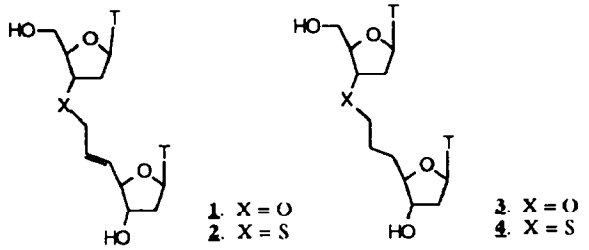
Abstract: A series of novel thymidine-thymidine dimers containing 3'-allylether, 3'-allylsulfide connections and their saturated derivatives have been prepared and incorporated into oligodeoxynucleotides (ODNs). The 3'-allylether analog results in only a modest destablization of double helix formation with a complementary ssRNA relative to the phosphodiester linkage. This fact coupled with its facile synthesis may point to an application of this linkage in oligonucleotide-based therapeutics.

Oligodeoxynucleotide (ODN) analogs bearing neutral phosphodiester mimics are of interest for theoretical and practical reasons[1]. Many achiral mimics have been synthesized[2] including 5'-sulfide,[3] siloxane,[4] sulfone,[5] methylhydroxylamine,[6] thio- and formacetal.[7] We now report the synthesis and the hybridization properties of ODNs containing thymidine-thymidine dimers bearing 3'-allylether **1**, 3'-allylsulfide **2**, and their saturated derivatives **3** and **4**,[8] replacing the phosphodiester linkages.



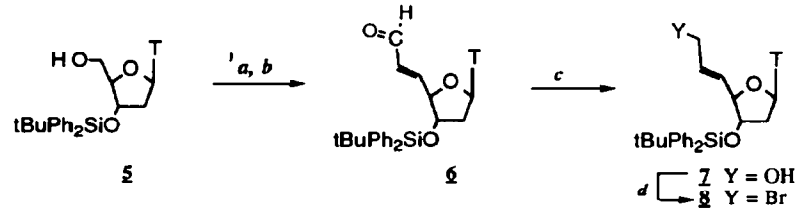
1. X = O
2. X = S

3. X = O
4. X = S

The 5' hydroxyl of 3'-O-*t*-butyldiphenylsilyl-thymidine **5** (**Scheme 1**) was oxidized to the aldehyde, and then condensed with Ph_3P=CHCHO to give the unsaturated aldehyde **6**.[9] Reduction of **6** with $NaBH_4/Et_3SiH$ in THF gave the allylic alcohol **7**. Compound **7** was then converted to the allyl bromide **8** using Ph_3P/CBr_4 in DMF. Condensation of the allyl bromide **8** and 5'-O-DMT-thymidine **9** (**Scheme 2**) was carried out by adding **8** to the solution of **9**, which was pretreated with five equivalents of NaH in THF at 0°C. The reaction gave the desired dinucleoside **11** (55% yield). Coupling of **10** with **8** to yield the 3'-allylsulfide **13** went smoothly in CH_2Cl_2 using diisopropylethylamine (DIPEA) as the base (75% yield). Both **11** and **13** were desilylated to **12**[10] and **14**[11] followed by conversion to the dinucleotides H-phosphonates **15** and **16**.

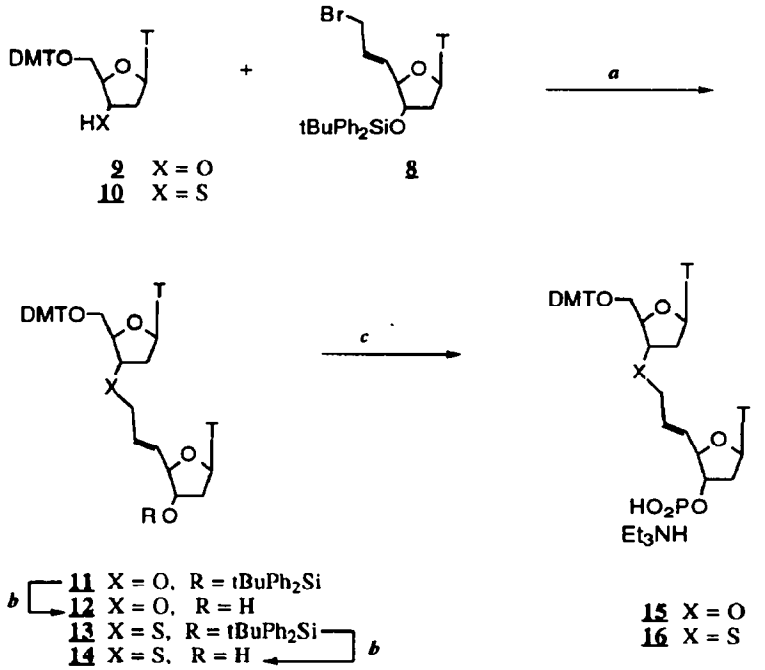
The saturated derivatives of the allylic dinucleoside **17**[12] and **22**[13] were synthesized in different manners (**Scheme 3** and **Scheme 4**). Compound **17** was obtained by ambient pressure hydrogenation of **12**. The saturated sulfur analog was prepared via a alternate route given sulfur would likely poison the hydrogenation catalyst. The allyl alcohol **7** was hydrogenated to **19** followed by bromination to the compound **20**. The bromide was condensed with mercaptan **10** using 1.0M sodium trimethylsilanolate in THF as the base to yield

Scheme 1



a. DMSO/DCC, *b*: $Ph_3P{=}CHCHO$, 65% for two steps; *c*. $NaBH_4/Et_3SiH$/THF, 90%; *d*. Ph_3P, CBr_4/DMF, 70%.

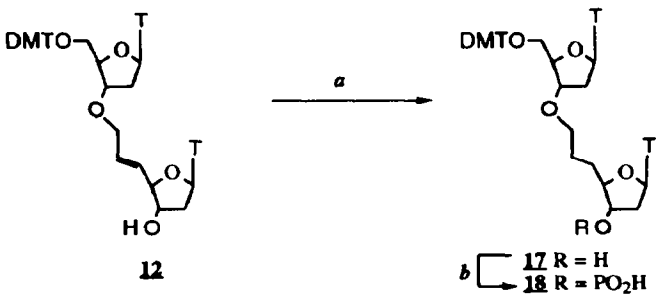
Scheme 2



a. For X = O: NaH/THF, 55%; for X = S: DIPEA/CH_2Cl_2, 75%; *b*. TBAF/THF, 70-80%; *c*. 2-chloro-4-H-1,3,2-benzodioxaphosphorin-4-one, pyridine/CH_2Cl_2, 75%-80%.

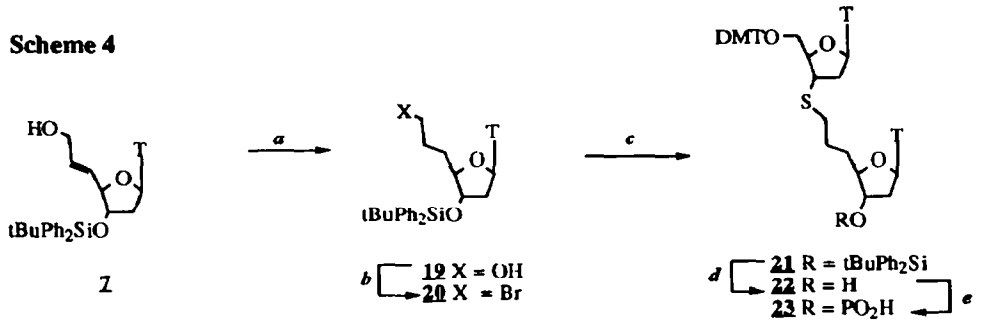
21 (80% yield), desilylated and converted to the H-phosphonate **23**.[14] The sequences listed in **Table 1** were prepared by standard H-phosphonate protocol[15] and characterized by digestion with nuclease and phosphatase followed by HPLC analysis of the monomers and dimers. All ODNs showed the expected ratios.

Scheme 3



a. H_2, Pd/C, 85%; *b*. 2-chloro-4-H-1,3,2-benzodioxaphosphorin-4-one, pyridine/CH_2Cl_2, 75%.

Scheme 4



a. H_2, Pd/C, EtOH, 95%; *b*. Ph_3P, CBr_4, DMF, 80%; *c*. 10, TMSONa, THF, 80%; *d*. TBAF, THF, 75%. *e*. 2-chloro-4-H-1,3,2-benzodioxaphosphorin-4-one, pyridine/CH_2Cl_2, 75%.

The hybridization properties were examined by thermal denaturation studies on the duplexes formed between the test ODN and its complement (**Table 1**). These data demonstrated that the allyl and propyl containing ODNs result in somewhat less affinity toward ssRNA relative to the control phophodiester. Further destablization resulted when the analogs were hybridized to ssDNA. This "selectivity" favoring RNA was recently also reported for a 5'-sulfide ODN analog.[16]

Table 1 Tm Analysis of ODNs Containing Backbone-Modified Dimeric Nucleotides

		ssRNA(ΔTm/subst.)	ssDNA(ΔTm/subst.)
Complement	3' ApGpApGpApGpApGpApGpApApApApA 5'		
phosphodiester	5' TpCpTpCpTpCpTpCpTpCpTpTpTpTpT 3'	62.5 ± 0.5°C	55.5 ± 0.5°C
Allylether	5' TpCpTpCpTpCpTpCpTpCpT*TpT*TpT 3'	60.5 (-1.0)	49.0(-3.25)
Allylsulfide	5' TpCpTpCpTpCpTpCpTpCpT*TpT*TpT 3'	59.5 (-1.5)	49.5(-3.0)
propylether	5' TpCpTpCpTpCpTpCpTpCpT*TpT*TpT 3'	58.5 (-2.0)	49.0(-3.25)
propyl sulfide	5' TpCpTpCpTpCpTpCpTpCpT*TpT*TpT 3'	59.0 (-1.75)	49.0(-3.25)

* = modified linkage; p = phosphodiester bond; C = 5 Methyl-2'-deoxycytidine

Among this series of novel thymidine-thymidine dimers, the 3'-allylether analog results in only a modest destablization of helix formation with a complementary ssRNA relative to phosphodiester linkages. This fact coupled with its facile synthesis may point to an application of this linkage in oligonucleotide-based therapeutics.

ACKNOWLEDGMENTS:

We thank T. Terhorst for ODN syntheses, C. Sueoka for base composition analyses, and S. Wadwani for Tm measurement.

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10. Compound **12**: ^{1}H-NMR δH(300MHz, $CDCl_3$) 9.60 (br., 1H); 9.80 (br., 1H); 7.63 (s, 1H); 6.70-7.46 (m, 14H); 6.38 (dd, 1H); 6.26 (t, 1H); 5.85-5.93 (m, 2H); 4.04-4.36 (m,6H); 3.82(s, 6H); 3.42 (ddd, 2H); 2.12-2.56 (m, 5H); 1.84 (s, 3H); 1.46 (s, 3H). MS: required 794.8, found M^+ 794.4.
11. Compound **14**: ^{1}H-NMR δH(300MHz, $CDCl_3$) 9.38 (br., 1H); 9.60 (br., 1H); 7.66 (s, 1H); 6.70-7.46 (m, 14H); 6.18 (t, 1H); 6.06 (dd, 1H); 5.65-5.90 (ddd, 2H); 3.98-4.24 (m, 5H); 3.78 (s, 6H); 3.58 (m,2H); 3.42 (ddd, 2H); 2.16-2.58 (m, 4H); 1.84 (s, 3H); 1.44 (s, 3H). MS: required 810.9, found M^+ 810.3.
12. Compound **17**: ^{1}H-NMR δH(300MHz, $CDCl_3$) 9.90 (br, 2H); 7.62 (s, 1H); 6.70-7.46 (m, 14H); 6.36(dd, 1H); 6.22 (t, 1H); 3.88-4.22 (m, 5H); 3.78 (s, 6H); 3.56 (m,2H); 3.38 (ddd, 2H); 2.16-2.56 (m, 4H); 1.94 (s, 3H); 1.70 (m, 4H); 1.48 (s, 3H). MS: required 796.9, found M^+ 796.3.
13. Compound **22**: ^{1}H-NMR δH(300MHz, $CDCl_3$) 9.92 (br, 2H); 7.76 (s, 1H); 6.70-7.46 (m, 14H); 6.18-6.22(m, 2H); 3.96-4.18 (m, 2H); 3.78 (s, 6H); 3.38-3.62 (m, 3H); 2.16-2.46 (m, 6H); 1.94 (s, 3H); 1.70 (m, 4H); 1.42 (s, 3H). MS: required 812.9, found M^+ 812.4.
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(*Received in USA* 22 *December* 1993; *revised* 26 *January* 1994; *accepted* 4 *February* 1994)