Tetrahedron Letters, Vol. 33, No. 19, pp. 2645-2648, 1992
Printed in Great Britain

0040-4039/92 $5.00 + .00
Pergamon Press Ltd

Intermolecular Radical C-C Bond Formation: Synthesis of a Novel Dinucleoside Linker for Non-anionic Antisense Oligonucleosides+

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Key Words: antisense oligonucleotides, nonionic backbone, intermolecular radical reaction

Abstract: **An efficient, stereoselective synthesis of a thymidine (T) nucleoside dimer (T-3'-CH_2-NH-O-5'-T) 1 has been accomplished via an intermolecular radical reaction. The new dimer and methodology is useful for the development of backbone modified antisense oligonucleosides.**

Modulation of the expression of genetic information by an antisense oligonucleotides is an exciting new drug design concept.[1] Development of therapeutic agents based on this technology requires modified oligonucleotides that are resistant to nucleolytic cleavage, penetrate cellular membranes, and hybridize, with appropriate affinity and specificity, to targeted RNA. The removal of the negative charge carried by the phosphodiester linkage of oligonucleotides may enhance each of these pharmacokinetic properties. However, efforts in this direction, such as substitutions on the prochiral phosphorus atom of the phosphodiester linkage, as represented by phosphotriesters, methylphosphonates, phosphoramidates, compromise binding affinity.[1] Several other approaches, that provide a neutral backbone, are the methylene,[2] carbonyl,[3] disubstituted silyl[4] modifications, which represent one to one atom replacements. A synthesis of a methylsulfonate as a two atom replacement, and a dimethylene sulfonate as a three atom replacement of the phosphodiester linkage has been reported.[5] We now report the replacement of the two atom moiety -3'-O-P(O)$^-_2$- of a T-T dinucleotide by the non-anionic 3'-CH_2-NH- moiety by a free radical, carbon-carbon bond formation between the nucleosides. The resulting hydroxylamine nucleoside dimer **1** is achiral, neutral,[6] and is expected to mimic a natural DNA phosphodiester linkage.[7]

Scheme I outlines the synthesis of novel target dinucleoside **1**, for which the key reaction is the stereoselective connection of 5'-*O*-(trityl)-3'-deoxy-3'-iodothymidine (**5**) and 5'-*O*-(methyleneamino)-3'-(*t*-butyldiphenylsilyl)thymidine (**9**). We chose to explore the possibility of achieving this connection via an intermolecular radical C-C bond formation. In recent years, considerable technology has been developed with radical reactions that result in a high degree of stereoselectivity.[8] In particular, our attention was drawn to the stereoselective synthesis of 3'-cyanomethyl- and 3'-cyano-3'-deoxythymidines by Tam,[9] and Parkes,[10] respectively. It is notable that these were stereoselective, high yield reactions which indicates the stability of the incipient 3'-radical of **3**.[11]

+We refer to modified oligonucleotides that have the phosphorus atom removed as *oligonucleosides*.

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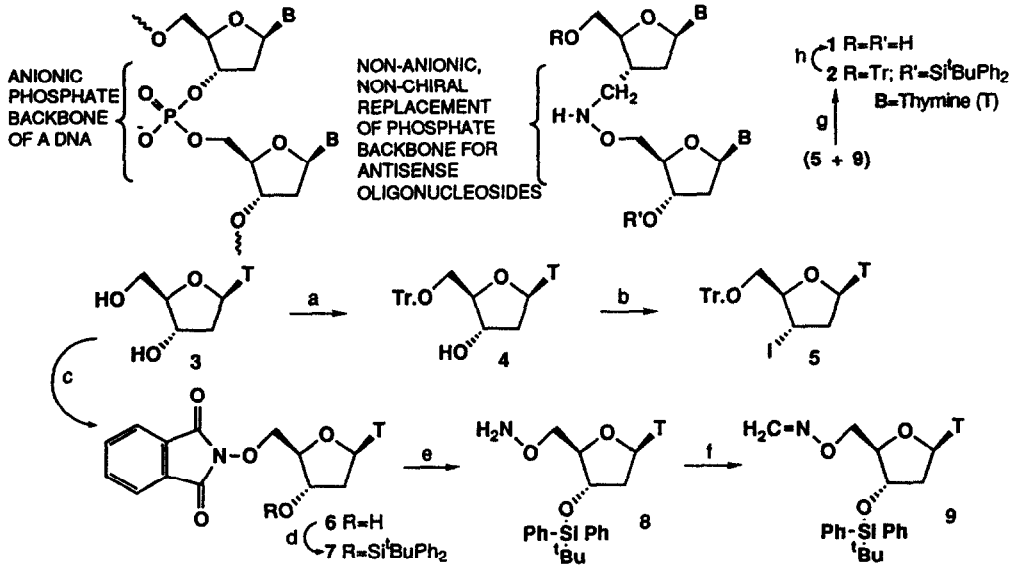
Scheme I. SYNTHESIS OF DINUCLEOSIDE 1[a]



[a] Reagents and conditions: (a) 1 equiv of 3, 1.15 equiv of TrCl, pyridine, reflux 1h, 79% of 4; (b) 1 equiv of 4, 2 equiv of $(PhO)_3MeP^+I^-$, DMF, room temperature,16h, 68% of 5; (c) 1 equiv of 3, 1.3 equiv of HONPhth, 1.3 equiv of Ph_3P, 1.5 equiv of DIPAD, DMF, 0°C -> room temperature, 15h, 80% of 6; (d) 1 equiv of 6, 1.3 equiv of $^tBuPh_2SiCl$, 2.6 equiv of imidazole, DMF, room temperature, 2h, 84% of 7; (e) 1 equiv of 7, 1.5 equiv of H_3CNHNH_2, CH_2Cl_2, room temperature, 2h, 79% of 8; (f) 1 equiv of 8, 1 equiv of aq. HCHO, dry MeOH, room temperature, 3h, 95% of 9; (g) 0.5 equiv of 5, 0.5 equiv of 9, 1.5 equiv of bis-(trimethylstannyl)benzopinacolate, 2 ml C_6H_6, 22h, 80°C, 30% of 2; (h) 1 equiv of 2, aq. HF 48% (5 ml) in CH_3CN (95 ml), 3h -> 1M nBu_4NF in pyridine (1 ml), pyridine (10 ml), room temperature, 30 min., 80% of 1.

Furthermore, the ready availability of the radical precursor, iodo nucleoside 5,[12] is very attractive. Oxime ethers have been reported to serve as radical acceptors for intramolecular cyclizations[13] and for intermolecular homologations.[14] This literature suggests that the oxime ether present in the novel nucleoside **9** should serve as an effective radical acceptor and provide the desired dinucleoside **1**. The synthesis of protected nucleoside **9** was achieved in four steps from readily available thymidine (**3**). A regioselective Mitsunobu reaction[15] of **3** with N-hydroxyphthalimide resulted in exclusive formation of novel 5'-*O*-(phthalimido)-thymidine (**6**)[16] in 80% yield. For larger-scale syntheses, crude **6** was silylated with *t*-$BuPh_2SiCl$ and purified by chromatography to furnish 5'-*O*-phthalimido-3'-*t*-butyldiphenylsilylthymidine (**7**) in 84% yield for two steps. Hydrazinolysis of **7** gave the 5'-*O*-aminothymidine derivative **8** in 79% yield.[17] The amino nucleoside **8** was found to be extremely reactive with aldehydes and ketones.[18] N-alkylation of **8** with an equivalent amount of formaldehyde led to formation of the desired oxime derivative **9** in 95% yield. A solution[19] of **5**, **9**, and bis(trimethylstannyl)benzopinacolate[20] in benzene was refluxed for 22 h to afford, after purification of the reaction mixture by silica gel chromatography, a 30% yield[21] of the protected dinucleoside **2**. The desired dimer **1** was obtained in 80% yield by a two-step deblocking (aq. HF/ nBu_4NF) of dinucleoside **2**.

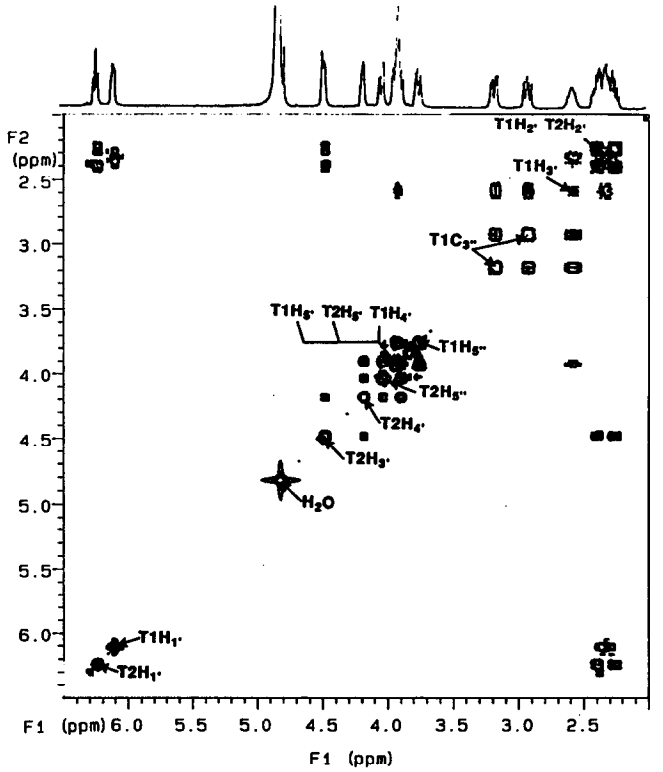


Figure 1
Partial COSY 400 MHz ^1H NMR of dinucleoside 1 in D_2O at 20°C

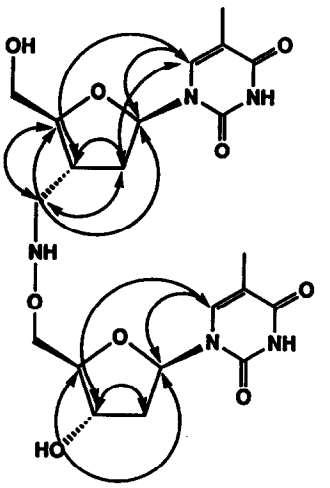


Figure 2

Observed NOEs in NOESY (↔) for dinucleoside 1

The structure of dinucleoside 1 resulting from the stereoselective radical reaction was established by ^1H, ^{13}C NMR, FAB MS and elemental analysis.[22] The assignment of each proton resonance in dimer 1 was carried out by COSY and NOESY techniques.[23] The spin systems of deoxysugar moieties [$H_{1'}$,2',3',(3"),4', and 5'] and of thymine (H6 and CH_3) were identified by spin-spin coupling with the COSY cross peaks. The figure 1 show an expansion of selected region of the COSY spectra of 1 with assignment of sugar protons. Sequential resonance assignments within the individual spin systems were determined by NOESY. The characteristic NOESY cross peaks between sugar and base protons are shown in figure 2. Thus, validating our assignment of the newly created C-C bond as having an α-configuration for $C_{3"}$ of the sugar ring.

The oxime ether radical linkage methodology developed from this research may have general application in constructing backbone modified antisense oligonucleosides. We are in process of incorporating the described dinucleoside 1 into oligonucleosides[24] for antisense evaluations.

Acknowledgements: We thank Dr. A. De Mesmaeker for helpful suggestions and Dr. Rich Griffey and Mr. Patrick Wheeler for ^1H NMR studies.

References and Notes

1. Selected review articles: Goodchild, J. *Bioconjugate Chem.* **1990**, *1*, 165. Uhlmann, E.; Peyman, A. *Chem. Rev.* 1990, 90, 543, Hélène, C.; Toulmé, J.J. *Biochim. Biophy. Acta* **1990**, *1049*, 99. Cohen, J.S. *Antiviral Res.* **1991**, *16*, 121. Matteucci, M.D.; Bischofberger, N. in *Ann. Rep. Med. Chem.* **1991**, *26*, 287. Cook, P.D. *Anticancer Drug Design* **1991**, *6*, 585. Chrisey, L.A. *Antisense Res. Develop.* **1991**, *1*, 65.
2. Matteucci, M.D. *Tetrahedron Lett.* **1990**, *31*, 2385. Matteucci, M.D.; Lin, K.-Y.; Butcher, S.; Moulds, C. *J. Am. Chem. Soc.* **1991**, *113*, 7767. Veeneman, G.H.; VanDer Marel, G.A.; Van Der Elst, H.; Van Boom, J. H. *Tetrahedron Lett.* **1991**, *47*, 1547. Quaedflieg, P.; VanDer Marel, G.A.; Kuyl-Yeheskiely, E.; Van Boom, J. H. *Recueil des Trav. Chim. des Pays-Bas* **1991**, *110*, 435-436.
3. Tittensor, J.R. *J. Chem. Soc.* (c) **1971**, 2656.
4. Cormier, J.F.; Ogilvie, K.K. *Nucleic Acids Res.* **1988**, *16*, 4583. Seliger, H.; Feger, G. *Nucleosides Nucleotides* **1987**, *6*, 483.
5. Musicki, B.; Widlanski, T.S. *Tetrahedron Lett.* **1991**, *32*, 1267. Huang, Z.; Schneider, K.C.; Benner, S.A. *J. Org. Chem.* **1991**, *56*, 3869.
6. We believe that the hydroxyamino linkage is not protonated at the physiological *pH* (pK_a of NH_2OH=5.96).
7. Molecular modeling of the described linkage and others have taken place and will be reported elsewhere.
8. For recent reviews of radicals in organic synthesis, see: Curran, D.P. *Radical Addition Reactions, Radical Cyclizations and Sequential Radical Reactions* In *Comprehensive Organic Synthesis*; Trost, B.M.; Fleming, Eds; Vol. 4, p 715-832, Pergamon Press, Oxford, 1991; Ramaiah, M. *Tetrahedron* **1987**, *43*, 3541-3676; Neumann, W.P. *Synthesis* **1987**, 665-683; Hart, D.J. *Science* **1984**, *223*, 883-887; Giese, B. *Agnew. Chem. Int.* Ed. **1985**, *97*, 555-567; Stork, G. *Bull Soc. Chim.* Fr. **1990**, *127*, 675-680; Giese, B. *Radicals in Organic Synthesis: Formation of Carbon-Carbon Bonds*, Pergamon Press, Oxford, 1986.
9. Fiandor, J.; Tam, S.Y. *Tetrahedron lett.* **1990**, *31*, 597-600. Fiandor, J.; Huryn, D.M.; Sluboski, B.; Todaro, L.J.; Tam, S.Y. *Nucleosides Nucleotides* **1989**, *8*, 1107-1108.
10. Parkes, K.E.B.; Taylor, K. *Tetrahedron lett.* **1988**, *29*, 2995-2996.
11. Wu, J.-C.; Xi, C.; Gioeli, C.; Chattopadhyaya, J. *Tetrahedron* **1991**, *47*, 2237-2254.
12. Verheyden, J.P.H.; Moffatt, J.G. *J. Org. Chem.* **1970**, *35*, 2868-2877.
13. Contelles, M.J.; Grau, M.A.; Bernabe, M.; Martin, N.; Seoane, C. *Synlett* **1991**, 165-167. Bartlett, P.A; McLaren, K.L.; Ting, P.C. *J. Am. Chem. Soc.* **1988**, *110*, 1633-1634.
14. Hart, D.J.; Seely, F.L. *J. Am. Chem. Soc.* **1988**, *110*, 1631-1633.
15. Mitsunobu, O. *Synthesis* **1981**, 1-28.
16. All new compounds exhibited satisfactory spectral and analytical and/or exact FAB mass data. Yields refer to spectroscopically and chromatographically homogeneous material.
17. Use of $MeNHNH_2/CH_2Cl_2$ allowed an easy separation of precipitated 1,2-dihydro-4-hydroxy-2-methyl-1-oxophthalazine by filtration from the product **8**.
18. Traces of acetone reacted with **8** to provide its isopropylidene derivative.
19. The flask was evacuated and flushed with argon 3-times.
20. Hillgartner, H.; Neumann, W.P.; Schroeder, B. *Liebigs. Ann. Chem.* **1975**, 586-599; We are indebted to Professor D. J. Hart for providing an improved procedure to make bis(trimethylstannyl)benzopinacolate.
21. Yield optimization of this reaction has not been completed. We made no attempts to characterize minor products obtained during this reaction.
22. Dinucleoside **1**: ^{1}H NMR (DMSO-d_6) δ 11.23 (br s, 2, 2N*H*), 7.83 and 7.49 (2s, 2, 2C_6*H*), 6.82 (t, 1, N*H*O), 6.14 (pseudo t, 1, *H*1', $J_{1',2'}$=7.6 Hz, $J_{1',2''}$=6.5 Hz), 5.96 (dd, 1, H1', $J_{1',2''}$=6.9 Hz, $J_{1',2''}$=4.3 Hz), 5.28 (s, 1, O*H*), 5.08 (s, 1, O*H*), 4.18 (m, 1, *H*3'), 3.89 (m, 1, *H*4'), 3.54-3.78 (m, 5, *H*5',5'', *H*4'), 2.76-2.94 (m, 2,*H*3''), 2.42 (m, 1, *H*3'), 2.0-2.17 (m, 4, *H*2',2''), 1.77 and 1.74 (2s, 6, 2 CH_3).
^{13}C (DMSO-d_6) δ 12.25, 35.62, 36.96, 45.47, 52.99, 61.3, 70.8, 73.5, 83.83, 84.5, 84.63, 108.67, 108.95, 109.59, 135.87, 136.28, 150.36, 150.45, 163.72, 163.83. FAB MS: M/z 496 $(M+H)^+$.
Anal Calcd for $C_{21}H_{29}N_5O_9 \cdot H_2O$: C, 49.12; H, 6.09; N, 13.64. Found: C, 48.99; H, 5.96; N, 13.49.
23. Wuthrich, K. "NMR of Proteins and Nucleic Acids", John Wiley & Sons, New York, 1986.
24. The synthesis and properties of novel oligonucleosides will be published elsewhere.

(Received in USA 14 January 1992)