The 4α-A and 4α-B/methylalumoxane catalysts ([Al]/[Zr] ≈ 1000) both produced isotactic polypropylene almost completely by means of enantiomorphic site control [activities at −30 °C ≈ 450, $\bar{M}_\eta \approx 260000$ (4α-A) and 470000 (4α-B)]. Most of the polymer (≥93%) was only soluble in boiling hexane. The ^{13}C NMR methyl pentad distribution (see Figure 1) indicated effective control of the CC coupling step by the chiral metal center of the active catalyst[7] (4α-B/$(MeAlO)_x$ 80% mmmm, $\langle m \rangle_{n,\alpha} \approx 24$).

The isotacticity achieved with the homogeneous Ziegler catalysts derived from the conformationally free nonbridged bent metallocene complexes **4** is still smaller and the activities are lower compared to some of the commonly employed *ansa*-metallocene/$(MeAlO)_x$ systems.[3,4,10,11] However, our study shows that there may be only gradual but not principal differences between the two types of catalyst systems. As extensive structural variation can be carried out much more easily with the nonbridged chiral metallocene precursors, we are optimistic that many of the current shortcomings of these new systems will be overcome in the future. From our preliminary studies it appears that enantiomerically pure catalyst systems are easily accessible from these nonbridged systems. We are currently looking for applications of such optically active homogeneous Ziegler systems in the enantioselective catalytic formation of monomeric organic target molecules.

Acknowledgment. Financial support from the Fonds der Chemischen Industrie, the Volkswagen-Stiftung, and the Alfried Krupp von Bohlen und Halbach-Stiftung is gratefully acknowledged.

Supplementary Material Available: Experimental details of the preparation and characterization of compounds **1–4** (10 pages). Ordering information is given on any current masthead page.

(11) See, for example: Collins, S.; Gauthier, W. J.; Holden, D. A.; Kuntz, B. A.; Taylor, N. J.; Ward, D. G. *Organometallics* **1991**, *10*, 2061 and references cited therein.

Oligonucleosides: Synthesis of a Novel Methylhydroxylamine-Linked Nucleoside Dimer and Its Incorporation into Antisense Sequences

Jean-Jacques Vasseur,† Françoise Debart,† Yogesh S. Sanghvi,* and P. Dan Cook

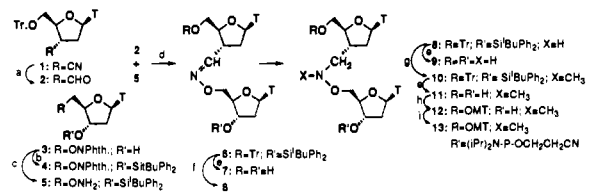
ISIS Pharmaceuticals, 2280 Faraday Avenue Carlsbad, California 92008

Received January 27, 1992

Modulation of gene expression by antisense technologies requires the development of modified oligonucleotides possessing enhanced cellular uptake, resistance toward degradation by nucleases, and appropriate hybridization to target RNAs.[1] These oligonucleotide pharmacokinetic design features are amenable to structure–activity relationship (SAR) studies and lead to antisense oligonucleotides modified in the heterocycle, sugar, phosphodiester linkage, and phosphorus atom.[1] Our research in this area has focused on the development of neutral or positively charged, achiral linkages between the 3′-carbon and the 4′-carbon of the sugars of an oligonucleoside.[2] Linkages of this type would circumvent the chirality problem found with phosphorus-modified oligonucleotides such as methyl phosphonates, phosphorothioates, and phosphoramidates[1h] and may provide resistance to enzymatic cleavage.[1f] Reasonable linkages that one may envisage of this type (four atoms, neutral or positively charged and achiral) would require the replacement of the phosphorus atom in the sugar–phosphate backbone of an oligonucleotide. One-to-one atom replacement of the phosphorus atom has been recently reported.[3] This communication describes the replacement of the anionic 3′-OP-$(O)_2^-$OCH$_2$-5′ linkage in an oligodeoxynucleotide by a neutral 3′-CH$_2$NH(Me)OCH$_2$-5′ linkage.[4]

Retrosynthetic analysis of desired dimer **11** indicated that 3′-deoxy-3′-*C*-formyl-5′-*O*-tritylthymidine (**2**) and 5′-*O*-amino-3′-*O*-(*tert*-butyldiphenylsilyl)thymidine (**5**) would serve as key building blocks (Scheme I). Thus, DIBAL-H reduction of 3′-*C*-cyano-3′-deoxy-5′-*O*-tritylthymidine[5] (**1**) led to the aldehyde **2**.[6] The α-stereochemistry of the 3′-CHO group in **2** was established by ^1H NOE experiments. A Mitsunobu reaction[7] of thymidine with *N*-hydroxyphthalimide resulted in the exclusive formation of 5′-*O*-phthalimidothymidine (**3**). Silylation of **3** followed by treatment with methylhydrazine provided **5**.[6] Hydroxylamine **5** was condensed with aldehyde **2** under acid catalysis to afford oxime dimer **6** (88%, mixture of *E*/*Z* isomers).[8] Dinucleoside **6** was reduced with NaBH$_3$CN/AcOH to provide protected dimer **8**[8] in 78% yield. Reductive alkylation of dimer **8** with HCHO/NaBH$_3$CN/AcOH furnished methylated dimer

Scheme I[a]



[a] (a) DIBAL/THF (55%). (b) *t*-BuPh$_2$SiCl/imidazole/DMF (92%). (c) MeNHNH$_2$/CH$_2$Cl$_2$ (89%). (d) 1.5% AcOH/CH$_2$Cl$_2$ (88%). (e) nBu$_4$NF/THF, 30 min → 0.14 M HCl/MeOH (89% of **7**, 90% of **9**, 87% of **11**). (f) NaBH$_3$CN/AcOH (78%). (g) HCHO/NaBH$_3$CN/AcOH (87%). (h) DMTCl/pyridine (85%). (i) 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite/*N*,*N*-diisopropylethylamine/THF (78%); DMT = 4,4′-dimethoxytrityl; T = thymine.

Table I. Hybridization Data on Oligonucleosides[a]

oligonucleoside	sequence (5′ → 3′)	T_m, °C[b]
i[c]	d(GpCpGpT*TpT*TpT*TpT*TpT*TpGpCpG)	50.8
ii[c]	d(CpTpCpGpTpApCpCpT*TpTpCpCpGpGpTpCpC)	64.9
iii[c]	d(CpTpCpGpTpApCpT*TpT*TpCpCpGpGpTpCpC)	57.3
iv[c]	d(CpGpApCpTpApTpGpCpApApTpT*TpC)	43.6

[a] Oligonucleosides i–iv were hybridized with complement RNA; p = 3′-OP(O)$_2$OCH$_2$-5′; * = 3′-CH$_2$N(Me)OCH$_2$-5′. [b] Absorbance vs temperature profiles were measured at 4 mM of each strand in 100 mM Na$^+$, 10 mM phosphate, 0.1 mM EDTA, pH 7.0 (see ref 11 for details). [c] T_m's of unmodified sequences: **i**, 50.2 °C; **ii**, 63.4 °C; **iii**, 56.3 °C; **iv**, 44.1 °C.

† Visiting Scientists from CNRS (France).

(1) Selected review articles: (a) Goodchild, J. *Bioconjugate Chem.* **1990**, *1*, 165. (b) Uhlmann, E.; Peyman, A. *Chem. Rev.* **1990**, *90*, 543. (c) Hélène, C.; Toulmé, J. J. *Biochim. Biophys. Acta* **1990**, *1049*, 99. (d) Cohen, J. S. *Antiviral Res.* **1991**, *16*, 121. (e) Matteucci, M. D.; Bischofberger, N. *Annu. Rep. Med. Chem.* **1991**, *26*, 287. (f) Cook, P. D. *Anticancer Drug Des.* **1991**, *6*, 585. (g) Chrisey, L. A. *Antisense Res. Develop.* **1991**, *1*, 65. (h) *Gene Regulation: Biology of Antisense RNA and DNA*; Erickson, R. P., Izant, J. G., Eds.; Raven Press: New York, 1992.

(2) We refer to modified oligonucleotides that lack the phosphorus atom in the backbone linkage as oligonucleosides. Designation of the backbone linkage as the moiety that connects the 3′-carbon of one furanosyl ring with the 4′-carbon of another furanosyl ring is generally applicable in describing various backbone linkages.

(3) Tittensor, J. R. *J. Chem. Soc. C* **1971**, 2656. Cormier, J. F.; Ogilvie, K. K. *Nucleic Acids Res.* **1988**, *16*, 4583. Seliger, H.; Feger, G. *Nucleosides Nucleotides* **1987**, *6*, 483. 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* **1991**, *47*, 1547. Quaedflieg, P.; VanDer Marel, G. A.; Kuyl-Yeheskiely, E.; Van Boom, J. H. *Recl. Trav. Chim. Pays-Bas* **1991**, *110*, 435.

(4) The pK_a of H$_3$CON(CH$_3$)$_2$ is 3.65 (Bissot, T. C.; Parry, R. W.; Campbell, D. H. *J. Am. Chem. Soc.* **1957**, *79*, 796), which would indicate that at physiological pH the dinucleoside methylhydroxylamine linkage would be neutral.

(5) Parkes, K. E. B.; Taylor, K. *Tetrahedron Lett.* **1988**, *29*, 2995. We thank Dr. Parkes for providing a preparation of **1**.

(6) All new compounds exhibited satisfactory spectral and analytical and/or exact FAB-MS data.

(7) Mitsunobu, O. *Synthesis* **1981**, 1.

(8) Dimers **6** and **8** were deprotected to give **7** and **9**, respectively, for complete characterization.

0002-7863/92/1514-4006$03.00/0

10 in 87% yield. Deprotection of 10 gave the novel dimer 11 (87%). Sequential dimethoxytritylation and phosphitylation of 11 following standard protocols provided protected dimer 13 in an overall yield of 82%.

Dimer 13 was inserted into a 16-mer standard oligonucleotide sequence [d(GpCpGpTpTpTpTpTpTpTpTpTpGpCpG)] 1-5 times and into antisense sequences in one or two positions (Table I) via phosphoramidite methodology.[9] The tritylated oligonucleosides possessing T*T linkages [* = 3'-$CH_2N(Me)OCH_2$-5'] were purified by reverse-phase HPLC and exhibited a single band on polyacrylamide gel electrophoresis. The structural identity of the oligonucleosides was indirectly confirmed by determining the structure of tetramer TpT*TpT by ^1H and ^{31}P NMR analysis. Furthermore, HPLC analysis of the enzymatic degradation[10] of d(GpCpGpTpTpTpTpT*TpTpTpTpTpGpCpG) indicated the expected ratios of nucleosides and the T*T dimer.

Hybridization studies indicated that incorporation of 1-5 modified linkages into the standard sequence had remarkably little effect on the stability of the duplexes formed between the oligonucleosides and their RNA complement (average ΔT_m/modification = -0.3 °C compared to the parent DNA:RNA duplex; data not shown).[11] Moreover, the studies suggest that the uniform distribution of T*T (oligonucleoside i) provided a more stable oligonucleoside/RNA duplex (ΔT_m/modification = +0.1 °C). The antisense sequences ii and iii with one or two linkage changes were slightly stabilized compared to their unmodified parent oligonucleotide. On examination of the base pair specificity of the 5'-T of the T*T dimer in ii, it was found that when matched to A in the RNA complement (T-rA) the duplex was more stable than duplexes having thymine mismatched with cytosine, guanine, or uracil (ΔT_m: T-rC, -10.1 °C; T-rG, -3.9 °C; T-rU, -10.3 °C). The average ΔT_m/mismatch (-7.3, ±3.4) was greater than the average ΔT_m/mismatch (-5.5, ±3.3) of the duplexes with thymine in the unmodified parent DNA against its mismatches in the RNA complement. These data indicate that the Watson-Crick base pair specificity of oligonucleosides containing T*T dimers is as good as or better than wild type DNA. Nuclease resistance study in HeLa cellular extracts showed that the half-life of full-length oligonucleoside i was 16 h, whereas the unmodified parent oligonucleotide had a $T_{1/2}$ of 0.5 h. The 3'-capped oligonucleoside iv had a $T_{1/2}$ of 14 h in 10% fetal calf serum.[12]

The synthesis of a T*T dimer possessing an achiral, neutral linkage replacing the negatively charged phosphodiester moeity of a natural oligonucleotide has been accomplished. Certain T*T-containing oligonucleosides were synthesized and were found to hybridize to their complementary RNAs as effectively as the unmodified parent DNAs. These oligonucleosides exhibit significant resistance to nucleases while maintaining a high level of base pair specificity.

Acknowledgment. We thank Maryann Zounes for performing the automated synthesis of oligonucleosides, Drs. Susan Freier and Elena Lesnik for hybridization studies, Drs. Glenn Hoke and Lendell Cummins for nuclease studies, and Patrick Wheeler for NMR studies.

Supplementary Material Available: Synthetic procedures and listings of spectroscopic and analytical data for compounds **1-5, 7, 9, 11,** and **13** (5 pages). Ordering information is given on any current masthead page.

(9) Oligonucleoside was synthesized on an ABI 380 B DNA synthesizer following the standard protocol (the average coupling efficiency for the T*T dimer in i was 98.6% and the overall yield was 86.7%).

(10) Oligonucleoside was digested (~90 h) with a mixture of spleen phosphodiesterase, snake venom phosphodiesterase, and bacterial alkaline phosphatase.

(11) See for experimental details: Freier, S. M.; Kierzek, R.; Jaeyer, J. A.; Sugimoto, N.; Caruthers, M. H.; Neilson, T. *Proc. Natl. Acad. Sci. USA* **1986**, *83*, 9373. Breslauer, K. J.; Frank, R.; Blocker, H.; Marky, L. A. *Proc. Natl. Acad. Sci. USA* **1991**, *88*, 3746.

(12) See for experimental details: Hoke, G. D.; Draper, K.; Freier, S. M.; Gonzalez, C.; Driver, V. B.; Zounes, M. C.; Ecker, D. J. *Nucleic Acids Res.* **1991**, *19*, 5743.

Synthesis and Radical-Induced Ring-Opening Reactions of 2'-Deoxyadenosine-2'-spirocyclopropane and Its Uridine Analogue. Mechanistic Probes for Ribonucleotide Reductases[1]

Vicente Samano and Morris J. Robins*

Department of Chemistry, Brigham Young University Provo, Utah 84602

Received February 6, 1992

Ribonucleoside di- and triphosphate reductases are metaloenzymes that catalyze the reduction of ribonucleotides to their 2'-deoxy-DNA components.[2] Inhibition of these reductases interferes with the replication of genetic material required for cancer cell division or viral genome biosynthesis. Stubbe and co-workers[3] have pursued elegant and extensive studies on molecular mechanisms of action of these enzymes. The first step in her working hypothesis involves the enzymatic removal of the H3' atom from a nucleoside 5'-di- or triphosphate to give a C3' radical intermediate. This radical then undergoes conversion into the corresponding 2'-deoxynucleotide via a series of enzyme-mediated steps that culminate in the return of the initially abstracted H3' atom to C3' with concomitant regeneration of the biological radical initiator.[3] Indirect evidence for the involvement of such radical species has been obtained by studies with isotopically labeled substrates and mechanism-based inhibitors.[3,4] However, direct attempts to observe the involvement of radicals in the dynamic enzyme process have been unsuccessful.

Ring opening of cyclopropylcarbinyl radicals to the corresponding 3-butenyl radicals occurs extremely rapidly. This radical clock[5a] has been used as a mechanistic probe to implicate radical intermediates in reaction pathways by detection of ring-opened products.[5b,c] The enhanced rates of ring opening of rigid spiro cyclopropylcarbinyl radicals have been attributed to the greater relief of ring strain and more favorable orbital alignment.[6] These considerations guided our design[7] of 2'-deoxynucleoside-2'-spirocyclopropanes as novel mechanistic probes for ribonucleotide reductases. We now describe the synthesis of 2'-deoxyadenosine-2'-spirocyclopropane (**5a**) and 2'-deoxyuridine-2'-spirocyclopropane (**5b**), their conversion to the thionoester (**7a** and **7b**) precursors of cyclopropylcarbinyl radicals, and the characterization of the respective 3-butenyl (**8** and **10**) and cyclonucleoside (**9** and **11**) ring-opening products.

Simmons-Smith[8] and related carbenoid methods for the synthesis of cyclopropanes failed to give the desired spirocyclopropyl nucleoside analogues. Treatment of 3',5'-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2'-deoxy-2'-methyleneadenosine[9] (**1a**) with excess diazomethane in diethyl ether for 48 h at ambient temperature gave a separable mixture of the microcrystalline

(1) Nucleic Acid Related Compounds. 75. Part 74: Robins, M. J.; Samano, V.; Zhang, W.; Balzarini, J.; De Clercq, E.; Borchardt, R. T.; Lee, Y.; Yuan, C.-S. *J. Med. Chem.*, in press.

(2) (a) Hogenkamp, H. P. C.; Sando, G. N. *Struct. Bonding (Berlin)* **1974**, *20*, 23-58. (b) Thelander, L.; Reichard, P. *Annu. Rev. Biochem.* **1979**, *48*, 133-158. (c) Lammers, M.; Follmann, H. *Struct. Bonding (Berlin)* **1983**, *54*, 27-91.

(3) Ashley, G. W.; Stubbe, J. In *Inhibitors of Ribonucleoside Diphosphate Reductase Activity*. International Encyclopedia of Pharmacology and Therapeutics, Section 128; Cory, J. G., Cory, A. H., Eds.; Pergamon Press: New York, 1989; pp 55-87 and references therein.

(4) Baker, C. H.; Banzon, J.; Bollinger, J. M.; Stubbe, J.; Samano, V.; Robins, M. J.; Lippert, B.; Jarvi, E.; Resvick, R. *J. Med. Chem.* **1991**, *34*, 1879-1884.

(5) (a) Griller, D.; Ingold, K. U. *Acc. Chem. Res.* **1980**, *13*, 317-323. (b) Beckwith, A. L. J.; Ingold, K. U. In *Rearrangements in Ground and Excited States*; De Mayo, P., Ed.; Academic Press: New York, 1980; Vol. I, Essay 4, pp 227-237. (c) Suckling, C. J. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 537-552.

(6) Roberts, C.; Walton, J. C. *J. Chem. Soc., Perkin Trans. 2* **1985**, 841-846.

(7) We thank Professor J. Stubbe for stimulating discussions.

(8) Simmons, H. E.; Cairns, T. L.; Vladuchick, S. A.; Hoiness, C. M. *Org. React.* **1973**, *20*, 1-131.

(9) Samano, V.; Robins, M. J. *J. Org. Chem.* **1991**, *56*, 7108-7113.

0002-7863/92/1514-4007$03.00/0