and J. A. Ibers, *Coord. Chem. Rev.* **100**, 223 (1990); M. G. Kanatzidis, *Comments Inorg. Chem.* **10**, 161 (1990).
11. P. Böttcher, *Angew. Chem. Int. Ed. Engl.* **27**, 759 (1988); J. Rouxel, *Acc. Chem. Res.* **25**, 328 (1992); S. A. Sunshine, D. A. Keszler, J. A. Ibers, *ibid.* **20**, 395 (1987); S. Dhingra and M. G. Kanatzidis, *Science* **258**, 1769 (1992).
12. M. G. Kanatzidis, *Chem. Mater.* **2**, 353 (1990).
13. _____ and Y. Park, *J. Am. Chem. Soc.* **111**, 3767 (1989).
14. D. B. Brown *et al.*, *Inorg. Chem.* **19**, 1945 (1980).
15. Y. Park and M. G. Kanatzidis, *Angew. Chem. Int. Ed. Engl.* **29**, 914 (1990).
16. R. Cava *et al.*, *Nature* **333**, 814 (1988).
17. W. K. Ham, G. F. Holland, A. M. Stacy, *J. Am. Chem. Soc.* **110**, 5214 (1988).
18. C. C. Torardi, M. A. Subramanian, J. Gopalakrishnan, A. W. Sleight, *Physica C* **158**, 465 (1989); M. A. Subramanian *et al.*, *Science* **240**, 495 (1988); H. Snibita, K. Kinoshita, T. Yamada, *Physica C* **170**, 411 (1990).
19. S. L. Stoll, C. C. Torardi, A. M. Stacy, in preparation.
20. R. J. Cava, *Science* **247**, 656 (1990).
21. J. D. Jorgensen *et al.*, *Phys. Rev. B* **36**, 3606 (1987).
22. D. Dimos, P. Chaudhari, H. Mannhart, *ibid.* **41**, 4093 (1990).
23. T. J. Richardson and L. C. deLonghe, *J. Mater. Res.* **5**, 2066 (1990).
24. D. Dimos, P. Chaudhari, H. Mannhart, F. K. LeGoues, *Phys. Rev. Lett.* **61**, 219 (1988).
25. L. N. Marquez, S. W. Keller, M. Fendorf, A. M. Stacy, in preparation.
26. M. J. Annen, M. E. Davis, J. B. Higgins, J. L. Schlenker, *J. Chem. Soc. Chem. Commun.* **1991**, 1175 (1991); M. J. Annen and M. E. Davis, in *Synthesis of Microporous Materials*, vol. 1, *Molecular Sieves*, M. L. Occelli and H. E. Robson, Eds. (Van Nostrand Reinhold, New York, 1992), pp. 349–358.
27. G. O. Brunner and W. M. Meier, *Nature* **337**, 146 (1989).
28. C. T. Kresge, M. E. Leonowicz, W. J. Roth, J. C. Vartuli, J. S. Beck, *ibid.* **359**, 710 (1992); C. T. Kresge *et al.*, *J. Am. Chem. Soc.* **114**, 10834 (1992).
29. G. D. Stucky and J. E. Mac Dougall, *Science* **247**, 669 (1990); G. A. Ozin, A. Kuperman, A. Stein, *Angew. Chem. Int. Ed. Engl.* **28**, 359 (1989); G. A. Ozin, *Adv. Mater.* **4**, 612 (1992); D. Rong *et al.*, *Coord. Chem. Rev.* **97**, 237 (1990); G. D. Stucky, *Progr. Inorg. Chem.* **40**, 99 (1992).
30. P. Enzel and T. Bein, *J. Phys. Chem.* **93**, 6270 (1989); *J. Chem. Soc. Chem. Commun.* **1989**, 1326 (1989); T. Bein and P. Enzel, *Angew. Chem. Int. Ed. Engl.* **28**, 1692 (1989); *Mol. Cryst. Liq. Cryst.* **181**, 315 (1990).
31. J. S. Krueger, J. E. Mayer, T. E. Mallouk, *J. Am. Chem. Soc.* **110**, 8232 (1988); Z. Li, C. Lai, T. E. Mallouk, *Inorg. Chem.* **28**, 178 (1989); D. Rong, Y. I. Kim, T. E. Mallouk, *ibid.* **29**, 1531 (1990).
32. R. C. Haushalter and L. A. Mundi, *Chem. Mater.* **4**, 31 (1992).
33. V. Soghomonian, Q. Chen, R. C. Haushalter, J. Zubieta, *Angew. Chem.*, in press.
34. R. Marchand, L. Brohan, M. Tournoux, *Mater. Res. Bull.* **15**, 1129 (1980).
35. M. Latroche, L. Brohan, R. Marchand, M. Tournoux, *J. Solid State Chem.* **81**, 78 (1989).
36. M. S. Whittingham and A. J. Jacobson, Eds., *Intercalation Chemistry* (Academic Press, London, 1982); M. S. Dresselhaus, *Mater. Sci. Eng. B* **1**, 259 (1988); J. Rouxel, *C. R. Acad. Sci. Ser. III Sci. Vie* **6**, 23 (1989); D. W. Murphy, in *Advances in the Synthesis and Reactivity of Solids*, T. E. Mallouk, Ed. (JAI Press, London, 1991), vol. 1, pp. 237–272.
37. X.-D. Xiang *et al.*, *Nature* **348**, 145 (1990); X.-D. Xiang *et al.*, *Phys. Rev. B* **43**, 11496 (1991); X.-D. Xiang *et al.*, *Science* **254**, 1487 (1991).
38. V. W. Day, W. G. Klemperer, V. V. Mainz, D. M. Millar, *J. Am. Chem. Soc.* **107**, 8262 (1985); P. A. Agaskar, V. W. Day, W. G. Klemperer, *ibid.* **109**, 5554 (1987).
39. F. J. Feher, T. A. Budzichowski, K. J. Weller, *ibid.* **111**, 7288 (1989); F. J. Feher, K. J. Weller, J. W. Ziller, *ibid.* **114**, 9686 (1992).
40. B. F. Hoskins and R. Robson, *ibid.* **112**, 1546 (1990); R. W. Gable, B. F. Hoskins, R. Robson, *J. Chem. Soc. Chem. Commun.* **1990**, 1677 (1990); B. F. Abrahams, B. F. Hoskins, R. Robson, *J. Am. Chem. Soc.* **113**, 3606 (1991); S. R. Batten, B. F. Hoskins, R. Robson, *J. Chem. Soc. Chem. Commun.* **1991**, 445 (1991).
41. P. Brandt, A. K. Brimah, R. D. Fischer, *Angew. Chem. Int. Ed. Engl.* **27**, 1521 (1988); U. Behrens *et al.*, *Organometallics* **11**, 1718 (1992).
42. C. M. Bell, S. W. Keller, V. M. Lynch, T. E. Mallouk, *Mater. Chem. Phys.*, in press.
43. We thank R. Haushalter and A. Stacy for communicating results to us before publication. This work was supported by grants from the National Science Foundation (CHE-9217719) and the Robert A. Welch Foundation. A.S. acknowledges support of a postdoctoral fellowship by the National Sciences and Engineering Research Council of Canada. T.E.M. also thanks the Camille and Henry Dreyfus Foundation for support in the form of a Teacher-Scholar Award.

Phosphorodithioate DNA as a Potential Therapeutic Drug

W. S. Marshall* and M. H. Caruthers†

This article summarizes methods for the synthesis of phosphorodithioate-linked deoxyoligonucleotides and details an analysis of one of the distinctive properties of phosphorodithioate DNA oligomers, their ability to strongly inhibit human immunodeficiency virus type–1 reverse transcriptase (HIV-1 RT). Mechanistic studies indicate that oligomers of this type interfere with enzyme function by binding tightly to the active site for primer-template, which results in low or subnanomolar inhibitory constants. Although many of these studies have used deoxyoligocytidine analogs, a rationally designed approach has led to the discovery of a very active phosphorodithioate deoxyoligonucleotide inhibitor. This type of inhibitor, which binds strongly to the primer-template active site of HIV-1 RT, provides another type of potential therapeutic agent against HIV-1.

The classical approach for discovering new drugs, such as 3′-α-azido-2′,3′-dideoxythymidine (AZT) for treating the debilitating consequences of acquired immunodeficiency syndrome (AIDS), involves the use of massive screening programs (*1*). A number often quoted is that on average 10,000 compounds must be tested in order to identify one new, biologically active substance. These are formidable statistics that have led many researchers to focus on more rational drug design strategies. The most successful have been those based upon understanding the biology, genetics, and biochemistry associated with a particular disease state. As a result of these efforts, many small organic molecules have been identified that inhibit cholesterol biosynthesis (*2*), nucleotide metabolism (*3*), and other disease-associated biological systems. Additionally, by using recombinant DNA technologies in association with a thorough understanding of the appropriate biology, proteins such as erythropoietin (*4*), granulocyte–colony-stimulating factor (*5*), insulin (*6*), and human growth hormone (*7*) have been shown to be effective for several therapeutic applications.

Primarily because of inherent disadvantages in these approaches such as the cost and uncertainties associated with massive screening programs and the instability of proteins, new drug design strategies have been explored. Two of these are based on the assumption that new drugs will interact with important binding sites on proteins or active sites of enzymes and thereby block the development of a specific disease state. For example, perhaps a molecule can be designed that binds within the deep clefts found on the surface of rhino- and picornoviruses and inhibits interactions of these viruses with host cells (*8*). These two strategies differ in how new drugs are identified. One uses the three-dimensional structure of a target protein in combination with computer modeling to identify potential drug candidates (*9*). The other, which does not require knowledge of tertiary structure, is based upon the vast information content of amino acid or nucleotide (nt) sequences. Libraries of completely random peptides or oligonucleotides are prepared from the monomers and then, through various selection protocols, highly specific, biologically active molecules are identified as potential drug candidates (*10–12*). Although these strategies appear very promising, success is still limited to the laboratory as clinical and even animal studies remain to be done.

An alternative new approach is to use the information content of the nucleic acids and knowledge of their interactions with

The authors are in the Department of Chemistry and Biochemistry, University of Colorado, Boulder, CO 80309.

*Present address: Amgen, Inc., Boulder, CO 80301.
†To whom correspondence should be addressed.

proteins or complementary polynucleotides as the basis for a new class of DNA therapeutic drugs. The elegance of this approach is simply that the structure of the potential drug is already known. For example, many proteins interact with high specificity to particular polynucleotide sequences. If the polynucleotide sequence is known for a drug-targeted protein, it may be possible to synthesize the appropriate oligonucleotide and use it as a drug to control an important biological function. Perhaps the first example of this type of regulation (albeit in a nontherapeutic drug setting) involved the use of multiple synthetic copies of the *lac* operator as part of a bacterial plasmid to derepress the *Escherichia coli lac* operon (*13–15*).

Oligonucleotides can also bind to target DNA or RNA through Watson-Crick or Hoogsteen and reverse Hoogsteen base-pairing. In the former case, the target is single-stranded RNA or DNA, whereas for the latter, it is the major groove of double-stranded DNA. Thus the complementary, or antisense, base sequence interacts by hydrogen bonding with a segment of cellular RNA or DNA to inhibit gene expression. If the cellular target for these antisense oligonucleotides is an oncogene or a viral gene, then the possibility exists that synthetic oligonucleotides could have therapeutic potential (*16–18*).

Specific inhibition of gene expression in cell culture by synthetic deoxyoligonucleotide was initially reported by Zamecnik and Stephenson (*19*). Following these early observations, much of the ensuing work with oligonucleotides established the principle of the approach and demonstrated the effect in model systems. Soon it became apparent that oligonucleotides functioning in an antisense manner could be used to map the biological function of various genes and to understand the molecular biology of gene expression. At the same time, the therapeutic potential of oligonucleotides became apparent. Today it is well established that oligonucleotides do indeed have antiviral activity and demonstrate an antiproliferative effect by blocking oncogenes and other cellular genes. The rapid expansion of this field has led to many excellent reviews on the chemistry and biology associated with the use of oligonucleotides as potential therapeutic drugs (*16–18, 20–26*).

Initial attempts to inhibit gene expression focused on normal deoxyoligonucleotides with phosphodiester linkages. However, several problems soon became apparent, including the rapid degradation of normal DNA by cellular nucleases, transport of deoxyoligonucleotides into cells and the proper cellular compartment, targeting of oligomers to the appropriate cell type within a living organism, and selection of the proper annealing site on the target nucleic acid. In order to meet at least some of these challenges, much effort has been directed toward various DNA analogs—specifically derivatives having phosphorus-modified internucleotide linkages because of their resistance to cellular nucleases (*17, 20, 27, 28*). An added feature is that derivatization at phosphorus is sequence nonspecific, so that the high degree of specificity encoded by the four nucleotide bases could be preserved.

Phosphorus-Modified DNA Analogs

Single substitutions at phosphorus. Perhaps the most studied modifications to date are the methylphosphonate, phosphorothioate, and phosphoramidate internucleotide linkages (Fig. 1), where a single, nonbridging oxygen atom is replaced with a methyl group, sulfur atom, or substituted amine group, respectively. All of these modifications generate certain desirable oligomer characteristics such as resistance towards nucleases and retention of the ability to form stable duplexes with natural RNA or DNA. Therefore, any of these derivatives could be used as steric blocks against gene expression. The phosphorothioate derivative also retains the ability to stimulate endogenous cellular ribonuclease (RNase) H activity, which allows for the specific degradation of the target RNA in hybrid duplexes. This very important property permits a deoxyoligonucleotide to act pseudo-

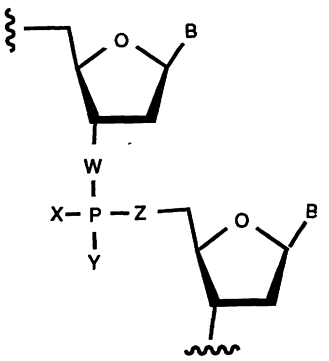

Fig. 1. Schematic of the DNA internucleotide linkage and its phosphorous-modified derivatives: W, X, Y, and Z = O, phosphodiester; W, X, and Z = O and Y = CH_3, methylphosphonate; W, X, and Z = O and Y = S, phosphorothioate; W, X, and Z = O and Y = NR_2, phosphoramidate, where R = alkyl or aryl substituents; W = S and X, Y, and Z = O, 3′-*S*-phosphorothioate; W = NH and X, Y, and Z = O, 3′-amino-phosphoramidate; W, X, and Y = O and Z = NH, 5′-amino-phosphoramidate; W, X, and Y = O and Z = CH_2, 5′-methylenephosphonate; and W and Z = O and X and Y = S, phosphorodithioate. Pyrimidine or purine base is denoted as B.

catalytically against many copies of a deleterious RNA. The utility of these derivatives is clearly demonstrated in the many potential antisense applications reported for deoxyoligonucleotides containing such modifications (*16–18, 20–28*). In addition, phosphorothioate analogs appear to exert effects independent of annealing that may be therapeutically beneficial against human immunodeficiency virus–type 1 (HIV-1) and herpes simplex virus (*1, 16*). Recently, favorable pharmacokinetic studies in animals have demonstrated that phosphorothioate analogs are rapidly dispersed to various tissues following uptake and cleared, primarily through the kidney, within acceptable time periods (*29, 30*).

There are, however, potential problems with these two analogs. Because the phosphorothioate derivative is partially susceptible to nucleases, the mononucleotide phosphorothioate degradation product could be incorporated into genomic DNA as it is known that the mononucleotide α-thiotriphosphates are substrates for DNA polymerases (*31*). These mononucleotide thiophosphates could also alter cellular metabolism by influencing the intracellular concentration of mononucleotide pools. A further complication arises because the phosphorus center is rendered chiral by phosphorothioate or methylphosphonate substitutions. For a deoxyoligonucleotide with n singly modified internucleotide linkages, there are 2^n stereoisomers with variable biophysical and biochemical properties. Recent attempts to overcome this problem have focused on developing chemistries that are either stereoselective and generate oligomers enriched in one diastereomer (*32, 33*) or are designed to synthesize new achiral phosphorus analogs.

Singly substituted, achiral phosphodiester analogs can be synthesized by introducing a chemical modification at a bridging position (W or Z in Fig. 1). Bridged derivatives such as the 3′ thiol (*34*), 3′ and 5′ amino (*35*), and 5′ methylene (*36*) deoxyoligonucleotide analogs have been synthesized and initially characterized. Another approach is to replace the phosphodiester bridge by an entirely different group. So far, a plethora of derivatives have been conceived, synthesized, and, in some cases, characterized relative to biophysical and biochemical properties. These include joining the nucleosides or nucleoside bases through siloxanes, methylene, carbonates, sulfates, sulfonamides, carbamates, amino acids, and several other linkages (*20, 37*). Although in some cases the biochemical results with these analogs appear promising, much work remains before their utility can be properly assessed.

Phosphorodithioate-linked deoxyoligonucleotides. Our research has focused on the development of achiral, closely related

mimics of natural DNA. A particularly promising derivative has a phosphorodithioate internucleotide bridge. Deoxyoligonucleotide phosphorodithioates are DNA analogs in which the internucleotide phosphodiester linkages have been replaced with dithiophosphodiesters (Fig. 1) (*38*). Replacement of the two nonbridging oxygen atoms with sulfur leads to a novel biopolymer that is isostructural and isopolar to natural phosphodiester containing DNA. Like natural DNA, it is achiral at phosphorus, but the introduction of the two sulfur moieties imparts useful properties to deoxyoligonucleotides. Recent research has demonstrated that this analog is completely resistant to nuclease degradation (*39–41*) and that oligomers bearing the modification form stable duplexes with unmodified nucleic acids (*42*). Importantly, dithioate DNA directs RNase H–mediated degradation of RNA in hybrid duplexes (*42*). In addition to these physicochemical and biochemical properties, dithioate deoxyoligocytidine analogs are active inhibitors of HIV-1 reverse transcriptase (RT) and viral replication (*43*). Further, recent results for phosphorodithioate deoxyoligonucleotides, which are designed as antisense agents against viral gene expression, indicate that they can specifically interfere with HIV-1 mRNA expression (*44*). Thus, the possibility that dithioate DNA oligomers may interfere with viral replication at several stages of the HIV-1 life cycle enhances their therapeutic potential. The mechanism of HIV-1 RT inhibition by deoxyoligocytidine phosphorodithioates and a rational search for more active inhibitors of HIV-1 RT is further described below. Because of these encouraging results, there have been numerous synthetic efforts directed toward the improvement of the chemistry used in synthesizing this analog. The current best method utilizes a silica-based support and deoxynucleoside phosphorothioamidites as synthons (*45–47*).

Preparation of 2′-deoxynucleoside 3′-phosphorothioamidites. Routine synthesis of deoxyoligonucleotide phosphorodithioates required the development of procedures that yield 2′-deoxynucleoside 3′-phosphorothioamidites in high yield and free of impurities. Appropriate protection of the sulfur moiety to reduce levels of phosphorothioate contamination in the polymer products was originally achieved with the 2,4-dichlorobenzyl group (*46*); however, our current method uses the β-thiobenzoylethyl, which can be removed during normal ammonolysis (*47*). Phosphitylation of the appropriately protected nucleoside was achieved with tris(pyrrolidino)phosphine under tetrazole catalysis to afford the bis(pyrrolidino)phosphite intermediate. Without isolation, this intermediate was converted to the phosphorothioamidite product by treatment with monobenzoylethanedithiol and additional tetrazole. After an aqueous work-up to remove tetrazole and tetrazolide salts, the 2′-deoxynucleoside 3′-phosphorothioamidite was isolated by precipitation from heptane in very good yields (85 to 90%). The precipitation step removes excess thiol and phosphines while yielding the phosphorothioamidite synthons as stable powders.

Synthesis of phosphorodithioate DNA. The solid-phase synthesis of phosphorodithioate DNA oligomers is similar to conventional procedures for preparing deoxyoligonucleotides (*48*) but there are a few important modifications (Fig. 2A). Following detritylation of a 2′-deoxynucleoside linked to controlled pore glass, the 2′-deoxynucleoside 3′-phosphorothioamidite synthons are coupled to the growing oligomers on the support by conventional tetrazole activation. The resulting thiophosphite triester is oxidized with elemental sulfur to the desired phosphorodithioate triester. Unreacted, support-linked 2′-deoxynucleoside is then capped by acylation with acetic anhydride, which completes the cycle for addition of one nucleotide. By using a pyrrolidine amide leaving group in the phosphorothioamidite synthon, the coupling rates are comparable to those observed with conventional 2′-deoxynucleoside 3′-diisopropylphosphoramidites. With slight modifications to the standard synthesis cycles, oligomers bearing both phosphodiesters and phosphorodithioates in any combination can be synthesized on automated DNA synthesizers.

Upon completion of the automated synthesis, ammonolysis converts the phosphorodithioate triester to the corresponding diester (Fig. 2B), removes the base-labile protecting groups, and cleaves the deoxyo-

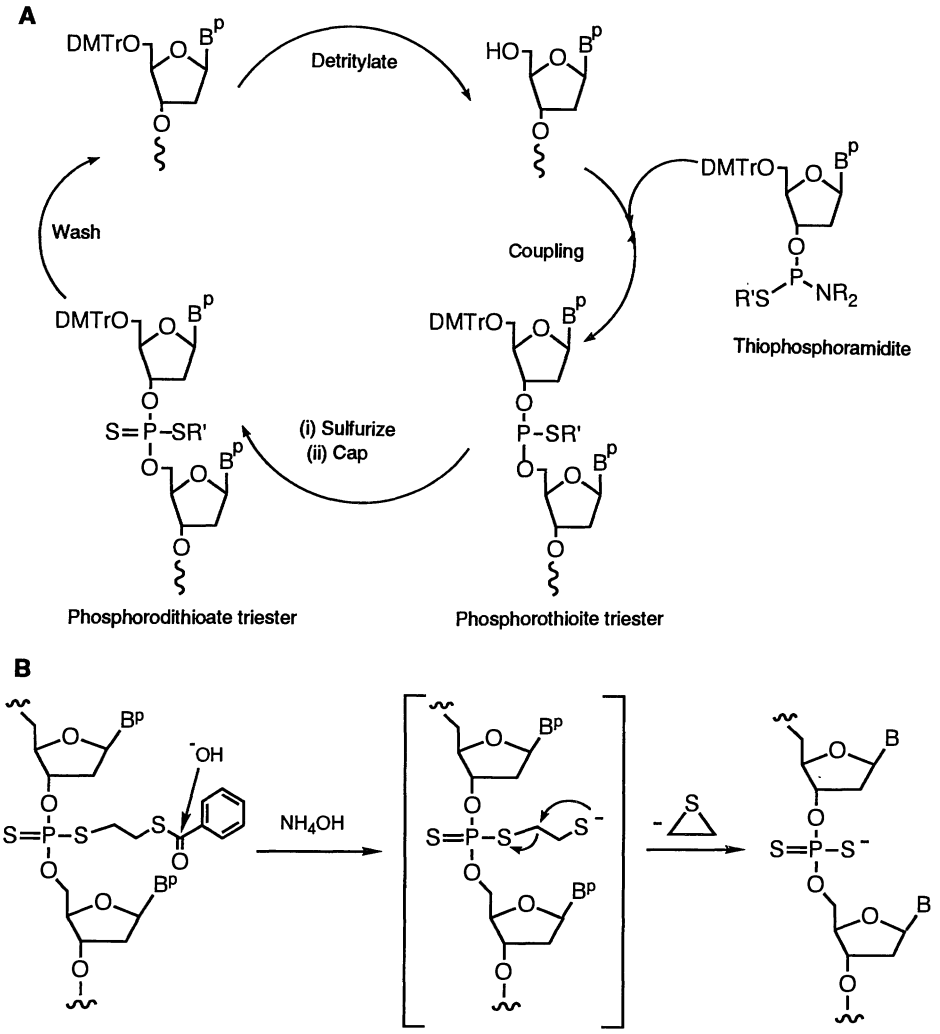

Fig. 2. (**A**) Synthesis cycle allowing for the production of phosphorodithioates during automated DNA synthesis: DMTr, dimethoxytrityl; NR_2, pyrolidino; and R′, 2,4-dichlorobenzyl (*46*), β-cyanoethyl (*45*), or β-thiobenzoyl (*47*); BP is an appropriately protected purine or pyrimidine base. (**B**) Deprotection mechanism of the β-thiobenzoyl sulfur protecting group during postsynthesis treatment with concentrated ammonium hydroxide.

ligonucleotide from the support. The mechanism by which the β-thiobenzoyl group is removed during ammonolysis involves a selective nucleophilic displacement similar to that observed for the 2,4-dichlorobenzyl group with thiophenolate. The selectivity of this reaction yields the same low levels of phosphorothioate contamination (2 to 5%) seen with the 2,4-dichlorobenzyl group. The β-thiobenzoylethyl can be considered a protected protecting group. Ammonolysis leads to rapid deacylation of the pendant thiol to yield a thiolate intermediate which, through intramolecular nucleophilic displacement, then causes elimination of ethylene sulfide to give the deprotected phosphorodithioate.

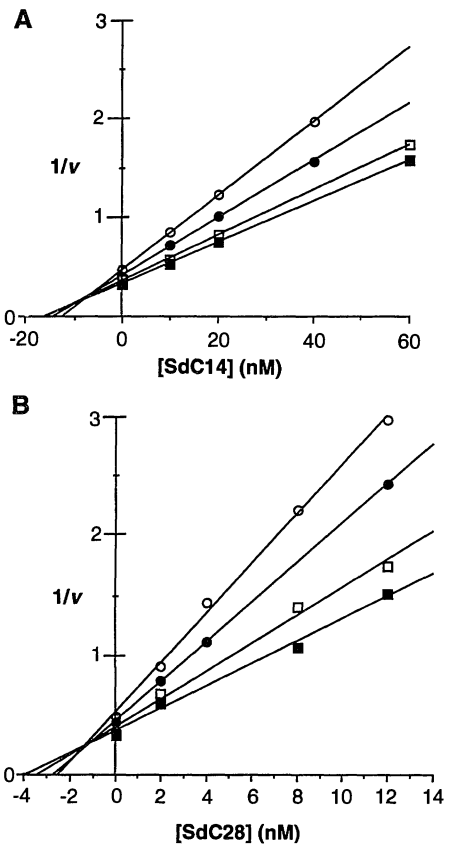

Fig. 3. Dixon plots obtained from kinetic analysis of inhibition of HIV-1 RT with respect to primer-template (PT). Determinations of K_i were similar to ID_{50} measurements (*43*) except that [PT] was simultaneously varied at the same time as inhibitor (*49*). Determination of the type of inhibition and K_i was conducted between 0.2 to 0.4 μM primer-template at constant [dNTP] = 0.25 mM. HIV-1 RT was present at ~2 nM, and the inhibitor concentration was varied in the range of K_i. Reported values are the result of simultaneous independent triplicate determinations and carry associated errors of ~35%. (**A**) Inhibition by tetradecadeoxycytidine ($S2dC_{14}$); $1/v$ has units of 10^{-3} μM^{-1} min μl^{-1}. Figure symbols: ○, [PT] = 0.2 μM; ●, [PT] = 0.25 μM; □, [PT] = 0.3 μM; and ■, [PT] = 0.4 μM. (**B**) Inhibition by a phosphorothioate deoxycytidine 28-nt oligomer (SdC_{28}). Figure symbols: ○, [PT] = 0.2 μM; ●, [PT] = 0.25 μM; □, [PT] = 0.3 μM; and ■, [PT] = 0.4 μM.

Inhibition of HIV-1 Reverse Transcriptase

Mechanism of inhibition of HIV-1 RT by dithioate DNA. We previously reported that phosphorodithioate-linked deoxyoligocytidine analogs are potent inhibitors of HIV-1 RT and other prokaryotic and eukaryotic DNA polymerases (*43*). To help better understand the nature of this inhibition, a kinetic analysis of enzyme inhibition was conducted to determine the mechanism by which phosphorodithioate oligomer analogs inhibit HIV-1 RT. Dixon plots of inhibitor concentration versus $1/v$ (where v is the reaction velocity) were used at a variety of substrate concentrations. For competitive inhibitors, this type of analysis will result in a family of lines all intersecting at the point $(-K_i, 1/V_{max})$, where K_i is the inhibition constant and V_{max} is the maximum velocity. Noncompetitive inhibitors give a family of lines intersecting at $(-K_i, 0)$ (*49*). This analysis was used to determine the pattern of inhibition with respect to primer-template for a number of deoxyoligocytidine analogs as well as the values for K_i.

A Dixon plot obtained for dithioate tetradecadeoxycytidine ($S2dC_{14}$) inhibition at a number of concentrations of primer-template is shown in Fig. 3A. This result clearly indicates that the oligomer is binding to HIV-1 RT in a manner that precludes binding of natural substrate, suggesting a competitive mechanism of inhibition with respect to primer-template. The resulting K_i value is 9.6 nM. A similar determination with a phosphorothioate deoxyoligocytidine of 28 nucleotides (SdC_{28}) is shown in Fig. 3B. It also gives a competitive pattern of inhibition with respect to primer-template (K_i = 1.8 nM), agreeing with an earlier report of competitive inhibition and a K_i of 2.8 nM (*50*). Values of K_i determined at two different oligomer lengths for phosphorothioate (S) and phosphorodithioate (S2) deoxyoligocytidine are summarized in Table 1. Interestingly, previous observations that thioate and dithioate oligomers of long lengths become more similar in their inhibitor properties (*43*) is confirmed in measurements of K_i. The inhibitory constants measured for all of the oligomers are quite low and indicate strong binding to HIV-1 RT (for competitive inhibitors, K_i is equal to the equilibrium dissociation constant K_d).

Table 1. Inhibitory constants measured for a number of dithioate deoxyoligonucleotide analogs with respect to primer template. Competitive inhibition was seen in each case. Inhibition constants are from independent triplicate experiments with associated errors of ~35% of reported values.

Deoxyoligonucleotide	K_i (nM)
$S2dC_{14}$	9.6
$S2dC_{28}$	1.3
SdC_{15}	180
SdC_{28}	1.8
$S2dKt_{14}$*	0.5
$S2dKR_{14}$*	4.0

*See Table 2 for sequences of oligomers.

Because HIV-1 RT is a bireactant enzyme, in order to complete the kinetic analysis the pattern of inhibition with respect to deoxynucleotide triphosphates (dNTPs) was determined for $S2dC_{14}$ by using a similar analysis (data not shown). In this case, the pattern of inhibition appears to be noncompetitive with an apparent K_i = 30 nM. The noncompetitive nature of the inhibition with respect to this substrate indicates that the dithioate oligomer is binding specifically to the primer-template active site. Although the K_i value differs somewhat from that observed for inhibition with respect to primer-template, it is important to remember that an observed inhibition constant in this case will be affected by the fixed concentration of primer-template because $S2dC_{14}$ appears to inhibit the enzyme competitively with respect to primer-template.

The results of the kinetic analysis suggest that both thioate and dithioate deoxyoligocytidine inhibit HIV-1 RT by specifically binding to the active site for primer-template, thereby precluding the binding of the natural substrate and subsequent polymerization. We recently verified this observation by using a gel mobility shift assay to directly measure binding of S2dC14 to HIV-1 RT. The results of such an analysis show that the oligomer binds to HIV-1 RT with K_d = 6.3 nM. The measured K_d value is essentially the same (within experimental error) as the reported K_i value (Table 1) and further indicates that phosphorodithioate oligomers are competitive inhibitors that bind to the enzyme's active site for primer-template.

Rational design of a potent deoxyoligonucleotide inhibitor of HIV-1 RT. The discovery of more effective dithioate hetero-deoxyoligonucleotide inhibitors of HIV-1 RT is complicated by the sheer size of the search base. Using the four bases normally found in DNA, a 14-nt analog can be arranged in ~270 million combinations. An antiviral screening of such proportions represents a formidable task to even the most prolific chemist. We therefore attempted to search for potent dithioate deoxyoligonucleotide inhibitors using a more directed approach.

In the course of the HIV-1 life cycle,

Table 2. ID_{50} values measured for 3′ end lysine tRNA oligomers, polypurine tract primer, and lysine tRNA anticodon region analogs. Half-inhibition values (ID_{50}) were measured as described in (*43*). Briefly, inhibition by deoxyoligonucleotides of HIV RT–catalyzed repair synthesis is measured on a synthetic DNA primer-template. The results as ID_{50} are the nanomolar concentration of deoxyoligonucleotide required to inhibit repair synthesis by HIV RT (10 nM) to 50% of an uninhibited control. Values are averages of triplicate independent determinations, the standard deviation of which is ≤20% of the reported value.

Deoxyoligonucleotide (5′–3′)		ID_{50} (nM)
$S2dKt_{14}$	(CTGTTCGGGCGCCA)	4.3
$S2dKt_{10}$	(TCGGGCGCCA)	30
$S2dKt_{8}$	(GGGCGCCA)	110
$SdKt_{14}$	(CTGTTCGGGCGCCA)	99
$S2dKR_{14}$	(GCTACGGCTCGCTG)	10
$S2dPPT_{14}$	(AAAAGAAAAGGGGG)	16
$S2dKAC_{14}$	(ACTCTTAATCTGAG)	26
$S2(dC_{14})$	(CCCCCCCCCCCCCC)	62
$S(dC_{15})$	(CCCCCCCCCCCCCCC)	1800

immediately after retroviral entry into the host cell, RT acts to produce a DNA version of the viral RNA genome. During reverse transcription, HIV-1 RT uses host-cell and viral nucleic acid sequences to initiate the synthesis of its genome (*51–53*). Because dithioate oligomers appear to inhibit HIV-1 RT by binding to its active site for primer-template, an examination of dithioate analogs of primers recognized by HIV-1 RT during the replicative cycle of the virus might lead to the discovery of very potent inhibitors. To this end we have examined the inhibitory properties of a number of oligomers that are dithioate DNA versions of the primers used by HIV-1 RT in the course of reverse transcription. A 14-nt oligomer based on the 3′ terminal end of human lysine tRNA ($S2dKt_{14}$), which is complementary to the 5′ end of the viral primer binding site (PBS), was examined because this particular primer has such a pivotal role in reverse transcription (*51, 52*). In addition, the anticodon region of the lysine tRNA ($S2dKAC_{14}$) was examined because of reports of an interaction between this region and HIV-1 RT (*54–56*). Finally, the primer derived by HIV-1 RT's RNase H activity in the polypurine tract ($S2dPPT_{14}$) was constructed as it functions in initiating viral minus strand synthesis (*57*).

Our analysis started by examining the dithioate DNA version of the far 3′ end of human lysine tRNA ($S2dKt_n$, Table 2). At $n = 14$ nt, we found that this oligomer was an extremely potent HIV-1 RT inhibitor and was 14 times as potent as $S2dC_{14}$ under identical conditions (*43*). The measured median inhibitory dose (ID_{50}) for $S2dKt_{14}$ may represent an upper limit of the oligomers potency because under the assay conditions (10 nM HIV-1 RT), half inhibition occurred at 4.3 nM, suggesting a titration of the enzyme (assuming that the HIV-1 RT:$S2dKt_{14}$ binding stoichiometry is 1:1).

Two 5′ truncated versions of this sequence (8 and 10 nt) were also examined (Table 2). The potency of inhibition, which drops off with a length dependence, supports earlier observations with dithioate-linked deoxyoligocytidine (*43*). Even these short oligomers are effective inhibitors with the 10-nt analog being twofold more active than $S2dC_{14}$.

A comparison with the phosphorothioate version of the tRNA 14-nt analog ($SdKt_{14}$) shows that it is also a much more potent inhibitor than the phosphorothioate deoxyoligocytidine of similar length (Table 2) (*43*). Thus this oligomer sequence causes a large increase in inhibitory effect that is independent of the type of phosphate modification. The increase in activity in going from thioate to dithioate is ~30-fold for deoxyoligocytidine, but the factor with this sequence is ~20-fold. This difference probably reflects the fact that the ID_{50} measured for $S2dKt_{14}$ does not fully indicate its inhibitory activity.

Because $S2dKt_{14}$ appeared to be such an effective inhibitor, we anticipated that this reflected some type of sequence-selective interaction with HIV-1 RT. To test this idea, another 14-nt oligomer of equivalent base composition, but with a randomly scrambled sequence, was constructed ($S2dKR_{14}$, Table 2). It was also a potent inhibitor (ID_{50} = 10 nM), but simply scrambling the sequence caused the half-inhibition value to more than double. Although this result is not sufficient to prove sequence-specific binding, it does indicate that the sequence of the bases is important for effective inhibition. A kinetic analysis was undertaken to help better address the relative efficacy of the defined and sequence-scrambled oligomers and is discussed below.

Interactions of HIV-1 RT with other regions of the human lysine tRNA would not be surprising given its role in reverse transcription. Inhibition of HIV-1 RT by lysine tRNA has been reported, and interactions with the anticodon region have been suggested to account for the inhibition (*55, 56*). The 14-nt oligomer implicated in this interaction was synthesized as dithioate DNA, and its inhibitory properties were monitored (Table 2). The measured ID_{50} was the highest value obtained for any heteropolymer examined and suggests that base composition may play an important role in the relative efficacy of heterosequences. Although we find that this sequence is not an effective inhibitor as a dithioate analog, the naturally occurring tRNA is highly modified in this region and can adopt structures, which may account for previously reported observations.

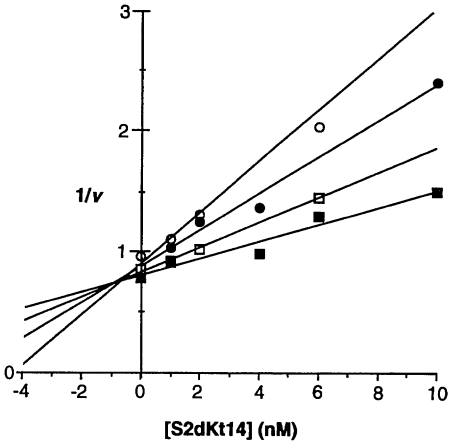

Fig. 4. Dixon plot for inhibition of HIV-1 RT by $S2dKt_{14}$, with respect to primer-template. Kinetics of inhibition were measured as described in Fig. 3. Measurements of the inhibitory constant were difficult because Dixon plots at various substrate concentrations intersect near the *y*-axis. Values for inhibition constants were therefore verified by replotting the slope of double reciprocal plots versus inhibitor concentration, which gave similar K_i values. The plot is from independent triplicate experiments. Associated errors are 35% of the reported values. Figure symbols: ○, [PT] = 0.2 μM; ●, [PT] = 0.25 μM; □, [PT] = 0.3 μM; and ■, [PT] = 0.4 μM.

An analog of the polypurine tract primer used by HIV-1 RT in plus strand DNA synthesis was next examined as an HIV-1 RT inhibitor. The sequence is shown in Table 2 along with its ID_{50}. This oligomer is interesting as it consists of two homopolymeric stretches. It is also an effective inhibitor (ID_{50} = 16 nM) but is intermediate in activity between other heterosequences and homopolymers; which is what one might expect for an oligomer of this base composition. Although $S2dPPT_{14}$ does not appear to be as effective an inhibitor as $S2dKt_{14}$, it may prove to be a potent inhibitor of HIV-1 RT's associated RNase H, because it is derived from this activity during the replicative cycle of the virus. Recent studies have indicated that deoxyoligonucleotide phosphorodithioates also strongly inhibit HIV-1 RT's RNase H activity (*44*).

Kinetics and mechanism of inhibition by $S2dKt_{14}$. To better address the relative potency of $S2dKt_{14}$, a Dixon analysis was performed to determine the type of inhibition and an inhibitory constant. A Dixon plot of inhibition by this oligomer at a number of primer-template concentrations is shown in Fig. 4. The pattern indicates competitive binding to the active site for primer-template (K_i = 0.5 nM). A compar-

ison of inhibition constants for $S2dKt_{14}$ and $S2dC_{14}$ shows that the lysine tRNA sequence is ~20-fold more effective (Table 1). It is also quite interesting to note that the inhibition constant measured for $S2dKt_{14}$ establishes it as the most potent deoxyoligonucleotide inhibitor examined, even more effective than $S2dC_{28}$. These kinetic results probably reflect the affinity of $S2dKt_{14}$ for HIV-1 RT but experiments that directly measure binding to HIV-1 RT have not been completed.

The kinetics of inhibition with $S2dKR_{14}$ (see Table 2 for sequence) were also examined to determine if scrambling the $S2dKt_{14}$ sequence would lead to a substantial difference in inhibition constants. It also inhibits competitively with respect to primer-template with an inhibition constant just greater than twofold lower than dithioate deoxyoligocytidine of the same length (Table 1). Scrambling the base sequence of $S2dKt_{14}$, reduced its efficacy by eightfold. This result strongly suggests that the base sequence is important for potent inhibition of HIV-1 RT. An examination of various primers used by HIV-1 RT during reverse transcription has led to the discovery of a number of potent enzyme inhibitors and the elucidation of a particularly effective deoxyoligonucleotide based inhibitor ($S2dKt_{14}$). In addition, this oligomer is complementary to a conserved region of the HIV-1 genome and could potentially exert an antisense effect against the virus.

Discussion

Interaction of deoxyoligonucleotide phosphorodithioates with proteins important to HIV-1 gene expression may be of therapeutic value. Initially we were very concerned about potential toxicity because of dithioate oligomer inhibition of host cell polymerases. However, cell culture studies have demonstrated very little toxicity (*43*). More recent studies with primary blood cultures indicate no toxicity at 10 μM deoxyoligonucleotide concentrations (*44*). It also does not appear that dithioate oligomers generally bind to proteins. The introduction of dithioate linkages into certain operator sequences causes the affinity for their nascent repressors to be dramatically decreased, and phosphorodithioate deoxyoligocytidine does not appear to inhibit binding of lambda *cro* protein to its operator sequence (*42*, *44*). Further studies of the anti-HIV activity of dithioate oligomers and any toxic side effects are necessary to fully evaluate their therapeutic potential.

A physicochemical explanation of the strong inhibition of HIV-1 RT by dithioate oligomers is still lacking. From our mechanistic studies of inhibition and equilibrium binding measurements, we know that dithioate oligomers interact with the active site for primer-template binding, but why do they bind to this site? Although we have not yet directly answered this question, insights into the physical properties of dithioate oligomers gained in the course of their purification and analysis has led to a hypothesis. The behavior of dithioate oligomers during reversed-phase high-performance liquid chromatography indicates that they are more hydrophobic than phosphodiesters or phosphorothioates, which is not surprising considering the chemical modification. Investigations of anion exchange chromatography of deoxyoligonucleotide phosphorodithioates under conditions, where reversed-phase effects should be abated, indicate very strong ionic interactions with column-linked amines. This amphipathic behavior is probably important to their inhibitory activity. Dithioate oligomers also have anomalous mobilities in native but not denaturing polyacrylamide gels, which may indicate some structural motif in aqueous solutions (*44*). Although the mobility of dithioate deoxyoligonucleotides is retarded approximately one nucleotide relative to a phosphodiester homolog in denaturing gels, the mobility of single-stranded dithioate oligomers in native gels is close to that of a duplexed oligomer of equivalent length. We interpret this result as being indicative of a rigid-rod–like structure or a highly defined multimer. Finally, from our substrate studies with dithioate deoxyoligonucleotide primers and reverse transcriptases, we know that these oligomers must be similar to phosphodiester-containing nucleic acids because they serve as active primer substrates when duplexed with a phosphodiester template (*44*).

One can imagine that the binding site for primer-template in HIV-1 RT probably consists of a relatively hydrophobic cleft in which basic amino acid side chains are extended to electrostatically interact with nucleic acids (*58*). Crystallographic studies have shown that HIV-1 RT does appear to have a large primer-template binding cleft (*59*). Dithioate oligomer analogs appear to be amphipathic, DNA-like rigid rods that interact strongly with amines and would therefore be ideally suited for binding in such a cleft. This property may be why dithioate oligomers interact so strongly with HIV-1 RT and are able to inhibit enzyme activity.

These results suggest that phosphorodithioate-containing deoxyoligonucleotides should be further explored as DNA therapeutic drugs. In addition to being readily available through automated synthesis protocols, these oligomers strongly inhibit HIV-1 RT, exert an antisense effect (*44*), and show very little toxicity to cells in culture (*43*). Although these results are encouraging, only future in vivo studies will present a clear evaluation of their therapeutic potential.

REFERENCES AND NOTES

1. H. Mitsuya, R. Yarchoan, S. Broder, *Science* **249**, 1533 (1990).
2. D. W. Bilheimer, S. M. Grundy, M. S. Brown, J. L. Goldstein, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 4124 (1983).
3. G. H. Hitchings, *Adv. Enzyme Regul.* **9**, 121 (1974).
4. F. K. Lin *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 7580 (1985).
5. L. M. Souza *et al.*, *Science* **232**, 61 (1986).
6. D. V. Goeddel *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 106 (1979).
7. D. V. Goeddel *et al.*, *Nature* **281**, 544 (1979).
8. M. G. Rossmann and J. E. Johnson, *Annu. Rev. Biochem.* **58**, 533 (1989).
9. S. Borman, *Chem. Eng. News* **70** (no. 32), 18 (1992).
10. C. Tuerk and L. Gold, *Science* **249**, 505 (1990); A. D. Ellington and J. W. Szostak, *Nature* **346**, 818 (1990).
11. L. C. Bock *et al.*, *ibid.* **355**, 564 (1992).
12. R. Owens *et al.*, *Biochem. Biophys. Res. Commun.* **181**, 402 (1991); K. S. Lam *et al.*, *Nature* **354**, 82 (1991); R. A. Houghten *et al.*, *ibid.*, p. 84.
13. K. J. Marians, R. Wu, J. Stawinski, T. Hozumi, S. A. Narang, *Nature* **263**, 744 (1976).
14. H. L. Heyneker *et al.*, *ibid.*, p. 748.
15. J. R. Sadler *et al.*, *Gene* **1**, 305 (1977).
16. S. Agarwal, *TIBTECH* **10**, 152 (1992).
17. C. A. Stein and J. S. Cohen, *Cancer Res.* **48**, 2659 (1988).
18. B. Calabretta, *ibid.* **51**, 4505 (1991).
19. P. C. Zamecnik and M. L. Stephenson, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 280 (1978).
20. E. Uhlmann and A. Peyman, *Chem. Rev.* **90**, 544 (1990).
21. A. R. van der Krol, J. N. Mol, A. R. Stuije, *Biotechniques* **6**, 958 (1988).
22. P. S. Miller and P. O. P. Ts'O, *Anti-Cancer Drug Des.* **2**, 117 (1987).
23. J. Goodchild, *Bioconjugate Chem.* **2**, 165 (1990).
24. D. Tidd, *Anticancer Res.* **10**, 1169 (1990).
25. C. Hélène and J. J. Toulmé, *Biochem. Biophys. Acta* **1049**, 99 (1990).
26. C. A. Stein, J. L. Tonkinson, L. Yakubov, *Pharmacol. Ther.* **52**, 365 (1991).
27. S. L. Beaucage and R. P. Iyer, *Tetrahedron* **48**, 2223 (1992).
28. U. Englisch and D. H. Gauss, *Angew. Chem. Int. Ed. Engl.* **30**, 613 (1991).
29. P. Iverson, *Anti-Cancer Drug Des.* **6**, 531 (1991).
30. S. Agarwal, J. Temsamani, J.-Y. Tang, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 7595 (1991).
31. F. Eckstein, *Annu. Rev. Biochem.* **54**, 367 (1985).
32. Z. J. Lesnikowski, M. Jaworska, W. J. Stec, *Nucleic Acids Res.* **18**, 2109 (1990).
33. W. J. Stec, A. Grajkowski, M. Koziolkiewicz, B. Uznanski, *ibid.* **19**, 5883 (1991).
34. R. Cosstick and J. S. Vyle, *ibid.* **18**, 829 (1990).
35. S. M. Gryaznov and R. L. Letsinger, *ibid.* **20**, 3403 (1992).
36. M. Boeringer and M. H. Caruthers, *Tetrahedron Lett.*, in press.
37. P. E. Nielsen, M. Egholm, R. H. Berg, O. Buchardt, *Science* **254**, 1497 (1991).
38. W. K.-D. Brill, J.-Y. Tang, Y.-X. Ma, M. H. Caruthers, *J. Am. Chem. Soc.* **113**, 3972 (1991).
39. J. Nielsen, W. K.-D. Brill, M. H. Caruthers, *Tetrahedron Lett.* **29**, 2911 (1988).
40. A. Grandas, W. S. Marshall, J. Nielsen, M. H. Caruthers, *ibid.* **30**, 543 (1989).
41. G. M. Porritt and C. B. Reese, *ibid.*, p. 4713.
42. M. H. Caruthers *et al.*, *Nucleosides Nucleotides* **10**, 47 (1991).
43. W. S. Marshall, G. Beaton, C. A. Stein, M. Matsukura, M. H. Caruthers, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 6265 (1992).
44. W. S. Marshall and M. H. Caruthers, unpublished data.

45. O. Dahl, *Sulfur Rep.* **11**, 167 (1991), and references therein.
46. G. Beaton, D. Dellinger, W. S. Marshall, M. H. Caruthers, in *Oligonucleotides and Analogues, A Practical Approach*, F. Eckstein, Ed. (IRL Press, Oxford, 1991), pp. 109–135.
47. W. T. Wiesler, W. S. Marshall, M. H. Caruthers, in *Methods in Molecular Biology: Oligonucleotide Synthesis*, S. Agrawal, Ed. (Humana, Totowa, NJ, in press).
48. M. H. Caruthers *et al.*, in *Methods Enzymol.* **154**, 287 (1987).
49. I. H. Segel, *Enzyme Kinetics. Behavior and Analysis of Rapid Equilibrium and Steady State Enzyme Systems* (Wiley, New York, 1975).
50. C. Majumdar, C. A. Stein, J. S. Cohen, S. Broder, S. Wilson, *Biochemistry* **28**, 1340 (1989).
51. E. Gilboa, S. W. Mitra, S. Goff, D. Baltimore, *Cell* **18**, 93 (1979).
52. S. Wain-Hobson, P. Sonigo, O. Danos, S. Cole, M. Alizon, *ibid.* **40**, 9 (1985).
53. H. Varmus, *Science* **240**, 1427 (1988).
54. C. Barat *et al.*, *EMBO J.* **8**, 3279 (1989).
55. B. Bordier *et al.*, *Nucleic Acids Res.* **18**, 429 (1990).
56. M.-L. Sallafraque-Andreola *et al.*, *Eur. J. Biochem.* **184**, 367 (1989).
57. B. M. Wohrl and K. Moelling, *Biochemistry* **29**, 10141 (1990).
58. D. H. Ohlendorf and J. B. Matthew, *Adv. Biophysics* **20**, 137 (1985).
59. L. A. Kohlstaedt, J. Wang, J. M. Friedman, P. A. Rice, T. A. Steitz, *Science* **256**, 1783 (1992).
60. We thank G. Beaton and W. Wiesler for their important efforts in the development of thiophosphoramidite chemistry and T. Jones for helpful comments on the manuscript. Supported by National Institutes of Health grants GM21120 and GM25680.

Molecular Interactions and Hydrogen Bond Tunneling Dynamics: Some New Perspectives

Richard J. Saykally* and Geoffrey A. Blake

The recent development of tunable far-infrared lasers and other high-resolution spectroscopic probes of weakly bound clusters is having a significant impact on our understanding of intermolecular forces and on the complex quantum tunneling dynamics that occur in hydrogen-bonded systems. Far-infrared studies of a variety of interactions are discussed, including several prototypical water-hydrophobe complexes, the water trimer, and the ammonia dimer. Particular attention is paid to the inversion of spectroscopic data to yield detailed intermolecular potential energy surfaces. Investigations of nonpairwise additivity are also described.

The widespread use of molecular modeling in modern science has lead to seminal insights into the nature of complex molecules and complicated chemical processes. As all such approaches are ultimately dependent on the "correctness" with which they represent molecular interactions, this latter subject has also received considerable recent attention. The strongest of these interactions, the hydrogen bond, is a highly revered dogma of modern science, transcending the boundaries of numerous disciplines. It is at once a structural model, an energy constraint, and an arena for an interesting kind of dynamics upon which a tremendous variety of natural phenomena depend. After many years of debate, the origin of hydrogen bonding is now generally understood to be primarily electrostatic, but with significant contributions arising from short-range charge transfer interactions. Typical hydrogen bond strengths in neutral systems fall in the range from 2 to 5 kcal/mol, whereas those involving ions are much stronger. The preponderance of modern research has shown that hydrogen bonds usually adopt a nearly linear M-H·····X: arrangement between a donor bond (M–H) and a proton acceptor (X:), typically a Lewis base. Weaker forces ("van der Waals interactions") are often assumed to be less "directional."

It is now possible to quantitatively test these long-held views. Classical routes to intermolecular potential energy surfaces (IPSs) have been augmented by powerful new spectroscopic methods that have been developed for investigating intermolecular forces and intramolecular dynamics at a vastly enhanced level of detail. The many results accumulated from these experiments have both dramatically enhanced our knowledge of molecular interactions and challenged time-honored theories and paradigms. For example, the microwave spectra of isolated clusters seem to indicate that some of our most basic notions of hydrogen bonding are not observed in several important prototypical systems (*1*).

In this article we describe one of the newest and most successful tools developed for studying molecular interactions, tunable far-infrared laser vibration-rotation-tunneling spectroscopy (FIR-VRTS) and we present some of the more interesting results that have recently been obtained with this method. By directly measuring the intermolecular vibrations of a weakly bound cluster (the term WBC is used here to denote both van der Waals and hydrogen-bonded complexes), we can resolve rotational and nuclear hyperfine structure and thus access the most sensitive measure of the IPS. These measurements have not been possible in the past simply because the necessary technology did not exist. Intermolecular vibrations typically lie in the FIR region of the electromagnetic spectrum (10 to 350 cm^{-1}), where high-performance light sources and detectors have been slow to evolve. The development of the tunable FIR laser and the emergence of improved detector materials has now revolutionized our capabilities in this spectral region.

In particular, by combining a tunable FIR laser spectrometer with a planar supersonic jet for generating WBCs, we have developed a very general tool for studying molecular interactions and hydrogen bond tunneling dynamics at an unprecedented level of detail. The origin and early development of FIR-VRTS have been reviewed previously (*2*), as have the much more advanced instruments currently in use in at least four independent laboratories (*3*–*5*). We present a general outline of the method in Fig. 1. Complementary results have been obtained for literally hundreds of clusters at both longer and shorter wavelengths. Interested readers may wish to consult recent reviews of these subjects (*6*, *7*).

Obtaining IPSs by Inverting FIR-VRT Spectra

The weak forces between molecules lead to shallow potential surfaces with a very rich and complex topology. Intermolecular vibrational, rotational, and tunneling states can therefore become quite strongly mixed, hence our general term of FIR-VRTS. It is vital that states spanning the complete range of intermolecular coordinates be measured due to the well-known uniqueness problem associated with the inversion of spectroscopic data. The VRT transitions sample particularly large regions of the IPSs, and their use in the determination of potentials for simple binary systems has been recently reviewed by Cohen and Saykally (*8*). The general approach developed at Berkeley for the inversion of VRT data makes use of the collocation method for solving the coupled channel equations that result from expressing the Schrödinger equation in scattering coordinates. Hutson has also recently reviewed the use of the more

R. J. Saykally is in the Department of Chemistry, University of California, Berkeley, CA 94720. G. A. Blake is in the Division of Geological and Planetary Sciences, 170-25, California Institute of Technology, Pasadena, CA 91125.

*To whom correspondence should be addressed.