Table I. ^1H and ^{31}P NMR Spectroscopic Data[a]

compound	P_A	P_B	$J_{P_A\text{-}Pt}$	$J_{P_B\text{-}Pt}$	H	$J_{H\text{-}P_{cis}}$	$J_{H\text{-}P_{trans}}$	$J_{H\text{-}Pt}$
$[(Cy_2P(CH_2)_2PCy_2)PtH_2]$ (1)	78.2		1826		+0.65	16	177	1098
$[(Cy_2P(CH_2)_3PCy_2)PtH_2]$ (2)	22.7		1901		−1.18	24	174	1069
$[(Cy_2P(CH_2)_4PCy_2)PtH_2]$ (3)	36.9		2010		−2.06	24	171	1045
$[(Cy_2P(CH_2)_2PCy)Pt(\mu\text{-}H)]_2$ (6)	83.6		73,2782		−2.48	37		481
$[(Cy_2P(CH_2)_3PCy_2)Pt^0(DEA)]$[b] (7)	17.9		3299					
$[(Cy_2P(CH_2)_3PCy_2)Pt^0(HC{\equiv}CPh)]$[c] (8)	17.8	20.1	3138	3212	8.54	15	22	15
$[(Cy_2P(CH_2)_3PCy_2)Pt(HC{\equiv}CCO_2Me)]$[d] (9)	18.9	20.0	3156	3404	8.66	19	19	7

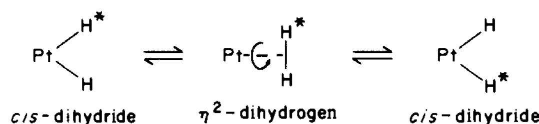
[a] P_A respresents the ^{31}P NMR resonance trans to an acetylenic hydrogen where relevant (i.e., 8 and 9). [b] DEA = $EtO_2CC{\equiv}CCO_2Et$. [c] $J_{P_A\text{-}P_B}$ = 9 Hz. [d] $J_{P_A\text{-}P_B}$ not resolved.

substituted phenyl or benzyl), in which we believed (on the basis of other evidence) that the ligand position exchange process involves SiR_3 and H interchange rather than PCy_3 interchange. To distinguish between these two possibilities we have prepared a series of chelating diphosphine complexes including $[H(Ph_3Si)Pt(Cy_2P(CH_2)_7PCy_2)]$,[11,12] which exhibit variable temperature ^1H NMR hydride line shapes similar to those of the parent PCy_3 complexes. Assuming no fluxionality of the chelating diphosphine ligands,[15] this confirms the idea of SiR_3 and H ligand position exchange. The same process was also observed for *cis*-$[H(R_3Si)Pt(PPh_3)_2]$ (R = Ph, C_6H_4Cl)[13] and more recently for $(PEt_3)_2Pt(\mu\text{-}H)(\mu\text{-}CO)Mn(CO)_4$.[14] In the case of the dihydride complexes 2 and 3, since *phosphine* ligand position exchange can presumably be precluded because of the chelating diphosphine ligands,[15] it is the hydride ligands that exchange positions. Alternatively, the hydride ligands may be regarded as an η^2-dihydrogen complex of platinum(0) in which the dihydrogen ligand is rotating about the Pt–H_2 bond, the H---H interaction presumably resulting from the steric constraints imposed by the bulky diphosphine ligand.[16]

Recently, a theoretical study[17] concluded that "oxidative addition" of H_2 to $Pt^0(PH_3)_2$ to form *cis*-$H_2Pt(PH_3)_2$ is not oxidative but rather an electronic promotion of platinum from the d^{10} ground state to the d^9s^1 state. Chemical confirmation that the hydride ligands are loosely bonded to platinum comes from the reaction of 2 with activated acetylenes. In this laboratory it has recently been shown[18] that activated acetylenes generally react with a close analogue of 2, namely *trans*-$H_2Pt(PCy_3)_2$, by insertion into Pt–H bonds to form the corresponding σ-alkenyl products *trans*-$H(RHC{=}CR)Pt(PCy_3)_2$. In contrast, addition of stoichiometric amounts of the acetylenes $EtO_2CC{\equiv}CCO_2Et$, $HC{\equiv}CPh$, and $HC{\equiv}CCO_2Me$ to benzene solutions of 2 results in a rapid evolution of gas at room temperature with complete displacement of the hydride ligands to form the species $[(Cy_2P(CH_2)_3PCy_2)Pt^0(RC{\equiv}CR')]$, 7, 8, and 9, respectively. For unsymmetrical acetylenes, two resonances in the $^{31}P\{^1H\}$ NMR spectra (with platinum satellites) indicate restricted rotation of the acetylene about the platinum center on the NMR time scale. The fact that the hydride ligands are so readily lost is consistent with their weak coordination to platinum.

Reversible H_2 coordination for the complex $[(t\text{-}BuPhP(CH_2)_2PPh\text{-}t\text{-}Bu)PtH_2]$[9] was demonstrated by a diminution of the hydride resonance in the ^1H NMR spectrum at 55 °C and a darkening of the solution to a red color, characteristic of the platinum(0) dimer $[(t\text{-}BuPhP(CH_2)_2PPh\text{-}t\text{-}Bu)Pt^0]_2$. In contrast, we observe no reduction in the ^1H NMR spectrum of the hydride signal intensity or darkening of a benzene solution of 3 on heating to 75 °C.

While the mechanistic details of the ligand position interchange process are still uncertain (e.g., a pseudotetrahedral or trigonal-planar intermediate), a sequence of events such as



can be envisaged and is consistent with the NMR data and the ease of displacement of both hydride ligands. We believe that the complexes described exist at an intermediate stage between dihydrides and η^2-dihydrogen complexes. We are currently studying the possibility of reversible *intermolecular* dihydrogen exchange in these compounds.

Registry No. 1, 102286-33-9; **2**, 102286-34-0; **3**, 102286-35-1; **4** (*n* = 2), 102286-36-2; **4** (*n* = 3), 102286-37-3; **4** (*n* = 4), 102286-38-4; **5** (*n* = 2), 102286-39-5; **5** (*n* = 3), 102286-40-8; **5** (*n* = 4), 102286-41-9; **6** (*n* = 2), 102286-42-0; **6** (*n* = 3), 102286-43-1; **6** (*n* = 4), 102286-44-2; **7**, 102286-45-3; **8**, 102286-46-4; **9**, 102286-47-5; $H(Ph_3Si)Pt(Cy_2P(CH_2)_7PCy_2)$, 102286-48-6; *trans*-$H_2Pt(PCy_3)_2$, 42764-83-0; H_2, 1333-74-0.

(12) Clark, H. C.; Hampden-Smith, M. J., unpublished results.
(13) Azizian, H.; Dixon, K. R.; Eaborn, C.; Pidcock, A.; Shuaib, N. M.; Vinaixa, J. *J. Chem. Soc., Chem. Commun.* **1982**, 1020–1022.
(14) Braunstein, P.; Geoffroy, G. L.; Metz, B. *Nouv. J. Chim.* **1985**, *9*, 221–223.
(15) Some examples do exist; e.g.: Hassan, F. S. M.; Markham, D. P.; Pringle, P. G.; Shaw, B. L. *J. Chem. Soc., Dalton Trans.* **1985**, 279–283.
(16) Since submission of this article, we have carried out an experiment in which D_2 gas was added to a benzene solution of 2. The reaction was followed by ^1H NMR spectroscopy which revealed steady diminution of the hydride resonance of 2 together with formation of a small amount of the hydride-bridged dimer (or its $(\mu\text{-}H)(\mu\text{-}D)$ analogue). Formation of $[(Cy_2P(CH_2)_3PCy_2)PtHD]$ (to obtain the value of $J_{H\text{-}D}$) was not observed. We are in the process of preparing the HD analogue of 2 from the reaction of 5 with deuterium hydride gas.
(17) Low, J. L.; Goddard, W. A. *J. Am. Chem. Soc.* **1984**, *106*, 6928–6937.
(18) Clark, H. C.; Janzen, E. J.; Ruegger, H.; Wong, C. S., unpublished results.

Dynamics and Design of Enzymes and Inhibitors

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The mutual recognition and binding of ligands and receptors represents the first step in many biochemical processes. The ability to predict changes in affinity that would result from modifications in a ligand or receptor would therefore be helpful in the design of molecules with specific activities.[1-4] Here, we describe the first application to biological molecules of a new computer simulation approach to such problems. We compute the relative affinity of two benzamidine inhibitors for trypsin and of benzamidine for native and a mutant trypsin. The agreement with experimental data is encouraging.

To compute relative affinities, we use the thermodynamic cycle–perturbation approach.[5,6] This has already been used suc-

(1) Beddell, C. R. *Chem. Soc. Rev.* **1984**, *13*, 279–319.
(2) Richards, W. G. *Endeavour* **1984**, *8*, 172–178.
(3) Kollman, P. *Acc. Chem. Res.* **1985**, *18*, 105–111.
(4) Smith, R. N.; Hansch, C.; Kim, K. H.; Omiya, B.; Fukumura, G.; Selassie, C. D.; Jow, P. Y. C.; Blaney, J. M.; Langridge, R. *Arch. Biochem. Biophys.* **1982**, *215*, 319–328.
(5) Tembe, B. L.; McCammon, J. A. *Comput. Chem.* **1984**, *8*, 281–283.
(6) McCammon, J. A.; Harvey, S. C. *Dynamics of Proteins and Nucleic Acids*; Cambridge: London, in press.

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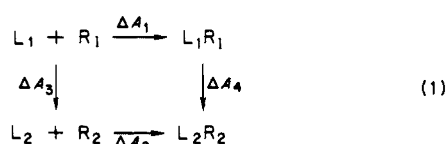
Table I. Molecular Dynamics Simulations[a]

simulation	solute	no. of water molecules	box dimens/nm	time/ps
1	benzamidine–inhibited trypsin	4785	4.92 × 5.44 × 6.43	28.8
2	benzamidine	212	1.86 × 1.86 × 1.86	64.0
3	native trypsin	4788	4.92 × 5.44 × 6.43	22.4

[a] Periodic boundary conditions were used. In simulation 2, all interactions were calculated between pairs of molecules having any heavy atoms closer than 0.8 nm. In simulations 1 and 3, this cutoff distance was maintained for Lennard-Jones interactions but was extended to 2.0 nm for Coulombic interactions. Covalent bond lengths were fixed by the SHAKE procedure.[13] Trajectories were calculated by using the Verlet algorithm with a time step of 2 fs.[14] The systems were coupled to a constant temperature bath (300 K) with a relaxation time of 0.1 ps.[15] Each simulation was preceded by a careful equilibration.

cessfully to predict the specificity of halide ion binding to an organic host in water.[7] The approach makes use of the cycle



$$\begin{array}{ccc} L_1 + R_1 & \xrightarrow{\Delta A_1} & L_1R_1 \\ \downarrow \Delta A_3 & & \downarrow \Delta A_4 \\ L_2 + R_2 & \xrightarrow{\Delta A_2} & L_2R_2 \end{array} \quad (1)$$

Here, L_1 and L_2 represent different modifications of a ligand, R_1 and R_2 represent different modifications of a receptor, and the ΔA's represent changes in the free energies for the indicated processes. Each of the four processes in the cycle can, in principle, be simulated by molecular dynamics calculations on model systems comprising ligand, receptor, and solvent.[6] Because such simulations reflect the thermal motion in the system, thermodynamic data such as free energies can be obtained.[5-7] The relative affinity of the two ligand receptor pairs is determined by $\Delta\Delta A = \Delta A_2 - \Delta A_1$. In practice, calculation of ΔA_1 and ΔA_2 is often very difficult, because the physical binding processes may involve slow desolvation steps or conformational changes. In the thermodynamic cycle–perturbation approach, these difficulties are circumvented by considering the nonphysical processes corresponding to ΔA_3 and ΔA_4. These molecular transmutations often involve only localized changes in the model systems. The quantities ΔA_3 and ΔA_4 can be calculated by statistical mechanical perturbation theory.[5-11] For example,

$$\Delta A_4 = k_B T \ln \langle \exp(V_{11} - V_{22})/k_B T \rangle_{11} \quad (2)$$

where $k_B T$ is Boltzmann's constant times temperature; V_{11} and V_{22} are the potential energy functions of the L_1R_1/solvent and L_2R_2/solvent systems, respectively; and $\langle\ \rangle_{11}$ indicates an average over representative atomic coordinate sets of the L_1R_1/solvent system for the given thermodynamic conditions. Reference to eq 1 shows that

$$\Delta\Delta A = \Delta A_4 - \Delta A_3 \quad (3)$$

which is the desired quantity, because free energies are state functions. In this difference, there is a cancellation of any formal contributions to ΔA_3 and ΔA_4 due to mass differences,[7b] nuclear changes, etc., produced by transmuation; these contributions are not displayed in eq 2.

In the present work, two different binding comparisons have been made. The first comparison is of p-fluorobenzamidine (L_2) vs. benzamidine (L_1) binding to trypsin ($R_1 = R_2$). The second comparison is of benzamidine ($L_1 = L_2$) binding to trypsin with Gly 216 replaced by Ala (R_2) vs. native trypsin (R_1). The benzamidines are protonated (benzamidinium ions), and the native enzyme is bovine pancreatic trypsin. The molecular dynamics simulations are carried out at constant temperature (300 K) and volume, so that the free energies obtained are Helmholtz energies. The simulations (Table I) were carried out on a CYBER 205 supercomputer by using the GROMOS molecular modeling package.[12] The initial coordinates for the inhibited enyzme were taken from the 0.17-nm resolution X-ray structure.[16,17] Those for the native enzyme were obtained by replacing the inhibitor with three water molecules.

Table II. Relative Free Energies of Binding

system	ΔA_3/kJ/mol	ΔA_4/kJ/mol	theor $\Delta\Delta A$/kJ/mol	exptl $\Delta\Delta A$/kJ/mol
L_1 = benzamidine, L_2 = p-fluorobenzamidine, $R_1 = R_2$ = trypsin	−3.5	0.3	3.8	2.1[a]
$L_1 = L_2$ = benzamidine, R_1 = trypsin, R_2 = trypsin (Gly 216→Ala)	2.3	7.9	5.6	~8.4[b]

[a] From ref 18. [b] From ref 19.

The results are displayed in Table II. The experimental result for the inhibitor modification is from a study of the same system considered here.[18] That for the enzyme modification is estimated from the Michaelis constants for native and mutant rat pancreatic trypsin II enzymes acting on arginine substrates.[19] Both the theoretical and experimental results are subject to some uncertainty. For the inhibitor modifications, the theoretical result has a standard deviation of 2.2 kJ/mol (based on separate analyses of 8-ps segments of the simulations), while that for the experimental result is 1.3 kJ/mol.[18] The uncertainities for the enzyme modifications are somewhat larger in the theoretical case because the simulation for ΔA_3 is shorter than for the inhibitor modification and in the experimental case because the result is from a homologous system.[20,21] Longer simulations are in progress to reduce the theoretical uncertainties.

The preliminary data given here allow us to draw several conclusions. First, the theoretical and experimental results agree that benzamidine binds somewhat more strongly than p-fluorobenzamidine to trypsin and that benzamidine binds more strongly to trypsin than to the mutant considered here. Second, the theoretical results for ΔA_3 and ΔA_4 aid in the interpretation of the net binding specificities. For the different inhibitors, $\Delta\Delta A$ is dominated by the negative value of ΔA_3. This indicates that the binding preference is determined by the unfavorable desolvation of p-fluorobenzamidine, which likely is due to the larger dipole moment of the latter in comparison to benzamidine. For the different enzymes, $\Delta\Delta A$ is dominated by the positive value of ΔA_4, which suggests that steric crowding due to the added methyl group in the mutant enzyme is the primary factor in determining binding

(7) (a) Lybrand, T. P.; McCammon, J. A.; Wipff, G. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 833–835. (b) Lybrand, T. P.; Ghosh, I.; McCammon, J. A. *J. Am. Chem. Soc.* **1985**, *107*, 7793–7794.
(8) McQuarrie, D. A. *Statistical Mechanics*; Harper & Row: New York, 1976.
(9) Postma, J. P. M.; Berendsen, H. J. C.; Haak, J. R. *Faraday Symp. Chem. Soc.* **1982**, *17*, 55–67.
(10) Jorgensen, W. L.; Ravimohan, C. *J. Chem. Phys.* **1985**, *83*, 3050–3054.
(11) Friedman, H. L. *A Course in Statistical Mechanics*; Prentice-Hall: Englewood Cliffs, NJ, 1985.
(12) Hermans, J.; Berendsen, H. J. C.; van Gunsteren, W.; Postma, J. P. M. *Biopolymers* **1984**, *23*, 1513–1518.
(13) Ryckaert, J. P.; Ciccotti, G.; Berendsen, H. J. C. *J. Comput. Phys.* **1977**, *23*, 327–341.
(14) Verlet, L. *Phys. Rev.* **1967**, *159*, 98–103.
(15) Berendsen, H. J. C.; Postma, J. P. M.; van Gunsteren, W. F.; DiNola, A.; Haak, J. R. *J. Chem. Phys.* **1984**, *81*, 3684–3690.
(16) Bode, W.; Schwager, P. *J. Mol. Biol.* **1975**, *98*, 693–717.
(17) Bernstein, F. C.; Koetzle, T. F.; Williams, G. J. B.; Meyer, E. F.; Brice, M. D.; Rodgers, J. R.; Kennard, O.; Shimanouchi, T.; Tasumi, M. *J. Mol. Biol.* **1977**, *112*, 535–542.
(18) Mares-Guia, M.; Nelson, D. L.; Rogana, E. *J. Am. Chem. Soc.* **1977**, *99*, 2331–2336.
(19) Craik, C. S.; Largmon, C.; Fletcher, T.; Roczniak, S.; Barr, P. J.; Fletterick, R.; Rutter, W. J. *Science (Washington, D.C.)* **1985**, *228*, 291–297.
(20) MacDonald, R. J.; Stary, S. J.; Swift, G. H. *J. Biol. Chem.* **1982**, *257*, 9724–9732.
(21) Craik, C. S.; Choo, Q.-L.; Swift, G. H.; Quinto, C.; MacDonald, R. J.; Rutter, W. J. *J. Biol. Chem.* **1984**, *259*, 14255–14264.

preference. Most importantly, we have shown that the thermodynamic cycle-perturbation approach is a feasible route for the analysis and prediction of affinity in large biomolecular systems.

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Registry No. Benzamidine, 618-39-3; *p*-fluorobenzamidine, 2339-59-5; trypsin, 9002-07-7.

Phosphorothioite Method. 1. A New Type of Internucleotidic Bond Formations Involving Simultaneous Oxidation Process by Use of Deoxyoligonucleoside Phosphorothioites as New Key Intermediates for Deoxyoligonucleotide Synthesis†

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In recent years, oligonucleotide synthesis has been markedly facilitated by developments of the phosphite and phosphoramidite approaches introduced by Letsinger[1] and Caruthers.[2] These methods have also been extended to the synthesis of nucleic acid analogues involving P—CH_3 and P=S bonds.[3]

Especially the phosphoramidite approach has proved to be practically useful for molecular biology.[4] However, the whole process for the phosphoramidite approach involves an additional oxidation step of the tervalent phosphite intermediates after condensation compared with the phosphotriester approach.

In this paper, we wish to report a more straightforward approach to the oligodeoxyribonucleotide synthesis involving a one-step reaction for both condensation and oxidation.

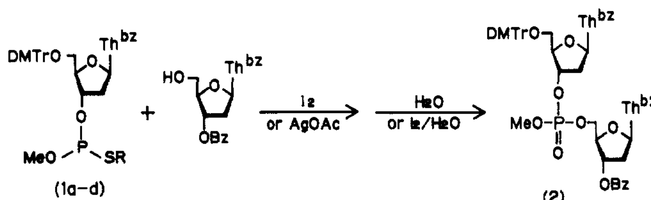
It is known the *S*-alkyl nucleoside phosphorothioates of the diester type were activated by iodine to give highly reactive metaphosphate intermediates.[5] Contrary to this fact, *S,S'*-diaryl nucleoside phosphorodithioates of the triester type did not react with iodine.[6] On the other hand, phosphite derivatives *S,S'*-diethyl alkyl phosphorodithioites were accessible to oxidative hydrolysis by means of aqueous iodine giving rise to monoalkyl phosphates.[7] The facile activation of tervalent P-S bonds with iodine suggested the possibility that if iodine was added to $(R'O)_2P{-}SR''$ in the presence of ROH under anhydrous conditions the SR″ group could be oxidatively replaced by the OR group to give the corresponding phosphotriesters, $(R'O)_2P(O)OR$.

Table I. Isolated Yields and ^{31}P NMR[a] Data of the Reported Compounds (**1**, **2**)

compd	yield/%	^{31}P NMR/ppm	yield of **2**/% I_2	yield of **2**/% AgOAc
1a	55	−139.26	42	66
1b	70	−138.63	72	88
1c	72	−139.12	78	78
1d	73	−139.12	80	81

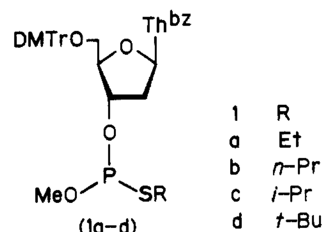
[a] Chemical shifts ($CDCl_3$/Py, 3:1, v/v) of compounds **1a–d** with respect to an external standard of 85% H_3PO_4 (aqueous). The lower field than the reference peak of 85% H_3PO_4 is described as the minus region.

Scheme I



In order to examine whether *S*-alkyl nucleoside phosphorothioites can be used as the starting units for oligodeoxyribonucleotide synthesis, several experiments have been conducted.

First, *S*-alkyl nucleoside phosphorothioites (**1a–d**) were synthesized by reaction of 5′-O-(dimethoxytrityl)-3-benzoylthymidine ($DMTrT^{bz}$) with an (alkylthio)methoxychlorophosphine.[8] A



typical procedure is shown as follows: [$CH_3OP(Cl)S$-*t*-Bu] was added dropwise to a solution of $DMTrT^{bz}$ in dry pyridine at room temperature. Aftr 10 min the solution was transferred with CH_2Cl_2 to a separatory funnel. The solution was washed 3 times with water. The organic phase was dried over anhydrous Na_2SO_4, filtrated, and evaporated under reduced pressure. The residue was separated by silica gel chromatography with CH_2Cl_2/hexane/pyridine (6:3:1, v/v/v). The purified product was dissolved in benzene and the solution was added dropwise to hexane while the mixture was stirred vigorously. The precipitate was collected and dried over P_4O_{10} under reduced pressure. The isolated yields of **1a–d** were 56–73% (Table I). These powders are found to be stable on storage at −30 °C for several months except for **1a**.

It was found that iodine or silver acetate could activate the P-S bond of **1a–d**. A mixture of compound **1** and 3,3′-O-dibenzoylthymidine was dissolved in a small amount of pyridine, coevaporated 3 times with dry pyridine, and dissolved in CH_2Cl_2/lutidine/NEt_3 (8:1:1, v/v/v). Iodine or AgOAc was added to the solution, and the mixture was vigorously stirred for 2 min. In the case of iodine, the 3′-5′ internucleotidic phosphate linkage was directly formed by addition of a small amount of water after the coupling reaction was completed. When AgOAc was used, dinucleoside methyl phosphite was initially formed and it required in situ treatment of the mixture with a solution (THF/2,6-lutidine/H_2O, 2:1:1, v/v/v) containing 0.2 M iodine for oxidation. After the usual workup followed by silica gel column chromatography, the coupling product (**2**) was isolated as shown in Table I (Scheme I).

Application of the present phosphorothioite approach to the synthesis of oligothymidylate on polymer support was also tested. Polystyrene (1% DVB cross-linking, 46 μmol/g) which was derived

† This paper is dedicated to Professor Morio Ikehara on the occasion of his retirement from Osaka University on March 1986.

(1) Letsinger, R. L.; Finnan, J. L.; Heavner, G. A.; Lunsford, W. B. *J. Am. Chem. Soc.* **1975**, *97*, 3278. Letsinger, R. L.; Lunsford, W. B. *J. Am. Chem. Soc.* **1976**, *98*, 3655.
(2) Beaucage, S. L.; Caruthers, M. H. *Tetrahedron Lett.* **1981**, *22*, 1859. McBride, L. J.; Caruthers, M. H. *Tetrahedron Lett.* **1983**, *24*, 245. McBride, L. J.; Caruthers, M. H. *Tetrahedron Lett.* **1983**, *24*, 2953.
(3) Stec, W. J.; Zon, G.; Egan, W.; Byrd, R. A.; Phillips, L. R.; Gallo, K. A. *J. Org. Chem.* **1985**, *50*, 3908. Stec, W. J.; Zon, G.; Egan, W.; Stec, B. *J. Am. Chem. Soc.* **1984**, *18*, 6077. Nobel, S. A.; Fisher, E. F.; Caruthers, M. H. *Nucleic Acids Res.* **1984**, *12*, 3387. Nemer, M. J.; Ogilvie, K. K. *Tetrahedron Lett.* **1980**, *21*, 4149. Nemer, M. J.; Ogilvie, K. K. *Tetrahedron Lett.* **1980**, *21*, 4153.
(4) Caruthers, M. H. *Science (Washington, D.C.)* **1985**, *230*, 281. Eckstein, F. *Angew. Chem., Int. Ed. Engl.* **1983**, *22*, 423. Connolly, B. A.; Potter, B. V. L.; Eckstein, F.; Pingoud, A.; Grotjahn, L. *Biochemistry* **1984**, *23*, 3443.
(5) Cock, A. F.; Holman, M. J.; Nussbaum, A. L. *J. Am. Chem. Soc.* **1969**, *91*, 1522. Cock, A. F.; Holman, M. J.; Nussbaum, A. L. *J. Am. Chem. Soc.* **1969**, *91*, 6479.
(6) Sekine, M.; Hamaoki, K.; Hata, T. *Bull. Chem. Soc. Jpn.* **1981**, *54*, 3815.
(7) Takaku, H.; Shimada, Y. *Tetrahedron Lett.* **1972**, *5*, 411.
(8) Schrader, G. Ger. Pat. 1 112 752, 1960; *Chem. Abstr.* **1962**, *56*, 2331c.

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