considerable attention recently,[13–16] but most of the discussion has centered around angles of 180° *vs.* 120°. There is mounting evidence[17–20] that angles in the 160° range are quite prevalent in metal nitrosyls. Intermediate angles such as these are obviously not easily rationalized; it may be that better overlap between the metal and ligand orbitals can be achieved through moderate bending.

**Acknowledgments.** We are grateful to the National Science Foundation (Grant No. GP-17472), the Petroleum Research Fund, administered by the American Chemical Society (Grant No. 2059-G3), and the Research Corporation for supporting this research. We would also like to thank the Computer Center of the University of Southern California for making available its IBM 360/65 computer for this work.

---

(13) D. J. Hodgson and J. A. Ibers, *Inorg. Chem.*, 7, 2345 (1968).

(14) C. G. Pierpont, D. G. Van der Veer, W. Durland, and R. Eisenberg, *J. Amer. Chem. Soc.*, 92, 4760 (1970).

(15) D. M. P. Mingos and J. A. Ibers, *Inorg. Chem.*, **10**, 1035 (1971).

(16) D. M. P. Mingos, W. T. Robinson, and J. A. Ibers, *ibid.*, 10, 1043 (1971).

(17) D. M. P. Mingos and J. A. Ibers, *ibid.*, 9, 1105 (1970).

(18) J. T. Thomas, J. H. Robertson, and E. G. Cox, *Acta Crystallogr.*, **11**, 599 (1958).

(19) G. R. Davies, R. H. B. Mais, and P. G. Owston, *Chem. Commun.*, 81 (1968).

(20) O. L. Carter, A. T. McPhail, and G. A. Sim, *J. Chem. Soc. A*, 1095 (1966).

**R. Bau,* I. H. Sabherwal, A. B. Burg**
*Department of Chemistry, University of Southern California*
*Los Angeles, California 90007*

*Received June 21, 1971*

## On the Action of Europium Shift Reagents

*Sir:*

Since the discovery by Hinckley[1] of the large shifts in nuclear magnetic resonances produced by interaction with compounds of trivalent europium, many studies which exploit the effect for determination of structure have been carried out. This communication is intended to remind people of the analysis carried out by Van Vleck[2] about 40 years ago of the paramagnetisms of Eu,$^{3+}$ and to emphasize its bearing on interpretation of the phenomena observed with the shift reagents.

At room temperature Eu$^{3+}$ is found in its lowest electronic state, $^7F_0$, and its first excited state at 200 cm$^{-1}$, $^7F_1$, with approximately equal occupation probabilities. The lowest state, $^7F_0$, is nondegenerate and has no Zeeman splitting. Hence the interactions usually cited—contact and pseudocontact—cannot operate in the lowest state. In the excited state $^7F_1$, where the crystal-field splittings are much smaller than $kT$, the pseudocontact and contact interactions may contribute to the shifts. The dominant contribution from the $^7F_0$ ground state is undoubtedly related to the second-order paramagnetism.

The static susceptibility of Eu$^{3+}$ should be almost independent of temperature below $\sim$100°K. At these temperatures, a shift independent of temperature is expected. It should have the $\langle r^{-3}\rangle$ dependence on distance exhibited by the pseudocontact shift. I would expect that, because of the very small crystal-field splittings of the excited state whose admixture produces the second-order paramagnetism, the temperature-independent part of the shift would be almost isotropic.

It is unfortunate that only meager data concerning the magnetic properties of the various rare earth ions in the compounds used as shift reagents are available. Their acquisition will probably enhance the usefulness of the method.

**Acknowledgment.** This work has been supported by the National Science Foundation and by the Petroleum Research Fund of the American Chemical Society.

**S. I. Weissman**
*Department of Chemistry, Washington University*
*St. Louis, Missouri 63130*

*Received August 16, 1971*

---

(1) C. C. Hinckley, *J. Amer. Chem. Soc.*, 91, 5160 (1969).

(2) J. H. Van Vleck, "The Theory of Electric and Magnetic Susceptibilities," Oxford University Press, London, 1932, Chapter IX.

## A Simple Protecting Group Protection–Purification "Handle" for Polynucleotide Synthesis. I

*Sir:*

In recent years chemical reactions have been carried out on polymer supports by a number of investigators with the objective of simplifying the procedures for the stepwise synthesis of complex substances such as polypeptides and polynucleotides. This method has proved a great success in the field of peptide synthesis.[1] More recently, the application of this procedure to oligonucleotide synthesis has been investigated in a number of laboratories.[2–7] However, desired sequences obtained through the present method of solid-phase synthesis are accompanied with several truncated sequences.[8]

Since it is evident that the formation of truncated sequences cannot be avoided in solid-phase synthesis, we hope to prepare desired sequences exclusively by taking advantage of separation on a solid phase, thereby overcoming the limitation of the solid-phase synthesis.

This report describes a new method of preparing oligothymidylate derivatives which may be applied to the preparation of other pure oligothymidylate sequences. The coupling reactions of nucleotides in this method were carried out in solution in the absence of polymer. In the next stage, the desired oligonucleotide was separated from the other by-products using an ion exchange resin employing salt formation or molecular adsorption between the protecting group and the ion exchange resin. A basic *N*,*N*-dimethyl-*p*-phenylenediamino group was chosen as a protecting group on the nucleoside 5′-phosphate end. This group was intro-

---

(1) R. B. Merrifield, *Advan. Enzymol.*, **32**, 221 (1969), and references cited therein.

(2) R. L. Letsinger and V. Mahadevan, *J. Amer. Chem. Soc.*, **87**, 3526 (1965); **88**, 5319 (1966).

(3) F. Cramer, R. Helbig, H. Hettler, K. H. Scheit, and H. Seliger, *Angew. Chem.*, **78**, 640 (1966); F. Cramer and H. Köster, *ibid.*, **80**, 488 (1968).

(4) H. Hayatsu and H. G. Khorana, *J. Amer. Chem. Soc.*, **88**, 3182 (1966); **89**, 3880 (1967).

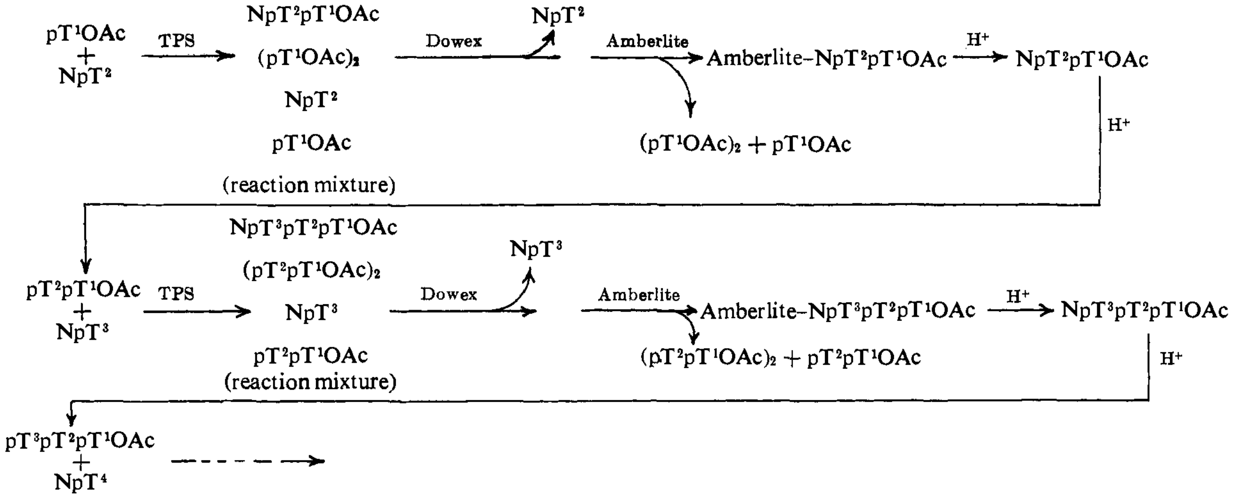
(5) L. R. Melby and D. R. Strobach, *ibid.*, **89**, 450 (1967); *J. Org. Chem.*, **34**, 421, 427 (1969).

(6) G. M. Blackburn, M. J. Brown, and M. R. Harris, *J. Chem. Soc. C*, 2483 (1967).

(7) T. Kusama and H. Hayatsu, *Chem. Pharm. Bull.*, **18**, 319 (1970).

(8) E. Bayer, H. Eckstein, K. Hägele, W. A. König, W. Brüning, H. Hagenmaier, and W. Parr, *J. Amer. Chem. Soc.*, **92**, 1735 (1970).

**Chart I.** General Synthetic Scheme



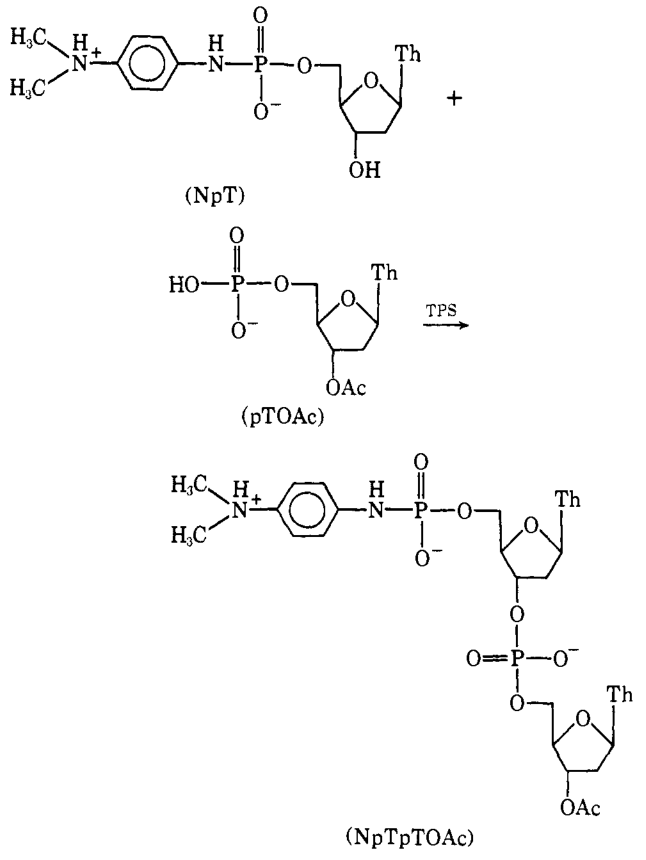
duced to form a phosphoroanilidate linkage which could be cleaved easily by treatment with 80% acetic acid at 80° for 3 hr.

The thymidine 5′-phosphoroanilidate (NpT) was obtained in 90% yield from *N*,*N*-dimethyl-*p*-phenylenediamine and thymidine 5′-phosphate (pT) by a modification of the procedure of Moffatt.[9] The NpT was selectively adsorbed on resin by the treatment of the reaction mixture with Dowex 50W-X2 ($H^+$ form)[10] and eluted by aqueous triethylamine (0.2 *M* solution).

The general synthetic scheme developed for oligothymidylate derivatives is outlined in Chart I.

When a mixture of NpT (1.3 mmol) and pTOAc (1.0 mmol) was treated with TPS (3.0 mmol)[11] in dry pyridine at room temperature for 13 hr, the corresponding dinucleotide derivative, NpTpTOAc, was obtained in 65% yield along with $(pTOAc)_2$, pTOAc, and NpT. After passing the mixture through a column (15 × 3 cm) of Dowex 50W-X2 ($H^+$ form), the eluate contains NpTpTOAc, $(pTOAc)_2$, and pTOAc since NpT is completely and selectively adsorbed on the resin as described above. The eluate was neutralized with 0.1 *N* sodium hydroxide and was passed through a column (20 × 3 cm) of Amberlite IR-45 (free base). The NpTpTOAc was selectively adsorbed on the resin which excluded the other compounds, $(pTOAc)_2$ and pTOAc.[12] After washing the column with water, NpTpTOAc was eluted with 80% acetic acid and the eluate was heated at 80° for 3 hr. The solution was concentrated to dryness and the residue was dissolved in water. After washing with ether, the aqueous solution of pTpTOAc was treated with Dowex 50W-X2 (pyridinium form) and concentrated to dryness. Evaporation was then repeated three times with dry pyridine. The pTpTOAc[13] was isolated in 60% yield based on pTOAc and stored in dry pyridine.

Furthermore, when a mixture of NpT (0.45 mmol) and pTpTOAc (0.15 mmol) in dry pyridine was treated with TPS (0.45 mmol) at room temperature for 14 hr, the corresponding trinucleotide derivative, NpTpTOAc,



(NpT)

(pTOAc)

(NpTpTOAc)

Th = thymine

was obtained in 34% yield along with NpT and $(pTpTOAc)_2$. After treating with a Dowex 50W-X2 ($H^+$ form) column (15 × 3 cm), NpT was removed and the eluate was neutralized with 0.1 *N* sodium hydroxide and passed through a column (15 × 3 cm) of Amberlite IR-45 (free base). The NpTpTpTOAc was adsorbed on the resin and eluted with 80% acetic acid. The eluate was heated at 80° for 3 hr and concentrated. The residue was dissolved in water and washed with

---

(9) J. G. Moffatt and H. G. Khorana, *J. Amer. Chem. Soc.*, **83**, 649 (1961).

(10) The phosphoroanilidate is perfectly stable to Dowex 50W-X2 ($H^+$ form) at 5° for 6 hr.

(11) In this notation pT refers to thymidine 5′-phosphate, N to *N*,*N*-dimethyl-*p*-phenylenediamino group, OAc to the 3′-*O*-acetyl group of thymidine, $(pTOAc)_2$ to $P^1,P^2$-di-3′-*O*-acetylthymidine 5′-pyrophosphate, and TPS to triisopropylbenzenesulfonyl chloride.

(12) It is possible that Amberlite IR-45, which contains many anilino groups, may be adsorbed by NpTpTOAc with the terminal *N*,*N*-dimethyl-*p*-phenylenediamino group by forces of molecular adsorption. This hypothesis is supported by the adsorption of *N*,*N*-dimethyl-*p*-phenylenediamine on Amberlite IR-45 resin.

(13) The hydrolysis of the acetyl group from pTpTOAc was observed under acidic conditions. Acetylation with acetic anhydride afforded pure pTpTOAc in quantitative yield.

ether. The pTpTpTOAc was isolated in 31% yield based on pTpTOAc.

Data on the chromatographic and spectral properties of the compounds which have been prepared are summarized in Table I. The yields of the compounds were determined spectrophotometrically.

**Table I.** Chromatographic and Spectral Properties of the Reported Compounds

| Compd | —Pc[a] solvent— A | B | Spectral data (in $H_2O$, pH = 9) $\lambda_{max}$ ($10^{-3}\epsilon$) | $\lambda_{min}$ |
|---|---|---|---|---|
| pT | 0.20 | 0.22 | | |
| NpT | 0.58 | | 251 (17.2) | 230 |
| pTOAc | 0.22 | 0.33 | | |
| NpTpTOAc | 0.45 | | 255 (25.0) | 235 |
| pTpTOAc | 0.12 | 0.29 | | |
| pTpT[b] | 0.09 | 0.19 | | |
| NpTpTpTOAc | 0.24 | | 266 (33.0) | 236 |
| pTpTpTOAc | 0.05 | 0.12 | | |
| pTpTpT[b] | 0.04 | 0.10 | | |

[a] Pc = paper chromatography. Paper chromatography was carried out by the descending technique using Toyo-Roshi no. 51 paper. Solvent systems used were: solvent A, isopropyl alcohol–concentrated ammonium hydroxide–water (7:1:2, v/v); solvent B, ethanol–0.5 *M* ammonium acetate, pH 3.8 (7:3, v/v). [b] The compound has been reported by Khorana and coworkers (P. T. Gilham and H. G. Khorana, *J. Amer. Chem. Soc.*, **80**, 6212 (1958); H. G. Khorana and J. P. Vizsolyi, *ibid.*, **83**, 675 (1961)). The $R_f$ values of the compounds agree well with those given in the literature and the oligothymidylates prepared by the present method were characterized by degradation to pT with snake venom phosphodiesterase.

**Tsujiaki Hata,* Kiyohiko Tajima, Teruaki Mukaiyama**
*Department of Chemistry, Tokyo Institute of Technology*
*Ookayama, Meguro-ku, Tokyo, Japan*

*Received January 25, 1971*

## A Comparison of the Reactivities of the Lowest Excited States of Nitrogen ($A^3\Sigma_u^+$) and of Carbon Monoxide ($a^3\Pi$)

*Sir:*

The long-lived lowest triplet states of nitrogen and carbon monoxide can be produced by discharge-flow techniques in an environment which is suitable for identification of their reactions and the measurement of rate constants.[1,2] Although both states have nearly the same amount of electronic energy, *i.e.*, CO($a^3\Pi$; $v' = 0$) = 48,474 cm$^{-1}$ and $N_2$($A^3\Sigma_u^+$; $v' = 0$) = 49,757 cm$^{-1}$, the CO state arises by excitation of an electron from the $5\sigma$ nonbonding to the $2\pi$ antibonding orbital, whereas the nitrogen state is formed by promotion of an electron from $1\pi_u$ bonding to the $2\pi_g$ antibonding orbital.[3] Both states acquire their metastable nature[4] from the prohibition of spin change with radiative decay. We wish to report on some interesting differences between the rates of quenching of these two excited states by several simple substrate molecules.

The excited states of $N_2$ and CO were generated by the interaction of ground-state $N_2$ and $CO_2$, respectively, with metastable Ar($^3P_{0,2}$) atoms (11.7 eV) in a discharge-flow apparatus; the technique has been described previously for both cases.[1,2] In the present application, the reaction vessel was a 28-mm i.d. Pyrex tube with three quartz observation windows. The interaction between the $CO_2$ or $N_2$ and the metastable argon atoms takes place at the entrance of the flow reactor, and the CO($a^3\Pi$) or $N_2$($A^3\Sigma_u^+$) molecules are carried downstream from the interaction zone by the argon carrier gas. The flow velocity was ~2 × $10^3$ cm sec$^{-1}$ at a flow tube pressure of 1.5–4.0 Torr. Reagents, diluted with 10 parts of pure argon and stored in 5-l. reservoirs, were added either 4.0 or 11.0 cm upstream of the last observation window. Observations were made through the quartz windows perpendicular to the gas flow with a 0.3-m McPherson scanning monochromator equipped with a HTV R212 photomultiplier tube and PAR phase lock detector. The CO($a^3\Pi$) concentration was monitored by observing the Cameron bands, CO($a^3\Pi \rightarrow X^1\Sigma^+$). In the present study the $N_2$($A^3\Sigma_u^+$) concentration was followed by direct observation of Vegard–Kaplan bands, $N_2$($A^3\Sigma_u^+ \rightarrow X^1\Sigma_g^+$). In our earlier work, the 2537-Å emission from Hg($^3P_1$), which is directly proportional to [$N_2$(A)],[1b,c,5] was employed to follow the $N_2$(A) concentration.

The quenching rate constants were measured by using the fixed-observation-point technique in which [$N_2$(A)] or [CO(a)] was monitored at a fixed distance downstream from the mixing point; we used the last window, 11 cm away from the uppermost addition jet through which quenching gases were added. First-order quenching plots, which followed $I = I_0 \exp(-k_Q \cdot [Q]\Delta t)$, were observed and the slopes of the lines shown in Figure 1 were used to obtain the quenching rate constant, $k_Q$. The method was checked by adding reagent at a jet 4.0 cm from the observation point, and good agreement was found for the rate constants of all quenching gases.

A summary of the rate constants for both metastables obtained at room temperature is shown in Table I. The rate constants for quenching of $N_2$(A) determined in this study were somewhat lower than those previously reported from this laboratory. A problem with incomplete mixing was determined to be the cause for this discrepancy. The rate constants measured in this work should be considered lower limits; however, they are probably good to within 50% of the actual values. Except for special cases mentioned in the footnotes of the table, the agreement between the $N_2$(A) rate constants measured here and the values in the literature is within experimental error.[1c,6,7] In any event, the relative quenching rate constants of CO(a) and $N_2$(A) measured in the present apparatus are not affected and may be considered accurate.

---

(1) (a) D. W. Setser, D. H. Stedman, and J. A. Coxon, *J. Chem. Phys.*, 53, 1004 (1970); (b) J. A. Meyer, D. W. Setser, and D. H. Stedman, *J. Phys. Chem.*, 74, 2238 (1970); (c) J. A. Meyer, D. W. Setser, and D. Klosterboer, *J. Chem. Phys.*, in press.

(2) G. W. Taylor and D. W. Setser, *Chem. Phys. Lett.*, **8**, 51 (1971).

(3) G. Herzberg, "Spectra of Diatomic Molecules," Van Nostrand, Princeton, N. J., 1950.

(4) (a) The radiative lifetime of the $N_2(A^3\Sigma_u^+)$ state is 1.3 and 2.6 sec for the $\Sigma = 0$ and ±1 substates, respectively: D. W. Shamansky and N. P. Carleton, *J. Chem. Phys.*, 51, 682 (1969). (b) The radiative lifetime of CO(a) is not firmly established, but a value of 7.5 msec recently has been reported: G. M. Lawrence, *Chem. Phys. Lett.*, **9**, 575 (1971); R. J. Donovan and D. Hussain, *Trans. Faraday Soc.*, **63**, 2879 (1967), reported a value of 12 msec.

(5) R. A. Young and G. A. St. John, *J. Chem. Phys.*, **48**, 2572 (1968).

(6) R. A. Young, G. Black, and T. G. Slanger, *ibid.*, **50**, 303 (1969).

(7) A. B. Callear and P. M. Wood, *Trans. Faraday Soc.*, **67**, 272 (1971).