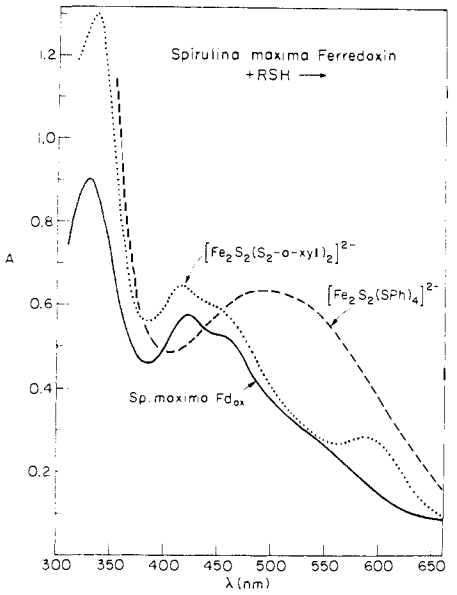


Figure 2. Spectrophotometric demonstration of two extrusion reactions of 2-Fd_{ox} in 80% DMSO. Product complexes were identified spectrally.[5,8]

are different from that of the protein and $[Fe_4S_4(SPh)_4]^{2-}$, and the same as those of the isolated complexes[5,8] measured separately.

With the demonstration of extrusion reactions of relatively simple proteins in hand, one of the potentially significant applications of reaction 1 would involve extension to complex Fe–S proteins and enzymes[10] in which the organization of active sites into 2-Fe and 4-Fe types cannot be satisfactorily established by spectroscopic methods. Several observations bearing on this point are noted. Experimental conditions for 2-Fd_{ox} extrusions must be carefully controlled. Preliminary kinetic measurements have shown that the dimer→tetramer conversion, reaction 2, is essentially

$$2[Fe_2S_2(SPh)_4]^{2-} \longrightarrow [Fe_4S_4(SPh)_4]^{2-} + PhSSPh + 2PhS^- \quad (2)$$

quantitative in 80% DMSO. Rates are dependent upon the pH of the aqueous component as the following dimer half-lives indicate: pH 8.5, 80 min; pH 7.9, 30 min (25°, dimer concentration *ca.* 10^{-4} *M*). The reaction is further retarded by excess thiol, such that, under the conditions employed, no significant amount of tetramer resulted. Reaction 2 must obviously be suppressed in order to prevent incorrect identification of the protein active site type when liberated as its arylthiolate derivative. A search has been made for thiol reagents capable of specific core extrusions. Although *o*-xyl$(SH)_2$ forms the stable binuclear complex $[Fe_2S_2(S_2$-*o*-xyl$)_2]^{2-}$, resistant to dimerization under extrusion conditions, its reaction with 8-Fd_{ox} and also $[Fe_4S_4(SEt)_4]^{2-}$ yields a product whose spectrum (Figure 1) indicates formation of an alkylthiolate tetramer,[2] presumably an oligomer of tetramers, $[Fe_4S_4(S_2$-*o*-xyl$)_2]_n^{2n-}$.[16] In contrast to the reactions with benzenethiol and *o*-xyl$(SH)_2$, treatment of both proteins with ethane-1,2-dithiol does not result in intact core extrusion. Instead the very stable *sulfide-free* dimer $[Fe_2(edt)_4]^{2-}$, previously characterized,[17] is obtained. The core structure of 8-Fd_{ox} is thus degraded, with the product complex obtained in reduced yield. With 2-Fd_{ox} the reaction proceeds through an unidentified intermediate species (λ_{max} 590 nm) and affords the dimer in high yield.

While no specific core extrusion reagents have been found, both benzenethiol and *o*-xyl$(SH)_2$ are capable of high-yield intact core removal from proteins in the form of spectrally distinguishable 2-Fe and 4-Fe complexes (Figure 1 and 2). Further, the electrochemical properties[5] of, *e.g.*, $[Fe_4S_4(SPh)_4]^{2-}$ and $[Fe_2S_2(SPh)_4]^{2-}$, are sufficiently different to permit analysis of solutions containing both species which might result from extrusion of a complex Fe–S protein. The use of the extrusion method to identify active sites in such molecules is under active investigation.[18] Any further elaboration of this method, especially with regard to the development of thiol reagents which afford protein core derivatives with more distinct or intense spectral features than those presented here, should be guided by the following observation. With all thiols thus far examined the reactions of the (readily accessible[5,7]) synthetic analogs $[Fe_2S_2(SR)_4]^{2-}$ and $[Fe_4S_4(SR)_4]^{2-}$ exactly parallel those of 2-Fd_{ox} and 8-Fd_{ox} proteins, respectively.

Acknowledgment. This research was supported at M.I.T. by National Institutes of Health Grant GP-19298. We thank R. Bare for experimental assistance.

References and Notes

(1) M. A. Bobrik, L. Que, Jr., and R. H. Holm, *J. Amer. Chem. Soc.*, **96**, 285 (1974).
(2) B. V. DePamphilis, B. A. Averill, T. Herskovitz, L. Que, Jr., and R. H. Holm, *J. Amer. Chem. Soc.*, **96**, 4159 (1974).
(3) L. Que, Jr., M. A. Bobrik, J. A. Ibers, and R. H. Holm, *J. Amer. Chem. Soc.*, **96**, 4168 (1974).
(4) L. Que, Jr., J. R. Anglin, M. A. Bobrik, A. Davison, and R. H. Holm, *J. Amer. Chem. Soc.*, **96**, 6042 (1974).
(5) J. J. Mayerle, S. E. Denmark, B. V. DePamphilis, J. A. Ibers, and R. H. Holm, *J. Amer. Chem. Soc.*, in press.
(6) T. Herskovitz, B. A. Averill, R. H. Holm, J. A. Ibers, W. D. Phillips, and J. F. Weiher, *Proc. Nat. Acad. Sci. U.S.*, **69**, 2437 (1972).
(7) B. A. Averill, T. Herskovitz, R. H. Holm, and J. A. Ibers, *J. Amer. Chem. Soc.*, **95**, 3523 (1973).
(8) J. J. Mayerle, R. B. Frankel, R. H. Holm, J. A. Ibers, W. D. Phillips, and J. F. Weiher, *Proc. Nat. Acad. Sci. U.S.*, **70**, 2429 (1973).
(9) R. H. Holm, W. D. Phillips, B. A. Averill, J. J. Mayerle, and T. Herskovitz, *J. Amer. Chem. Soc.*, **96**, 2109 (1974).
(10) W. H. Orme-Johnson, *Annu. Rev. Biochem.*, **42**, 159 (1973).
(11) Numbers refer to iron content per mole. Proteins were isolated according to published procedures: 8-Fd_{ox}, L. E. Mortenson, *Biochim. Biophys. Acta*, **81**, 71 (1964); 2-Fd_{ox}, D. O. Hall, K. K. Rao, and R. Cammack, *Biochem. Biophys. Res. Commun.*, **47**, 798 (1972).
(12) J. R. Bale and W. H. Orme-Johnson, *Proc. Nat. Acad. Sci. U.S.*, submitted for publication.
(13) C. C. McDonald, W. D. Phillips, W. Lovenberg, and R. H. Holm, *Ann. N.Y. Acad. Sci.*, **222**, 789 (1973).
(14) R. Cammack, *Biochem. Biophys. Res. Commun.*, **54**, 548 (1973).
(15) G. R. Dukes and R. H. Holm, *J. Amer. Chem. Soc.*, in press.
(16) The ligand bite distance appears too small to span the *ca.* 7 Å between thiolate coordination positions *within* a tetramer. The oligomeric product has not been isolated in crystalline form.
(17) M. R. Snow and J. A. Ibers, *Inorg. Chem.*, **12**, 249 (1973); T. Herskovitz, B. V. DePamphilis, W. O. Gillum, and R. H. Holm, *Inorg. Chem.*, in press.
(18) W. H. Orme-Johnson, private communication.

L. Que, Jr., R. H. Holm*
Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

L. E. Mortenson
Department of Biological Sciences, Purdue University Lafayette, Indiana 47907

Received October 25, 1974

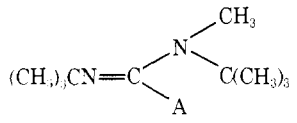
Dehydrations with *N*-Methyl-*N,N'*-di-*tert*-butylcarbodiimidium Ion

Sir:

In view of the broad utility of N,N'-disubstituted carbodiimides (**1**) for effecting molecular dehydrations,[1] it becomes of interest to evaluate the *N,N,N'*-trialkylcarbodi-

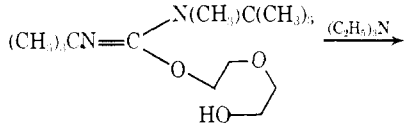
$$RN{=}C{=}NR \qquad RN{=}C{=}\overset{+}{N}(R')R$$
1 **2**

imidium ion (**2**), which, because of the quaternary nitrogen present, might act as an even more potent agent for such reactions. A preliminary survey reveals that *N*-methyl-*N,N'*-di-*tert*-butylcarbodiimidium tetrafluoroborate (**3**)[2] not only permits overall conversion of protected amino acids to peptide and nucleotide to pyrophosphate but also aliphatic glycol to cyclic ether, all reactions apparently involving initial rapid addition of alcohol or acid (AH) to the reagent, giving **4**, which is then transformed to final product.[3]

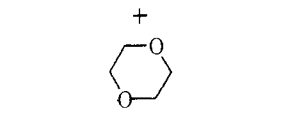


4

In the ether synthesis, 1.0 equiv of reagent **3** is added slowly to diethylene glycol in $CHCl_3$–$C_6H_5NO_2$ at room temperature. After addition of 1.2 equiv of triethylamine, the mixture is heated to 130° while the product, *p*-dioxane (55%), is collected by distillation over a 2-hr period. Under similar conditions, 1,4-butanediol generates tetrahydrofuran (64%), and 1.6-hexanediol provides oxepane (22%).[4] In our hands no dioxane is formed from equimolar amounts of glycol and tosylchloride in pyridine (with or without triethylamine), while 13% of this ether was detected when dicyclohexylcarbodiimide (DCC)-*p*-toluenesulfonic acid in $CHCl_3$ was employed. The rapid (<5 min), quantitative addition of methanol at 20° to **3** to give adduct **4** (A = OCH_3), mp 98.5–99.5° (ir (CH_2Cl_2) 3.4, 6.12 μ; nmr CD_3NO_2 δ 1.45 (s, 9 H), 1.50 (s, 9 H), 3.08 (3 H), 4.20 (3 H)), suggests that in the ether synthesis glycol addition initially provides **5**, which under the influence of the added amine undergoes internal displacement of trialkylurea by alkoxide ion.[5,6]

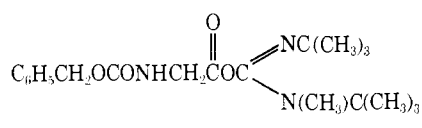


5

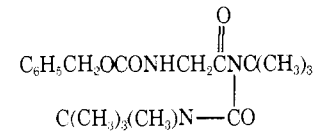


In the peptide synthesis experiments, equimolar amounts of *N*-acylamino acid and amino acid ester hydrochloride or amine were stirred in $C_2H_4Cl_2$ or CH_2Cl_2 at 0 or −78° under N_2 while salt **3** was added. During work-up, hexane–ether was used to remove urea by-product, and the desired amide was obtained by normal peptide isolation procedures. By such means, the expected amide was prepared from benzylamine and *N*-carbobenzoxyglycine (77%), and peptide was secured from *N*-benzoyl-L-leucine and ethyl glycinate (69%, product 86% optically pure), and from *N*-carbobenzoxy-L-valine and ethyl glycinate (69%). In the absence of amine, *N*-carbobenzoxyglycine is converted, apparently *via* O → N acyl migration of **6**, to the *N*-acylurea **7** (ir (CCl_4) 5.79, 5.93, 5.98 μ; nmr ($CDCl_3$) δ 1.43 (18 H), 3.03 (3 H), 3.86 (d, 2 H), 5.12 (2 H), 5.43–5.83 (m, 1 H), 7.31 (5 H)), which is inert to primary amines or glacial acetic acid for 3 hr at 70°.

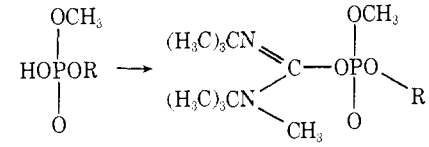
Symmetrical pyrophosphate results from the action of **3** on nucleotide, for example, 2',3'-isopropylidineuridine 5'-phosphate (trimethylamine salt) (DMF at 20°; 73% yield, after cellulose chromatography and ion exchange). However, experiments designed to give a dinucleotide from suitably protected nucleotide and nucleoside provided only a few per cent of the expected product after 2 days at 20°. Carbodiimidium reagent **3** converted methyluridine 5'-phosphate **8** to a product regarded as urea **10** (nmr (D_2O) δ 0.9–1.3 (m, 18 H), 2.45 (3 H), 3.40 (d(J = 11 Hz), 3 H), 3.8–4.3 (m, 5 H), 5.6–6.0 (m, 2 H); 7.73 (d(J = 8 Hz), 1 H)), arising by O → N migration of the phosphate unit in intermediate **9**. Similar behavior of nucleotide diesters with DCC has been reported by Khorana and coworkers.[7]



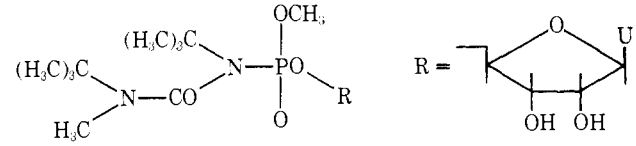
6



7



8 9



10

Acknowledgment. Financial support was provided by National Institutes of Health Grant GM20677.

References and Notes

(1) H. G. Khorana, *Chem. Rev.*, **53**, 145 (1953); F. Kurzer and K. Douraghi-Zadeh, *ibid.*, **67**, 107 (1967).
(2) K. Hartke, F. Rossbach, and M. Radau, *Justus Liebigs Ann. Chem.*, **762**, 167 (1972).
(3) The only other, previously reported reaction of trialkylcarbodiimidium ion involves use of the iodide salt for conversion of an alcohol to the corresponding iodide. See R. Scheffold and E. Saladin, *Angew. Chem., Int. Ed. Engl.*, **11**, 229 (1972).
(4) The sulfurane procedure of J. C. Martin, J. A. Franz, and R. J. Arhart, *J. Amer. Chem. Soc.*, **96**, 4605 (1974), gave 0% oxepane from 1,6-hexanediol, 40% *p*-dioxane from diethylene glycol, and 72% THF from 1,4-butanediol.
(5) On treatment of **3** with pyridine or trimethylamine in CH_2Cl_2 or anhydrous K_2CO_3 in $C_2H_4Cl_2$, isobutylene and methyl-*tert*-butylcyanamide are formed.
(6) The preparation of aryl alkyl ethers from phenols and primary alcohols through the agency of dicyclohexylcarbodiimide has been reported by E. Vorwinkel, *Ber. Deut. Chem. Ges.*, **99**, 42 (1966).
(7) M. Smith, J. G. Moffatt, and H. G. Khorana, *J. Amer. Chem. Soc.*, **80**, 6204 (1958).

R. C. Schnur, E. E. van Tamelen*
Department of Chemistry, Stanford University
Stanford, California 94305
Received November 4, 1974

Syntheses with Tungsten and Molybdenum Atoms. Reactions with Cyclopentadiene and Cycloheptatriene

Sir:

We report here syntheses of several organometallic compounds by reaction of free tungsten and molybdenum atoms with cyclopentadiene and cycloheptatriene. These one step reactions proceed in good yields offering convenient means for preparing several hundred milligrams of products.