Acknowledgment. I am indebted to Dr. B. R. Kennedy for assistance with the computer simulation and helpful suggestions and to Dr. S. W. Nicksic, who obtained the ESR spectra.

References and Notes

(1) L. de Vries, *J. Org. Chem.*, **38**, 2604, 4357 (1973).
(2) H. Gold and O. Bayer, *Chem. Ber.*, **94**, 2594 (1961).
(3) All new compounds gave satisfactory analysis and spectral data consistent with the structure. For further details, structure proof, or mode of formation, see L. de Vries, to be published.
(4) B. de B. Darwent, "Bond Dissociation Energies in Simple Molecules", U.S. Department of Commerce, National Bureau of Standards, NSRDS-NBS 31 (1970).
(5) R. W. Baldock, P. Hudson, A. R. Katritzky, and F. Soti, *J. Chem. Soc., Perkin Trans. 1*, 1422, 1427 (1974).
(6) R. A. Kaba and K. U. Ingold, *J. Am. Chem. Soc.*, **98**, 523 (1976).
(7) (a) D. E. Wood and R. V. Lloyd, *J. Chem. Phys.*, **52**, 3840 (1970); (b) W. C. Danen and R. C. Rickard, *J. Am. Chem. Soc.*, **94**, 3254 (1972).
(8) K. Wallenfels, K. Friedrich, J. Rieser, W. Erbel, and H. K. Thieme, *Angew. Chem., Int. Ed. Engl.*, **15**, 261 (1976).
(9) R. B. Woodward and R. Hoffmann, "The Conservation of Orbital Symmetry", Verlag Chemie GmbH, 1970, p 171.
(10) C. J. Jameson and W. Yang, *J. Theor. Biol.*, **35**, 248 (1972); J. B. Moffat, *J. Chem. Soc., Chem. Commun.*, 888 (1975); J. B. Moffat and K. F. Tang, *J. Theor. Biol.*, **58**, 83 (1976).
(11) W. A. Lathan, L. A. Curtiss, W. J. Hehre, J. B. Lisle, and J. A. Pople, *Prog. Phys. Org. Chem.*, 11, 175 (1974); D. Booth and J. W. Murrell, *Mol. Phys.*, **24**, 1117 (1972).
(12) The sole example may be the direct fluorination of hydrocarbons, i.e., RH + FF → R· + HF + F·. Here this process is exceptionally favored by the dissociation energies: 135 kcal for HF and 37 kcal for F_2.[4]
(13) R. W. Begland, D. R. Harter, F. N. Jones, D. J. Sam, W. H. Sheppard, O. W. Webster, and F. J. Weigert, *J. Org. Chem.*, **39**, 2341 (1974).
(14) M. Calvin, "Chemical Evolution", Oxford University Press, New York, N.Y., 1969, 135–142.
(15) J. P. Ferris, D. B. Donner, and W. Lotz, *J. Am. Chem. Soc.*, **94**, 6968 (1972).

Louis de Vries
Chevron Research Co.
Richmond, California 94802
Received August 9, 1976

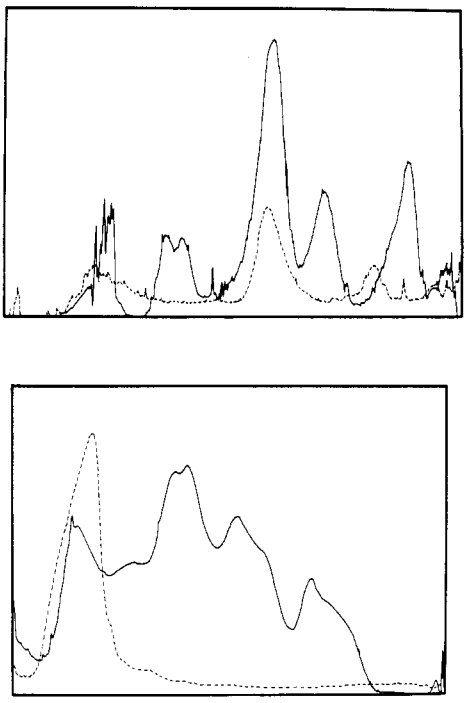


Figure 1. Superimposed densitometer tracings of polyarcylamide gels from SDS disk gel electrophoresis (1% mercaptoethanol; stained with coomassie blue and scanned at 550 nm). Gels scanned left to right—high to low molecular weight. Trace of standard reaction mixture with protein each treated in 0.1 M acetic acid 0.5% SDS, 0.001% EDTA, 12 h, r.t. 1 h, 80 °C without quinonediimide 1 (···) and with quinonediimide (—): top, partially reduced bovine pancreatic ribonuclease (Worthington); bottom, ovalbumin. The profile of ovalbumin is unchanged if the reaction is not heated after treatment with quinonediimide.

Cysteine Modification and Cleavage of Proteins with 2-Methyl-N^1-benzenesulfonyl-N^4-bromoacetylquinonediimide

Sir:

The determination of protein sequence has been greatly facilitated by cleavage techniques which cause fragmentation of the macromolecular peptide chain in a specific manner.[1,2] The use of peptidases for this purpose has been supplemented in recent years by a number of chemical cleavage techniques.[3-5] Nevertheless, the majority of chemical methods proposed for this purpose (based on studies of model substrates) have not been applied successfully to proteins. We wish to report the design and development of a new, cysteine-selective modification and cleavage agent, 2-methyl-N^1-benzenesulfonyl-N^4-bromoacetylquinonediimide (**1**),[6] which has been observed to cause fragmentation of both ovalbumin and reduced bovine pancreatic ribonuclease,[7] as well as model substrates.

Incubation of these proteins in 0.1 N acetic acid solution containing 0.5% SDS and 0.001 M EDTA (ovalbumin 4.5×10^{-5} M; ribonuclease 6.7×10^{-5} M) with an excess of **1** (as a 0.08 M solution in acetone) at room temperature for 12 h followed by heating at 80 °C for 1 h resulted in chain cleavage, which was detected by SDS gel electrophoresis[8] (see Figure 1). In each case, although under these particular conditions some uncleaved protein is observed,[8] a number of lower molecular weight protein fragments are produced upon such treatment. This fragmentation is proposed to occur via the route illustrated in Scheme I.

Initial 1,4-addition of the cysteine sulfhydryl function is directed "para" relative to the 2-methyl substituent, to form an aromatic thioether (**2**).[9,10] This primary regiospecific alkylation (easily observed and monitored by UV at 305 nm) permits subsequent alkylation of the sulfur atom by the adjacent bromoacetamido function to generate a labile sulfonium

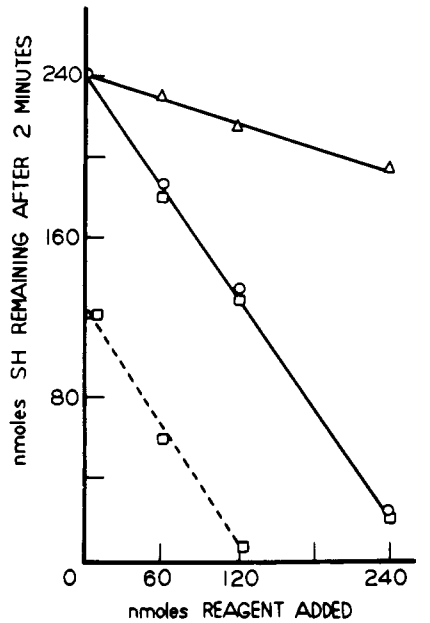
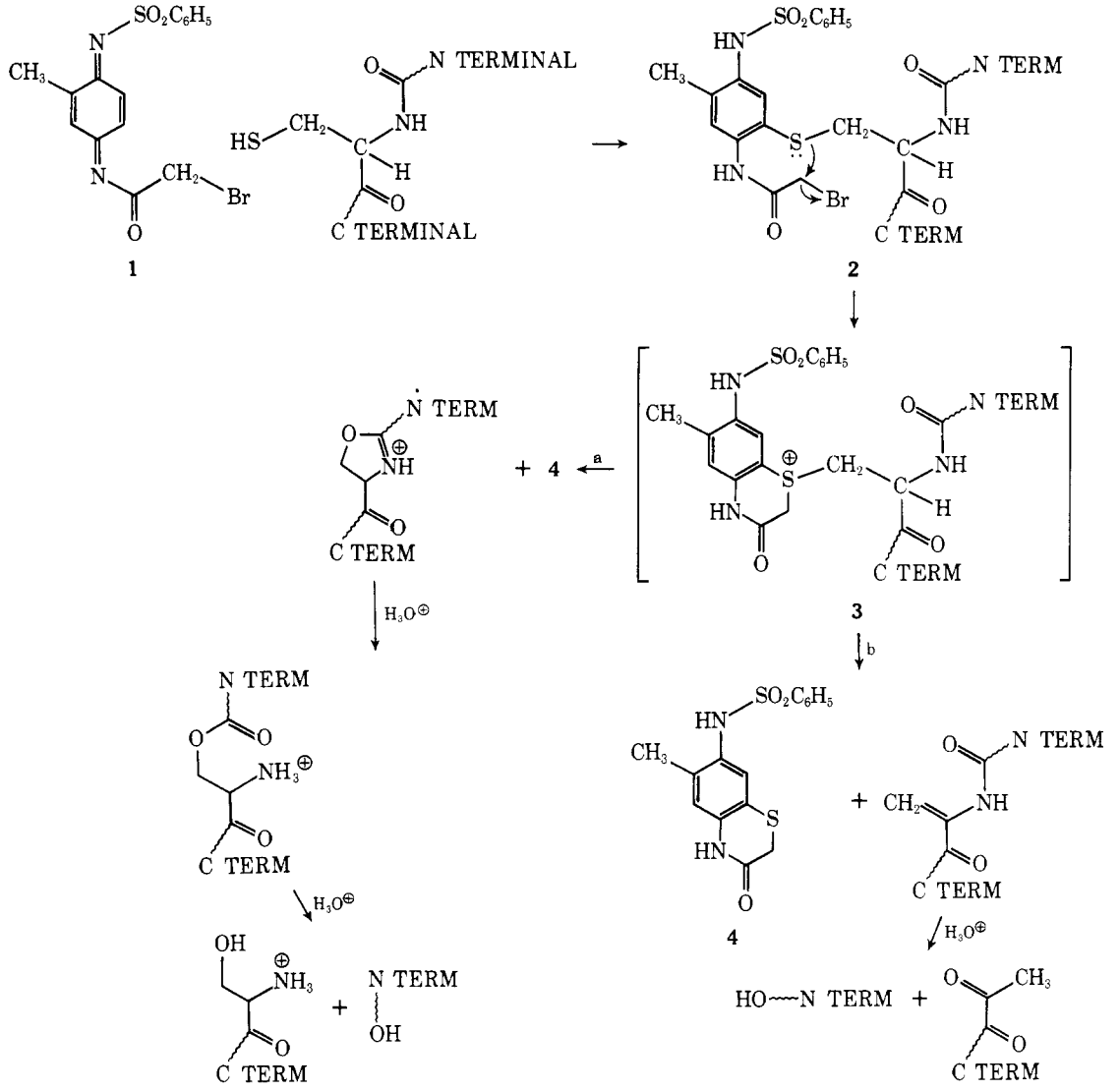


Figure 2. Loss of sulfhydryl of *N*-acetylcysteine (6.2×10^{-5} M) (—) and ovalbumin (1.25×10^{-5} M) (- - -) in 4.0 mL of 0.1 M phosphate buffer, pH 8, containing 0.5% SDS and 0.001 M (EDTA) after 2 min reaction time with 2-methyl-N'-benzenesulfonyl-N'-4-bromoacetylquinonediimide, **1** (□), or with 2-methyl-N'-benzenesulfonyl-N-4-acetylquinonediimide, **1a** (Br = H) (○), or with reduced reagent 2-methyl-4-bromoacetamidobenzenesulfonanidide (△). Sulfhydryl was determined by incubation with Ellman's reagent (DTNB) and recording absorbance at 420 nm after 20 min.

Scheme I



species (**3**). Degradation of this species (**3**) would be expected to occur via either of the two routes indicated: (a) by participation of the adjacent cysteinyl *N*-peptide bond to yield a hydrolyzable oxazoline, or (b) by β-elimination of 6-methyl-7-benzenesulfonamido-1,4-benzothiazine-3(4*H*)-one (**4**) to form an acid labile dehydroalanine (DHA) moiety.[3] Either of these routes would ultimately result in specific cleavage of the polypeptide chain adjacent to the cysteine residues. We anticipated that interference by nonspecific alkylation of other nucleophilic sites in the protein by either the bromoacetyl or quinone moieties[10] would be suppressed by maintenance of relatively low pH and mild conditions.[11]

This proposed reaction pathway is supported by the observed reactivity of **1** with the model substrate, *N*-acetylcysteine (N terminal = CH_3; C terminal = OH). As shown in Figure 2, the quinonediimide moiety (**1** and **1a**) is very sensitive to 1,4-addition of the cysteine sulfhydryl function at room temperature, in marked contrast to the slower alkylation of the α-bromoacetamido function of the reduced reagent (2′-methyl-4′-bromoacetamidobenzenesulfonanilide). Furthermore, the reaction of **1** with *N*-acetylcysteine in glacial acetic acid (10% water added after a short initial incubation) at 80 °C has been observed to produce the expected fragment (**4**) from bisalkylation of the cysteinyl thiol function (up to 75% isolated yield; mp 218–220 °C dec; ^{1}H NMR (acetone-d_6, Me_4Si) 2.05 (s, 3), 3.43 (s, 2), 6.89 (s, 1), 7.07 (s, 1), 7.33–7.90 (m, 6), 8.3 (bs, 1), 9.49 (bs, 1); IR (KBr) 3220, 1650, 1325, 1160 cm^{-1}; *m/e* 334 (M^+), 193 (base, $M^+ - C_6H_5SO_4$); λ_{max}^{EtOH} 220, 250 nm. Anal. ($C_{15}H_{14}N_2O_3S_2$): C, 53.98; H, 4.38; N, 8.43). In this model reaction, small quantities of both serine and acetylserine produced by path a are easily observed (TLC, ninhydrin). Acetaldehyde apparently produced through a decarboxylative elimination and hydrolysis (analogous to path b) was also detected (as DNPH, TLC comparison). Neither intermediate thioether (**2**) nor other aromatic thioethers which might result from nonregiospecific addition have been observed. However, under similar conditions to those above, the non-brominated quinonediimide (**1a**, Br = H), which possesses no capability for secondary alkylation, was observed to produce the analogous aromatic thioether (**2a**, Br = H, N term = CH_3, C term = OH) (63% isolated yield; mp 187–189 °C dec; ^{1}H NMR (acetone-d_6 with 10% Me_2SO-d_6 v/v to increase solubility) 1.96 (s, 3), 2.03 (s, 3), 2.11 (s, 3), 3.0 (m, 2), 4.36 (m, 1), 7.08 (s, 1), 7.3–7.9 (m, 6); *m/e* 465 (M^+), 318, 177 (base). Anal. ($C_{20}H_{23}N_4O_6S_2$): C, 51.37; H, 4.70; N, 8.92).

Reduction of the quinonediimide to an aromatic species with concomitant thiol oxidation to the corresponding disulfide has been observed to compete with the initial addition and subsequent cleavage reaction in certain cases. This phenomenon had been recognized previously by Adams in his experiments with quinonediimides.[11] Such redox interference was found to be particularly predominant in cleavage attempts with reduced glutathione, but less so with *N*-acetylcysteine or proteins such

as ovalbumin (see Figure 2). The investigation of other 2-substituted bisalkylating quinonediimides (2-chloro, MeO and CF_3) having different redox potentials has so far revealed no advantages. However, this redox reaction does not appear to be a major drawback to cleavage of reduced, denatured proteins, where fragments may be easily separated from oxidized proteins. In addition, preliminary data suggest this redox pathway may be controlled by radical chain inhibitors such as 2,6-di-*tert*-butylphenol.

Further corroboration of the selectivity of this reagent was observed by automated amino acid analyses of substrates treated with **1**.[12] Compared to samples treated under the same conditions without reagent, the destruction of cysteine (observed as $CySO_3H$) in peptide and proteins treated with **1** was as follows: *N*-acetylcysteine (48%); reduced glutathione (42%); ovalbumin (12%); and partially reduced bovine pancreatic ribonuclease[7] (50%). The conditions for oxidative hydrolyses of these proteins are quite strenuous (24–48 h at 110 °C, 6.2 N HCl, excess Me_2SO) and preclude the precise evaluation of nonspecific reagent interactions with certain residues such as histidine, tyrosine, serine, and methionine. However, homoserine from methionine alkylation and cleavage was not observed, and both lysine and arginine residues were unaffected by the reagent in these cases. We do know that certain tryptophan (indole) residues can react. They do not seem to give cleavage of the protein chain. Ammonia production (above that produced by substrate or reagent alone) was clearly observed in the analyses of each of the reaction mixtures. This observation, the model studies, and our negative attempts to dansyl label[13] new N-termini produced in the fragmentation of ovalbumin using **1** support dehydroalanine formation and hydrolysis as the predominant pathway of degradation. The formation of dehydroalanine residues from cysteine sulfonium species has been observed previously by Gross to be favored over amide bond participation at elevated temperatures and, therefore, is not unexpected with reagent **1** under the prescribed conditions (80 °C).[14] The advantage of our reagent is that it can be employed under mild acidic reaction conditions and thus suppress the complications (particularly lysinoalanine formation) observed when DHA residues are generated under strongly alkaline conditions.[15] Further, the fragmentation does not occur at methionine and the pyruvoyl peptide fragments produced by hydrolysis are amenable to sequence analysis.[16] We believe reagents such as **1** will have significant application in protein modification and structure determination.

Acknowledgments. The authors wish to acknowledge the generous assistance of Dr. Charles H. Williams, Jr., of the Department of Biological Chemistry, University of Michigan, and Veterans Administration Hospital, Ann Arbor, for obtaining the amino acid analysis. Financial support (T.J.H., Jr.) under NDEA Title IV (1971–74) and NIH Traineeship (1974–1975) programs is gratefully acknowledged.

References and Notes

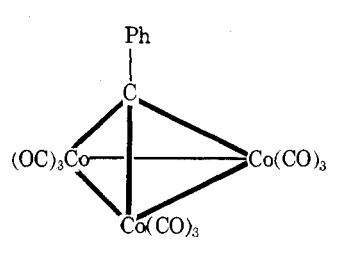
(1) C. H. W. Hirs and S. G. Timasheff, Ed., *Methods Enzymol.*, **25** 1972.
(2) A. Fontana and E. Scoffone in "Elucidation of Organic Structures by Physical and Chemical Methods", Part II, 2nd ed, K. W. Bentley and G. W. Kirby, Ed., Wiley-Interscience, New York, N.Y., 1973, p 452.
(3) T. F. Spande, B. Witkop, V. Degani, and A. Patchornik, *Adv. Protein Chem.*, **24**, 97 (1970).
(4) G. R. Jacobson, M. H. Schaffer, G. R. Stark, and T. C. Vanaman, *J. Biol. Chem.*, **248**, 6583 (1973).
(5) Y. Degani and A. Patchornik, *Biochemistry*, **13**, 1 (1974).
(6) This reagent (**1**) was obtained by lead tetraacetate oxidation of 2'-methyl-4'-bromoacetamidobenzenesulfonanilide and was isolated as a stable, yellow crystalline solid (mp 118–120 °C; UV_{max} 305 nm (ϵ 16 000); ^1H NMR ($CDCl_3$, Me_4Si) δ 2.03 (d, J = 1 Hz, 3), 4.03 (s, 2), 6.70–7.00 (m, 2), 7.33–8.07 (m, 6); IR ($CHCl_3$) 1690, 1590 cm^{-1}. Anal. ($C_{15}H_{13}N_2O_3SBr$): C, 47.25; H, 3.48; N, 7.43; S, 8.50; Br, 21.09). The reagent has been tagged with the acronym Cyssor I (Cysteine specific scission by organic reagent).
(7) Reduction of bovine pancreatic ribonuclease (Worthington) as described by F. H. White, Jr., *J. Biol. Chem.*, **235**, 383 (1960), provided an enzyme with six free sulfhydryl functions according to DTNB assay.
(8) Reaction mixtures were applied directly to gels after quenching with aqueous 1% SDS–1% 2-mercaptoethanol and adjusting the pH to the bromophenol blue endpoint with ammonium hydroxide solution. Relatively large quantities of protein were employed to detect low levels of cleavage. Ovalbumin reactions were analyzed according to K. Weber and M. Osborn, *J. Biol. Chem.*, **244**, 4406 (1969); ribonuclease reactions were analyzed on "Biophore Gels" (Bio-Rad Laboratories). A variety of stepwise and individual schemes may be optimized to yield better cleavage and/or separations. This procedure required minimal sample manipulation and was used to allow the identification of cleavage with a range of substrates. A methodology of pH 7 for the initial addition, G-25 Sephadex separation of protein at pH 5 and hydrolysis with *p*-toluene sulfonic acid (0.1 M) 80 °C, 10 min, is convenient if the initial trials show cleavage.
(9) H. S. Wilgus, III, E. Frauenglass, E. T. Jones, R. F. Porter, and J. W. Gates, Jr., *J. Org. Chem.*, **29**, 594 (1964).
(10) See G. Toennies and J. J. Kolb, *J. Am. Chem. Soc.*, **67**, 849 (1945); H. G. Gundlach, S. Moore, and W. Stein, *J. Biol. Chem*, **231**, 1761 (1959); A. M. Crestfield, W. H. Stein, and S. Moore, *J. Biol. Chem.*, **238**, 2413 (1963).
(11) R. Adams and W. Reifschneider, *Bull. Soc. Chim. Fr.*, 23 (1958).
(12) All amino acid analyses were performed on a modified Beckman Model B analyzer. Reaction mixtures were hydrolyzed (6.2 N HCl at 110 °C) in the presence of an excess of Me_2SO to convert all cysteine and cystine residues to equivalent amounts of cysteic acid ($CySO_3H$).
(13) A. M. Weiner, T. Platt, and K. Weber, *J. Biol. Chem.*, **247**, 3242 (1972).
(14) E. Gross, J. L. Morell, and P. Q. Lee, Abstracts, Proceedings of the 7th International Congress of Biochemistry, Tokyo, 1967, Pt. XI, p 535.
(15) A. Patchornik and M. Sokolovsky, *J. Am. Chem. Soc.*, **86**, 1860 (1964).
(16) H. B. F. Dixon and V. Moret, *Biochem. J.*, **94**, 463 (1965). See also H. B. F. Dixon and R. Fields, *Methods Enzymol.*, **25B** (1972).

Thomas J. Holmes, Jr., Richard G. Lawton*
Chemistry Department and
Interdepartmental Program in Medicinal Chemistry
University of Michigan
Ann Arbor, Michigan 48109
Received March 9, 1976

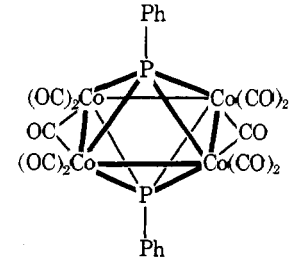
Metal Cluster Catalysis. 1. Hydroformylations of 1- and 2-Pentene Catalyzed by Two Cobalt Carbonyl Clusters: $Co_3(CO)_9(\mu_3\text{-}CC_6H_5)$ and $Co_4(CO)_8(\mu_2\text{-}CO)_2(\mu_4\text{-}PC_6H_5)_2$

Sir:

Muetterties' studies of acetylene[1,2] and butadiene[1] cyclizations, catalyzed by $Ni_4(CNR)_4(\mu_3\text{-}CNR)_3$, and the reduction of synthesis gas to methane[3] by $Ir_4(CO)_{12}$ and $Os_3(CO)_{12}$ (along with Roundhill's[4] oxidations of carbon monoxide and cyclohexane to carbon dioxide and adipic acid, respectively) has focused attention on the use of discrete metal clusters as models for heterogeneous metal catalysis.[5] However, few organometallic clusters have ever been reported as homogeneous catalysts,[5] and systematic studies of clusters under a variety of catalytic conditions do not exist. Thus, we present the results of 1- and 2-pentene hydroformylations[6] (eq 1) catalyzed by two cobalt carbonyl clusters: $Co_3(CO)_9(\mu_3\text{-}CC_6H_5)$, **1**,[7] and $Co_4(CO)_8(\mu_2\text{-}CO)_2(\mu_4\text{-}PC_6H_5)_2$, **2**.[8]



1



2

Hydroformylation of 1- and 2-pentene to aldehydes in high yields[9] with a fairly high normal-to-branched selectivity[10] was achieved under mild conditions. In addition, the hydrogenation of 1- and 2-pentyne and 1- and 2-pentene was effected.[11] Clusters **1** and **2** were recovered, unchanged,[12] in high yields from these reactions. Since **1** is bonded together by carbon–