the SP receptor with an IC_{50} of 60 nM. In a functional assay it inhibited SP-mediated inositol phosphate production.[10] Further, **IV** is highly selective for the substance P receptor and does not interact with some 50 other receptors, including the SRIF and β-adrenergic receptors at concentrations of 1 μM.[11] That such a subtle modification can produce this switch in binding affinity is surprising; in addition, **IV** is to our knowledge the first neutral SP antagonist.[12]

It is of interest that the SRIF, β-adrenergic, and SP receptors utilize G-protein-mediated signal transduction. Such receptors share structural as well as functional similarities characterized by seven hydrophobic transmembrane domains connected by hydrophilic extramembranous loops.[13,14] The interaction of **II** and **III** with three different G-protein-coupled receptors, as well as the highly lipophilic nature of these glycosides, suggests that the binding may involve similar interactions within the conserved hydrophobic domains of the three receptors.[15] The high affinity and selectivity of **IV** for the SP receptor must reflect some specific interactions of the compound with a binding domain of that receptor. While a structural relationship between SP and **IV** is not obvious, it is tempting to speculate that the benzyl substituents on the glucose scaffold might be binding to the site in the receptor normally occupied by the two phenylalanine residues near the C-terminus of substance P and/or one or more of the three phenyl groups of CP-96,345[16] or RP6758,[17] both substance P antagonists.

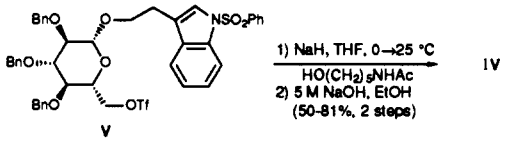
Ariëns had previously called attention to the importance in receptor binding of hydrophobic double ring systems.[18] More recently, Tanford[19] and Wiley and Rich[20] have discussed the interactions whereby hydrophobic peptides and other flexible hydrophobic organic molecules assume a stabilized conformation in aqueous medium, a phenomenon which Rich termed hydrophobic collapse. This may play a role in the high affinity of **IV** for the substance P receptor.

In summary, we have observed that β-D-glucose can serve as a scaffold for molecules that can bind to G-protein-coupled receptors. The unexpected observation that compounds designed to act at the somatostatin receptor are also antagonists of the β-adrenergic and substance P receptors underscores the structural similarities in the binding domains of the family of G-protein-coupled receptors. That the seemingly subtle structural differences between **II** and **IV** produced such a dramatic change in biological profile was completely unexpected, and it suggests the possibility that agonists and antagonists of other G-protein-linked receptors can be found by the strategy described herein. Further work to design high-affinity ligands around the β-D-glucose template are underway.

Acknowledgment. We gratefully acknowledge support from NIH Grant GM-41821-01-03, Bachem, Inc. (CA), Sterling Winthrop, Inc., and Merck & Co., Inc. We are grateful to L. Shoots and S. Sadowski for assistance with receptor assays and to P. Friese for typing the manuscript.

(8) A peptide which blocked the in vivo effects of somatostatin on GH release (Fries, J. L.; Murphy, W. A.; Sueras-Diaz, J.; Coy, D. A. *Peptides* **1982**, *3*, 811) was subsequently found to involve receptors other than the pituitary somatostatin receptor (Coy, D. A. Personal communication to R.H.).

(9) The synthesis of the novel **IV** unexpectedly could not be accomplished via acetylation of **II** due to partial indole acylation giving gross mixtures even with a stoichiometric amount of acetic anhydride. This is in contrast to the fact that the solid-phase peptide synthesis of Trp-containing peptides does not require deactivation of the indole heterocyclic ring and that **I** was selectively acetylated on the primary amine. **IV** was prepared as shown.



(10) Berridge, M. J.; Downes, C. P.; Hanley, M. R. *Biochem. J.* **1982**, *206*, 587.

(11) We are indebted to Drs. L. Iversen and S. Freedman (Merck Research Laboratories, Terlings Park, Harlow, UK) and Dr. D. J. Pettibone (Department of Biomedical Pharmacology, Merck Research Laboratories, West Point, PA) for these results. We are also grateful to the Merck Research Laboratories for making **IV** available for broad screening at Panlabs, Inc.

(12) Interestingly, the N-acetylated SRIF agonist MK 678[2] c-(*N*-Me-Ala-Tyr-D-Trp-Lys-Val-Phe) does not bind to the SP receptor. Thus nonpeptidal peptidomimetics can disclose structural relationships between receptors not revealed by their peptidal counterparts.

(13) Dohlman, H. G.; Thorner, J. M.; Caron, M. G.; Lefkowitz, R. J. *Annu. Rev. Biochem.* **1991**, *60*, 653.

(14) Yamada, Y.; Post, S.; Wang, K.; Tager, H.; Bell, G.; Seino, S. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 251.

(15) It seems likely that as we learn more about the similarities between the primary and tertiary structures of diverse receptors, the changes in biological profile resulting from seemingly minor alterations in molecular structure of small molecules [e.g., valium → tifluadom (Römer, D.; Büscher, H. H.; Hill, R. C.; Maurer, R.; Petcher, T. J.; Zeugner, H.; Benson, W.; Finner, E.; Milkowski, W.; Thies, P. W. *Nature* **1982**, *298*, 759)] will become more understandable [Hirschmann, R. Review of the Fogarty International Center Conference, NIH: Peptides in Health and Disease. *Int. J. Pept. Protein Res.*, in press].

(16) (a) Snider, R. M.; Constantine, J. W.; Lowe, J. A., III; Longo, K. P.; Lebel, W. S.; Woody, H. A.; Drozda, S. E.; Desai, M. C.; Vinick, F. J.; Spencer, R. W.; Hess, H.-J. *Science* **1991**, *251*, 435. (b) McLean, S.; Ganong, A. H.; Seeger, T. F.; Bryce, D. K.; Pratt, K. G.; Reynolds, L. S.; Siok, C. J.; Lowe, J. A., III; Heym, J. *Science* **1991**, *251*, 437.

(17) Garret, C.; Carruette, A.; Fardin, V.; Moussaoui, S.; Peyronel, J.-F.; Blanchard, J.-C.; Laduron, P. M. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 10208.

(18) Ariëns, E. J.; Beld, A. J.; Rodrigues de Miranda, J. F.; Simonis, A. M. In *The Receptors: A Comprehensive Treatise*; O'Brien, R. D., Ed.; Plenum: New York; Vol 1, p 33.

(19) Tanford, C. In *The Hydrophobic Effect: Formation of Micelles and Biological Membranes*; Wiley-Interscience: New York, 1980.

(20) Wiley, R. A.; Rich, D. H. *Med. Res. Rev.*, in press.

On the Origins of the DNA Sequence Selectivity of Mitomycin Monoalkylation Transformations

Harold Kohn,*,† Ven-Shun Li,† Pascal Schiltz,† and Moon-shong Tang‡

Department of Chemistry, University of Houston Houston, Texas 77204-5641
The University of Texas System M. D. Anderson Cancer Center, Science Park—Research Division Smithville, Texas 78957

Received July 10, 1992

The cytotoxicity and antitumor activity of mitomycin C (**1a**), a clinically important anticancer agent,[1] have been associated with the DNA–drug bonding process.[2] UVRABC nuclease assay on mitomycin C–DNA monoadduct sites revealed that DNA modification occurred predominantly at 5′CG* sequences (G*, site of drug lesion) and that 5′CG*G sequences were the preferred trinucleotide units for monoalkylation transformations.[3] Analysis of the data and computer-aided model building studies indicated that two key hydrogen bonds contributed to the sequence selectivity.[3b] We have proposed that both the C(10) oxygen of the carbamate group[4] and the N(2) ammonium group in the activated mitomycin species (**2a**) are well-situated to hydrogen bond to the

† University of Houston.

‡ The University of Texas System M. D. Anderson Cancer Center.

(1) Carter, S. K.; Crooke, S. T. *Mitomycin C. Current Status and New Developments*; Academic Press: New York, 1979.

(2) (a) Iyer, V. N.; Szybalski, W. *Science* **1964**, *145*, 55. (b) Remers, W. A. *The Chemistry of Antitumor Antibiotics*; Wiley: New York, 1979; Vol. 1, pp 221–276. (c) Franck, R. W.; Tomasz, M. In *The Chemistry of Antitumor Agents*; Wilman, D. E. V., Ed.; Blackie and Sons, Ltd.: Glasgow, Scotland, 1990; pp 379–394. (d) Fisher, J. F.; Aristoff, P. A. *Prog. Drug Res.* **1988**, *32*, 411. (e) Moore, H. W.; Czerniak, R. *Med. Res. Rev.* **1981**, *1*, 249. (f) Zwelling, L. A.; Anderson, T.; Kohn, K. W. *Cancer Res.* **1979**, *39*, 365.

(3) (a) Li, V.-S.; Kohn, H. *J. Am. Chem. Soc.* **1991**, *113*, 275. (b) Kohn, H.; Li, V.-S.; Tang, M.-s. *J. Am. Chem. Soc.* **1992**, *114*, 5501.

(4) For a comparable discussion concerning the role of the C(10) oxygen in **1a**–DNA bonding processes, see ref 5.

(5) Kumar, S.; Lipman, R.; Tomasz, M. *Biochemistry* **1992**, *31*, 1399.

0002-7863/92/1514-9218$03.00/0

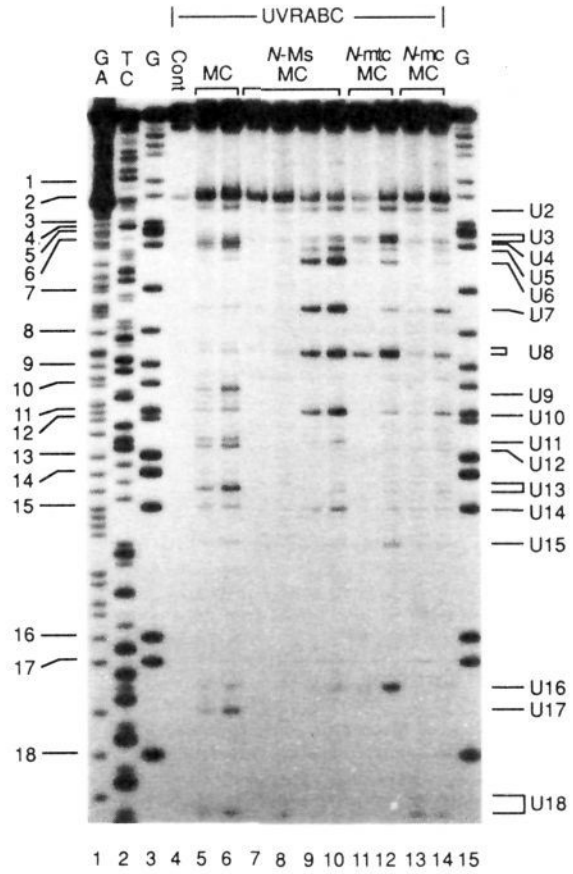
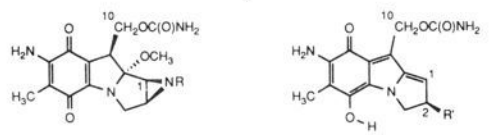


Figure 1. Autoradiogram of UVRABC nuclease cutting of the mitomycin-modified 3′-end-labeled 129 base pair fragment from pBR322 plasmid (top strand): lanes 1–3 and 15, Maxam–Gilbert chemical sequencing reactions of AG, TC, G, and G, respectively; lane 4, DNA treated with NADH-xanthine oxidase (XO) without drug (control); lanes 5 and 6, DNA modified with 0.30 and 0.45 mM **1a**, respectively, after reductive activation with NADH-XO; lanes 7–10, DNA modified with 0.06, 0.10, 0.15, 0.30 mM **1d**, respectively, after reductive activation with NADH-XO; lanes 11 and 12, DNA modified with 0.15 and 0.30 mM **1f**, respectively, after reductive activation with NADH-XO; lanes 13 and 14, DNA modified with 0.15 and 0.30 mM **1e**, respectively, after reductive activation with NADH-XO. The drug modification induced UVRABC nuclease incision bands (U_2–U_{18}) are labeled on the right side of the panel, and the numbers correspond to the specific guanine residues that are listed on the left side of the panel.

N(2) amino guanine on the complementary strand and the N(3) on the 3′ adjacent guanine (*G*) on the bonding strand, respectively.[3b] We tested this hypothesis in part by examining the DNA site selectivity for mitomycins **1a–f** in which the aziridine N-substituent has been selectively modified.



The 129 base pair fragment from pBR322 plasmid was modified with the mitomycin using xanthine oxidase/NADH,[6] and then the site of DNA bonding was determined by the *Escherichia coli* UVRABC nuclease assay.[7,8] Autoradiograms (Figure 1 and

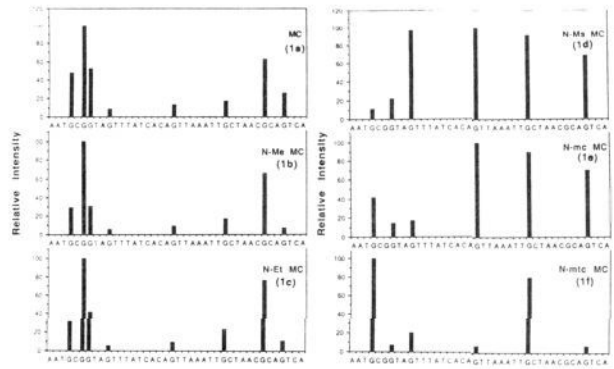


Figure 2. Relative intensities (RI) of UVRABC nuclease incision of mitomycin **1a–f**–DNA adducts of 40 base region within the 3′-end-labeled (*Bst*NI-*Eco*RI) 129 base pair sequence from pBR322 plasmid. The concentration was 0.45 mM for **1a–c**, 0.15 mM for **1d**, and 0.30 mM for **1e** and **1f**. The intensities were normalized to 100% for the most intense band within each experiment.

supplementary figure S1) and the corresponding densitometric data (Figure 2 and supplementary figure S2) showed that bonding was drug-dose dependent and that pronounced changes in the hierarchy of the preferred trinucleotide bonding sites were observed as the aziridine N-substituent was changed. The favored bonding site for **1a**, *N*-methylmitomycin C[9] (**1b**), and *N*-ethylmitomycin C (**1c**) was 5′CG*G. By comparison, DNA bonding of *N*-[(methylthio)carbonyl]mitomycin C[10] (**1f**) took place most frequently at 5′TG*C sites. No modification of the 5′CG*G site was observed. Finally, with *N*-(methylsulfonyl)mitomycin C[11] (**1d**) and *N*-(methoxycarbonyl)mitomycin C[10] (**1e**), DNA alkylation occurred predominantly at the 5′TG*C and 5′AG*T sequences.

Experiments with **1d**-modified DNA provided information on the specific site of guanine modification. First, neither thermal (DSC buffer, 90 °C, 15–45 min)[12] nor piperidine (90 °C, 30 min)[12b,d] treatment of the mitomycin-modified labeled DNA produced detectable radiolabeled DNA fragments (data not shown). Second, when DNA incubated with distamycin A (12.5–100 μM) was treated with reductively activated **1d** and then digested with UVRABC, the intensity of the **1d** bonding sites decreased (data not shown). Moreover, the most intense **1d**–DNA alkylation sites were not in proximity to established distamycin A–DNA binding regions for this restriction fragment. Previous studies report that distamycin A binds to DNA in the minor groove at adenine- and thymine-rich regions.[13] These experiments are consistent with the notion that **1d**, like **1a**,[14] bonds to guanine at the C(2) amino site.

The composite data revealed that **1a–f** selectively alkylated DNA at guanines. The marked variation in the preferred trinucleotide recognition site observed for **1a–f** has been attributed to the necessary molecular complementarity between the activated drug (**2**) and the DNA for bonding to occur and supports our

(6) (a) Phillips, D. R.; White, R. J.; Cullinane, C. *FEBS* **1989**, *246*, 233. (b) McGuinness, B. F.; Lipman, R.; Goldstein, J.; Nakanishi, K.; Tomasz, M. *Biochemistry* **1991**, *30*, 6444.

(7) Friedberg, E. C. *DNA Repair*; W. H. Freeman: New York, 1984.

(8) (a) Sancar, A.; Sancar, G. B. *Annu. Rev. Biochem.* **1988**, *57*, 29. (b) Van Houten, B. *Microbiol. Rev.* **1990**, *54*, 18.

(9) (a) Kinoshita, S.; Uzu, K.; Nakano, K.; Shimizu, M.; Takahashi, T. *J. Med. Chem.* **1971**, *14*, 103. (b) Hong, Y. P.; Kohn, H. *J. Am. Chem. Soc.* **1991**, *113*, 4634.

(10) Fishbein, P. L.; Kohn, H. *J. Med. Chem.* **1987**, *30*, 1767.

(11) (a) Kyowa Fermentation Industry Netherlands Patent Appl. 6,412,941; *Chem. Abstr.* **1966**, *65*, 13712h. (b) Han, I.; Russell, D. J.; Kohn, H. *J. Org. Chem.* **1992**, *57*, 1799.

(12) (a) Maxam, A. M.; Gilbert, W. *Methods Enzymol.* **1980**, *65*, 499. (b) Reynolds, V. L.; Molineux, I. J.; Kaplan, D. J.; Swenson, D. H.; Hurley, L. H. *Biochemistry* **1985**, *24*, 6228. (c) Hurley, L. H.; Lee, C.-S.; McGovren, J. P.; Warpehoski, M. A.; Mitchell, M. A.; Kelley, R. C.; Aristoff, P. A. *Biochemistry* **1988**, *27*, 3886. (d) Suzuki, T.; Kuwahara, J.; Sugiura, Y. *Biochem. Biophys. Res. Commun.* **1983**, *117*, 916.

(13) (a) Van Dyke, M. W.; Dervan, P. B. *Nucleic Acids Res.* **1983**, *11*, 5555. (b) Fox, K. R.; Waring, M. J. *Nucleic Acids Res.* **1984**, *12*, 9271. (c) Uesugi, M.; Sekida, T.; Matsuki, S.; Sugiura, Y. *Biochemistry* **1991**, *30*, 6711.

(14) Tomasz, M.; Lipman, R.; Chowdary, D.; Pawlak, J.; Verdine, G. L.; Nakanishi, K. *Science* **1987**, *235*, 1204.

previous notion about the key interactions responsible for the formation of specific mitomycin C-DNA monoadducts.[3b] These results may provide a molecular basis for future drug design. Currently studies are in progress to determine the specific site of drug modification and the consensus bonding sequences for **1b-f** and to model the binding process for select mitomycins to aid in identifying the specific interactions responsible for the sequence selectivity.

Acknowledgment. We thank Dr. M. Nazimiec and Ms. A. Pao for UVR protein purification and Dr. A. Sancar (University of North Carolina) for providing *E. coli* strains containing UVR gene plasmids. We also thank Drs. A. M. Casazza and W. Rose and the Bristol-Myers Squibb Co. for a gift of **1a**. This work was supported by NIH Grants RO1CA29756 (H.K.) and ES03124 (M.-s.T.), the Robert A. Welch Foundation (E607, H.K.), and the American Cancer Society (CH-485, M.-s.T.).

Supplementary Material Available: Copies of ^1H and ^{13}C NMR and mass spectra for **1c**, autoradiograms of UVRABC nuclease cutting of the **1b-d**-modified 3'-end-labeled 129 base pair fragment from pBR322 plasmid (Figure S1), and full histograms depicting the relative intensities of UVRABC nuclease incision sites for **1a-f**-DNA adducts in the 3'-end-labeled 129 base pair fragment from pBR322 plasmid (Figure S2) (7 pages). Ordering information is given on any current masthead page.

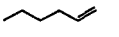
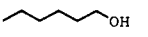
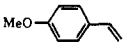
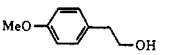
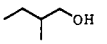
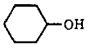
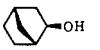
Organolanthanide-Catalyzed Hydroboration of Olefins

Karl N. Harrison and Tobin J. Marks*

Department of Chemistry
Northwestern University
Evanston, Illinois 60208-3113
Received July 21, 1992

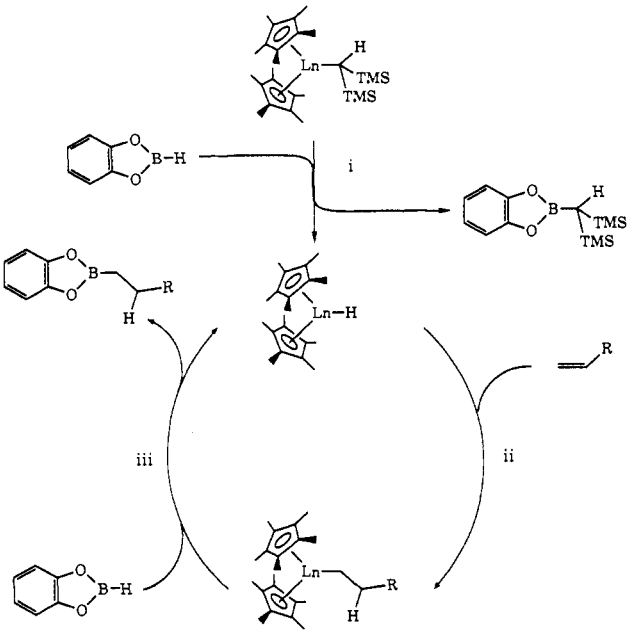
The great utility of hydroboration in organic synthesis[1] has recently been enhanced by transition-metal-catalyzed processes which afford increased rates and substantially modified regio- and enantioselectivities.[2,3] Mechanistic results have identified the central role of oxidative addition/reductive elimination sequences at the electron-rich group 9 metal centers in mediating R_2B-H/olefin addition.[2,3] Distinctive reactivity patterns at organolanthanide centers include facile olefin insertion, σ-bond metathesis, and hydrocarbyl protonolysis. Combinations of these transformations have recently been shown to effect the efficient and selective catalysis of a variety of olefin transformations including hydrogenation,[4] oligomerization/polymerization/cyclization,[5] hydroamination,[6] hydrosilylation,[7] and hydrophosphination,[8] via unconventional electrophilic pathways (not involving change in metal formal oxidation state). We now report that organolanthanides are effective homogeneous catalysts for olefin hydroboration and disclose initial observations on scope, selectivity, and mechanism.

Table I. Organolanthanide-Catalyzed Olefin Hydroboration with Catecholborane[a]

Entry	Substrate	Product	Isolated Yield [%]
1			78
2			89
3			71
4			95
5			71
6			61
7			79
8			73

[a] Procedures given in ref 12.

Scheme I. Proposed Mechanism of Homogeneous Organolanthanide-Catalyzed Olefin Hydroboration



(1) Pelter, A.; Smith, K.; Brown, H. C. *Borane Reagents*; Academic Press: New York, 1988.

(2) (a) Burgess, K.; Ohlmeyer, M. J. *Chem. Rev.* **1991**, *91*, 1179-1191 and references therein. (b) Zhang, J.; Luo, B.; Guo, G.; Dai, L. *J. Org. Chem.* **1991**, *56*, 1670-1672. (c) Hayashi, T.; Matsumoto, Y.; Ito, T. *J. Am. Chem. Soc.* **1989**, *111*, 3426-3428. (d) Burgess, K.; Ohlmeyer, M. J. *J. Org. Chem.* **1988**, *53*, 5179-5181. (e) Mannig, D.; Nöth, H. *Angew. Chem., Int. Ed. Engl.* **1985**, *25*, 878-879. (f) Hewes, J. D.; Kreimendahl, C. W.; Marder, T. B.; Hawthorne, M. F. *J. Am. Chem. Soc.*, **1984**, *106*, 5757-5759.

(3) (a) Evans, D. A.; Fu, G. C.; Hoveyda, A. H. *J. Am. Chem. Soc.* **1992**, *114*, 6671-6679. (b) Evans, D. A.; Fu, G. C.; Anderson, B. A. *J. Am. Chem. Soc.* **1992**, *114*, 6679-6685. (c) Westcott, S. A.; Taylor, N. J.; Marder, T. B.; Baker, R. T.; Jones, N. J.; Calabrese, J. C. *J. Chem. Soc., Chem. Commun.* **1991**, 304-305. (d) Knorr, J. R.; Merola, J. S. *Organometallics* **1990**, *9*, 3008-3010. (e) Baker, R. T.; Overnall, D. W.; Calabrese, J. C.; Wescott, S. A.; Taylor, N. J.; Williams, I. D.; Marder, T. B. *J. Am. Chem. Soc.* **1990**, *112*, 9399-9400.

(4) (a) Conticello, V. P.; Brard, L.; Giardello, M. A.; Tsujii, Y.; Sabat, M.; Stern, C. L.; Marks, T. J. *J. Am. Chem. Soc.* **1992**, *114*, 2761-2762. (b) Molander, G. A.; Hobert, J. O. *J. Org. Chem.* **1992**, *57*, 3326-3268. (c) Jeske, G.; Lauke, H.; Mauermann, H.; Schumann, H.; Marks, T. J. *J. Am. Chem. Soc.* **1985**, *107*, 8111-8118. (d) Mauermann, H.; Sweptson, P. N.; Marks, T. J. *Organometallics* **1985**, *4*, 200-202.

(5) (a) Molander, G. A.; Hoberg, J. O. *J. Am. Chem. Soc.* **1992**, *114*, 3123-3125. (b) Jeske, G.; Lauke, H.; Mauermann, H.; Sweptson, P. N.; Schumann, H.; Marks, T. J. *J. Am. Chem. Soc.* **1985**, *107*, 8091-8103. (c) Jeske, G.; Schock, L. E.; Sweptson, P. N.; Schumann, H.; Marks, T. J. *J. Am. Chem. Soc.* **1985**, *107*, 8103-8110. (d) Watson, P. L.; Parshall, G. W. *Acc. Chem. Res.* **1985**, *18*, 51-55.

(6) (a) Gagné, M. R.; Brard, L.; Conticello, V. P.; Giardello, M. A.; Stern, C. L.; Marks, T. J. *Organometallics* **1992**, *11*, 2003-2005. (b) Gagné, M. R.; Stern, C. L.; Marks, T. J. *J. Am. Chem. Soc.* **1992**, *114*, 275-294. (c) Gagné, M. R.; Nolan, S. P.; Marks, T. J. *Organometallics* **1990**, *9*, 1716-1718. (d) Gagné, M. R.; Marks, T. J. *J. Am. Chem. Soc.* **1989**, *111*, 4108-4109.

(7) (a) Nolan, S. P.; Porchia, M.; Marks, T. J. *Organometallics* **1991**, *10*, 1450-1457. (b) Sakakura, T.; Lautenschlager, H.-J.; Tanaka, M. *J. Chem. Soc., Chem. Commun.* **1991**, 40-41. (c) Brard, L.; Forsyth, C. M.; Nolan, S. P.; Marks, T. J. Manuscript in preparation.

(8) Giardello, M. A.; King, W. A.; Nolan, S. P.; Porchia, M.; Sishta, C.; Marks, T. J. In *Energetics of Organometallic Species*; Martinho Simoes, J. A., Ed.; Kuwer: Dordrecht, 1992; pp 35-51.

0002-7863/92/1514-9220$03.00/0