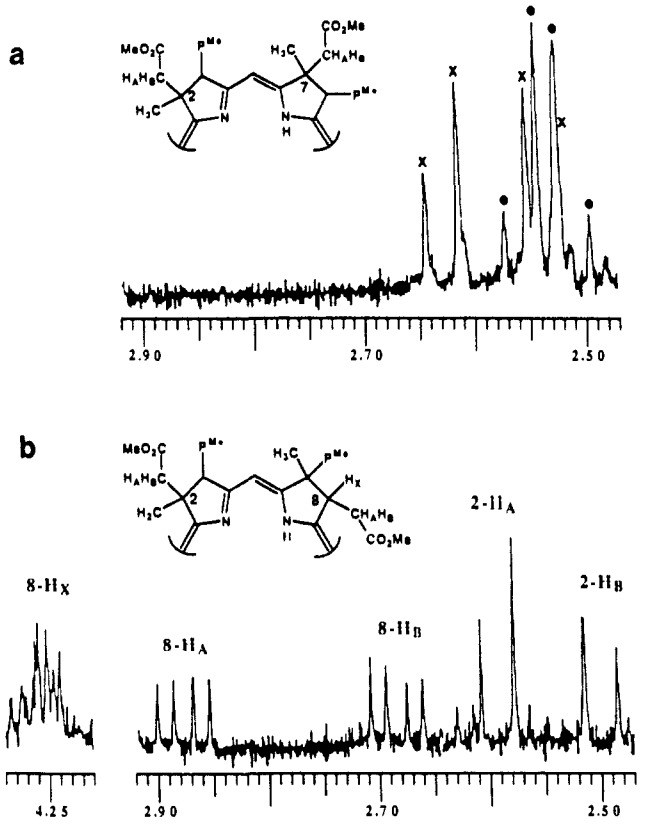
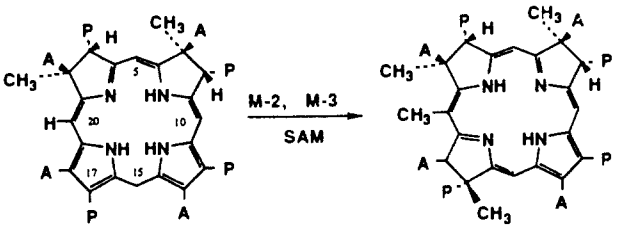


Figure 1. 500-MHz proton NMR spectra of the region 2.4–2.9 ppm of (a) sirohydrochlorin IV (**9**), showing the two AB systems (× and ●) arising from the methylene protons of the acetate side chains attached to positions 2 and 7, and (b) sirohydrochlorin II (**12**), showing an AB system (2.4–2.6 ppm) arising from the methylene protons 2-H$_{A,B}$ of the acetate side chain attached to position 2 which is clearly distinguishable from the ABX system due to the presence of the C-8 proton 8-H$_X$ which resonates at δ = 4.25 ppm (see insert).

expected from the acetate methylene protons, as seen in sirohydrochlorin IV (**9**) (Figure 1a), occurred as an ABX system (Figure 1b). COSY and homonuclear decoupling experiments demonstrated that a proton was indeed adjacent to an acetate side chain, indicating that methylation could not have occurred at that terminus. One of the ^{13}C resonances of the two methyl groups was also shifted from the normal position between 19.5 and 20.1 ppm, lending further support for the idea that the second methyl group had been added at C-7, i.e., at a propionate- rather than an acetate-bearing carbon. This was confirmed by a NOESY experiment where correlation was observed between the C-20 meso proton (δ = 7.53 ppm) and the methyl signal at δ = 1.79 ppm and between the C-5 meso proton (δ = 6.71 ppm) and the methyl signal at δ = 1.23 ppm. Collectively these data prove that methylation had occurred at the acetate (C-2) and propionate termini (C-7), leading to structure **12** for sirohydrochlorin II, incorporating the same overall stereochemistry as **3**, **6**, and **9**.

The ramifications of these results are multiple. Firstly, the enzyme M-1 is capable of C-methylating unnatural substrates to yield nonphysiological intermediates, suggesting that, during molecular recognition, the order of the carboxylic side chains serves only to position the substrate for the first methylation event. Methylation occurs sequentially with the addition of a methyl group first to an acetate terminus,[15,16] as indicated by the isolation of monomethyl chlorins (corresponding to oxidized **8** and **11**), followed by C-methylation at the first side-chain terminus of the adjacent pyrrole ring reached in a clockwise direction in **8** and **11**, regardless of whether the side chain is acetate or propionate. Thus the pattern of C-methylation by the enzyme is identical with that observed for chemical methylation[17] ($C_2 > C_7 > C_{12}$) which identified the chromophoric array of the substrate as the major criterion for regioselectivity. Secondly, the fact that M-1 can methylate at a propionate side chain indicates that the mode of action of methylase-3, responsible for C-methylation at C-17 of precorrin-3[10] to form the hypothetical pyrrocorphin "precorrin-4", is probably similar to that of uroporphyrinogen methylase (M-1), and that the methylases used for tetrapyrrole modification may have evolved from a common ancestor. Thirdly, the products of these reactions can now be tested as substrates for a study of the further methylating enzymes required for B_{12} synthesis and of the enzymes necessary for the corrin ring contraction process. Thus the type-IV counterpart (**13** ≡ dihydro-**9**) of precorrin-2[10] (**2**) should be a substrate for the enzymes required to methylate positions 20 and 17 (M-2, M-3). The absence of an acetate group at position 12 would preclude decarboxylation and hence further methylation, yielding "precorrin-4"[10] (**14**). Similarly the type-II precorrin-2 (dihydro-**12**) should be an excellent candidate for further methylation and may possibly yield a complete type-II corrinoid.[10] These novel synthetic ideas are now under experimental test.



13 **14**

Acknowledgment. We thank the National Institutes of Health for financial support of this work.

(16) Preferentially this acetate corresponds to the second "A" of the sequence *PA*, *AP*. If such an acetate cannot be found, as in the case of uroporphyrinogen I (**4**), a random selection of acetate *AP*, *AP* is made. In each case the second methylation occurs on the C-7 position of the adjacent ring regardless of whether it is *AP* or *PA*.

(17) Biomimetic chemical methylation of hexahydroporphinoids has been described: Eschenmoser, A. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 5. See also: Leumann, C.; Hilpert, H.; Schreiber, A.; Eschenmoser, A. *J. Chem. Soc., Chem. Commun.* **1983**, 1404.

Solid-Phase-Mediated Peptide Heterodisulfide Formation[1]

Berta Ponsati, Mariano Ruiz-Gayo,[2] Ernest Giralt, Fernando Albericio,* and David Andreu*

Department of Organic Chemistry
University of Barcelona, 08028 Barcelona, Spain

Received January 31, 1990
Revised Manuscript Received April 2, 1990

Formation of an asymmetric disulfide bridge between two peptide chains is often one of the most problematic aspects of peptide synthesis.[3] Since cooxidation of two cysteine-containing peptides gives mixtures of homo- and heterodimers, directed methods for the formation of heterodisulfides are required.[4] A

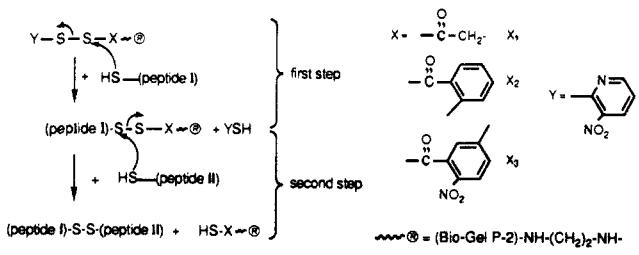
(1) Taken in part from the Ph.D. dissertations of B.P. and M.R.-G. (University of Barcelona).

(2) Present address: Université René Descartes (Paris V), UER des Sciences Pharmaceutiques et Biologiques, Departement de Chimie Organique; 4, Avenue de l'Observatoire, 75270 Paris, France.

(3) For reviews, see: (a) Barany, G.; Merrifield, R. B. In *The Peptides*; Gross, E., Meienhofer, J., Eds.; Academic Press: New York, 1979; Vol. 2, pp 1–284. (b) Hiskey, R. G. In *The Peptides*; Gross, E., Meienhofer, J., Eds.; Academic Press: New York, 1981; Vol. 3, pp 137–167. (c) König, W.; Geiger, R. In *Perspectives in Peptide Chemistry*; Eberle, A., Geiger, R., Wieland, T., Eds.; Karger: Basel, 1981; pp 31–44.

0002-7863/90/1512-5345$02.50/0

Scheme I



common approach to this problem has been (i) synthesis of the two cysteine-protected peptide moieties, (ii) deprotection and purification to give the free-thiol peptides, and (iii) selective cysteine protection–activation[5] of one of the monomers,[6] which undergoes (iv) nucleophilic attack by the other, free-thiol-containing monomer to give the disulfide. A noteworthy exception to this strategy is the use of the 3-nitro-2-pyridylsulfenyl group (Npys)[7,8] as cysteine protection, which has the unusual attraction of allowing selective disulfide formation at acidic pH.

In this work, we introduce a new strategy for heterodisulfide formation in which activation of one of the cysteine components is achieved by its anchoring to an appropriately functionalized polymer support. Our approach is influenced by the work of Whitesides,[9] who has shown that the exchange reaction between a free thiol, RSH, and a preformed disulfide, R'SSR'', to give RSSR' is favored if the acidity of the attacking species is less than that of the conjugate acid of the potential leaving group, R''S$^-$. Thus, in Scheme I, YSH and R'''XSH need to be more acidic than the cysteine thiol groups of the incoming peptides I and II, respectively. (R''' in the text of this paper represents encircled R in Scheme I.) Furthermore, the regiospecific attack that results in the anchoring of peptide I to the polymer matrix requires that YSH be in turn more acidic than R'''XSH. Our scheme thus combines the potential of directed disulfide formation described above with the known advantages of solid-phase chemistry.

Three mercapto acids, 2-mercaptoacetic acid (X_1SH, $pK_a(SH)$ = 9.8), 2-mercaptobenzoic acid (X_2SH, $pK_a(SH)$ = 4.9), and 2-nitro-5-mercaptobenzoic acid (X_3SH, $pK_a(SH)$ = 4.1) were selected as XS moieties and readily anchored to ethylenediamine-modified Bio-Gel P-2.[10] For the remaining YS moiety we chose in all cases the Npys group, the conjugate acid of which has a rather low $pK_a(SH)$ value of ca. 2.2.[11] Upon this starting polymer[12] the radiolabeled, free-thiol-containing peptide [^{3}H]-Ac-Ala-Phe-Cys(H)-NH_2 was readily anchored by vigorous shaking in 0.1 M HOAc for 80 min. At this stage, peptide I/polymer molar ratios influence both the product distribution and overall yield of the process. Thus, if substoichiometric amounts of I were used, the partially unreacted polymer gave rise to significant levels of homodimer II in the second step. On the other hand, if excess I was used, attack of the soluble form on its polymer-activated counterpart gave homodimer I, with simultaneous polymer defunctionalization. In our hands, and for the chosen solid support, slight excesses of I (ca. 1.1 equiv) seemed to be an acceptable compromise.

For the second step, peptide II (Ac-Tyr-Gly-Cys(H)-NH_2) in 0.05 M citrate, pH 5, was used as the sulfur nucleophile with the three different forms of polymer-activated peptide I. For X_1S as spacer (derived from 2-mercaptoacetic acid), the reaction was too slow to be detectable by HPLC or radiometric analysis of the supernatant even after 48 h. The other two forms of polymer-activated I, X_2S and X_3S (derived from 2-mercaptobenzoic acid and 2-nitro-5-mercaptobenzoic acid, respectively), gave good yields (60–70%) of the desired heterodimer[13] under similar conditions, with faster reaction for X_3.[14]

In conclusion, an advantageous new approach to peptide heterodisulfide formation has been demonstrated, with the following features: (i) the starting functionalized polymer has general applicability and is readily available and easily regenerated; (ii) both peptide components are introduced as free thiols, thereby avoiding unnecessary derivatization and purification steps; and (iii) incorporation of the first peptide unit is accompanied by a significant degree of purification from unwanted oxidation by-

(4) For recent examples, see: (a) Sieber, P.; Kamber, B.; Hartmann, A.; Jöhl, A.; Riniker, B.; Rittel, W. *Helv. Chim. Acta* **1977**, *60*, 27–37. (b) Mott, A. W.; Slomczynska, U.; Barany, G. In *Forum Peptides*; Castro, B., Martinez, J., Eds.; Les Impressions Dohr: Nancy, France, 1986; pp 321–324. (c) Chino, N.; Yoshizawa-Kumagaye, K.; Noda, Y.; Watanabe, T.; Kimura, T.; Sakakibara, S. *Biochem. Biophys. Res. Commun.* **1986**, *141*, 665–672. (d) Bernatowicz, M.; Matsueda, R.; Matsueda, G. R. *Int. J. Pept. Protein Res.* **1986**, *28*, 107–112. (e) Yajima, H.; Fujii, N.; Funakoshi, S.; Watanabe, T.; Murayama, E.; Otaka, A. *Tetrahedron* **1988**, *44*, 805–819. (f) Ruiz-Gayo, M.; Albericio, F.; Pons, M.; Royo, M.; Pedroso, E.; Giralt, E. *Tetrahedron Lett.* **1988**, *29*, 3845–3848. (g) Albericio, F.; Ruiz-Gayo, M.; Pedroso, E.; Giralt, E. *React. Polym.* **1989**, *10*, 259–268. For an elegant approach to peptide synthesis also using asymmetric disulfides, see: (h) Fotohoui, N.; Galakatos, N. G.; Kemp, D. S. *J. Org. Chem.* **1989**, *54*, 2803–2817.

(5) This is usually accomplished by using sulfenyl-type derivatives; see refs 4b,d and see: (a) Kamber, B. *Helv. Chim. Acta* **1973**, *56*, 1370–1381. (b) Hiskey, R. G.; Li, C. D.; Vunnam, R. R. *J. Org. Chem.* **1975**, *40*, 3697–3703. (c) Le Nguyen, D.; Rivier, J. *Int. J. Pept. Protein Res.* **1987**, *29*, 98–106. (d) Romani, S.; Moroder, L.; Wünsch, E. *Int. J. Pept. Protein Res.* **1987**, *29*, 98–106. (e) Kemp, D. S.; Carey, R. I. *J. Org. Chem.* **1989**, *54*, 3640–3646.

(6) The acetamidomethyl group (Veber, D. F.; Milkowski, J. D.; Varga, S. L.; Denkewalter, R. G.; Hirschmann, R. *J. Am. Chem. Soc.* **1972**, *94*, 5456–5461) allows combination of steps ii and iii into a single procedure by means of protecting-group exchange reactions; see, e.g., refs 4b,d.

(7) (a) Matsueda, R.; Higashida, S.; Ridge, R. J.; Matsueda, G. R. *Chem. Lett.* **1982**, 921–924. (b) References 4c,f. (c) Ploux, O.; Chassaing, G.; Marquet, A. *Int. J. Pept. Protein Res.* **1987**, *29*, 162–169.

(8) The Npys group is fully compatible with Boc/Bzl-type protection schemes of peptide synthesis, although it is not appropriate for use with Fmoc-based synthetic protocols; see: Albericio, F.; Andreu, D.; Giralt, E.; Navalpotro, C.; Pedroso, E.; Ponsati, B.; Ruiz-Gayo, M. *Int. J. Pept. Protein Res.* **1989**, *34*, 124–128.

(9) (a) Whitesides, G. M.; Lilburn, J. E.; Szajewski, R. P. *J. Org. Chem.* **1977**, *42*, 332–338. (b) Whitesides, G. M.; Houk, J.; Patterson, M. A. K. *J. Org. Chem.* **1983**, *48*, 112–115. (c) Houk, J.; Whitesides, G. M. *J. Am. Chem. Soc.* **1987**, *109*, 6825–6836.

(10) Four grams of commercial Bio-Gel P-2 was suspended in 80 mL of ethylenediamine and stirred at 90 °C for 1 h. The resin was filtered and washed with 1 N aqueous HCl and water. A picric acid titration (Gisin, B. F. *Anal. Chim. Acta* **1972**, *58*, 548–549) gave an amine substitution of 0.6 mmol/g. Bio-Gel P-4, controlled-pore glass, and polyacrylamide resins were also tested but were found to be unsatisfactory.

(11) Calculated from Hammet equation data; see: Perrin, D. D.; Dempsey, B.; Serjeant, E. P. *pK_a Prediction for Organic Acids and Bases*; Chapman and Hall: New York, 1981.

(12) General protocol for the preparation of the starting polymer-bound disulfide was as follows: the modified polymer (0.3 mmol of NH_2, see ref 11), swollen in 0.1 M Tris, pH 8, and XSH (1.5 mmol) in 3:1 0.1 M Tris/DMF, pH 8, were reacted in the presence of 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide (1.5 mmol) for 48 h at room temperature. Unreacted amine groups were capped with Ac_2O. Prior to this, the free thiols were temporarily protected by reaction with 5,5'-dithiobis(2-nitrobenzoic acid). After acetylation, they were regenerated by treatment with aqueous 2-mercaptoethanol and then reacted with NpysCl in 1:1 0.1 M Tris/DMF, pH 8, for 4 h at room temperature.

(13) Preparation of [^{3}H]Ac-Ala-Phe-Cys-NH_2 Ac-Tyr-Gly-Cys-NH_2 was as follows. Npys-S-S-X_3-NH$(CH_2)_2$NH-Bio-Gel P-2 (6.9 mg, 0.69 μmol) was swollen in an Eppendorf tube by repeated treatments with 0.1 M HOAc (1 mL) for 15 min, followed by centrifugation and removal of supernatant. [^{3}H]Ac-Ala-Phe-Cys(H)-NH_2 was dissolved in 0.1 M HOAc to a concentration of 3.8×10^{-2} M (determined according to Ellman: Ellman, G. L. *Arch. Biochem. Biophys.* **1959**, *82*, 70–77), and its homogeneity (i.e., absence of dimeric form) was checked by analytical HPLC (Vydac C_{18}-silica, 5 μm; 4 × 250 mm column, eluted with a 5–65% (v/v) linear acetonitrile gradient in 0.045% TFA; 1 mL/min). Nineteen microliters of this solution (0.72 μmol peptide) was added to the previously swollen resin, resuspended in 1 mL of 0.1 M HOAc, and shaken vigorously for 80 min at 25 °C. At this time, the reaction mixture had developed a yellowish color (release of 3-nitro-2-thiopyridone), and HPLC analysis of the supernatant (same conditions as above) indicated total disappearance of the starting peptide from solution. The polymer was then submitted to another wash/centrifugation cycle with 0.1 M HOAc (3 × 1 mL) and with 0.05 M sodium citrate, pH 5, then resuspended in 1 mL of this buffer, and treated with a 4.4×10^{-2} M solution of Ac-Tyr-Gly-Cys(H)-NH_2 in the same buffer (15 μL, 0.96 equiv) with vigorous stirring. After 5 h at 25 °C, no Ac-Tyr-Gly-Cys(H)-NH_2 was detectable by HPLC in the supernatant whereas two new peaks, in a 38:62 ratio, assigned respectively to Ac-Tyr-Gly-Cys-NH_2 Ac-Tyr-Gly-Cys-NH_2 (nonradioactive) and to [^{3}H]Ac-Ala-Phe-Cys-NH_2 Ac-Tyr-Gly-Cys-NH_2 (radioactive) were observed. The homodimer was identified by coelution with an independent sample prepared by air oxidation of Ac-Tyr-Gly-Cys(H)-NH_2. Confirmation of the identity of the heterodimer was further achieved by amino acid analysis and FAB-MS.

(14) Five hours for X_3S vs 12 h for X_2S.

products and other synthetic impurities.[15]

Acknowledgment. This work was supported by funds from CIRIT (fellowships to B.P. and M.R.-G.) and CICYT (BT86-18).

Registry No. [^3H]Ac-Ala-Phe-Cys(H)-NH_2, 127279-51-0; Ac-Tyr-Gly-Cys(H)-NH_2, 127279-52-1; (Ac-Tyr-Gly-Cys-NH_2)$_2$ (disulfide linkage), 127279-53-2; [^3H]Ac-Ala-Phe-Cys-NH_2, Ac-Tyr-Gly-Cys-NH_2 (disulfide linkage), 127258-03-1; $HSCH_2COOH$, 68-11-1; 2-HSC_6H_4COOH, 147-93-3; 2-(NO_2)-5-$(HS)C_6H_4COOH$, 15139-21-6.

(15) For a related application of the same principle, see: Ponsati, B.; Giralt, E.; Andreu, D. *Anal. Biochem.* **1989**, *181*, 389-395.

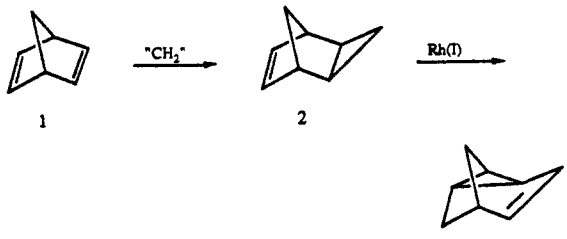
Platina(IV) Cyclobutane Chemistry: On the Mechanism of the Ring Homologation Reaction

J. O. Hoberg and P. W. Jennings*

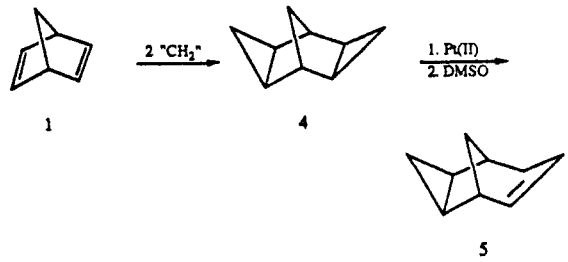
Gaines Hall Laboratory
Department of Chemistry
Montana State University
Bozeman, Montana 59717

Received December 15, 1989

Ring or chain homologation of olefinic compounds by direct transformations using the methodology of cyclopropanation and subsequent ring cleavage has not been generally successful.[1] Thus, with the reactions shown in eq 1[2] and 2,[3] there appears to be an opportunity to facilitate this transformation. We wish to report the results of mechanistic studies involving ^2H, ^{13}C, and substitution labeling experiments with these two reactions using *platinum* as the transformation catalyst.



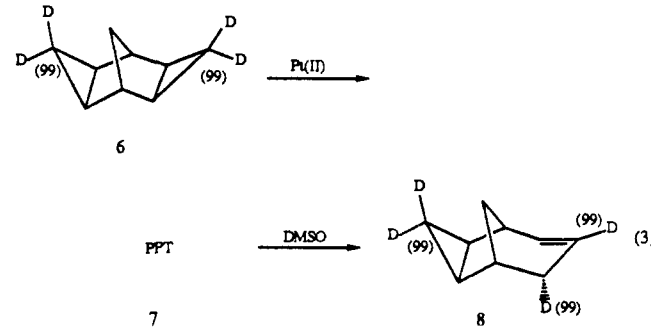
(1)



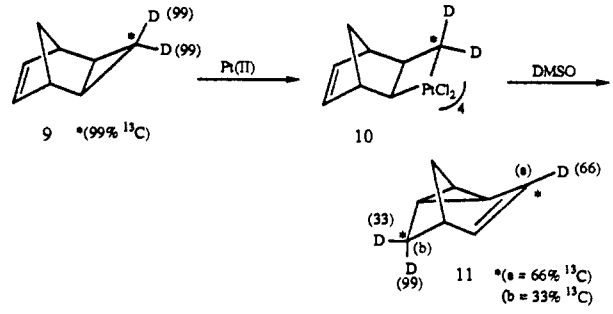
(2)

Treatment of **2** or **4** with Zeise's dimer $(C_2H_4PtCl_2)_2$ produces a light yellow solid tetrameric complex[4,5] having the platina(IV)cyclobutane moiety as shown for **10**.[6] Reaction of these metallacyclic *precipitates* from **2** and **4** with DMSO gave excellent yields of **3** (75%) and **5** (90%), respectively.[7]

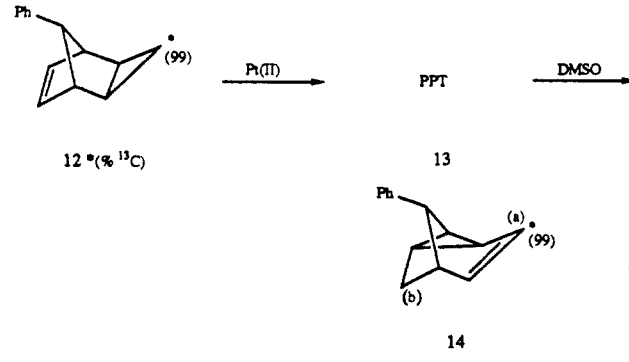
Results of the transformation with labeled substrates[8] are shown in eqs 3, 4, and 5. Numbers in parentheses represent the percent label observed by ^1H, ^2H, and/or ^{13}C NMR spectroscopy. A mechanism for reaction 3 which adequately accounts for the observed distribution of ^2H and ^{13}C in the product is shown in Scheme I.



(3)



(4)



(5)

For reaction 4, a sequence proposed earlier by Katz[2] using Rh(I) would predict 99% deuterium and 100% ^{13}C at carbon (a) and 99% deuterium only in the endo configuration at carbon (b) for product **11**. This was not observed, eq 4. Thus, the three reaction pathways shown in Scheme II were considered to explain the labeling results. Path A, **16**-**18**, represents 66% of the reaction course following a path analogous to that observed by Katz with rhodium.[2] Pathway B results from bridge migration and subsequent steps. Pathway C branches from path A at intermediate **16** leading to the same product as from path B.[11] Either path B and/or C could place 33% ^{13}C at carbon b (eq 4) and 33% ^2H

(1) March, J. *Advanced Organic Chemistry: Reactions, Mechanisms, and Structure*; John Wiley and Sons: New York, see index on ring expansion.
(2) Katz, T. J.; Cerefice, S. A. *J. Am. Chem. Soc.* **1969**, *91*, 2405 and **1971**, *93*, 1049.
(3) Johnson, T. H.; Cheng, S. S. *Synth. Commun.* **1980**, *10*, 381.
(4) Binns, S. E.; Cragg, R. H.; Gillard, R. D.; Heaton, B. T.; Pilbrow, M. F. *J. Chem. Soc. A* **1969**, 1227.
(5) Puddephatt, R. J. *Coord. Chem. Rev.* **1980**, *33*, 149.
(6) Waddington, M.; Jennings, P. W. *Organometallics* **1982**, *1*, 385 and 1370.

(7) In this reaction, we have recently found that higher yields are obtained when the DMSO reaction is given more time to react. It is slow at room temperature with $\kappa = 2.6 \times 10^{-4}$ s^{-1} with a $t_{1/2} = 44$ min. Thus, complete reaction of 3.4×10^{-4} moles of platinacycle required about 5 h.
(8) Compounds **2** and **4** were prepared with and without deuterium at the cyclopropyl apex methylene by use of diiodomethane (99.4% d_2) and the Simmons-Smith reagent. Further, compounds **2** and **4** were prepared with ^{13}C at the apical carbon by using diazomethane (99%^{13}C) and $Pd(OAc)_2$. All compounds were purified by preparative gas chromatography and analyzed by GC-MS and NMR spectroscopy. The proton and carbon assignments for these compounds have been thoroughly analyzed assuring adequate interpretation.[6,9,10]
(9) Campbell, W. H.; Jennings, P. W. *Organometallics* **1982**, *1*, 1071.
(10) Campbell, W. H.; Jennings, P. W. *Organometallics* **1983**, *2*, 1460.
(11) The authors are indebted to an astute referee for pointing out the possibility of path C.

0002-7863/90/1512-5347$02.50/0