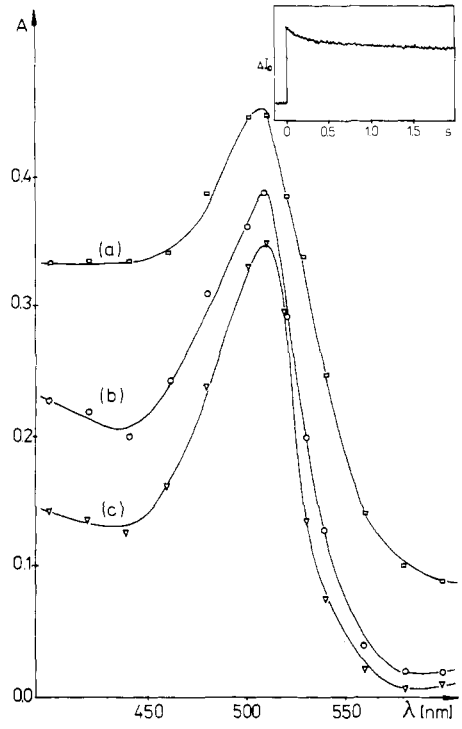


Figure 2. Transient absorption spectra of HMB solution at 93 K obtained (a) 7×10^{-4} s, (b) 0.25 s, and (c) 2.5 s after the 4 μs-electron pulse delivering a dose of 600 Gy. The sample contained HMB (0.02 M) and 1-butyl chloride (1 M) in 3-methylpentane. Inset: scope trace at 510 nm.

The time-resolved spectra for the HMDB system are very much different. The spectrum determined after the pulse has practically no absorption in the observation range (Figure 3). However, the $HMB^{\bullet+}$ absorption at 510 nm appeared to grow with time, and 0.25 s after the pulse a spectrum with a maximum at 510 nm was clearly seen.[11] The evident growth of this absorption was noticed both at 77 and 93 K (insets in Figures 1 and 3). The delayed formation of a signal at 510 nm can be assigned to the unimolecular valence isomerization of $HMDB^{\bullet+}$ (reaction 1). This picture is also consistent with the steady-state measurements. Ignoring the decay of $HMB^{\bullet+}$ ($k_2 \ll k_1$) one can calculate the rate constant k_1. At 77 K this assumption is even unnecessary since k_2 is practically zero. The calculated values of k_1 are 1.71 and 0.015 s^{-1} at temperatures of 93 and 77 K, respectively. Activation parameters associated with the isomerization process were calculated to be $E_A = 17.6$ kJ/mol and $A = 1.3 \times 10^{10}$ s^{-1}. We believe that these values are related to the intrinsic process of valence isomerization, and they are not associated with softening of the matrix, which controls the decay of $HMB^{\bullet+}$.[3] The processes concerning dissipation of the excess energy in rigid matrices are faster and do not coincide with our observation.[13-15] This lends support to a view that the reaction studied involves vibrationally relaxed radical ions.

Our efforts to monitor directly the absorption of $HMDB^{\bullet+}$ have not been successful. If the absorption of $HMDB^{\bullet+}$ lies below 350 nm the detection is difficult or even impossible since that range is obscure by the strong absorption from the radicals. In the region of 350-700 nm the absorption of $HMDB^{\bullet+}$ might escape from the detection only when it is very weak, i.e., $\epsilon < 100$. We have not searched for $HMDB^{\bullet+}$ in the region of $\lambda > 700$ nm.

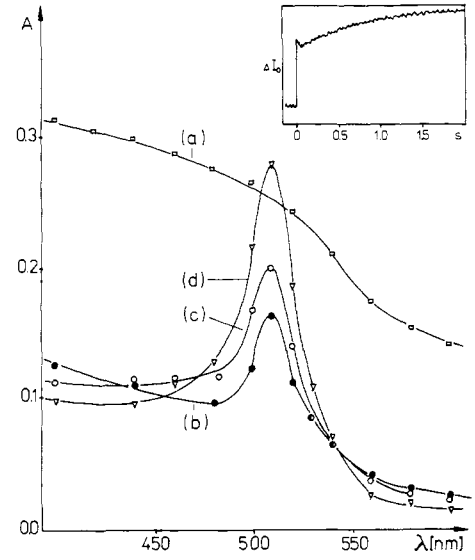


Figure 3. Transient absorption spectra of HMDB solution at 93 K obtained (a) 7×10^{-4} s, (b) 0.25 s, (c) 0.5 s, and (d) 2.5 s after the 4 μs-electron pulse delivering a dose of 600 Gy. The sample contained HMDB (0.02 M) and 1-butyl chloride (1 M) in 3-methylpentane. Inset: scope trace at 510 nm.

Acknowledgment. We acknowledge a cooperation of the staff of the accelerator laboratory. This work was supported by the research program CPBP 01.19.

Registry No. $HMDB^{\bullet+}$, 85293-78-3; $HMB^{\bullet+}$, 34473-51-3; HMDB, 7641-77-2; HMB, 87-85-4.

(10) Kato, N.; Miyazaki, T.; Fueki, K.; Miyata, S.; Kawai, Y. *J. Phys. Chem.* **1984**, *88*, 1445-1449.
(11) The $HMB^{\bullet+}$ absorption generated from the HMDB solution (Figure 3) seems to have a slightly different shape, particularly at high-energy side, as compared to the absorption of $HMB^{\bullet+}$ generated from HMB (Figure 2). This might be due to a contribution of dimer cation to the spectra presented in Figure 2, which absorbs at 480 nm.[12]
(12) Badger, B.; Brocklehurst, B. *Trans. Faraday Soc.* **1969**, *65*, 2582-2587.
(13) Dubost, H. *Ber. Bunsenges. Phys. Chem.* **1978**, *85*, 112-121.
(14) Wiesenfeld, J. M.; Moore, C. B. *J. Chem. Phys.* **1979**, *70*, 930-946.
(15) Bondybey, V. E. *Adv. Chem. Phys.* **1981**, *47*, 521-533.

Phospholipids Chiral at Phosphorus. 18. Stereochemistry of Phosphatidylinositide-Specific Phospholipase C[1]

Gialih Lin and Ming-Daw Tsai*

Department of Chemistry, The Ohio State University Columbus, Ohio 43210
Received January 18, 1989

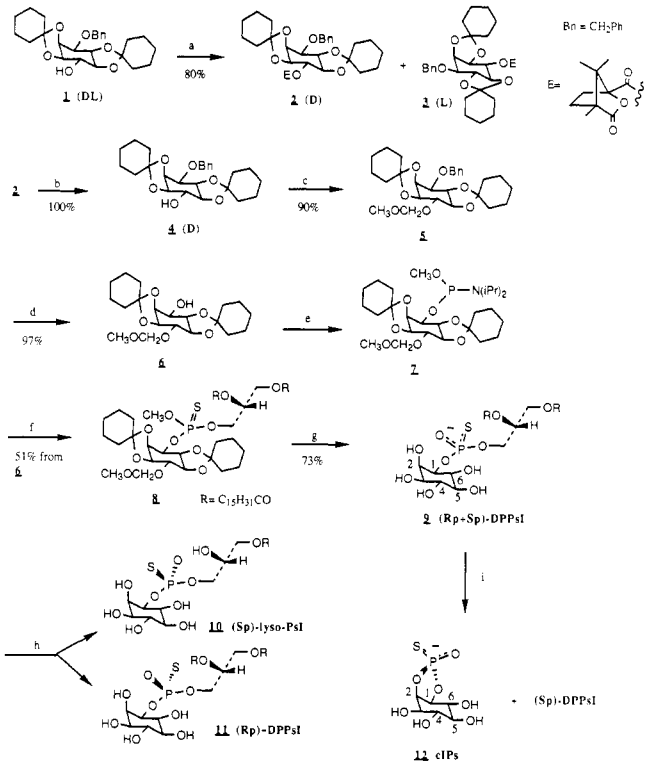
Phosphatidylinositides-specific phospholipase C (PI-PLC), a key enzyme in the metabolism of phosphatidylinositides, catalyzes the formation of three second messengers: diacylglycerol, inositol 1,4,5-trisphosphate, and inositol 1,2-cyclic 4,5-trisphosphate.[2-4] Despite its biological significance and its mechanistic uniqueness in producing both cyclic and open inositol phosphates simultaneously, little mechanistic information about this enzyme has been available. We report the stereochemical mechanism of PI-PLC from *Bacillus cereus*.

Scheme I outlines the synthesis of R_p and S_p isomers of 1,2-dipalmitoyl-*sn*-glycero-3-thiophosphoinositol (DPPsI). The starting material **1** (DL) was synthesized from *myo*-inositol as described by Garegg et al.[5] Resolution of D and L enantiomers was achieved by derivatization with (-)-camphanic acid chloride

(1) Supported by research Grant GM 30327 from NIH. Paper 17: Sarvis, H. E.; Loffredo, W.; Dluhy, R. A.; Hernqvist, L.; Wisner, D.; Tsai, M.-D. *Biochemistry* **1988**, *27*, 4625-4631.
(2) Michell, R. H. *Biochim. Biophys. Acta* **1975**, *415*, 81-147.
(3) (a) Shukla, S. D. *Life Sci.* **1982**, *30*, 1325-1335. (b) Majerus, P. W.; Connolly, T. M.; Deckmyn, H.; Ross, T. S.; Bross, T. E.; Ishii, H.; Bansal, V. S.; Wilson, D. B. *Science* **1986**, *234*, 1519-1526. (c) Majerus, P. W.; Connolly, T. M.; Bansal, V. S.; Inhorn, R. C.; Ross, T. S.; Lips, D. L. *J. Biol. Chem.* **1988**, *263*, 3051-3054. (d) Dawson, R. M.; Freinkel, N. B.; Clarke, N. *Biochem. J.* **1971**, *122*, 605-607.
(4) (a) Berridge, M. J. *Biochem. J.* **1984**, *220*, 345-360. (b) Berridge, M. J. *Ann. Rev. Biochem.* **1987**, *56*, 159-193.
(5) Garegg, P. J.; Iversen, T.; Johansson, R.; Lindberg, B. *Carbohydr. Res.* **1984**, *130*, 322-326.

0002-7863/89/1511-3099$01.50/0

Scheme I. The Synthesis and Reactions of DPPsI[a]



[a] Reagents and conditions: (a) (−)-camphanic acid chloride, Et_3N, 4-(dimethylamino)pyridine, CH_2Cl_2, 25 °C, 7 h; (b) LiOH, THF–H_2O (2:1), 25 °C, 2 h; (c) CH_3OCH_2Cl, iPr_2NEt, CH_2Cl_2, 25 °C, 17 h; (d) Li, THF–NH_3, −78 °C, 0.5 h; (e) $ClP(OCH_3)N(iPr)_2$, Et_3N, CH_2Cl_2, 25 °C, 0.5 h; (f) (i) 1,2-dipalmitoyl-*sn*-glycerol, tetrazole, THF–CH_3CN, 25 °C, 24 h; (ii) S_8, toluene, 25 °C, 47 h; (g) (i) 80% HOAc, 90–100 °C, 2–3 h; (ii) NMe_3, toluene, 25 °C, 15 h; (h) PLA2; (i) PI-PLC.

followed by chromatographic separation of **2** and **3**.[6,7] Deprotection of **2** gave **4**, which was reprotected with chloromethyl methyl ether[8] to give **5**. Debenzylation of **5** gave **6**, which was phosphorylated with $ClP(OCH_3)N(iPr)_2$ to give **7**. The phosphite **7** was converted to **8** directly (without purification) by treating with 1,2-dipalmitoyl-*sn*-glycerol and tetrazole, followed with excess S_8 in toluene.[9] The presence of two diastereomers of **8** was demonstrated by two equal intensity resonances in ^{31}P NMR (101.256 MHz, $CDCl_3$) at 67.63 and 67.93 ppm. A separate sample of **8** derived from DL-**4** gave two additional signals at 67.69 and 67.86 ppm. All intermediates were characterized by ^1H and ^{13}C NMR. (R_p+S_p)-DPPsI (**9**) was obtained by deprotection of D-**8** with acetic acid followed with demethylation with trimethylamine and characterized by ^1H and ^{13}C NMR and fast atom bombardment mass spectroscopy. The ^{31}P NMR spectrum of **9** (δ 55.15 and 55.56 ppm) is shown in Figure 1A.

Assignment of the resonances in Figure 1A was based on the observation that the isomer at 55.56 ppm was hydrolyzed by bee venom phospholipase A_2 (PLA2), with concomitant formation of lyso-DPPsI (**10**) at 55.15 ppm (spectrum not shown). It has been established that PLA2 specifically hydrolyzes the R_p isomer of thiophosphatidylcholine and thiophosphatidylethanolamine.[10]

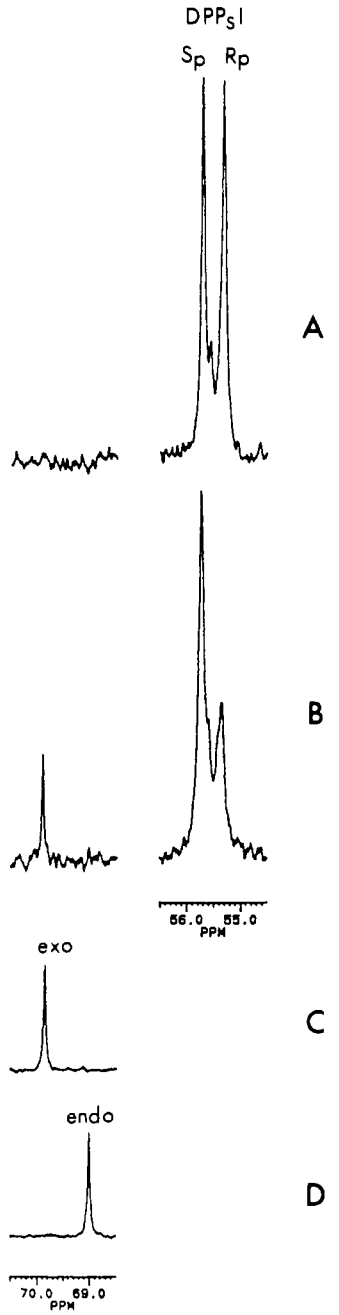


Figure 1. Use of ^{31}P NMR (101.2 MHz) to show the stereospecificity of PI-PLC. (A) 7.5 mg of (R_p+S_p)-DPPsI in D_2O containing 5% Triton X-100, 50 mM HEPES buffer, pD 7.2, 2.5 mM Ca^{2+}, and 0.25 mM EDTA. (B) After addition of PI-PLC. (C) **19a** (*exo*-DL-cIPs). (D) **19b** (*endo*-DL-cIPs). The minor peak in A and B (and another one with similar intensity, unresolved in the present spectra) can be attributed to a small amount of DPPsI derived from contaminating L-**3**.[7]

However, it should be noted that *due to a change in priority, the relative configurations of* (R_p)- *and* (S_p)-*DPPsI correspond to those of the* S_p *and* R_p *isomers, respectively, of thiophosphatidylcholine.* Thus the downfield resonance is assigned the S_p isomer.

Figure 1B shows that PI-PLC from *Bacillus cereus*[11–13] spe-

(6) (a) Vacca, J. P.; deSolms, S. J.; Huff, J. R. *J. Am. Chem. Soc.* **1987**, *109*, 3478–3479. (b) Billington, D. C.; Baker, R.; Kulagowski, J.; Mawer, I. M. *J. Chem. Soc., Chem. Commun.* **1987**, 314–316.

(7) The percent diastereomeric excess (% de) of **2** was determined to be 91% from ^{13}C NMR (75.48 MHz) under nonsaturating conditions. However, the sample actually used for the large-scale synthesis of **9** was less pure (ca. 70% de).

(8) (a) Corey, E. J.; Pan, B.-C.; Hua, D. H.; Deardorff, D. R. *J. Am. Chem. Soc.* **1982**, *104*, 6816–6818. (b) Corey, E. J.; Hua, D. H.; Pan, B.-C.; Steitz, S. P. *J. Am. Chem. Soc.* **1982**, *104*, 6818–6820. (c) Stork, G.; Takahashi, T. *J. Am. Chem. Soc.* **1977**, *99*, 1275–1276.

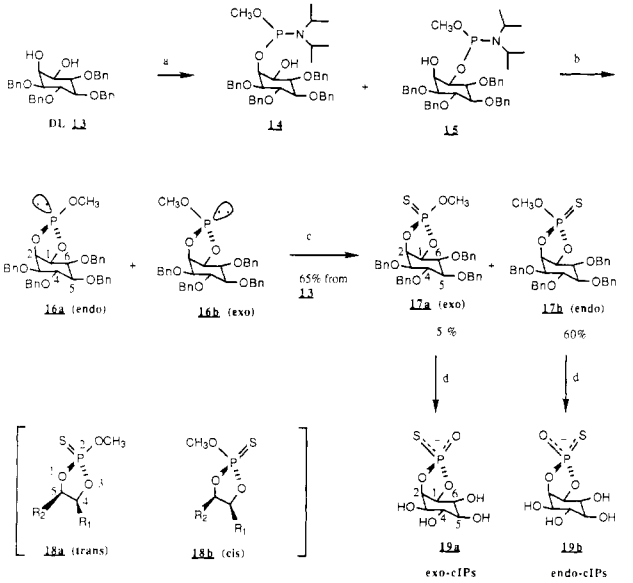
(9) Bruzik, K. S.; Salamonczyk, G.; Stec, W. J. *J. Org. Chem.* **1986**, *51*, 2368–2370.

(10) (a) Bruzik, K.; Gupte, S. M.; Tsai, M.-D. *J. Am. Chem. Soc.* **1982**, *104*, 4682–4684. (b) Orr, G. A.; Brewer, C. F.; Heney, G. *Biochemistry* **1982**, *21*, 3202–3206. (c) Bruzik, K.; Jiang, R.-T.; Tsai, M.-D. *Biochemistry* **1983**, *22*, 2478–2486. (d) Jiang, R.-T.; Shyy, Y.-J.; Tsai, M.-D. *Biochemistry* **1984**, *23*, 1661–1667. (e) Tsai, T.-C.; Hart, J.; Jiang, R.-T.; Bruzik, K.; Tsai, M.-D. *Biochemistry* **1985**, *24*, 3180–3188.

(11) Ikezawa, H.; Yamanegi, M.; Taguchi, R.; Miyashita, T.; Ohyabu, T. *Biochim. Biophys. Acta* **1976**, *450*, 154–164.

(12) Ikezawa, H.; Taguchi, R. *Methods Enzymol.* **1981**, *84*, 731–741.

Scheme II. The Synthesis of Endo and Exo cIPs (DL Mixtures Were Used, but Only D-Forms Are Shown)[a]



[a] Reagents and conditions: (a) 1.2 equiv $ClP(OCH_3)N(iPr)_2$, iPr_2NEt, CH_2Cl_2, 25 °C, 0.5 h; (b) 4 equiv tetrazole, THF–CH_3CN, 25 °C, 18 h; (c) excess S_8, toluene, 25 °C, 48 h; (d) 40 equiv Li, THF–NH_3, −78 °C, 5 min.

cifically converts the R_p isomer of DPPsI to inositol 1,2-cyclic thiophosphate (cIPs) (**12**) (^{31}P δ 69.89 ppm, characteristic of cyclic thiophosphates) as the predominant product. Thus despite differences in substrate specificity, structure, and function, PI-PLC exhibits the same stereospecificity as phosphatidylcholine-specific PLC (PC-PLC), which prefers the S_p isomer of thiophosphatidylcholine.[10b-d]

To elucidate the steric course of PI-PLC requires cIPs with known configuration. Thus, DL-cIPs was synthesized according to Scheme II. DL-1,4,5,6-Tetra-*O*-benzyl-*myo*-inositol (**13**; prepared by established procedures[14]) was phosphorylated by $ClP(OCH_3)N(iPr)_2$ to give **14** and **15**, which were then treated with tetrazole in THF–CH_3CN to produce **16(a+b)** via a novel intramolecular cyclization.[15] Without isolation, **16** was treated with an excess of S_8 in toluene to give **17a** (^{31}P δ 84.41 ppm, *exo*-DL, i.e. D-R_p + L-S_p)[16] and **17b** (^{31}P δ 82.65 ppm, *endo*-DL, i.e. D-S_p + L-R_p), which were separated by chromatography. Assignments of the configurations of **17a** and **17b** were based on four criteria, the first three of which had been established previously on model compounds **18a**, **18b**, and related systems: (i) The predominant form **17b** should be endo since the predominant form of the phosphite **16** should be the least sterically hindered form **16b**,[17] and oxidation by sulfur is known to proceed with retention of configuration at phosphorus.[18] (ii) The relative ^{31}P δ of **17a** and **17b** thus assigned are consistent with that of **18a** and **18b** (83.0 and 80.5 ppm, respectively, when $R_1 = R_2 = CH_3$) in that the trans (exo) form is more downfield.[17b,19] (iii) The three-bond coupling constants between P and 1-H are 18.4 and 9.7 Hz for **17a** and **17b**, respectively. These are consistent with the data for **18a**, **18b**, and related compounds ($^3J_{H-C(4)-O-P}$ is **a** > **b**), and with the empirical rule that the OCH_3 group is "axial seeking" in these systems.[19,20] (iv) Irradiation of 2-H resulted in detectable nuclear Overhauser effect on the methyl proton resonance in **17b** but not **17a**. Detailed NMR assignments and conformational analysis will be presented later.

The synthesis was completed by treating **17a** and **17b** with Li in THF–NH_3(l) to give **19a** (exo[16], ^{31}P δ 69.85 ppm, Figure 1C) and **19b** (endo, ^{31}P δ 69.00 ppm, Figure 1D), respectively. The ^{31}P δ of **19a** coincides with that of **12**, which was further confirmed by addition of **19a** to the reaction mixture in Figure 1B (spectrum not shown). Thus the configuration of **12** should be D-R_p, and the steric course should be *inversion* at phosphorus. The result suggests that the conversion of PI to cIP catalyzed by PI-PLC from *B. cereus* involves direct attack of the 2-OH group to displace the diacylglycerol moiety of the substrate. The steric course of the formation of the noncyclic IP awaits future studies.

Application of phosphorothioates on PI-related systems has also been realized by other groups recently. Chemical synthesis of DL-cIPs[21] by a different procedure has been reported, but the configuration was not determined. The phosphorothioate analogues of DL-*myo*-inositol phosphates have been synthesized[22] and shown to be resistant to hydrolysis by phosphatases.[22c]

(13) The PI-PLC used in this work was obtained from Sigma (which consists of a mixture of PC-PLC, PI-PLC, and sphingomyelinase) and further purified by fast protein liquid chromatography. Sundler, R.; Alberts, A. W.; Vagelos, P. R. *J. Biol. Chem.* **1978**, *253*, 4175-4179.

(14) (a) Angyal, S. J.; Tate, M. E.; Gero, S. D. *J. Chem. Soc.* **1961**, 4116-4122. (b) Gigg, R.; Warren, C. D. *J. Chem. Soc.* **1969**, 2367-2371. (c) Watanabe, Y.; Ogasawara, T.; Shiotani, N.; Ozaki, S. *Tetrahedron Lett.* **1987**, *28*, 2607-2610.

(15) To the best of our knowledge, this is the first example of using $ClP(OCH_3)N(iPr)_2$ as a phosphorylating and intramolecular cyclization agent.

(16) The exo form of **17** and **19** is defined as the form in which sulfur and the inositol ring are on the opposite side of the five-membered ring. In the *R/S* designation, the axial position has higher priority than the equatorial position when all things are equal.

(17) (a) Denney, D. Z.; Chen, G. Y.; Denney, D. B. *J. Am. Chem. Soc.* **1969**, *91*, 6838-6841. (b) Mikolajczyk, M.; Witczak, M. *J. Chem. Soc., Perkin Trans. 1* **1976**, 371-377. (c) Cox, R. H.; Newton, M. G. *J. Am. Chem. Soc.* **1972**, *94*, 4212-4217. (d) Newton, M. G.; Campbell, B. S. *J. Am. Chem. Soc.* **1974**, *96*, 7790-7797. (e) Tan, H.-W.; Bentrude, W. G. *Tetrahedron Lett.* **1975**, 619-622. (f) Bentrude, W. G.; Tan, H.-W. *J. Am. Chem. Soc.* **1976**, *98*, 1850-1859. Our MM-2 calculation also indicates that **16b** (85.3 kcal/mol) is more stable than **16a** (88.5 kcal/mol).

(18) McEven, W. C. *Top. Phosphorus Chem.* **1965**, *2*, 1-41.

(19) Mikolajczyk, M.; Witczak, M. *J. Chem. Soc., Perkin Trans. 1* **1977**, 2213-2222.

(20) (a) Bentrude, W. G.; Setzer, W. N. In *Phosphorus-31 NMR Spectroscopy in Stereochemical Analysis*; Verkade, J. G., Quin, L. D., Eds.; VCH Publishers, Inc.: Deerfield Beach, FL, 1987; pp 365-389. (b) Lee, C.-H.; Sarma, R. H. *J. Am. Chem. Soc.* **1976**, *98*, 3541-3548. (c) Cooper, D. B.; Hall, C. R.; Harrison, J. M.; Inch, T. D. *J. Chem. Soc., Perkin Trans. 1* **1977**, 1969-1980.

(21) Schultz, C.; Metschies, T.; Jastorff, B. *Tetrahedron Lett.* **1988**, *29*, 3919-3920.

(22) (a) Cooke, A. M.; Gigg, R.; Potter, B. V. *J. Chem. Soc., Chem. Commun.* **1987**, 1525-1526. (b) Metschies, T.; Schultz, C.; Jastorff, B. *Tetrahedron Lett.* **1988**, *29*, 3921-3922. (c) Taylor, C. W.; Berridge, M. J.; Brown, K. D.; Cooke, A. M.; Potter, B. V. L. *Biochem. Biophys. Res. Commun.* **1988**, *150*, 626-632.

Novel Regioselectivity and C–F Bond Cleavage in the Reactions of Alkylplatinum(II) Complexes with Amide and Alkoxide Anions

Soonheum Park, M. Pontier-Johnson, and D. Max Roundhill*

Department of Chemistry, Tulane University New Orleans, Louisiana 70118

Received December 12, 1988

Recently there has been a surge of interest in the chemistry of complexes formed between amide or alkoxide anions and transition metals of the platinum group.[1] Previous synthesis had avoided such complexes because the "hard and soft" acid and base concept had predicted weak metal–ligand bonding. Recent solution equilibrium data, however, have shown that these complexes have bond enthalpies comparable with those of alkyl complexes.[2] This communication reports some novel regioselectivities discovered from reacting amides with platinum(II) complexes and

(1) Bryndza, H. E.; Tam, W. *Chem. Rev.* **1988**, *88*, 1163-1188.

(2) Bryndza, H. E.; Fong, L. K.; Paciello, R. A.; Tam, W.; Bercaw, J. E. *J. Am. Chem. Soc.* **1987**, *109*, 1444-1456. Erikson, T. K.; Bryan, J. C.; Mayer, J. M. *Organometallics* **1988**, *7*, 1930-1938.

0002-7863/89/1511-3101$01.50/0