Co(III) method was as follows. To a stirred solution of H-Phe-Leu-OAz (0.5 g) in dry MeOH (3 cm^3) was added 1.0 g of $[Co(en)_2(Gly\text{-}OCH_3)](CF_3SO_3)_3$ in 0.1-g aliquots. The reaction was followed by TLC (silica gel, ethanol); after 15 min all of the H-Phe-Leu-OAz had reacted. The red-orange solution was diluted with water (3 cm^3), NaCN (0.1 g) was added, and the solution was stirred for 5 min. H-Gly-Phe-Leu-OAz which had been displaced from the complex (reaction 3) was isolated by extraction

$$[Co(en)_2(Gly\text{-}Phe\text{-}Leu\text{-}OAz)]^{3+} + CN^- \text{ (excess)} \rightarrow CoCN_6^{3-} + {}^+H_2\text{-}Gly\text{-}Phe\text{-}Leu\text{-}OAz + 2enH_2^{2+} \quad (3)$$

into $CHCl_3$ and chromatography on silica gel. Subsequent couplings were carried out in a similar manner.[8] All peptide intermediates were shown to be homogeneous by TLC, spectral techniques (IR and ^1H NMR), and amino acid analysis. Catalytic hydrogenation[9] of the protected intermediate VII (Scheme I) produced [Leu^5]enkephalin in 24% overall yield. This was shown to be homogeneous by TLC and HPLC;[10] the amino acid composition was $Gly_{2.1}Leu_{1.0}Phe_{1.0}Tyr_{0.95}$, and it was shown to be identical with the product obtained by an alternative solution synthesis.[2]

(8) Percentage yields of the various intermediate peptides were as follows: III, 75%; IV, 95%; V, 82%; VI, 75%; VII, 55%. Yields were estimated by wt % recovery following silica gel chromatography and recrystallization. Amino acid analysis was carried out at each stage. VII exhibited poor solubility in the cyanide displacement reaction (despite the addition of 2 cm^3 of Me_2SO), and this is probably responsible for the lower recovery in this case.

(9) A solution of protected peptide (VII, 0.05 g) and 10% Pd/C (0.03 g) in methanol (20 mL) was shaken with hydrogen at 50 psi for 10 h. Following removal of catalyst and solvent, the product was chromatographed on Biogel P-2 (2 mol dm^{-3} of HOAc eluant).

(10) M. T. W. Hearn, C. A. Bishop, W. S. Hancock, D. R. K. Harding, and G. D. Reynolds, *J. Liq. Chromatogr.*, **2**, 1 (1979).

Total Synthesis of FK-156 Isolated from a *Streptomyces* as an Immunostimulating Peptide: Application of a Novel Copper Chelate Amino Protection

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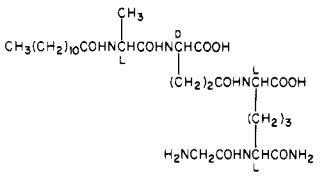
In recent years, considerable attention has been focused on the peptidoglycan fragments of bacterial cell walls because of their unique immunostimulating activity.[1] Recently, Imanaka et al.[2] isolated FK-156 as a metabolite possessing an activity similar to that of peptidoglycans from *Streptomyces olivaceogriseus* sp. nov.[3]

(1) For reviews, see, e.g.: (a) Chedid, L.; Lederer, E. *Biochem. Pharmacol.* **1978**, *27*, 2183. (b) Duker, P.; Tarcsay, L.; Baschang, G. *Annu. Rep. Med. Chem.* **1979**, *14*, 146. (c) Lederer, E. *J. Med. Chem.* **1980**, *23*, 819.

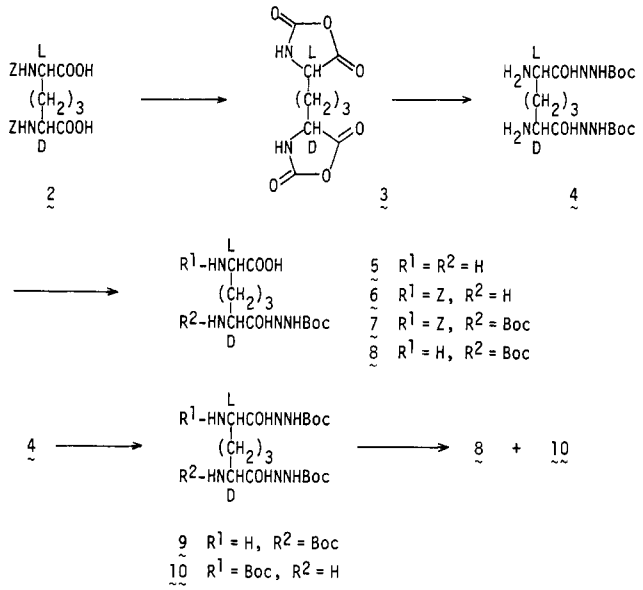
(2) Gotoh, T.; Kuroda, Y.; Okuhara, M.; Tanaka, H.; Nishiura, T.; Kohsaka, M.; Aoki, H.; Imanaka, H. "Abstract", *21st Interscience Conference Antimicrobial Agents and Chemotherapy*, Chicago, IL, 1981; in press.

(3) Migliore-Samour et al. have also reported an adjuvant activity of the peptidolipid i prepared by lauroylation of a cell-wall tetrapeptide isolated from *Streptomyces stimulosus*: Migliore-Samour, D.; Bauchaudon, J.; Floc'h, F.; Zerial, A.; Ninet, L.; Werner, G. H.; Jollès, P. *C. R. Hebd. Seances Acad. Sci., Ser. D* **1979**, *289*, 473. *Life Sci.* **1980**, *26*, 889.

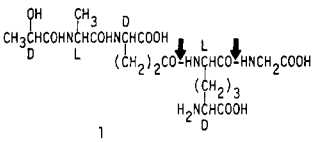


i

Scheme I



and proposed its structure to be **1**. Herein we report the total synthesis of **1** which finally confirmed the proposed structure.



The key in the synthesis of **1** is to create the peptide bonds at the positions marked by arrows with proper differentiation between the two pairs of the amino acid functions in *meso*-2,2′-diaminopimelic acid. This differentiation was cleanly performed by a sequence of reactions involving, as key steps, an enzyme-mediated asymmetric hydrolysis (**4** → **5**) followed by a selective carbobenzyloxylation using a copper chelate procedure (**5** → **6**).

The synthetically available di-Z-*meso*-2,2′-diaminopimelic acid (**2**)[4,5] was treated with PCl_5 (2.2 equiv) in CH_2Cl_2 (Scheme I) (0 °C → reflux, 1 h) to form the crystalline bis(*N*-carboxyanhydride) **3** [mp >250 °C; IR (Nujol) 1840, 1765 cm^{-1}] in 95% yield, which was subsequently allowed to react with *tert*-butyl carbazate as follows: A solution of **3** in MeCN and a solution of *tert*-butyl carbazate (2.0 equiv) and oxalic acid dihydrate (2.0 equiv) in MeOH were mixed at room temperature and vigorously stirred for 30 min to provide an 86% yield of the bis(Boc-hydrazide) **4**[6] [dioxalate; mp 130–135 °C dec; R_f = 0.39 (A)[8]].

Enzymatic hydrolysis of **4** to **5** was accomplished by using an aminopeptidase produced by *Streptomyces sapporonensis*.[9] After conversion to the free base, **4** was dissolved in Tris buffer ($^1/_{20}$ M, pH 7.5), and a 0.25 M solution was incubated at 37 °C in the presence of a crude powder of the enzyme [activity, 5.9 units/mg (protein);[10] application quantity, 100 units/g (sub-

(4) Wade, R.; Birnbaum, S. M.; Winitz, M.; Koegel, R. J.; Greenstein, J. P. *J. Am. Chem. Soc.* **1957**, *79*, 648.

(5) Work, E.; Birnbaum, S. M.; Winitz, M.; Greenstein, J. P. *J. Am. Chem. Soc.* **1955**, *77*, 1916.

(6) This compound **4** had been prepared, by Bricas et al.,[7] from **2** via coupling with *tert*-butyl carbazate by a mixed anhydride method using *i*-BuOCOCl followed by hydrogenolysis (lit. mp 128–130 °C). As compared to this, our method is apparently superior because of the simplicity in synthetic manipulations and the adaptability to a large scale preparation of **4**.

(7) Dezélée, P.; Bricas, E. *Bull. Soc. Chim. Biol.* **1967**, *44*, 1579.

(8) Analytical TLC was performed with silica gel 60-F_{254} (E. Merck AG) using the following solvent systems: (A) *n*-BuOH–AcOH–H_2O (4:1:5, upper phase); (B) *n*-BuOH–AcOH–H_2O (2:1:1); (C) AcOEt–AcOH (10:1); (D) *n*-PrOH–H_2O (3:2).

(9) Isolation and characterization of this enzyme will be reported by Imanaka et al. in due course.

(10) One unit was defined as the quantity capable of hydrolyzing 1.0 μmol of L-leucine *p*-nitroaniline per min at pH 7.0 at 37 °C.

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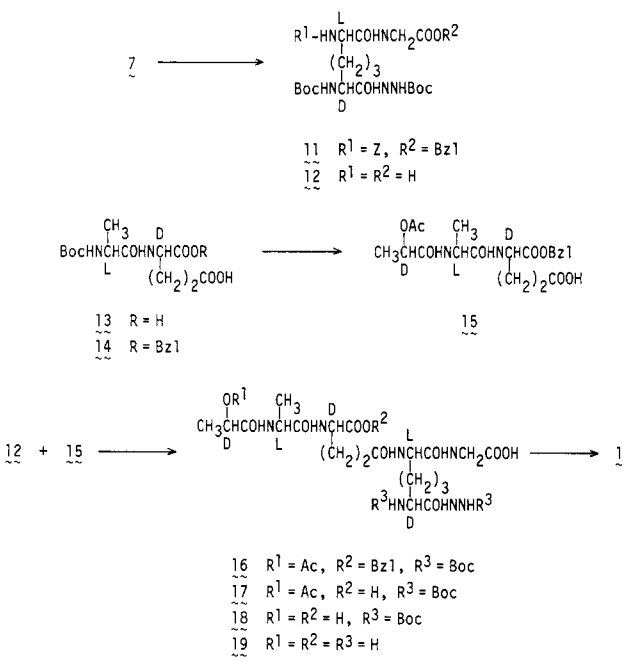
Scheme II



strate)]. The mixture was purified by chromatography using Dia-ion HP-20[11] to afford an 84% yield of **5** [powder; $[\alpha]_D$ −19.8° (*c* 1.0, H_2O); pK_a = 2.0, 7.1, and 9.6 (H_2O); R_f = 0.25 (A); retention time, 6.5 min (high pressure LC)[12]]. The stereoselectivity was identified by comparison of **5** with the sample prepared via the leucine aminopeptidase mediated hydrolysis.[7] The above microorganism-originated enzyme is inexhaustible and sufficiently adaptable to the preparative purpose in this special case.

For selective protection of the two amino groups in **5**, we examined carbobenzyloxylation of **5** under copper chelate conditions [$C_6H_5CH_2OCOCl$ (1.1 equiv)/$CuCl_2$ (0.5 equiv)/H_2O, pH 9–10 with 2 N NaOH, 0 °C] and observed a novel and preferred selectivity in the introduction of the mono-Z group to **5**, thus giving rise to, after purification by chromatography (Dia-ion HP-20), an 85% yield of **6** [mp 194–196 °C dec; $[\alpha]_D$ −20.8° (*c* 0.5, MeOH); R_f = 0.62 (A)],[13] whose structure was assigned on the basis of its pK_a values [3.4 and 7.5 (H_2O)].[15]

Further corroboration of **6** was obtained by conversion into **8**. *tert*-Butoxycarbonylation of **6** [$(Boc)_2O/Et_3N$/dioxane–H_2O] to **7** [mp 146–148 °C; $[\alpha]_D$ +18.8° (*c* 1.0, MeOH); 93%] and removal of the Z group of **7** by hydrogenolysis (10% Pd–C/AcOH) gave **8** [mp 200 °C dec; $[\alpha]_D$ +29.5° (*c* 0.5, MeOH); pK_a = 2.3 and 9.7 (H_2O);[17] R_f = 0.55 (A)]. For a final confirmation on the structure of **8**, **4** (free base) was partially protected with the Boc group [$(Boc)_2O$ (1.2 equiv)/dioxane–H_2O] and the resulting racemic mono-Boc derivatives **9** and **10** were subjected to the leucine aminopeptidase hydrolysis.[14] Only **9** possessing the free amino group at the L asymmetric center underwent the enzyme action to give **8** identical in all respects with the sample described above, together with unchanged **10**.

The unique selectivity in the above copper chelate controlled carbobenzyloxylation is quite remarkable. To our knowledge, this is the first example demonstrating that the copper complex of α-amino acid hydrazides is more stable than that of α-amino acids themselves.[18]

Having secured a method for the differentiation of the divalent amino groups in *meso*-diaminopimelic acid, we proceeded then to the synthesis of **1** (Scheme II). Using **7** as the key intermediate, condensation with benzyl glycinate was accomplished using *i*-BuOCOCl (*N*-methylmorpholine/CH_2Cl_2, −10 to −15 °C, 20 min; GlyOBz, −10 → 0 °C, 1.5 h) to produce **11** [mp 85–87 °C; $[\alpha]_D$ +6.4° (*c* 1.0, MeOH)] in 90% yield. Hydrogenolysis of **11** (10% Pd–C/MeOH–AcOH) yielded **12** [mp 130–138 °C dec; $[\alpha]_D$ +46.2° (*c* 0.5, MeOH); R_f = 0.50 (A); 89%], thus providing for the condensation with the residual, appropriately protected lactoyl dipeptide **15**.

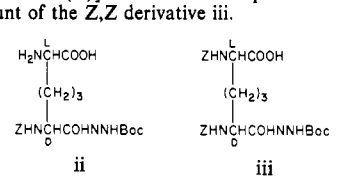
The fragment **15** was synthesized using Boc-L-alanyl-D-glutamic acid (**13**) [mp 152–154 °C; $[\alpha]_D$ −17.6° (*c* 1.0, MeOH); R_f = 0.35 (C)], prepared from Boc-L-alanine and D-glutamic acid by the standard manner. Treatment with benzyl bromide [1.2 equiv/Et_3N (1.2 equiv)/DMF, 0 → 50 °C, 2 h][19] gave, after purification principally by recrystallization, a 50% yield of **14** [mp 84–86 °C; $[\alpha]_D$ −3.1° (*c* 0.4, MeOH); R_f = 0.56 (C)]. Particular advantages of this strategy for the preparation of **14** are the shorter route and the better total yield as compared with the stepwise synthesis via α-benzyl D-glutamate.[14] The Boc group of **14** was removed by treatment with TFA (25 °C, 15 min) followed by acylation with *O*-acetyl-D-lactoyl chloride[20] (BSA/CH_2Cl_2, −15 → 0 °C, 1 h), furnishing **15** [mp 105–106 °C dec; $[\alpha]_D$ −16.1° (*c* 1.0, MeOH); R_f = 0.48 (C)] in 90% yield.

Finally, **15** was preactivated with *i*-BuOCOCl (*N*-methylmorpholine/CH_2Cl_2, −10 to −15 °C, 30 min) and coupled, at −10 to 0 °C for 2 h, to the trimethylsilyl ester of **12**, prepared in situ by treatment with BSA (CH_2Cl_2–DMF), resulting in an 89% yield of **16** [mp ~113 °C dec; $[\alpha]_D$ −4.5° (*c* 0.5, MeOH)]. The protecting groups in **16** were removed by (a) hydrogenolysis (**16** → **17**) over 10% Pd–C (MeOH–H_2O), (b) hydrolysis (**17** → **18**) with aqueous K_2CO_3 (pH 10), (c) treatment (**18** → **19**) with TFA (0–25 °C), and (d) oxidation with $NaIO_4$ (2.5 equiv, H_2O, pH 1 with 0.1 N H_2SO_4, 0 °C) followed by a spontaneously occurring hydrolysis (**19** → **1**). The final product was purified by chromatography (Dia-ion HP-20) and lyophilized to afford pure **1** [powder; $[\alpha]_D$ −30.0° (*c* 0.4, H_2O); R_f = 0.21 (B), 0.60 (D); retention time, 13.5 min (high pressure LC)[22]][23] in 61% yield from

(11) A nonionic macroporous adsorption resin (Mitsubishi Chemical Industry Co., Ltd.).

(12) Performed on a Merck RP-18 reverse-phase column (4-mm i.d. × 150 mm) using a KH_2PO_4–H_3PO_4 buffer (pH 2.0) as eluant (flow rate, 1.2 mL/min).

(13) Although Bricas et al.[14] had reported that carbobenzyloxylation of **5** at pH 9 under Schotten–Baumann conditions gave **6** as the major product (lit. mp 180–182 °C dec), we observed that their conditions gave rise to its counterpart ii [mp 213–215 °C dec; $[\alpha]_D$ +26.1° (*c* 0.5, MeOH); pK_a = 2.0 and 9.5 (H_2O); R_f = 0.55 (A)] almost as the sole product except the formation of a certain amount of the Z,Z derivative iii.



ii iii

(14) Dezélée, P.; Bricas, E. *Biochemistry* **1970**, *9*, 823.

(15) Corresponding to those of the carboxy group of acylated α-amino acids and the amino group of amidated α-amino acids, respectively.[16] In this regard, BocAla and AlaNHNHBoc showed pK_a = 3.8 and 7.5, respectively, in our experiments.

(16) Greenstein, J. P.; Winitz, M. "Chemistry of the Amino Acids"; Wiley: New York, 1969; Vol. 1, pp 475–500.

(17) Corresponding to those of the unprotected α-amino acid moiety.[16] In contrast to BocAla and AlaNHNHBoc (cf. ref 15), Ala itself showed pK_a = 2.3 and 9.7.

(18) This behavior was ascertained by measurement of the circular dichroism of D-AlaNHNHBoc in comparison with that of L-Ala: the copper complexes of D-AlaNHNHBoc and L-Ala showed $[\theta]_{522}$ +1.19 × 10^3 and $[\theta]_{630}$ −2.6 × 10^2 at pH 9, respectively, whereas a 1:1 equiv mixture of these in the presence of Cu^{2+} (0.5 equiv) under the same conditions exhibited a positive Cotton effect with nearly the same amplitude ($[\theta]_{522}$ +1.09 × 10^3) as that of D-AlaNHNHBoc alone, indicating that AlaNHNHBoc formed the complex in preference to Ala itself. Deservingly, **5** also showed a positive Cotton effect ($[\theta]_{530}$ +1.01 × 10^3) in the presence of 0.5 equiv of Cu^{2+} at pH 9.

(19) Nefkens, G. H. L.; Nivard, R. J. F. *Recl. Trav. Chim. Pays-Bas* **1964**, *83*, 199.

(20) Prepared from D-Ala according to the procedure reported in the literature:[21] bp 64–65 °C (22 torr); $[\alpha]_D$ +31.0° (*c* 4.0, $CHCl_3$).

(21) Koga, K.; Yamada, S.; Yoh, M.; Mizoguchi, T. *Carbohydr. Res.* **1974**, *36*, C9.

(22) Performed on a Umetani ODS-5 reverse-phase column (4-mm i.d. × 150 mm) using a KH_2PO_4–H_3PO_4 buffer (pH 2.0) as eluant (flow rate, 0.5 mL/min).

16. This synthetic sample was shown to be identical with the material from nature by comparison of their physical data and biological potencies in stimulation of carbon clearance, stimulation of delayed-type hypersensitivity reaction and antibody production, mitogenic effect on mouse spleen lymphocytes, protective effect against bacterial infections, etc.,[24] thereby confirming the structure of **1**.

This synthesis of **1** is unique in the two pivotal steps: the asymmetric hydrolysis (**4** → **5**) by the microorganism-originated aminopeptidase and the selective carbobenzyloxylation (**5** → **6**) via the novel copper chelate amino protection. In particular, the feature of the latter reaction may provide significant implications in the amino acid and peptide chemistry fields. Moreover, this synthetic route to **1** is capable of providing the amounts necessary for detailed biological tests, instead of having to resort to a tedious isolation process from the natural source, and may also be followed for the preparation of analogous compounds.[25]

Acknowledgment. We are indebted to Y. Miyazaki and T. Nakamura for their technical assistance during the course of this work.

(23) ^{1}H NMR spectrum (100 MHz, D_2O), amino acid analysis, and elemental analysis data of **1** were as follows: δ 1.2-2.5 (m, 10 H), 1.40 (d, J = 7 Hz, 3 H), 1.46 (d, J = 7 Hz, 3 H), 3.88 (t, J = 6 Hz, 1 H), 4.02 (s, 2 H); Ala 1.00, Glu 1.01, Gly 0.97, *meso*-2,2′-diaminopimelic acid 1.06. Anal. Calcd. for $C_{20}H_{33}O_{11}N_5 \cdot 2H_2O$: C, 43.24; H, 6.71; N, 12.61; H_2O, 6.71. Found: C, 43.47; H, 6.57; N, 12.57; H_2O, 6.37.

(24) The biological data of **1** will be reported by Nishida et al. elsewhere.

(25) We also synthesized some stereoisomers of **1** for ascertainment of the chiral and geometric centers in **1** and for a structure–activity relationship study. These will be reported in forthcoming full papers.

Direct Evidence for Solvent Coordination in Migratory CO Insertion

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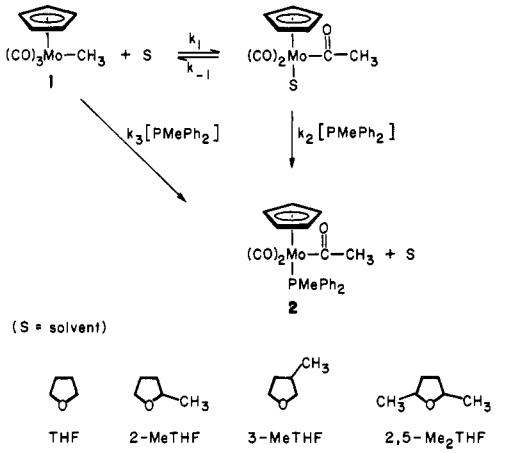
Migratory CO insertion, perhaps the most thoroughly studied process in organotransition metal chemistry,[1] is subject to solvent effects which in some cases are quite large. In early work on the carbonylation of pentacarbonylmethylmanganese(I), Calderazzo and Cotton[2] observed enhanced rates in polar, donating solvents which they attributed to variations in dielectric constant. Since that time, other systems have been found to exhibit similar behavior.[1] The stereochemistry of CO migratory insertion also is affected markedly by changes in solvent. As Flood and his coworkers have pointed out recently,[3] two conflicting explanations have been postulated for these effects: (a) generalized stabilization of the migratory insertion transition state by solvation and (b) direct attack of solvent at the metal center. Neither experimental work nor semi-empirical theory[4-6] has provided a convincing means of distinguishing between these two models.

Scheme I



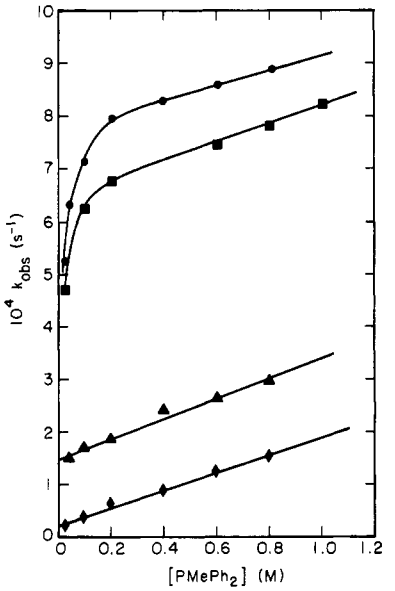


Figure 1. Dependence of the pseudo-first-order rate constant k_{obsd}, for conversion of **1** to **2** in excess $PMePh_2$, upon phosphine concentration in the following solvents: (●) THF; (■) 3-MeTHF; (▲) 2-MeTHF; (♦) 2,5-Me_2THF.

Complicating interpretation of rate data are changes in kinetic order encountered when alkylcarbonyl complexes are treated with nucleophiles in various solvents. Butler, Basolo, and Pearson found that the rate of reaction of $CpMo(CO)_3CH_3$ (**1**, Cp = η^5-C_5H_5) is a linear function of triphenylphosphine concentration in benzene but is independent of the amount of phosphine present in tetrahydrofuran (THF).[7] In chloroform, both of these types of behavior are exhibited simultaneously by the ethyl analogue of **1**.[8] Pentacarbonylmethylmanganese(I) reacts according to either first or second order or saturation kinetics, depending upon the nucleophile and solvent used.[9]

The cyclopentadienyltricarbonyl(alkyl)molybdenum insertion has been the object of intense study and displays large medium effects. In this paper we report the application of a technique to this system which distinguishes between the two solvent-involvement models mentioned above. We believe our results offer the first truly compelling support for the original hypothesis of Mawby, Basolo, and Pearson:[9,10] in this case *the effect of solvent*

(1) For leading references, see: (a) Collman, J. P.; Hegedus, L. S. "Principles and Applications of Organotransition Metal Chemistry"; University Science Books: Mill Valley, CA, 1980; pp 260-288. (b) Kuhlman, E. J.; Alexander, J. J. *Coord. Chem. Rev.* **1980**, *33*, 195-225.

(2) Calderazzo, F.; Cotton, F. A. *Inorg. Chem.* **1962**, *1*, 30-36.

(3) Flood, T. C.; Jensen, J. E.; Statler, J. A. *J. Am. Chem. Soc.* **1981**, *103*, 4410-4414.

(4) Berke, H.; Hoffmann, R. *J. Am. Chem. Soc.* **1978**, *100*, 7224-7236.

(5) Saddia, D.; Freund, H. J.; Hohlneicher, G. *J. Organomet. Chem.* **1980**, *186*, 63-75.

(6) Ruiz, M. E.; Flores-Riveros, H. J.; Novaro, O. *J. Catal.* **1980**, *64*, 1-12.

(7) Butler, I. S.; Basolo, F.; Pearson, R. G. *Inorg. Chem.* **1967**, *6*, 2074-2079.

(8) Craig, P. J.; Green, M. *J. Chem. Soc. A* **1968**, 1978-1981.

(9) Mawby, R. J.; Basolo, F.; Pearson, R. G. *J. Am. Chem. Soc.* **1964**, *86*, 3994-3999.

(10) Cotton, J. D.; Crisp, G. T.; Daly, V. A. *Inorg. Chim. Acta* **1981**, *47*, 165-169.

0002-7863/81/1503-7028$01.25/0