Table I. Catalytic Aldol Reactions of Silyl Ketene Acetal 1 with Aldehydes Mediated by Borane Complexes of 6a and 6b[a]

entry	aldehyde	ligand	yield of 5a, %	ee,[b] % (config)
1	PhCHO	6a	80	84 (R)[c]
2	PhCHO	6b	83	91 (R)[c]
3	c-C_6H_{11}CHO	6a	68	91 (R)[d]
4	c-C_6H_{11}CHO	6b	59	96 (R)[d]
5	$CH_3(CH_2)_2CHO$	6a	81	>98[e]
6	$CH_3(CH_2)_2CHO$	6b	82	>98[e]
7	$(CH_3)_2CHCH_2CHO$	6a	87	97[e]
8	$(CH_3)_2CHCH_2CHO$	6b	89	>98[e]
9	$Ph(CH_2)_2CHO$	6a	83	>98[e]
10	$Ph(CH_2)_2CHO$	6b	83	>98[e]
11	$BnO(CH_2)_2CHO$	6a	86	99[e]

[a] Experimental conditions as in text. [b] Enantiomeric excesses determined by chiral Daicel OD HPLC column (entries 1 and 2) or ^1H (300 MHz) and ^{19}F NMR analysis of the (S)-MTPA esters derived from 5a. [c] Absolute configuration determined by hydrolysis of the β-hydroxy ester to the corresponding carboxylic acid and comparison of optical rotations with literature values.[11] [d] Absolute configuration determined by comparison with authentic material derived from reduction (H_2/Rh-Al_2O_3) of (R)-(−)-5a (R = Ph). [e] Absolute configuration undetermined.

Complete consumption of benzaldehyde using α,α-disubstituted glycine derivatives has led to the selection of ligands 6a and 6b, both of which are readily available in enantiopure form from known amino acids.[7] Slow addition of benzaldehyde over a 3.5-h period resulted in an increase in the enantioselectivity from 57 to 84% ee for the catalytic (20 mol %)[8] reaction using ligand 6a, and hence a standard procedure was established as defined below. Furthermore, modification of the *p*-tolylsulfonyl moiety of ligand 6a provided the following enantiomeric excesses with varying Ar: Ar = 3,5-bis(trifluoromethyl)phenyl (52%), mesityl (53%), α-naphthyl (67%), β-naphthyl (78%), 4-*tert*-butylphenyl (81%), phenyl (83%), 4-methoxyphenyl (86%), 4-acetamidophenyl (86%). Since these modifications did not offer a substantial improvement in ee over 6a, the *p*-toluenesulfonamide derivatives were selected as ligands.[9] Aldol reactions with 6a and 6b indeed proceeded smoothly, with high chemical yields and, pleasingly, with high enantioselection as well, for a number of typical aldehydes (Table I). Note that the reactions of primary aldehydes led to the exclusive formation of a single enantiomer through the use of either ligand 6a or 6b (entries 5 and 6 and 8–10).

Although the hypothetical Scheme I has served as a guide for successfully designing the catalytic process, its mechanistic course is undoubtedly complicated, and even the structure of the catalyst tentatively formulated as 7 is yet to be established.[10] Preliminary results, however, indicate that an *intramolecular* B/Si-exchange reaction features in the catalytic cycle, and the same holds true for the asymmetric aldol reaction reported by the Yamamoto group.[5g] In the past we have attempted in vain to construct cycles with an *intermolecular* metal-exchange reaction, and the success outlined above hints at the direction of future catalyst design.

A standard procedure for aldol reactions is as follows. The ligand 6 (0.1 mmol, 0.2 equiv) in propionitrile (1.5 mL) was treated with BH_3·THF complex (100 μL of a 1 M solution in THF, 0.1 mmol, 0.2 equiv). The solution was warmed to 45 °C for 1 h and cooled to −78 °C before the addition of the ketene acetal (126 μL, 0.6 mmol, 1.2 equiv). The aldehyde (0.5 mmol) was then added as a solution in propionitrile (1 mL) over 3.5 h (syringe pump) and the reaction mixture stirred a further 1 h at the same temperature before being poured into pH 7 buffer at 0 °C. Usual workup, yielding silyl ether 5 accompanied by a small amount of β-hydroxy ester 5a, was followed by hydrolysis (1 N HCl/THF) and column chromatography to give pure 5a.

Acknowledgment. This work was supported by a grant from the NIH (GM35879). E.R.P. and O.T. are holders of SERC/NATO postdoctoral and Merck Fellowships, respectively.

Supplementary Material Available: Experimental details of the synthesis of 6a and 6b (5 pages). Ordering information is given on any current masthead page.

(7) (a) Slates, H. L.; Taub, D.; Kuo, C. H.; Wendler, N. L. *J. Org. Chem.* **1964**, *29*, 1424. (b) Cremlyn, R. J. W.; Chisholm, M. *J. Chem. Soc. C* **1967**, 1762. (c) Munday, L. *J. Chem. Soc.* **1961**, 4372. (d) Stereochemistry of 6b: Sacripante, G.; Edward, J. T. *Can. J. Chem.* **1982**, *60*, 1982. (e) For general and specific synthetic procedures, see: Williams, R. M. *Synthesis of Optically Active α-Amino Acids*; Pergamon Press: Oxford, 1989. (f) Galdecki, Z.; Karolak-Wojciechowska, J. *J. Crystallogr. Spectrosc. Res.* **1986**, *16*, 467.

(8) The use of 10 mol % catalyst derived from ligand 6a did not result in a significant loss of enantioselectivity or reactivity.

(9) The ligands are recoverable.

(10) The ^{11}B NMR spectra of the borane complexes derived from 6a and α-methylalanine sulfonamide exhibit a single broad peak centered at ≈+6 ppm (reference = BF_3·OEt_2).

(11) (a) Matell, M. *Ark. Kemi* **1949**, *1*, 455. (b) Guette, M.; Capillon, J.; Guette, J.-P. *Tetrahedron* **1973**, *29*, 3659. (c) Kende, A. S.; Kawamura, K.; Orwat, M. J. *Tetrahedron Lett.* **1989**, *30*, 5821.

Induced Stereoselectivity and Substrate Selectivity of Bio-Imprinted α-Chymotrypsin in Anhydrous Organic Media

Marianne Ståhl, Ulla Jeppsson-Wistrand, Mats-Olle Månsson, and Klaus Mosbach*

Pure and Applied Biochemistry
Chemical Center, University of Lund
P.O.B. 124, S-221 00 Lund, Sweden

Received June 7, 1991

In recent years much work has focused on attempts to change the properties of antibodies[1] or of proteins and enzymes in water-poor media where rigidity of conformation is the basis for the new properties.[2] In this communication we describe how precipitation of α-chymotrypsin with 1-propanol in the presence of *N*-acetyl-D-tryptophan, *N*-acetyl-D-phenylalanine, or *N*-acetyl-D-tyrosine, followed by drying of the precipitate, appears to induce a new conformation of the active site. In an anhydrous solvent α-chymotrypsin prepared in this way exhibits high selectivity in the synthesis of the D-form of the ethyl ester of the N-acetylated amino acid present during precipitation. This is not possible with α-chymotrypsin precipitated in the presence of the L-form or in the absence of the D-isomer. We have coined this method, leading to a change of the conformation of the active site, "bio-imprinting", in analogy to molecular imprinting in synthetic polymers.[3]

To gain a better understanding of the underlying mechanism, α-chymotrypsin was bio-imprinted with *N*-acetyl-D-tryptophan and *N*-acetyl-L-tryptophan, and the synthesis of *N*-acetyl-D-tryptophan ethyl ester and *N*-acetyl-L-tryptophan ethyl ester, as influenced by small additions of water to the reaction solution, was investigated. The activity of the enzyme in the synthesis of *N*-acetyl-L-tryptophan ethyl ester increased rapidly as the water concentration was increased (Figure 1A), whereas the opposite was the case in the synthesis of *N*-acetyl-D-tryptophan ethyl ester (Figure 1B). The effects of water on catalysis described in Figure 1A have been observed earlier[6] and was interpreted as an effect

(1) (a) Benkovic, J. S.; Adams, J. A.; Borders, C. I.; Jr.; Janda, K. D.; Lerner, R. A. *Science* **1990**, *250*, 1135–1139. (b) Shokat, K. M.; Ko, M. K.; Scanlan, T. S.; Kochersperger, L.; Yonkovich, S.; Thaisrivongs, S.; Schultz, P. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 1296–1303.

(2) (a) Ståhl, M.; Månsson, M. O.; Mosbach, K. *Swedish patent application no. 89040215-4* **1989**. (b) Braco, L.; Dabulis, K.; Klibanov, A. M. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 274–277. (c) Ståhl, M.; Månsson, M. O.; Mosbach, K. *Biotechnol. Lett.* **1990**, *12*, 161–166. (d) Zaks, A.; Klibanov, A. M. *J. Biol. Chem.* **1988**, *263*, 3194–3201.

(3) Ekberg, B.; Mosbach, K. *TIBTECH* **1989**, *7*, 92–96.

(4) Initial water content of the reaction mixture was 10 mM and after equilibration with the enzyme 20 mM, indicating that water had been removed from the enzyme and into the solvent (measured using the Fisher method). Water content of the dried enzyme (measured gravimetrically) was 12.2% (w/w).

0002-7863/91/1513-9366$02.50/0

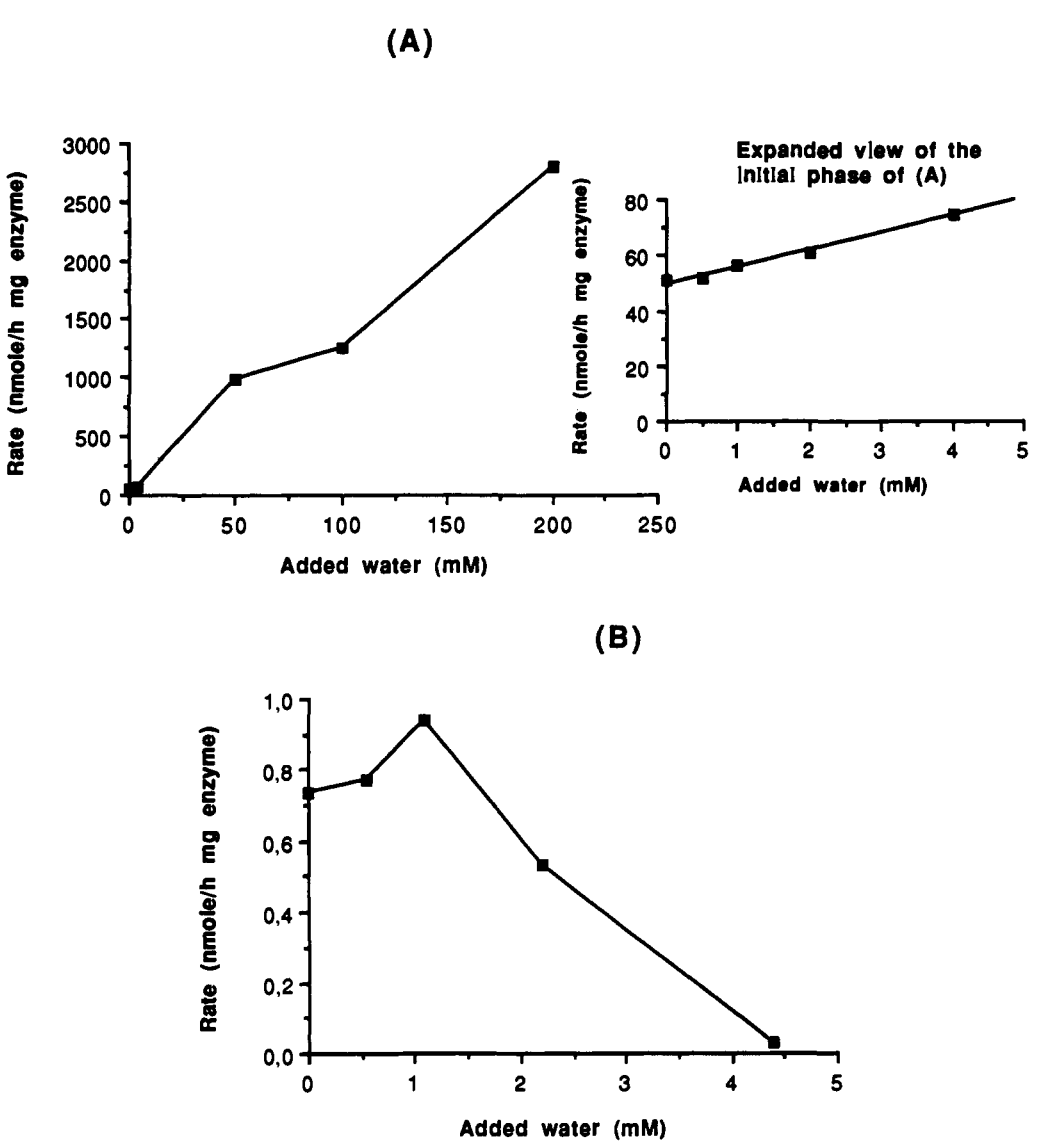


Figure 1. Effect of water[4] on the synthesis of (A) *N*-acetyl-L-tryptophan ethyl ester with α-chymotrypsin bio-imprinted[5] with *N*-acetyl-L-tryptophan and (B) *N*-acetyl-D-tryptophan ethyl ester with α-chymotrypsin bio-imprinted with *N*-acetyl-D-tryptophan.

of water acting as a molecular lubricant, increasing the mobility of the enzyme. A possible explanation of the results described in Figure 1B can be that the conformation of α-chymotrypsin obtained upon bio-imprinting is stable only below a certain water concentration. When the water concentration is increased above this level, the interactions between the enzyme and the surrounding water molecules are such that the original native enzyme conformation, active only toward the L-form, is regained. When the total water concentration was increased by only 4 mM the D-specific activity was totally lost. How the added water partitions between the enzyme and the solvent is not known and merits further study as does possible effects of ethanol and remaining 1-propanol acting as water mimics[7] in the interactions between solvent and enzyme.

α-Chymotrypsin was also bio-imprinted with the D- and L-forms of *N*-acetylphenylalanine and *N*-acetyltyrosine. The initial rates are summarized in Table I and show that besides the new D-stereospecificity a remarkably high degree of substrate selectivity was obtained. The conformation on the active site was restricted to the one complementary to the ligand present during bio-imprinting. α-Chymotrypsin bio-imprinted with the D-form was still active toward the L-form of the substrate but at a lower rate (10–20% of the rate obtained with α-chymotrypsin bio-imprinted with the L-form).

The presence of water in the active site of the enzyme when no ligand is present during precipitation seems to provide a certain

Table I. Initial Rates (nmol/h·mg enzyme) during Synthesis of Ethyl Esters of L- and D-Forms of *N*-Acetyltryptophan, *N*-Acetylphenylalanine, and *N*-Acetyltyrosine with Bio-imprinted α-Chymotrypsin[8,a]

	N-acetylated ligand present during bio-imprinting			
substrate	L-Trp	L-Phe	L-Tyr	no ligand
N-Ac-L-Trp	72	0	0	24
N-Ac-L-Phe	0	398	0	131
N-Ac-L-Tyr	0	0	306	183
substrate	D-Trp	D-Phe	D-Tyr	no ligand
N-Ac-D-Trp	1.9	0	0	0
N-Ac-D-Phe	0	1.7	0	n.d.
N-Ac-D-Tyr	0	0	0.8	n.d.

[a] Zero means below the detection limit (supplementary material); n.d. = not determined.

(5) α-Chymotrypsin (30 mg) was dissolved in 1 mL of 10 mM sodium phosphate buffer pH 7.8 containing the amino acid derivative (20 mM). The enzyme solution was cooled to 0 °C and equilibrated for 30 min, and then 4 mL of 1-propanol (−20 °C) was added. The precipitate formed was recovered by centrifugation after 30 min on ice. After three washes with 1-propanol the precipitate was dried under vacuum. The dried precipitate contained 3.5 mol amino acid derivative/mol α-chymotrypsin.

(6) (a) Reslow, M.; Adlercreutz, P.; Mattiasson, B. *Appl. Microbiol. Biotechnol.* **1987**, *26*, 1–8. (b) Zaks, A.; Klibanov, A. M. *J. Biol. Chem.* **1988**, *263*, 8017–8021.

(7) Kitaguchi, H.; Klibanov, A. M. *J. Am. Chem. Soc.* **1989**, *111*, 9272–9273.

conformational flexibility to the active site allowing different substrates to enter the catalytic center of the enzyme as shown for the L-enantiomers in Table I.

The higher initial rates obtained with *N*-acetyl-L-amino acids present during the bio-imprinting procedure than those obtained without ligand are noteworthy.

After an initial lag phase (24–36 h for D-ester synthesis and 1–3 h for L-ester synthesis) the enzyme lost some of its induced substrate specificity and was able to also synthesize the esters of the two N-acetylated amino acids not present during the bio-imprinting. However, this took place at a lower rate, 2–30% of the rate obtained when the substrate was used during the bio-imprinting. The lost specificity might be due to conformational changes which may occur due to water molecules produced during the enzymatic reaction. For the L-ester synthesis it was shown in preliminary experiments that small additions of water at the outset reduced the lag phase considerably (data not shown). This is in accordance with the discussion above concerning water addition.

Two-thirds (68%) of the active sites were accessible to the substrates as determined with active site titration with *trans*-cinnamoylimidazole.[2d,9] Furthermore, α-chymotrypsin irreversibly inhibited with phenylmethylsulfonyl fluoride[10] prior to bio-imprinting was completely inactive in the synthesis of ester indicating that the active site serine is involved in catalysis. The effects of bio-imprinting are thus active-site related and not a general protein-related property.

Acknowledgment. The authors are grateful to the Swedish Natural Science Research Council (NFR 2616-309) for its financial support.

Supplementary Material Available: Bio-imprinting procedure, product determination, and identification (2 pages). Ordering information is given on any current masthead page.

(8) Bio-imprinted (8 mg) α-chymotrypsin was suspended in 2 mL of cyclohexane and sonicated (1 min). Ethanol (0.5 ml) with 0.1 M N-acetylated amino acid was added. Product formation was followed with HPLC. Optical purity of the product was determined to be at least 98% as judged by measuring the specific rotation polarimetrically.

(9) Schonbaum, G. R.; Zerner, B.; Bender, M. L. *J. Biol. Chem.* **1961**, *236*, 2930–2935.

(10) Gold, A. M. *Methods Enzymol.* **1967**, *11*, 706–711.

1,4-Silylstannation of 1,3-Dienes Catalyzed by Platinum Complex

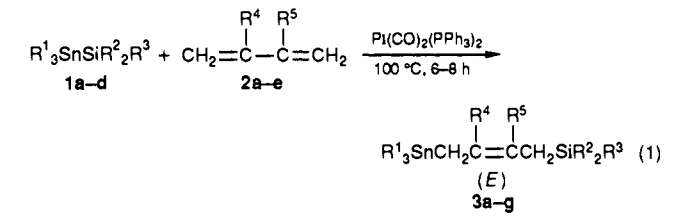
Yasushi Tsuji* and Yasushi Obora

Department of Chemistry, Faculty of Engineering
Gifu University, Gifu 501-11, Japan

Received August 5, 1991

Transformations of typical metal reagents by transition-metal catalysis provide new methodology for selective organic synthesis.[1] Recently, considerable attention has been paid to organosilylstannanes, R_3SnSiR_3' (**1**).[2-5] Silylstannanes (**1**) add to 1-alkynes in the presence of $Pd(PPh_3)_4$ to give alkenes having vicinal silyl and stannyl substituents.[4] The same catalyst induces the insertion of isonitriles into the Sn–Si bond of **1**.[5] The stannyl and silyl moieties in those products[4,5] can be utilized in further functionalizations.[4b,6] However, to our knowledge, there have been no reports on the addition of **1** to dienes and unactivated olefins.

We disclose here the first example of 1,4-silylstannation of 1,3-dienes (eq 1). The reaction is highly regio- and stereoselective.



1a–d **2a–e** **3a–g** (1)

All the products in this study are new compounds, which possess allylic silane and allylic stannane functionalities in the same molecule sharing the same carbon–carbon double bond. The allylic silanes[7] and allylic stannanes[8] are extremely important in selective organic synthesis. Therefore, the present 1,4-silylstannation will offer a new class of versatile building block.

When (trimethylsilyl)tributylstannane (**1a**) was allowed to react with 3 equiv of 1,3-butadiene (**2a**) in the presence of a catalytic amount (5 mol %) of $Pt(CO)_2(PPh_3)_2$ in toluene at 100 °C for 6 h, the 1,4-silylstannation product (**3a**) was obtained as a single isomer in excellent yield (Table I, entry 1).[9] All the spectral data[10] show that the adduct has exclusively (*E*)-1,4 structure. The nature of the catalyst precursor has a critical effect on the reaction. The palladium(0) complexes, which were the most effective catalyst precursors in the precedent studies using **1**,[2a,3-5] are almost inactive with the substrates reported here (yield of **3a**: $Pd(PPh_3)_4$ trace (entry 3), $Pd(CO)(PPh_3)_3$ 2%). Among the platinum complexes examined, $Pt(CO)_2(PPh_3)_2$ gave the best result. Other platinum complexes give less favorable results (yield of **3a**: $Pt(PPh_3)_4$ 29%, $Pt(C_2H_4)(PPh_3)_2$ 13%, $PtCl_2(PPh_3)_2$ trace, $Pt(DBA)_2$ 0%, $Pt(DBA)_2 + 2PPh_3$ 12%).[12]

(1) (a) Collman, J. P.; Hegedus, L. S.; Norton, J. R.; Finke, R. G. *Principle and Applications of Organotransition Metal Chemistry*; University Science Books: Mill Valley, CA, 1987; pp 704–720. (b) Negishi, E.-I. *Advances in Metal-Organic Chemistry*; Liebeskind, L. S., Ed.; JAI Press: Greenwich, CT, 1988; Vol. 1, pp 177–207.

(2) (a) Kosugi, M.; Ohya, T.; Migita, T. *Bull. Chem. Soc. Jpn.* **1983**, *56*, 3539. (b) Lipshuta, B. H.; Reuter, D. C.; Ellsworth, E. L. *J. Org. Chem.* **1989**, *54*, 4975.

(3) Mori, M.; Kaneda, N.; Shibasaki, M. *J. Org. Chem.* **1991**, *56*, 3486.

(4) (a) Mitchell, T. N.; Killing, H.; Dicke, R.; Wickenkamp, R. *J. Chem. Soc., Chem. Commun.* **1985**, 354. (b) Mitchel, T. N.; Wickenkamp, R.; Amamria, A.; Dicke, R.; Schneider, U. *J. Org. Chem.* **1987**, *52*, 4868. (c) Chenard, B. L.; Laganis, E. D.; Davidson, F.; RajanBabu, T. V. *J. Org. Chem.* **1985**, *50*, 3666. (d) Chenard, B. L.; Van Zyl, C. M. *J. Org. Chem.* **1986**, *51*, 3561. (e) Murakami, M.; Morita, Y.; Ito, Y. *J. Chem. Soc., Chem. Commun.* **1990**, 428.

(5) (a) Ito, Y.; Bando, T.; Matsuura, T.; Ishikawa, M. *J. Chem. Soc., Chem. Commun.* **1986**, 980. (b) Ito, Y. *Pure Appl. Chem.* **1990**, *62*, 583.

(6) (a) Ito, Y.; Matsuura, T.; Murakami, M. *J. Am. Chem. Soc.* **1987**, *109*, 7888. (b) Murakami, M.; Matsuura, T.; Ito, Y. *Tetrahedron Lett.* **1988**, *29*, 355. (c) Chenard, B. L.; Van Zyl, C.; Sanderson, D. R. *Tetrahedron Lett.* **1986**, *27*, 2801.

(7) (a) Colvin, E. W. *Silicon Reagents in Organic Synthesis*; Academic: London, 1988; pp 25–37. (b) Weber, W. P. *Silicon Reagents for Organic Synthesis*; Springer: Berlin, 1983; pp 173–205. (c) Colvin, E. W. *Silicon in Organic Synthesis*; Butterworths: London, 1981; pp 97–124.

(8) Pereyre, M.; Quintard, J.-P.; Rahm, A. *Tin in Organic Synthesis*; Butterworths: London, 1987; pp 185–258.

(9) A typical procedure is as follows: 1,3-Butadiene (1.5 mmol, 0.94 mL of 1.6 M stock solution in toluene), $Pt(CO)_2(PPh_3)_2$ (19 mg, 0.025 mmol), **1a** (181 mg, 0.5 mmol), toluene (2.0 mL), and a magnetic stirring bar were placed under argon flow in a 30 mL stainless steel autoclave containing an inserted glass tube. An air purge was confirmed by three pressurization (20 atm)–depressurization sequences with argon. After the reaction, the mixture was passed through a short Florisil column (8 mm i.d. × 50 mm) and the product (**3a**) was isolated by Kugelrohr distillation (pot temperature 115 °C (0.5 mmHg); 175 mg, 84%).

(10) **3a**: ^1H NMR ($CDCl_3$) δ −0.02 (s, 9 H), 0.82–0.94 (m, 15 H), 1.24–1.55 (m, 12 H), 1.48 (d, 2 H), 1.70 (d, 2 H, $^2J_{Sn-H}$ = 58 Hz), 5.19 (dt, 1 H, *J* = 15 Hz, 7 Hz), 5.38 (dt, 1 H, *J* = 15 Hz, 7 Hz); ^{13}C NMR ($CDCl_3$) δ −1.87 (q), 9.15 (t, $^1J_{Sn-C}$ = 296 Hz, 310 Hz), 13.74 (q), 14.26 (t), 22.61 (t, $^1J_{Sn-C}$ = 261, 270 Hz), 27.42 (t, $^2J_{Sn-C}$ = 65 Hz), 29.26 (t, $^3J_{Sn-C}$ = 24 Hz), 121.5 (d, J_{Sn-C} = 48 Hz), 127.6 (d, J_{Sn-C} = 44 Hz); ^{119}Sn NMR (C_6D_6) −17.74 ppm; MS (EI) *m/e* 418 (M^+). Anal. Found: C, 54.40; H, 10.08. Calcd for $C_{19}H_{42}SnSi$: C, 54.68; H, 10.14. **3b**: ^1H NMR ($CDCl_3$) δ −0.01 (s, 9 H), 0.83–0.96 (m, 15 H), 1.24–1.56 (m, 12 H), 1.50 (d, 2 H), 1.55 (s, 3 H), 1.77 (s, 2 H, $^2J_{Sn-H}$ = 59 Hz[11]), 5.03 (t, 1 H); ^{13}C NMR ($CDCl_3$) δ −1.57 (q), 9.54 (t, $^1J_{Sn-C}$ = 292, 306 Hz), 13.77 (q), 18.46 (q), 18.73 (t), 22.25 (t, $^1J_{Sn-C}$ = 261, 271 Hz), 27.55 (t, $^2J_{Sn-C}$ = 54 Hz), 29.32 (t, $^3J_{Sn-C}$ = 24 Hz), 115.9 (d, $^3J_{Sn-C}$ = 44 Hz), 132.3 (s, $^2J_{Sn-C}$ = 44 Hz); ^{119}Sn NMR −16.04 ppm; MS (EI) *m/e* 432 (M^+). Anal. Found: C, 55.40; H, 10.52. Calcd for $C_{20}H_{44}SnSi$: C, 55.69; H, 10.28.

(11) Only one side (lower field) of the satellite peaks was observed. The pair (higher field) to the observed side could not be distinguished because of overlap with other proton resonances.

0002-7863/91/1513-9368$02.50/0