

6 nM for **3**, which equals almost exactly the  $K_d$  measured for **1**,  $99 \pm 6$  nM. The  $K_d$  increases with tether length for both classes of probes:  $K_d(\mathbf{4}) = 276 \pm 12$  nM,  $K_d(\mathbf{2}) = 263 \pm 8$  nM. All tethered probes bind significantly better than oligonucleotides **8** and **9** [ $K_d(\mathbf{8}) = 17\,500 \pm 2200$  nM,  $K_d(\mathbf{9}) = 135\,000 \pm 19\,000$  nM]. Therefore, two oligonucleotides united by a neutral tether designed to minimize electrostatic effects<sup>20</sup> recognize RNA molecules on the basis of both sequence and structure. This observation is very intriguing, since it suggests that TOPs could function as *molecular rulers for RNA*.

**Acknowledgment.** We thank J. Bruzik for the SL RNA construct, R. Gregorian and D. Jeruzalmi for gifts of T7 RNA polymerase, D. Crothers for providing use of the Betascope, and T. Shrader and B. Honig for helpful discussions. S.T.C. thanks the Department of Education for a Predoctoral Fellowship. This work was supported by the National Institutes of Health (GM43501) and by Merck & Co., Inc. A.S. is a recipient of a David and Lucile Packard Foundation Fellowship and a Presidential Young Investigator Award.

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### Tris(phenylimido) Complexes of Tungsten: Preparation and Properties of the d<sup>0</sup> W(=NR)<sub>3</sub> Functional Group

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Despite their notoriety as inert ligands, highly reactive early-transition-metal imido<sup>1</sup> moieties (M=NR) may be generated—even toward C-H activation<sup>2,3</sup>—if sufficient electron density resides on the imido nitrogen.<sup>3</sup> One potential means to achieve this condition is to lade the metal center with strong  $\pi$ -donor ligands and thereby induce a competition for available d{ $\pi$ }-p{ $\pi$ } interactions.<sup>2-4</sup> Such " $\pi$ -loading" may result from environments that employ multiple imido coordination, i.e., additional [NR]<sup>2-</sup> groups

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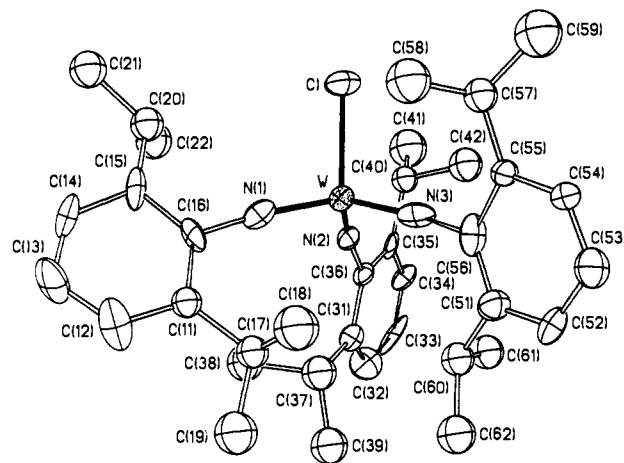


Figure 1. Molecular structure of the [W(NAr)<sub>3</sub>Cl]<sup>-</sup> anion in [Li(THF)<sub>4</sub>][W(NAr)<sub>3</sub>Cl] (**3**, Ar = 2,6-diisopropylphenyl) with atoms shown as 50% probability ellipsoids.

as the ancillary  $\pi$ -donors.<sup>5</sup> Established imido functional groups of d<sup>0</sup> tungsten include W=NR,<sup>6</sup> W(=NR)<sub>2</sub>,<sup>7</sup> and [W(=NR)<sub>4</sub>]<sup>2-</sup>,<sup>8</sup> but the tris(imido) complexes are conspicuously absent from this series. Herein, we report the preparation of the d<sup>0</sup> W(=NR)<sub>3</sub> functional group and demonstrate an electronic and structural analogy to related M( $\pi$ -donor)<sub>3</sub> complexes.

Upon reaction of W(NAr)Cl<sub>4</sub>(THF)<sup>9</sup> with 2 equiv of Me<sub>3</sub>SINHAr in THF (45 °C, 24 h, Ar = 2,6-diisopropylphenyl), red orange W(NAr)<sub>2</sub>Cl<sub>2</sub>(THF)<sub>2</sub> (**1**) forms in 87% yield. NMR data for **1** reveal<sup>10</sup> equivalent imido and THF ligands, thus a structure parallel to reported W(NR)<sub>2</sub>Cl<sub>2</sub>(L-L) chelate adducts is proposed (Scheme 1).<sup>7c,9,11</sup> W(NAr)<sub>2</sub>Cl<sub>2</sub>(THF)<sub>2</sub> (**1**) is readily functionalized by using excess Me<sub>3</sub>SiNEt<sub>2</sub> (in Et<sub>2</sub>O) to provide orange crystals of W(NAr)<sub>2</sub>(NEt<sub>2</sub>)Cl (**2**). Upon reaction of W(NAr)<sub>2</sub>Cl<sub>2</sub>(THF)<sub>2</sub> (**1**) with 2 equiv of LiNHAr in THF (room temperature, 12 h), yellow crystalline **3** is obtained in high yield. The absence of a  $\nu$ (N-H) mode in the IR spectrum of **3**, the lack of NH resonances in its <sup>1</sup>H NMR spectrum, its elemental analysis, and its reactivity (vide infra) all support the formulation of **3** as the tris(imido) complex [Li(THF)<sub>4</sub>][W(NAr)<sub>3</sub>Cl].<sup>12</sup>

The structure of the [W(NAr)<sub>3</sub>Cl]<sup>-</sup> anion in [Li(THF)<sub>4</sub>][W(NAr)<sub>3</sub>Cl] (**3**, Figure 1)<sup>13,14</sup> reveals that the tungsten atom is

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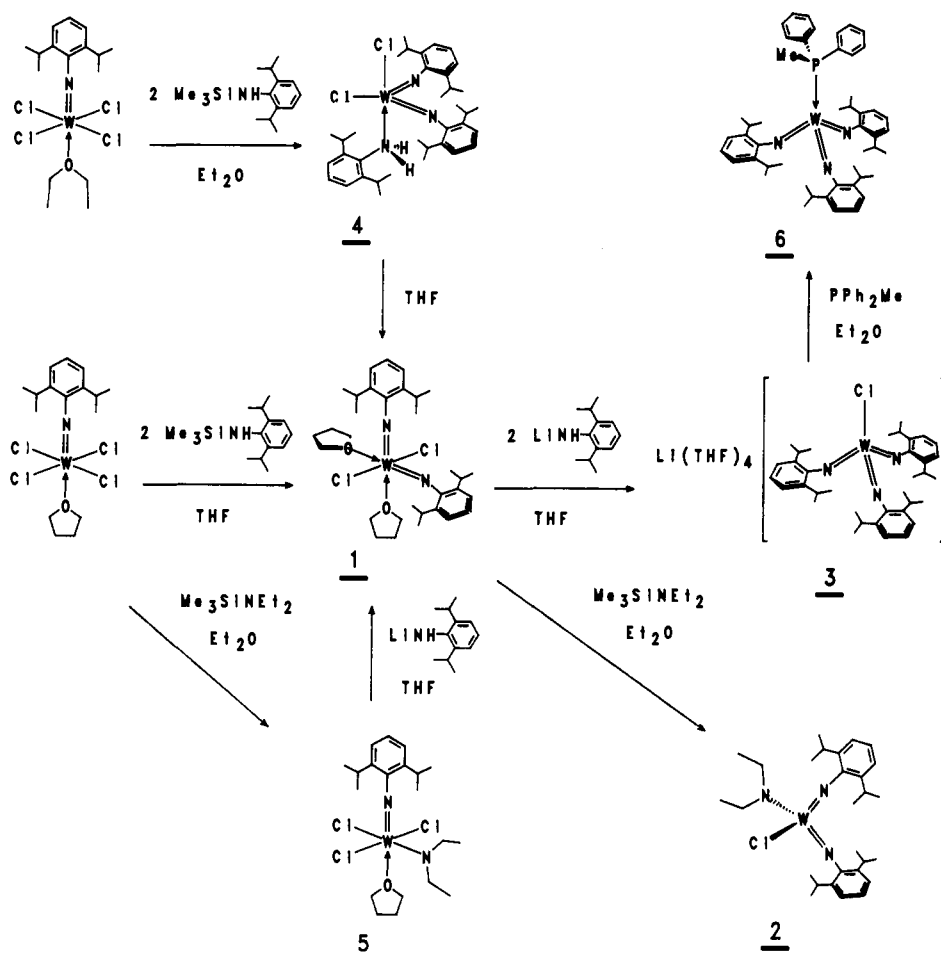
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(10) Analytical and spectroscopic data for compounds 1-6 are available as supplementary material.

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(12) [Li(THF)<sub>4</sub>][W(NAr)<sub>3</sub>Cl] (**3**): <sup>1</sup>H NMR (CD<sub>2</sub>Cl<sub>2</sub>)  $\delta$  6.97-6.65 (A<sub>2</sub>B mult, 9 H, H<sub>arip</sub>), 3.71 (m, 16 H, C<sub>2</sub>H, THF), 3.57 (spt, 6 H, CHMe<sub>2</sub>), 1.90 (m, 16 H, C<sub>2</sub>H, THF), 1.06 (d, 36 H, CHMe<sub>2</sub>); <sup>13</sup>C NMR (CD<sub>2</sub>Cl<sub>2</sub>)  $\delta$  140.4 (C<sub>q</sub>), 125.1 (C<sub>ipso</sub>), 122.0 (overlapping C<sub>ar</sub> and C<sub>p</sub>), 68.7 (C<sub>ar</sub>, THF), 28.3 (CHMe<sub>2</sub>), 25.8 (C<sub>2</sub>, THF), 23.8 (CHMe<sub>2</sub>). Anal. Calcd for C<sub>52</sub>H<sub>83</sub>ClLiN<sub>3</sub>O<sub>4</sub>W: C, 60.03; H, 8.04; N, 4.04; Cl, 3.41. Found: C, 59.54; H, 8.44; N, 4.15; Cl, 2.81.

Scheme I



tetrahedrally coordinated with three essentially identical imido nitrogen atoms. The W-N-C<sub>ipso</sub> bond angles are close to linear (average 170.8 (15)°), and the average W-N bond length is 1.782 (15) Å. In C<sub>3v</sub> symmetry, the six π molecular orbitals of the [NR]<sup>2-</sup> ligands (derived from each nitrogen's p<sub>x</sub> and p<sub>y</sub>) transform as a<sub>1</sub> + a<sub>2</sub> + 2e. Since the metal has no orbital of a<sub>2</sub> symmetry, two electrons must occupy a ligand-based, nonbonding a<sub>2</sub> MO comprised of the nitrogen p orbitals lying perpendicular to the C<sub>3</sub> axis. Because only 10 of the 12 [NR]<sup>2-</sup> π electrons are available for interactions with the metal, [W(NAr)<sub>3</sub>Cl]<sup>-</sup> is an 18-electron complex (not 20), electronically (and structurally) analogous to the classic "20 electron" W(RC≡CR)<sub>3</sub>(CO) species<sup>15</sup> and neutral Re(NR)<sub>3</sub>X (X = OSiMe<sub>3</sub>,<sup>7a</sup> Cl<sup>16</sup>) compounds. Likewise, recently reported trigonal-planar Os(NAr)<sub>3</sub> seems to have two electrons in a ligand-based a<sub>2</sub>' MO and is also an 18-electron compound.<sup>17</sup>

(13) Crystal data for C<sub>52</sub>H<sub>83</sub>Cl<sub>4</sub>LiN<sub>3</sub>O<sub>4</sub>W (3): yellow, monoclinic, P2<sub>1</sub>/n, a = 13.787 (4) Å, b = 17.348 (5) Å, c = 22.781 (8) Å, β = 90.43 (3)°, V = 5448.5 (30) Å<sup>3</sup>, Z = 4, D(calcd) = 1.268 g cm<sup>-3</sup>, μ(Mo Kα) = 23.29 cm<sup>-1</sup> (T = 296 K); 6532 independent reflections with 4° < 2θ < 45° were collected, of which 2938 reflections with F<sub>o</sub> > 5σ(F<sub>o</sub>) were used in refinement. R = 6.77%, R<sub>w</sub> = 6.39%, GOF = 1.23.

(14) Selected bond lengths (Å) and angles (deg) for 3: W-Cl = 2.343 (6), W-N(1) = 1.777 (15), W-N(2) = 1.763 (15), W-N(3) = 1.805 (18); Cl-W-N(1) = 106.0 (6), Cl-W-N(2) = 104.7 (5), Cl-W-N(3) = 107.2 (6), N(1)-W-N(2) = 112.5 (7), N(2)-W-N(3) = 112.1 (7), N(1)-W-N(3) = 113.5 (7), W-N(1)-C(16) = 173.4 (15), W-N(2)-C(36) = 167.7 (14), W-N(3)-C(56) = 171.4 (15).

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(16) Horton, A. D.; Schrock, R. R. *Polyhedron* **1988**, *7*, 1841.

(17) Anhaus, J. T.; Kee, T. P.; Schofield, M. H.; Schrock, R. R. *J. Am. Chem. Soc.* **1990**, *112*, 1642.

To more firmly establish how compounds 1 and 3 arise, we report the results of the following experiments (Scheme I). (i) When W(NAr)Cl<sub>4</sub>(OEt)<sub>2</sub> reacts with 2 equiv of Me<sub>3</sub>SiNHAr in a weakly coordinating solvent, viz., Et<sub>2</sub>O, the five-coordinate adduct W(NAr)<sub>2</sub>Cl<sub>2</sub>(NH<sub>2</sub>Ar) (4) is isolated in 55% yield, presumably via the bis(amide) "W(NAr)(NHAr)<sub>2</sub>Cl<sub>2</sub>". Compound 4 is readily converted to 1 upon treatment with neat THF. From the precedence provided by d<sup>0</sup> TBP complexes with two π-donor ligands,<sup>18</sup> 4 is formulated structurally with two equatorial imido groups and an axial H<sub>2</sub>NAr ligand. (ii) Upon reaction of W(NAr)Cl<sub>4</sub>(THF) with Me<sub>3</sub>SiNEt<sub>2</sub> (≥ 2 equiv) in Et<sub>2</sub>O, orange crystalline W(NAr)(NEt<sub>2</sub>)Cl<sub>3</sub>(THF) (5) forms in 77% yield. W(NAr)<sub>2</sub>Cl<sub>2</sub>(THF)<sub>2</sub> (1) is afforded in quantitative yield upon reacting 5 with 1 equiv of LiNHAr (in THF), presumably via incipient "W(NAr)(NHAr)(NEt<sub>2</sub>)Cl<sub>2</sub>(THF)<sub>x</sub>". When monitoring this latter reaction in THF-d<sub>8</sub> (over 48 h, room temperature), exactly 1 equiv of HNEt<sub>2</sub> is produced per 1 equiv of W(NAr)<sub>2</sub>Cl<sub>2</sub>(THF)<sub>2</sub> (1) formed (<sup>1</sup>H NMR). (iii) The reaction of [Li(THF)<sub>4</sub>][W(NAr)<sub>3</sub>Cl] (3) with PPh<sub>2</sub>Me in Et<sub>2</sub>O or C<sub>6</sub>H<sub>6</sub> affords dark red crystals of neutral W(NAr)<sub>3</sub>(PPh<sub>2</sub>Me) (6). Solutions of W(NAr)<sub>3</sub>(PPh<sub>2</sub>Me) and [<sup>n</sup>Bu<sub>4</sub>N]Br (1:1) establish the equilibrium W(NAr)<sub>3</sub>(PPh<sub>2</sub>Me) + Br<sup>-</sup> ⇌ [W(NAr)<sub>3</sub>Br]<sup>-</sup> + PPh<sub>2</sub>Me, which is favored far to the right in both THF-d<sub>8</sub> and C<sub>6</sub>D<sub>6</sub> (<sup>1</sup>H NMR). (iv) Complex 3 has not been observed nor isolated from the reaction of W(NAr)<sub>2</sub>(NEt<sub>2</sub>)Cl (2) with LiNHAr in THF. On the basis of these experiments, 1 is proposed to arise through an intramolecular α hydrogen abstraction sequence with the concomitant loss of HNR<sub>2</sub> (R = Et or 2,6-C<sub>6</sub>H<sub>3</sub>Pr<sub>2</sub>). How-

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ever, the formation of **3** from **1** does not require such a scheme, since nascent "W(NAr)<sub>2</sub>(NHAr)Cl" could be deprotonated *intermolecularly* and since the reaction of halide ion with neutral "W(NAr)<sub>3</sub>L" (formed by any reaction sequence) appears facile.

These experiments underscore the use of highly basic amido ligands in a sacrificial sense to effect sequential  $\alpha$  hydrogen abstractions, a task that is often consigned to carbanion equivalents.<sup>8,19</sup> Of particular interest will be the reactivity of the W(=NR)<sub>3</sub> functional group if 16-electron, presumably trigonal-planar W(NAr)<sub>3</sub> can be prepared.

**Acknowledgment.** D.E.W. thanks the National Science Foundation (CHE-8919367) for support. Acknowledgment is also made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, for partial support of this research and the Biomedical Research Support Grant Program, Division of Research Resources, National Institutes of Health, for partial support (BRSG S07RR07002).

**Supplementary Material Available:** Analytical and spectroscopic data for compounds **1-6** and tables of crystal data, data collection parameters, atomic positional and thermal parameters, bond distances, and bond angles for [Li(THF)<sub>4</sub>][W(NAr)<sub>3</sub>Cl] (**3**) (Ar = 2,6-diisopropylphenyl) (8 pages); listing of observed and calculated structure factors for **3** (9 pages). Ordering information is given on any current masthead page.

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## Selective Acylation of Peptides Catalyzed by Lipases in Organic Solvents

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The covalent attachment of carboxylic acids is one of the most ubiquitous and important posttranslational modifications of peptides *in vivo*.<sup>1</sup> In order to better understand the function and biochemical significance of such common acylations<sup>1</sup> as acetylation, myristoylation, and palmitoylation, the *in vitro* synthesis of selectively acylated peptides, with the possibility of varying the modification sites, should be very helpful. In addition, peptides acylated with fatty acids become capable of being anchored to liposomes, translocating across lipid membranes, penetrating intact cells, and penetrating through the blood-brain barrier.<sup>2</sup> However, selective acylation is a formidable task to a chemist due to the presence of numerous reactive groups in peptides and the complexity of the enzymatic systems involved.<sup>1</sup>

We report herein a new approach to this problem which is based on our finding<sup>3</sup> that lipases, when acting in organic solvents, can catalyze amide-bond formation. The selectivity of lipases in the aminolysis of esters in anhydrous media has been profitably used for asymmetric transformations<sup>4</sup> and peptide synthesis.<sup>5</sup> It is now

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applied to selective acylation of peptides.

We prepared<sup>6</sup> as the initial target molecule, the dipeptide L-Phe- $\alpha$ -L-Lys-O-*t*-Bu (**1**). It has two primary amino groups, the  $\alpha$ -NH<sub>2</sub> group of Phe and the  $\epsilon$ -NH<sub>2</sub> group of Lys, and thus offers a challenge to selective acylation. This dipeptide (5  $\mu$ mol) and the activated ester trifluoroethyl acetate<sup>7</sup> (50  $\mu$ mol) were dissolved in 1 mL of anhydrous acetonitrile,<sup>8</sup> and then 50 mg of one of 15 commercially available lipases<sup>9</sup> was added to each reaction mixture, followed by vigorous shaking at 45 °C; the reaction progress was monitored by HPLC. After 24 h, in 12 out of 15 reaction mixtures an appreciable disappearance of **1** was observed; in five, the conversion exceeded 50%, and in three, **1**'s HPLC peak completely vanished and a new peak appeared. With the three lipases affording the complete conversion (those from *Pseudomonas* sp., *Aspergillus niger*, and *Chromobacterium viscosum*), the reactions were scaled up 10-fold, and the products were purified by silica gel chromatography (MeOH/CHCl<sub>3</sub>, 1:9, as the eluent) and identified by <sup>1</sup>H NMR. All three enzymatic reactions were found<sup>10</sup> to result in a single product, *N*- $\epsilon$ -monoacetyl-**1**. Thus all three lipases are highly efficient and regioselective catalysts of acetylation of **1**. In contrast, when this dipeptide was subjected to chemical acetylation (a slight molar excess of acetic anhydride under the same conditions), the product mixture consisted of<sup>10</sup> 73% of *N*- $\epsilon$ -monoacetyl-**1**, 4% of *N*- $\alpha$ -monoacetyl-**1**, and 23% of *N,N*- $\alpha,\epsilon$ -diacetyl-**1**. The lipases'  $\epsilon$ -regioselectivity is particularly impressive considering that the enzymatic acetylation of the  $\alpha$ -NH<sub>2</sub> group did not occur even though a large excess of trifluoroethyl acetate was still present at the end of the reaction.

*Pseudomonas* sp. lipase,<sup>11</sup> which afforded complete  $\epsilon$ -monoacetylation of **1** even after a 2-h reaction, was selected for further experimentation. It was established that acetonitrile was not a unique medium for the lipase-catalyzed acetylation: after 24 h the enzymatic reaction was also complete in *tert*-amyl alcohol, tetrahydrofuran, and dichloromethane; significantly, the same exquisite regioselectivity was retained in all the solvents.

The foregoing lipase-catalyzed peptide modification was successfully applied to acyl moieties other than acetyl: under the same experimental conditions as those employed for the acety-

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(6) This dipeptide (and others used in this study) was synthesized via the classical DCC coupling (Sheehan, J. C.; Hess, G. P. *J. Am. Chem. Soc.* **1955**, *77*, 1067) of *N*-CBZ-L-Phe and *N*- $\epsilon$ -CBZ-L-Lys-O-*t*-Bu (prepared as described by Roeske, R. *J. Org. Chem.* **1963**, *28*, 1251), followed by deprotection via catalytic hydrogenation. All structures were confirmed by 250-MHz <sup>1</sup>H NMR.

(7) All 2,2,2-trifluoroethyl esters were synthesized as described previously (Riva, S.; Klibanov, A. M. *J. Am. Chem. Soc.* **1988**, *110*, 3291).

(8) All the solvents used as reaction media were extensively dried by shaking with 3-Å molecular sieves (which brings the water content below 0.01%) in order to avoid hydrolysis of activated esters.

(9) Lipases from porcine pancreas, *Candida cylindracea*, *Candida lipolytica*, *Pseudomonas* sp., *Aspergillus niger*, *Chromobacterium viscosum*, wheat germ, *Rhizopus arrhizus*, *Rhizopus delemar*, *Rhizopus japonicus*, *Geotrichum candidum*, *Humicola lanuginosa*, *Mucor meheii*, *Mucor javanicus*, and *Penicillium cyclopium*.

(10) All structure elucidations were accomplished by 250-MHz <sup>1</sup>H NMR. Formation of the amide bond through the  $\epsilon$ -NH<sub>2</sub> group of **1** resulted in the downfield shift of the  $\epsilon$ -protons from 2.64 to 3.15 ppm (and no effect on the Phe's  $\alpha$ -proton). In contrast, the spectrum of *N*- $\alpha$ -monoacetyl-**1** (independently synthesized<sup>5c</sup> by us) showed a downfield shift of the  $\alpha$ -proton from 3.61 to 4.31 ppm (and no effect on the Lys's  $\epsilon$ -protons). Note that both isomers of monoacetylated **1** and *N,N*- $\alpha,\epsilon$ -diacetyl-**1** (prepared by exhaustive chemical acetylation) were readily distinguishable by HPLC, thereby providing a simple routine selectivity-monitoring technique. With the Ala-Lys and Phe-Ser dipeptides, the analysis was similar. In the latter case, acetylation of the Ser's OH group resulted in the downfield shift of the  $\beta$ -protons from 3.86 to 4.87 ppm and a split of the signal of the Ser's  $\alpha$ -proton due to its coupling with the  $\beta$ -protons.

(11) Lipoprotein lipase (LPL, hereafter referred to as "lipase") obtained from Amano International Enzyme Co. (Troy, VA) and dried under vacuum prior to use.