ever, the formation of **3** from **1** does not require such a scheme, since nascent "$W(NAr)_2(NHAr)Cl$" could be deprotonated *intermolecularly* and since the reaction of halide ion with neutral "$W(NAr)_3L$" (formed by any reaction sequence) appears facile.

These experiments underscore the use of highly basic amido ligands in a sacrificial sense to effect sequential α hydrogen abstractions, a task that is often consigned to carbanion equivalents.[8,19] Of particular interest will be the reactivity of the $W(=NR)_3$ functional group if 16-electron, presumably trigonal-planar $W(NAr)_3$ can be prepared.

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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 $[Li(THF)_4][W(NAr)_3Cl]$ (**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.[1] In order to better understand the function and biochemical significance of such common acylations[1] 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.[2] 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.[1]

We report herein a new approach to this problem which is based on our finding[3] 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[4] and peptide synthesis.[5] It is now applied to selective acylation of peptides.

We prepared,[6] as the initial target molecule, the dipeptide L-Phe-α-L-Lys-O-*t*-Bu (**1**). It has two primary amino groups, the α-NH_2 group of Phe and the ϵ-NH_2 group of Lys, and thus offers a challenge to selective acylation. This dipeptide (5 μmol) and the activated ester trifluoroethyl acetate[7] (50 μmol) were dissolved in 1 mL of anhydrous acetonitrile,[8] and then 50 mg of one of 15 commercially available lipases[9] 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 ($MeOH/CHCl_3$, 1:9, as the eluent) and identified by ^1H NMR. All three enzymatic reactions were found[10] to result in a single product, *N*-ϵ-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[10] 73% of *N*-ϵ-monoacetyl-**1**, 4% of *N*-α-monoacetyl-**1**, and 23% of *N*,*N*-α,ϵ-diacetyl-**1**. The lipases' ϵ-regioselectivity is particularly impressive considering that the enzymatic acetylation of the α-NH_2 group did not occur even though a large excess of trifluoroethyl acetate was still present at the end of the reaction.

Pseudomonas sp. lipase,[11] which afforded complete ϵ-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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(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 languinosa*, *Mucor meheii*, *Mucor javanicus*, and *Penicillium cyclopium*.

(10) All structure elucidations were accomplished by 250-MHz ^1H NMR. Formation of the amide bond through the ϵ-NH_2 group of **1** resulted in the downfield shift of the ϵ-protons from 2.64 to 3.15 ppm (and no effect on the Phe's α-proton). In contrast, the spectrum of *N*-α-monoacetyl-**1** (independently synthesized[5c] by us) showed a downfield shift of the α-proton from 3.61 to 4.31 ppm (and no effect on the Lys's ϵ-protons). Note that both isomers of monoacetylated **1** and *N*,*N*-α,ϵ-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 β-protons from 3.86 to 4.87 ppm and a split of the signal of the Ser's α-proton due to its coupling with the β-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.

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lation, **1** was fully acylated after a 24-h incubation with trifluoroethyl esters of both longer aliphatic (octanoic, myristic, and palmitic) and aromatic (phenylacetic) acids. Analysis of the products[10] revealed that in all cases only *N*-ε-monoacyl-**1** compounds were formed.

In order to explain the striking ε-specificity of lipase with **1**, we examined the acetylation of several other peptides in acetonitrile. It was found that L-Ala-α-L-Lys-O-*t*-Bu had essentially the same reactivity as **1** and the product of the enzymatic reaction was the *N*-ε-monoacetyl dipeptide. Hence the phenyl ring in **1** is not responsible for the low reactivity of the α-NH_2 group compared to ε. However, the reactivity of the α-NH_2 group in L-Phe-NH_2 was (i) just 3 times lower than that of the ε-NH_2 group in **1**, but (ii) 180 times greater than that of the α-NH_2 group in L-Phe-O-*t*-Bu. These data suggest that the lipase is intolerant of a bulky main (but not side) chain of the peptide. This factor, however, plays no role in the reactivity of the ε-NH_2 group (presumably due to its remoteness from the main chain), for the rates of enzymatic acetylation of **1** and of the smaller *N*-α-acetyl-L-Lys-$NHCH_3$ were identical.

Lipase was also found to selectively esterify Ser in a peptide. In fact, the acylation of Ser in the model peptide L-Phe-L-Ser-NH-β-Naph (**2**) was even faster than that of Lys in **1**: the enzymatic conversions in *tert*-amyl alcohol (the former peptide is insoluble in acetonitrile) after 1.5 h were 98% and 52%, respectively. The NMR analysis[10] of the product revealed it to be exclusively *O*-monoacetyl-**2**, thus pointing to lipase's overwhelming preference for Ser's OH vs (chemically more reactive) Phe's NH_2 group.[12] The same result was obtained in the preparative enzymatic palmitoylation of the dipeptide.[12] In contrast, chemical acetylation with equimolar acetic anhydride yielded approximately 50% of the *N*,*O*-diacetyl-**2**, with the rest being the unreacted dipeptide.

In closing, we have developed a facile methodology for regio- and chemoselective enzymatic incorporation of various acyl moieties into short peptides. We are currently exploring its extension to longer peptides and to proteins.

(12) Thus allowing for the direct acylation of a hydroxyl group without protecting an amino group first.

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Solid-State Photochemical Generation of A Very Stable Phenoxyl–Phenoxyl Radical Pair

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We have recently reported our finding that aryl oxalate derivatives are convenient, effective unimolecular photochemical sources of aryloxyl radicals.[1-3] As a result, it is now possible to explore aspects of aryloxy radical chemistry that were not easily probed by the standard solution bimolecular methods for generating such radicals.[4] In this paper we report the generation, electron spin resonance (ESR) observation, and thermal stability of remarkably persistent phenoxyl–phenoxyl π-radical pairs generated by polycrystalline solid-state photolysis of bis(2,6-di-*tert*-butyl-4-methoxyphenyl) oxalate.

Generation and direct cryogenic observation of radical pairs trapped in proximity in the solid state is a well-established phenomenon.[5] However, radical pairs typically recombine or react upon warming, and are not readily kept at room temperature. In a case closely related to our work, McRae and Symons[6] found that 77 K solid-state γ radiolysis (but *not* UV–vis photolysis) of diaryl carbonates produced both isolated and triplet-paired phenoxyl radicals, which disappeared on warming.

We found upon quartz-filtered xenon-arc UV–vis photolysis[7] of a powder sample of bis(2,6-di-*tert*-butyl-4-methoxyphenyl) oxalate **1** at 77 K under vacuum for ca. 3 min, the production of a reddish sample having a strong central ESR peak[8] with g = 2.0051, attributable to isolated 2,6-di-*tert*-butyl-4-methoxyphenyl radical (2,6-Bu-4-OMe-Phen). In addition, we were able clearly to observe six peaks consistent with the pattern expected for a randomly oriented triplet sample having zero-field-splitting (zfs) parameters $|D'|$ = 116 G, $|E'|$ = 6.0 G, with g_{xx} = 2.0060, g_{yy} = 2.0057, and g_{zz} = 2.0040. The presence of a $\Delta M_s = 2$ transition in the $g = 4$ region confirms the presence of a triplet state species, which we attribute to interaction of a geminate pair of 2,6-Bu-4-OMe-Phen radicals, constrained in the crystal matrix of the precursor diaryl oxalate (DAO) after double decarbonylation (Scheme I).

The line shape of the triplet ESR spectrum was simulated by the method of Kottis and Levebvre[9,10] based upon the above values and is shown as curve b of Figure 1. The reddish color and g values are consistent[11] with generation of 2,6-Bu-4-OMe-Phen radicals. After the sample was annealed to room temperature and recooled, spectrum a (Figure 1) changed and a new radical-pair spectrum became evident. An example of the new spectrum obtained without a contaminating component of spectrum a is shown in Figure 1 as spectrum c, characterized by zfs parameters $|D'|$ = 133 G, $|E'|$ = 6.7 G, with g_{xx} = 2.0060, g_{yy} = 2.0056, and g_{zz} = 2.0044 (see simulated curve d). We attribute spectrum a to an initially formed geminate radical pair after photolysis that upon annealing reorganizes to a more stable geometric arrangement in the crystal to give spectrum c, which remains stable for days at room temperature under vacuum. The

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