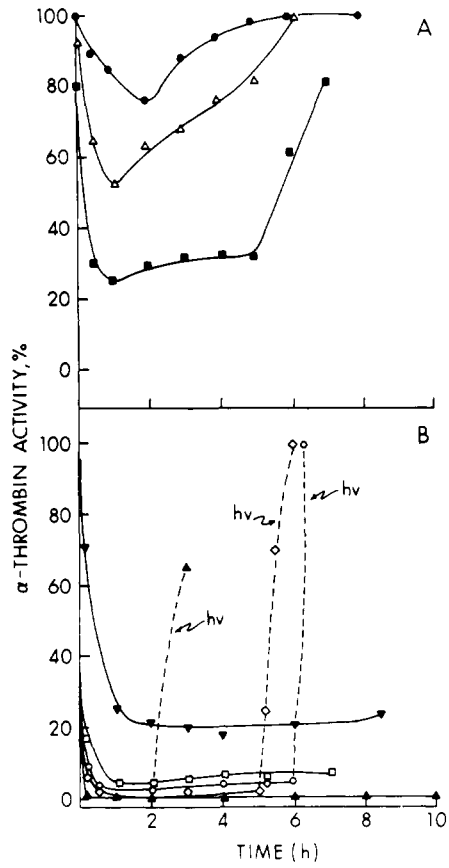

Figure 1. Time courses for the inhibition and photoreactivation of human α-thrombin with *p*-amidinophenyl *o*-hydroxy-α-methylcinnamate. The broken lines indicate reactivation of photolyzed samples: (A) 0.19 (●), 0.39 (▲), 0.78 μM (■) *o*-hydroxy-α-methylcinnamate; (B) 1.5 (▼), 2.4 (□), 9.7 (○), 49 (◇), and 97 μM (▲) *o*-hydroxy-α-methylcinnamate. Enzyme concentration is 0.12 μM.

The 1:1 acyl-thrombin complex was isolated by gel filtration chromatography. This inactive complex displayed no change in enzyme activity for at least 26 h in the absence of light even when stored at room temperature. Photolysis of the acyl-thrombin, however, resulted in fully reactivated α-thrombin in approximately 15 min.

Other approaches have been taken to photosensitize enzymatic processes and most of these studies of enzyme photoregulation have involved the cis/trans photoisomerization of substituted alkenes.[9,10] All of these approaches rely solely on steric effects to differentiate photoisomers, and both cis and trans acyl-enzyme complexes usually display measurable deacylation rates at ambient temperature and moderate pH. The approach described here is an active and perhaps general approach to photocontrol of enzyme activity. Acyl-enzyme stability can be built into the substrate by substituting at the α-center (steric effects), and photodeacylation can potentially be regulated by manipulating nucleophilicity of the ortho substituent involved in intramolecular deacylation.[11] It also seems likely that the scope of such a strategy could be broadened to include other enzyme active-site nucleophilic centers such as amines and thiol functionalities.[12]

Acknowledgment. This work was supported by grants from the NIH (HL17921, HL24066, and HL31932).

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(12) Compound **1** acts as a photoreversible inhibitor with factor Xa, trypsin, and α-chymotrypsin. The 1:1 acyl complex can be isolated with factor Xa and trypsin but cannot be purified with chymotrypsin. The details of these experiments will be reported in due course.

Nitrogen-15-Labeled Deoxynucleosides. Synthesis of [6-^{15}N]- and [1-^{15}N]Deoxyadenosines from Deoxyadenosine†

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The approaches used to-date for introduction of ^{15}N into nucleosides have been largely based on de novo synthesis of the desired heterocyclic base followed by coupling with an appropriate sugar. This has been most successful in the pyrimidine series, where ^{15}N-labeled thymidine,[1] uridine,[2] and cytidine[3] derivatives have been prepared. In the purine series, chemically synthesized N^1-labeled hypoxanthine was incorporated into a yeast tRNA by fermentation and was successfully used as a ^{15}N NMR probe[4]. Several ^{15}N-labeled adenines were synthesized by Leonard and co-workers but were not converted to nucleosides.[5,6] In order to generate sufficient quantities of ^{15}N-labeled deoxynucleosides for incorporation into oligonucleotides by chemical synthesis, we have sought to develop alternative routes. Our approach has been to employ transformation of an intact deoxynucleoside, rather than a de novo synthesis, based on the assumption that the high cost of the deoxynucleoside would be more than offset by the simplification of the overall synthesis. At this time we wish to report the syntheses of [6-^{15}N]deoxyadenosine and, from [1-^{15}N]-deoxyadenosine as well as characterization by ^{1}H and ^{15}N NMR and mass spectrometry.

Two routes were explored for the introduction of ^{15}N, as shown in Scheme I. In one approach, deoxyadenosine (**1a**) was first enzymatically deaminated to give deoxyinosine (**5a**), which was acetylated to give **5c** and reacted with triisopropylbenzenesulfonyl chloride (TPS-Cl) to give **6c**. This sulfonylation reaction, unlike the analogous O^6-sulfonylation of guanine derivatives, gives a nearly equal amount of an N-TPS derivative in addition to the desired O^6-derivative. Careful chromatography was then required to obtain pure **6c** in only 33% yield. Displacement of the O^6-TPS group of **6c** with benzylamine occurs smoothly at room temperature. In the reaction with [^{15}N]benzylamine, which was generated in situ from the hydrochloride[7] by addition of DBU (1,8-diazabicyclo[5.4.0]undec-7-ene), we used a 2:1 excess of **6c**.

The alternative route shown in Scheme I begins with nonaqueous deamination to generate a 6-chloro intermediate (**2b**).[8,9] Deoxyadenosine (**1a**) was protected as the 3',5'-*O*-bis(*tert*-butyldimethylsilyl) derivative **1b** and reacted with *tert*-butyl nitrite in a mixture of CH_2Cl_2 and CCl_4 containing 4 equiv of tetraethylammonium chloride. The 6-chloro compound **2b** was produced rapidly, along with a nearly equal amount of the deoxyinosine derivative **5b** as the major byproduct. The estimated yield of **2b**, which could only be obtained as a gum, was 50–60%. An excess of **2b** was then reacted with [^{15}N]benzylamine as described above for **6c**. The displacement again took place readily.

Debenzylation was attempted under a variety of reductive conditions, none of which proved to be successful. Although

† Preliminary accounts of this work were presented at the Fourth Conversation in Biomolecular Stereodynamics, Albany, NY, June 1985, and at the VIIth International Round Table on Nucleosides, Nucleotides, and Their Biological Applications, Konstanz, W. Germany, Oct. 1986.

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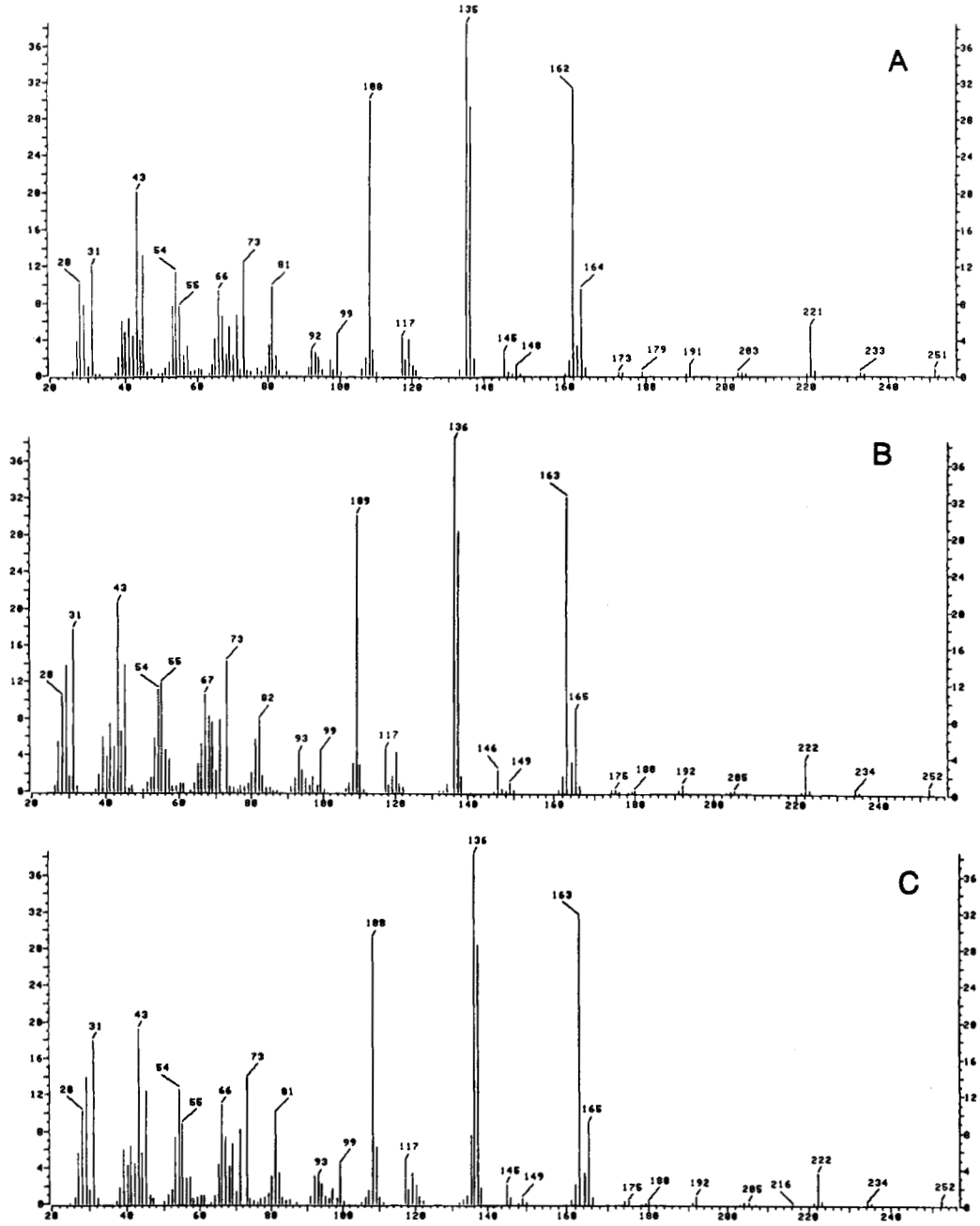

Figure 1. Mass spectra: (A) 5'-deoxyadenosine; (B) [6-^{15}N]deoxyadenosine (**4a**); (C) [1-^{15}N]deoxyadenosine (**9a**).

reductive cleavage of benzyl groups is most common, cleavage of benzyl ethers via oxidation to the corresponding benzoyl esters has been reported.[10] Reaction of **3b** or **3c** with 4 equiv of $NaIO_4$ and 0.02 equiv of $RuO_2 \cdot 2H_2O$ in a mixture of CH_2Cl_2/CH_3CN/H_2O (2:2:3)[11] effected quantitative oxidation. Treatment with aqueous ammonia then completed deprotection giving **4a** or **4b** in an overall yield of 61–70% based on the [^{15}N]benzylamine hydrochloride.

Transformation of [6-^{15}N]deoxyadenosine (**4a**) to [1-^{15}N]-deoxyadenosine (**9a**) was carried out as shown in Scheme II. The reaction of **4b** with benzyl bromide followed by Dimroth rearrangement[12] using methanolic dimethylamine (1:1) gave **8b** in 89% yield.

The mass spectra of [6-^{15}N]deoxyadenosine (**4a**), [1-^{15}N]-deoxyadenosine (**9a**), and for comparison deoxyadenosine (**1a**) are shown in Figure 1. The presence of one atom of ^{15}N is seen in the molecular ion, which has a m/z of 252 for **4a** and **9a**. Similarly, ions resulting from fragmentation of the sugar occur at m/e 136, 137, 163, 165, and 222 for **4a** and **9a**, one unit larger than for **1a**. These correspond to the base + H and base + 2H

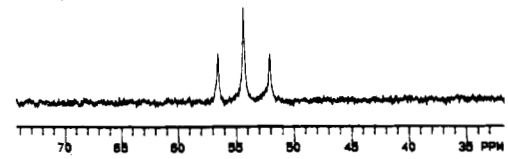

Figure 2. ^{15}N NMR spectrum of [6-^{15}N]deoxyadenosine (**4a**) without ^{1}H decoupling. The spectrum was obtained at a concentration of 6 mM in 20% ^2H_2O (0.1 M NaCl, 10 mM NaH_2PO_4, 0.1 mM EDTA, pH 6.5) at 25 °C; 30 FID's were collected with a pulse length of 22.5 μs. The spectrum was line broadened by 5 Hz; [^{15}N]NH_4Cl in 10% HCl was used a the reference.

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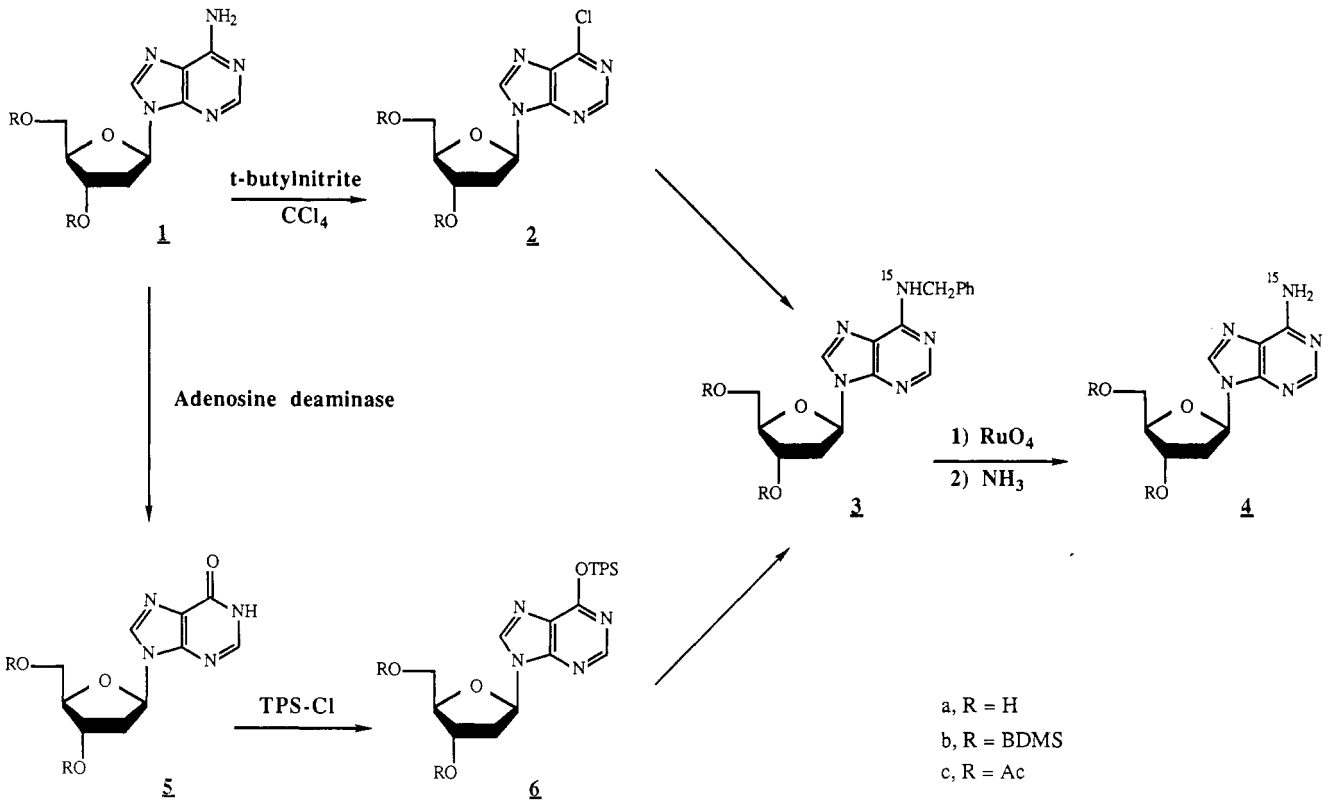
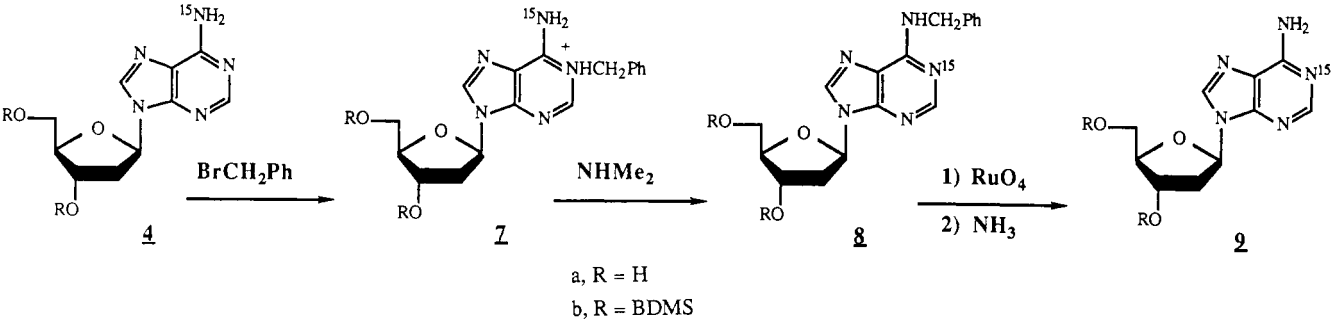
Scheme I



Scheme II



ions and ions d, h, and c,[13] respectively, resulting from partial fragmentation of the deoxyribose. Fragmentation of the b + H ion proceeds by successive losses of HCN. The first loss involves mainly N^1, since **9a** shows this ion at m/z 108, like **1a**, while in **4a** it has m/z 109. The second HCN then includes mainly N^7, rather than N^6, since it shows up at m/z 82 for **4a**, but at m/z 81 for **1a** and **9a**. This is in accord with earlier observations of adenine fragmentation.[5,6,13] One minor exception to the existing literature that we noted is in ion n. This generally small ion would logically correspond to loss of the N^6 moiety from the b + H ion. For **1a** this gives m/z 119 and would be expected to show up at 119 for **4a** and at 120 for **9a**. Instead we find peaks at both m/z 119 and 120 for both **4a** and **9a**, with the 119 peak being the larger in **9a** and the 120 peak the larger in **4a**. This would suggest that, for deoxyadenosine, ion n results mainly from the loss of N^1, presumably through complex secondary reactions and rearrangements, rather than the conceptually more facile loss of N^6.

In the ^1H NMR spectra the amino protons of **4a** give a doublet with the expected large one-bond ^{15}N–^1H coupling of 91 Hz, while the H^2 resonance of **9a** is a doublet with the smaller two-bond coupling of 13 Hz (data not shown). Figure 2 shows the ^{15}H NMR spectrum of **4a** without ^1H decoupling. The ^{15}N resonance is a triplet at 54.3 ppm[14] with the same ^{15}N–^1H coupling (91 Hz) seen in the ^1H NMR. Similarly, the ^1H-coupled ^{15}N spectrum of **9a** (Figure 3) shows a doublet (13 Hz) for N^1 at 203.9 ppm.[14]

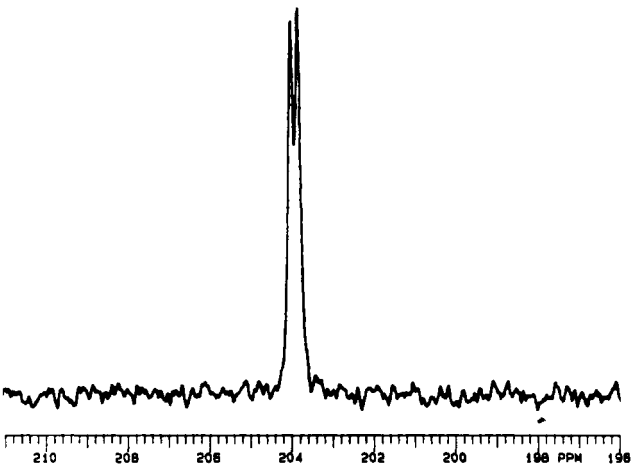


Figure 3. ^{15}N NMR spectrum of [1-^{15}N]deoxyadenosine (**9a**) without ^1H decoupling. The spectrum was obtained as indicated for Figure 2 except that 160 FID's were collected by using a DEPT pulse sequence with a pulse length of 25 μs.

We have developed an efficient synthesis of deoxyadenosine ^{15}N-labeled at N^6 or N^1. These are the first ^{15}N-labeled purine

(14) The ^{15}N chemical shifts are relative to [^{15}N]NH_4Cl in 10% HCl.

deoxynucleosides to be reported. The N^6-labeled derivative requires five steps, while the N^1-labeled compound requires eight steps. Moreover, the lowest yield reactions are all carried out before introduction of ^{15}N. Each of the reactions carried out after introduction of the ^{15}N label proceed in high yield. The reactions themselves, and the purification steps, are all straightforward and amenable to synthesis of adequate amounts of material for subsequent incorporation into synthetic oligonucleotides where the ^{15}N label can be used as a ^{15}N NMR probe.[15]

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Supplementary Material Available: Experimental section for **6c**, **4a**, **2b**, and **9b** and comparison ^1H NMR spectra of **1a** with **4a** and **9a** (6 pages). Ordering information is given on any current masthead page.

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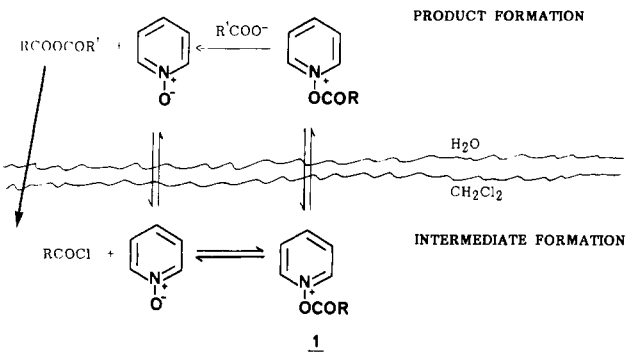
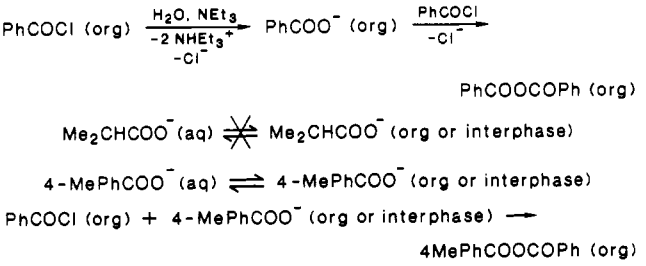
Scheme I



Scheme II



Inverse Phase-Transfer Catalysis: Probing Its Mechanism with Competitive Transacylation

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The utility of two-phase water–organic solvent media for organic synthesis is widely recognized. Nearly all reported examples of this methodology involve transport of a reactant from the water phase into the organic phase where it encounters a second reactant to effect reaction. This process commonly known as phase-transfer catalysis (PTC) is the subject of numerous reports and reviews.[2-5] The literature includes a few examples of a complementary synthetic procedure in which an organic solvent soluble reagent is activated by conversion to an ionic intermediate and transported to the aqueous phase for reaction.[6-9] The recent report by Mathias and Vaidya describes a new example of this virtually unexplored methodology, which they have named inverse phase-transfer catalysis (IPTC).[9]

We report here some preliminary results from our continuing investigation[10,11] of multiple-phase systems as media for organic reactions which provide significant new insight into the IPTC process. The reaction in Scheme I requires a nucleophilic phase-transfer agent/catalyst, and results indicate that pyridine 1-oxide (PNO) is a highly effective catalyst in this process. This anhydride-forming procedure provides a powerful, yet simple probe for investigating the nature of inverse phase-transfer catalysis. The presumed intermediate is the 1-(acyloxy)pyridinium ion, **1**. This ion and the related 1-acyl-4-(dimethylamino)pyridinium ion, **2**, form readily in the organic phase by reaction between acid chlorides and PNO[6,8] or 4-(dimethylamino)pyridine (DMAP),[12,13] eq 1. These ions are highly water soluble and sufficiently stable

$$\mathrm{RCOCl} + \mathrm{Nu} \xrightarrow[\mathrm{CH_2Cl_2}]{} \mathrm{RCO\overset{+}{N}u}\ \mathrm{Cl^-} \qquad (1)$$

1, Nu = PNO
2, Nu = DMAP

that reaction with carboxylate ions can be carried out in water. In fact, reaction mixtures may include the reactive acylating ion and two or more carboxylate ions, eq 2. If reaction between these

$$\underset{1}{\mathrm{RCO\overset{+}{N}u}} + \begin{matrix}\mathrm{4\text{-}MePhCOO^-} \\ \mathrm{Me_2CHCOO^-}\end{matrix} \xrightarrow{\mathrm{H_2O}} \begin{matrix}\mathrm{4\text{-}MePhCOOCOR} \\ \mathrm{Me_2CHCOOCOR}\end{matrix} + \mathrm{Nu} \qquad (2)$$

oppositely charged ions is occurring exclusively in the aqueous phase, product formation is expected to be statistically controlled.[14,15] Thus, the composition of product mixtures should reflect the relative concentrations of the carboxylate ions. This prediction has been verified by treating preformed acylating agent, **1** (R = phenyl), with varying proportions of sodium *p*-toluate and

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