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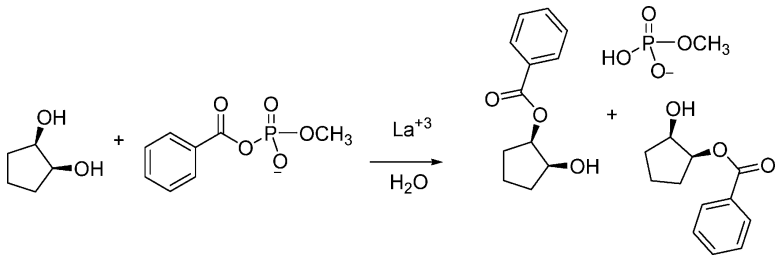

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Biomimetic Monoacylation of Diols in Water. Lanthanide-Promoted Reactions of Methyl Benzoyl Phosphate

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Abstract: The direct monoacylation of diols by acyl phosphate monoesters in water is a biomimetic analogy to the enzymic aminoacylation of tRNA by aminoacyl adenylates. Without catalysis, acyl phosphate monoesters react rapidly with amines but very slowly with water and alcohols. Lanthanide ions dramatically and selectively facilitate the base-catalyzed monoacylation of diols in water by methyl benzoyl phosphate (MBP), a typical acyl phosphate monoester, in neutral solutions where reactive amines are protonated and unreactive. The reaction patterns and reactivity of various diols with MBP in the presence of lanthanides are consistent with a mechanism that involves internal addition from the conjugate base of the bis-bidentate complex of the lanthanide with the diol and MBP. The method is also applicable to reactions of nucleosides as evidenced by the selective monoacylation of the 2′- or 3′-hydroxyl group of adenosine, without reaction of the 5′-hydroxyl group or the 6-amino group. Analogues of adenosine without the diol are unreactive. This suggests that the method will selectively monoacylate the hydroxyl groups at the unique diol in tRNA that forms the 3′-terminus.

Amino acids are activated for protein synthesis as esters of a 3′-terminal hydroxyl group of transfer RNA (tRNA), catalyzed by aminoacyl tRNA synthetases.[1] The pathway involves initial formation of an acyl phosphate monoester (aminoacyl adenylate),[2] which then aminoacylates the terminal hydroxyl group (Scheme 1).

The preparation of tRNAs acylated with unnatural amino acids is an important step in the Hecht−Schultz scheme that uses the ribosome to produce protein-specific residues with novel side chains.[3−5] A biomimetic system would utilize synthetic aminoacyl phosphate monoesters[6,7] to acylate hydroxyls at the 3′-terminal of tRNAs.

Acyl (and aminoacyl) phosphate monoesters are water-soluble and can be prepared by convenient synthetic routes.[8] They are generally unreactive toward attack by hydroxyl groups, as exemplified by their very slow hydrolysis in neutral solution.[9,10] It is of interest that enzymic aminoacylation of hydroxyl groups in tRNA is promoted primarily by the enzymes binding the reactants in reactive proximity.[11] We considered that metal cations, which coordinate to the anionic phosphate oxygens of acyl phosphate monoesters,[12,13] would activate the adjacent carbonyl group through bidentate coordination and charge neutralization.[14] However, divalent cations only weakly enhance the hydrolysis of acyl phosphate monoesters through such complexation.[6] We reasoned that trivalent lanthanide ions would be more likely to promote the desired reactions based on their size, lability, and charge. Indeed, we found that lanthanide ions dramatically accelerate base-promoted hydrolysis and methanolysis of methyl benzoyl phosphate (MBP) in water.[14] This established the general activation pattern and suggests a mechanism in which the coordinated conjugate base of the hydroxyl group is the target of acyl transfer from the coordinated acyl phosphate monoester. We assumed that lanthanide ions coordinate both the phosphate and the carbonyl oxygens of MBP.[14] Because the desired acylation sites in tRNA are the vicinal hydroxyl groups in the unique diol functionality at the 3′-terminus of tRNA, we examined the reaction patterns of diols with MBP and lanthanide ions and have found that monoacylation of appropriate diols occurs readily and selectively.

Experimental Section

UV kinetic measurements were performed in triplicate in the temperature-controlled cell compartment maintained within 0.1 °C. HPLC analysis was performed with a C18 reverse phase analytical column and eluted with HPLC-grade acetonitrile. The methanolysis of MBP was following at 240 nm. Cells containing 2.85 mL of dry methanol were equilibrated at 25 °C for 30 min. MBP was dissolved in methanol, and 0.15 mL of this solution was immediately added to

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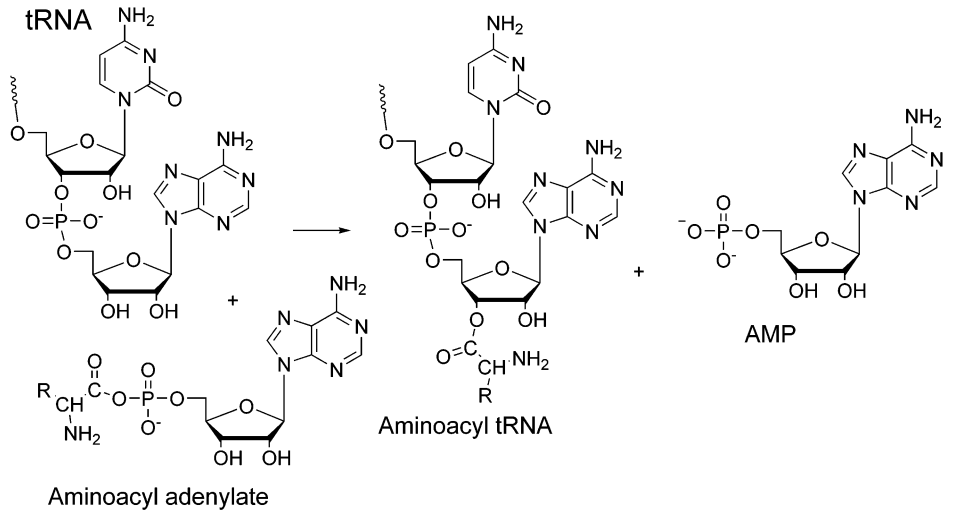
10.1021/ja049538l CCC: $27.50

Scheme 1



the cuvette with a concentration of MBP of 5×10^{-5} M. The spontaneous methanolysis of MBP follows an approximately straight-line change in concentration versus time between 0 and 100 min, which amounts to less than 1% conversion. Using this slope, the observed first-order rate constant was determined by dividing the initial rate by the initial concentration of MBP. The lanthanide concentration dependence of the methanol reaction was studied by adding different amounts of lanthanum trifluoromethanesulfonate (0.01 M in methanol) to MBP in methanol. Lanthanide catalysis was studied over a small concentration range (2.5×10^{-5} to 1×10^{-4} M) because at higher concentrations the reactions are very fast. Scans of the product mixtures show a shift of λ_{max} from that of MBP at 234 nm to 227 nm, which is the λ_{max} of methyl benzoate in methanol. Reactions were followed to at least five half-lives.

The addition reactions of other alcohols and MBP in the presence of lanthanide and EPPS buffer were also followed via monitoring of the decrease of absorbance from MBP at 240 nm. Fresh solutions of EPPS buffer and lanthanum salts were prepared and adjusted to pH 7 at 25 °C with 0.25 M ethanol, ethylene glycol, glycerol, D-glucose, ethanolamine, methylamine, or diethylamine. The alcohol concentrations varied from 0 to 2 M with methanol and 0 to 0.1 M with the other alcohols. Stock solutions of amines were adjusted to pH 7. First-order rate coefficients for the lanthanide-catalyzed reaction of MBP in the presence of added alcohols were calculated from nonlinear regression fitting of the absorbance versus time data to the equation for a first-order decay. The observed first-order rate constants were plotted against alcohol concentration, and the second-order rate constants for alcoholysis were obtained from the slopes.

We have previously reported that lanthanides promote the formation of methyl benzoate from methanol, MBP, and lanthanides in water.[14] The products resulting from reaction of MBP with other alcohols in the presence of lanthanide ions in aqueous solution were analyzed by HPLC and comparison with genuine samples of likely products. The alcohols utilized were ethanol, *n*-propanol, 2-propanol, 1,2-propanediol, 1,3-propanediol, ethylene glycol, glycerol, *cis*-1,2-cyclopentanediol, *trans*-1,2-cyclopentanediol, 1,3-cyclopentanediol, and cyclopentanol. Stock solutions of lanthanum trifluoromethanesulfonate (0.01 M), EPPS (0.1 M), and MBP (5×10^{-3} M) were prepared as described for kinetic studies. The final reagent concentrations were 1 M alcohol, 1×10^{-3} M $La(OTf)_3$, 0.01 M EPPS (pH 8), and 5×10^{-4} M MBP. The solution pH was varied from 7 to 8 and the concentration of lanthanum trifluoromethanesulfonate was varied from 5×10^{-4} to 0.01 M, to study the effect of pH and lanthanum concentration on the product ratio. The pH range that we used was limited because at higher pH the lanthanide salts precipitate. Europium ion (as europium chloride) was also studied in some cases, giving results that were essentially identical to those with lanthanum salts. Europium triflate gave the same results as europium chloride.

We performed HPLC analysis with a reverse phase column. The eluent was a mixture of water and acetonitrile containing 0.1% trifluoroacetic acid (TFA) with a flow rate of 1 mL/min. The eluting species were detected at 230 nm. The column was equilibrated in a solution of 5% acetonitrile in water, and a linear gradient of acetonitrile (2%/min) was initiated after injection of the sample (25 μL). In the case of the reactions of 1,2-propanediol, two monoester products formed (the hydroxyl groups are not equivalent). An isocratic eluent, 25% acetonitrile in water with 0.1% TFA, was used to improve the separation of the two esters in the HPLC for the 1,2-propanediol reaction. The identities of the resulting acid and esters were tested by HPLC by co-injecting genuine samples with the lanthanide-catalyzed reaction solutions. Stock solutions (5×10^{-3} M) of benzoic acid, methyl benzoate, ethyl benzoate, 2-hydroxyethyl benzoate, and *cis*-1,2-hydroxycyclopentyl benzoate were prepared.[15] The solutions were added to a lanthanide-containing reaction mixture after absorbance from MBP had decreased to less than 1% of its original value and the combined solution was injected into the HPLC. Overlap on elution of the genuine sample and the product peaks was used to identify the reaction product. The ^{1}H NMR spectra of genuine samples were also compared to those obtained in the lanthanide-catalyzed reaction of the diols and MBP.

We also used HPLC analysis to determine the products from the reaction of adenosine and its analogues as the diol component with MBP. On the basis of the selectivity we observed in the reactions of MBP with diols and lanthanides (and the suppressed reactions of amines), we extended our studies to include reactions of the nucleoside at the 3′-terminal of tRNA, adenosine, and some analogues: uridine, 2′-deoxyadenosine, 2′3′-isopropylideneadenosine, and 5′-chloro-5′-deoxyadenosine.[16] The reaction mixture contained 2 mM nucleoside or nucleoside derivative, 0.5 mM MBP, 1 mM $La(OTf)_3$, 10 mM pH 8 EPPS. Products were monitored by HPLC, using an isocratic eluent (25% acetonitrile in water with 0.1% trifluoroacetic acid), at a flow rate of 1 mL/min with a 25 μL injection volume. The eluting species were detected at 230 nm.

Results

Diols undergo efficient lanthanide-enhanced monoacylation in water, while esterification of ethanol and larger monofunctional alcohols in water does not compete effectively with

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Table 1. Lanthanum Ion-Catalyzed Formation of Monoesters and Competing Hydrolysis of MBP from Alcohols[a]

alcohol	product ratio acid:ester	ester
methanol	1:0.36	methyl benzoate
ethanol	1:0.03	ethyl benzoate
ethylene glycol	1:3.1	2-hydroxyethyl benzoate
1,2-propanediol	1:1.5:0.2	2-hydroxy-1-propyl benzoate, 1-hydroxy-2-propyl benzoate
cis-1,2-cyclopentanediol	1:0.84	(D,L)-*cis*-2-hydroxycyclopentyl benzoate
trans-1,2-cyclopentanediol		no ester formed
1,3-propanediol	1:0.31	3-hydroxy-1-propyl benzoate
1,3-cyclopentanediol[b]	1:0.9	*trans*-3-hydroxycyclopentyl benzoate

[a] See Experimental Section for conditions. [b] Mixture of cis and trans isomers.

hydrolysis. Due to the large excess of water as compared to diol, hydrolysis of MBP does accompany monobenzoylation of the diols. The monoesters themselves are stable both to hydrolysis and to further benzoylation. This pattern indicates that the use of excess acylating agent will overcome competition from hydrolysis when the hydroxyl substrate is limiting. The reaction pattern is predictive of appropriate biomimetic reactivity with tRNA and agrees with patterns reported by Clarke for reactions between diols and acetic anhydride in organic solvents.[17−19]

We also find that lanthanide ions do not promote competing amide-forming reactions of amines and MBP. The uncatalyzed reactions of amines with MBP depend on the concentration of free amine and would normally be done at pH 9 where basic amines would not be heavily protonated.[20] The weakly basic amino group of adenosine (pK_a of the conjugate acid is 3.6) would be unlikely to compete as the rate of amide formation from acyl phosphate monoesters is directly proportional to the basicity of the amino group with $\beta_{nuc} \approx 1$. For example, the rate constant for aminolysis MBP with free *n*-propylamine is 1.1 M^{-1} s^{-1} (pK_a of conjugate acid = 10.5), while for 2,2-difluoroethylamine, $k = 1.7 \times 10^{-3}$ M^{-1} s^{-1} (pK_a of conjugate acid = 7.5).

In our initial screening of reaction patterns, we found that the combination of lanthanide ion, MBP, and hydroxyl group is most effective for reactions of (*cis*)-1,2-diol substrates. These are converted to monobenzoyl esters in water (Table 1) and were identified as described in the Experimental Section. While the reactions of MBP with water and with methanol are accelerated, we observed no ester formation for higher monofunctional alcohols (propanol, butanol, cyclopentanol) and only a slow reaction with ethanol that does not compete effectively with hydrolysis. The reactions of diols are sufficiently accelerated to permit them to be converted to monoesters in water by the combination of acyl phosphate monoester and lanthanide.

Ethylene glycol, 1,2-propanediol, and *cis*-1,2-cyclopentanediol react rapidly with MBP. Symmetrical diols react to give only one product, the monobenzoyl ester. 1,2-Propanediol, which has heterotopic hydroxyl groups, gives two products that form at different rates, with the primary hydroxyl group being more reactive (initial product ratio is 92:8 in favor of the primary ester). This is consistent with a steric preference. While the 1,3-substituted diols also react readily with MBP in the presence of lanthanum ion, the results in Table 1 indicate that the hydroxyl groups of the cyclic diol are esterified in preference to hydrolysis by ratios of 49 and 17 as compared to the similar groups in the linear 1,3-diol. Because 1,3-cyclopentanediol is a mixture of cis and trans isomers, we did not determine individual reactivities.

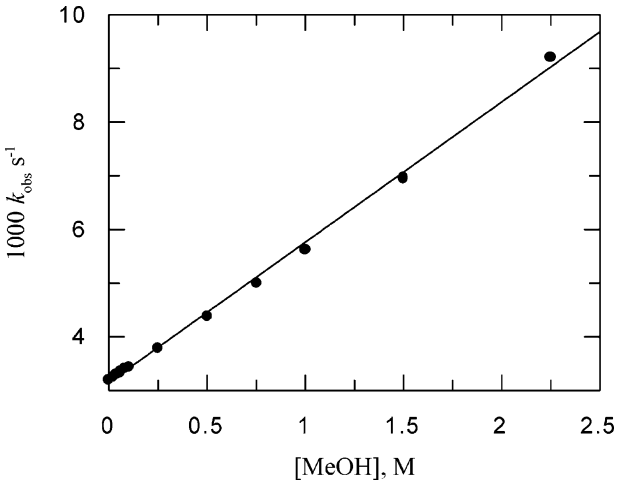

Figure 1. Formation of methyl benzoate from MBP as a function of methanol concentration in pH 7, 0.01 M EPPS, with 2.5×10^{-3} M $EuCl_3$, 25 °C. Similar results were obtained with lanthanum triflate in place of europium chloride.

Table 2. Second-Order Rate Constants (M^{-1} s^{-1}) for the Lanthanum-Catalyzed Reaction of MBP with Alcohols, 25 °C[a]

alcohol	k
methanol	$(2.6 \pm 0.04) \times 10^{-3}$
ethanol	no reaction observed
ethylene glycol	$(1.2 \pm 0.04) \times 10^{-2}$
glycerol	$(3.0 \pm 0.02) \times 10^{-2}$
D-glucose	$(6.2 \pm 0.5) \times 10^{-3}$

[a] See Experimental Section for conditions.

The products from the reactions of glycerol and D-glucose elute in the HPLC with retention times similar to those of MBP and benzoic acid. Broad peaks are consistent with a mixture of isomeric products. It is likely that the products are monoesters because we do not observe products eluting with the longer retention times expected for higher weight materials.

Kinetic measurements of the disappearance of MBP in water containing reactive alcohols were performed with methanol, ethylene glycol, glycerol, and glucose. The reactions are all first order in alcohol for $k_{obs} = k_{hyd} + k_{alc}[ROH]$. The linear dependence on methanol concentration between 0 and 2 M is shown in Figure 1. Second-order rate constants for the reactions were obtained from the slope of the linear dependences of k_{obs} on alcohol concentrations (Table 2). The reaction with glucose is complicated by ring and anomeric equilibria. We have undertaken a new set of studies with specific cyclic glycosides to get a clearer picture of the specificity of the reaction.

Amines inhibit the lanthanide-catalyzed hydrolysis of MBP, suggesting that they coordinate to the metal ion in competition with the alcohols. In no case did we observe formation of amides, which occurs in solutions where the amines are present to a significant extent in an unprotonated state (pH > 8).[21] For example, methylamine and ethanolamine reduce the observed

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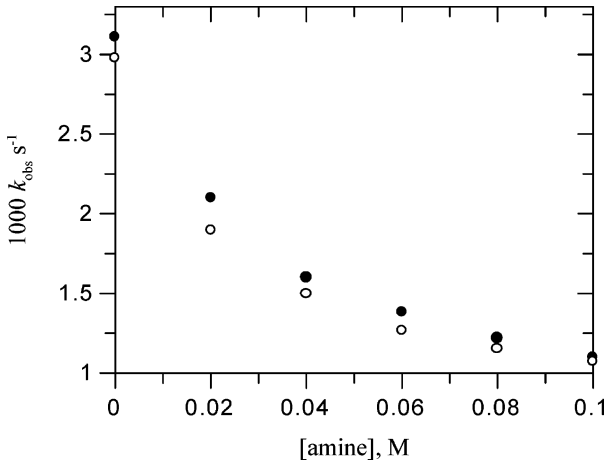

Figure 2. Dependence of k_{obs} for lanthanide-catalyzed hydrolysis of MBP on methylamine (●), and ethanolamine (○) concentration. 0.01 M EPPS, pH 7, containing 2.5×10^{-3} M lanthanum triflate, repeated with europium trichloride, 25 °C.

Table 3. Effect of Lanthanum Concentration on the Products from MBP and 1 M Ethylene Glycol in Water Containing EPPS Buffer

pH	10^3 [La^{3+}], M	acid:ester
7.0	0.50	1:1.3
	1.0	1:3.6
	2.0	1:6.2
8.0	1.0	1:5.3
	2.0	1:5.3

first-order rate coefficient for hydrolysis of MBP (Figure 2). Ethanolamine did not give any ester or amide product. Isopropylamine, diisopropylamine, diethylamine, and triethylamine also depress MBP hydrolysis. In a separate study, we examined the effects of ionic strength upon the rate and observed a depressing salt effect that is more evident at higher concentrations, following the Debye−Hückel expression. Thus, a portion of the depression is due to a nonspecific salt effect.

Changing the pH of the reaction solution from 7 to 8 increases the rates of reaction of MBP but does not affect the relative amounts of alcoholysis and hydrolysis. Increasing the concentration of lanthanum ion results in a slight increase in the rate of the MBP reaction, suggesting that the concentrations are nearly saturating. In all cases but one, increasing the lanthanide concentration has no effect on the product ratio. The exception is that in the reaction of ethylene glycol, an increase in lanthanum concentration results in a modest increase in the rate of alcoholysis at pH 7 but not at pH 8 (Table 3).

Diol-Selective Monobenzoylation of Adenosine. The selectivity we have seen in the reactions of lanthanides and MBP with diols in the presence of amines indicates that a reaction with adenosine would be selective for monoacylation of its 2′- or 3′-hydroxyl group, avoiding reaction of the 6-amino group exocyclic to the purine and the 5′-hydroxyl group. To test this prediction, we examined the reaction of adenosine and analogues of adenosine with lanthanides and MBP (Figure 3). Lanthanide ion catalyzes the reaction of MBP with adenosine at pH 7 and pH 8 (in the absence of lanthanide ions, MBP does not react with adenosine). The reaction solution contained 5×10^{-4} M MBP, 1×10^{-3} M $La(OTf)_3$, 0.01 M EPPS, pH 8. MBP gives

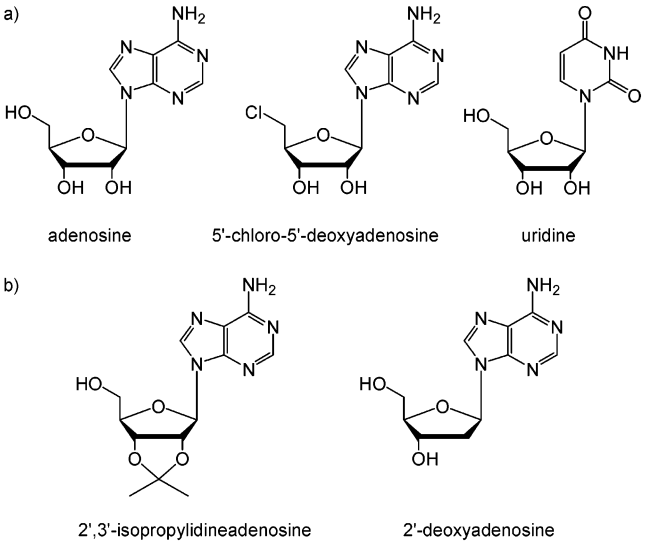

Figure 3. (a) Adenosine and derivatives that react with MBP and lanthanides to give two acylation products. (b) Adenosine derivatives that do not react with MBP and lanthanides.

benzoic acid and two benzoyl esters of adenosine. The reaction of MBP with water is slower than its reaction with the nucleoside.

Four compounds were studied for comparison with adenosine to establish the site of reaction because our attempts at NMR-based identification did not provide a definitive assignment. Two analogues contain the 2′- and 3′-hydroxyl groups but lack other potential nucleophiles (group "a"): 5′-chloro-5′-deoxyadenosine, which lacks the primary hydroxyl group, and uridine, which lacks an amino group. We also examined the reactions of compounds that do not contain either or both the 2′- and 3′-hydroxyl groups but which do contain the other potential nucleophiles found in adenosine (group "b"): 2′,3′-isopropylidine-adenosine has a 5′-hydroxyl group and a 6-amino group; 2′-deoxyadenosine has 5′- and 3′-hydroxyl groups (nonadjacent) and the 6-amino group.

As in the case of adenosine, the lanthanide-catalyzed reactions of MBP with uridine and 5′-chloro-5′deoxyadenosine give two monoacylation products in addition to benzoic acid (from competing hydrolysis), a pattern similar to that of adenosine. No esters are observed in the lanthanide-catalyzed reaction of MBP with 2′-deoxyadenosine and 2′,3′-isopropylideneadenosine, both of which contain the other two potentially nucleophilic sites but no vicinal diols.

On the basis of a referee's suggestion, we also tested the effects of amino acids on the reaction with adenosine. We used the same conditions for the lanthanum-promoted benzoylation of adenosine with alanine and serine in concentrations equal to that of adenosine. In each case, the amino acid had no effect on the yield of 2′- and 3′-benzoyl adenosines (R. Ren, unpublished).

Discussion

The enhanced reactivity of diols with MBP in the presence of lanthanides is consistent with coordination of the diol being advantageous for lanthanide catalysis in water, as was observed by Clarke for reactions of diols with acetic anhydride in organic solvents.[19] Lanthanides should preferentially coordinate appropriate diols due to the entropic advantage of chelation.

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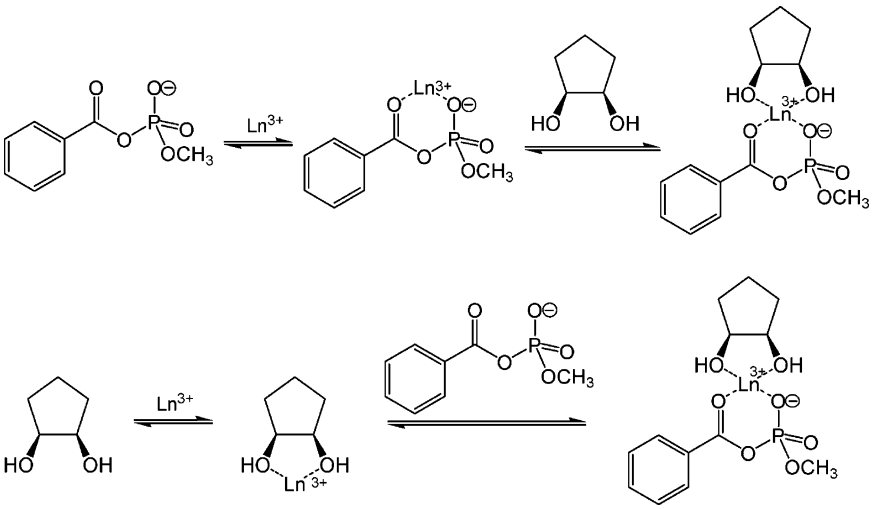
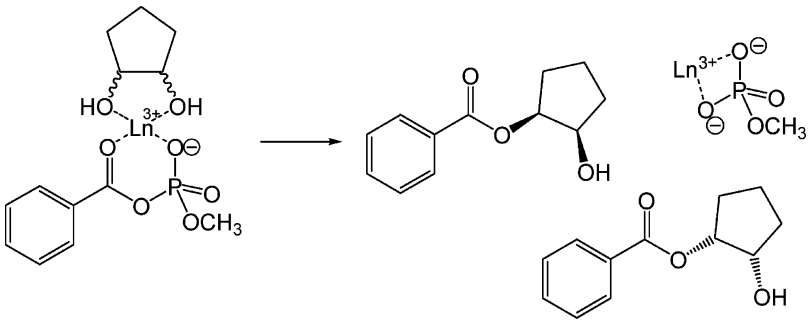
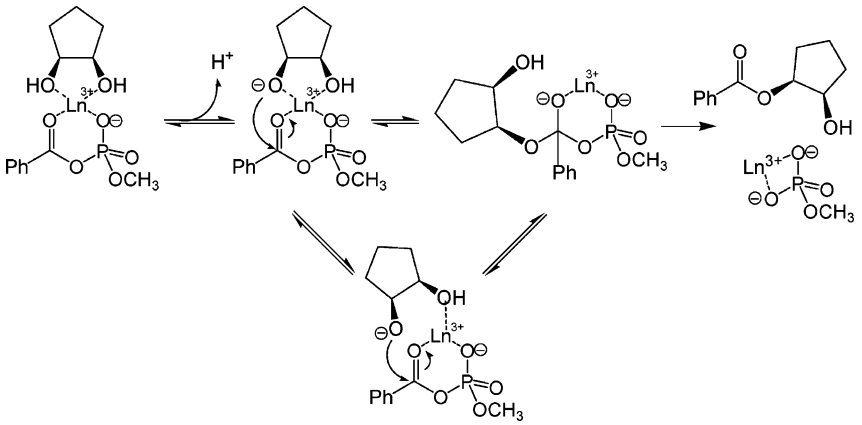
Scheme 2



Scheme 3



Scheme 4



Although the diol is competing with MBP for binding, bis-chelation of the lanthanide ion by the electrophile and nucleophile would be likely to facilitate the reaction between the coordinated components. A mechanism for the reaction of *cis*-1,2-cyclopentanediol to form a coordinated intermediate is shown in Scheme 2 and is based on Clarke's proposal.[19]

This intermediate will give a mixture of enantiomeric esters and a lanthanide-stabilized methyl phosphate as shown in Scheme 3.

Of course, the mechanism of conversion of the complex to the products remains to be determined. The properties that make lanthanides versatile catalysts, such as the lability of the complexes, highly variable coordination geometry, and coordination number, provide many potential routes.[22,23] Scheme 4 shows a possible detailed route to the products with ionization of the coordinated hydroxyl undergoing internal addition.

The geometry of the cyclic diols affects the reactivity of the hydroxyl-derived nucleophile. *trans*-1,2-Cyclopentanediol is the only diol we studied that did not react. While Clarke and co-workers report the acylation of the *trans*-diol by acetic anhydride with $YbCl_3$ in THF,[24] the yield is significantly lower than that with other diols. As well, this is the only substrate they report that gave significant amounts of the bis-acylation product. Because their reaction conditions do not involve competing

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hydrolysis, it is likely that a slower monodentate route is responsible. In our work, the addition of *trans*-1,2-cyclopentanediol slows the rate at which MBP is hydrolyzed (roughly 25% hydrolysis at 1 h, as compared to roughly 100% hydrolysis in the absence of the *trans*-diol), as is observed for the *cis*-diol. This suggests the lanthanide is coordinating the *trans*-diol but that the interaction does not lead to acylation.

In the absence of lanthanides, unprotonated amino groups are rapidly converted to amides by acyl phosphate monoesters, while reactions with oxygen-centered nucleophiles are very slow.[8] The lanthanide-promoted reactions of diols occur in neutral solution where free amine concentrations are very low. While lanthanides form weak complexes with amines, this would not promote their nucleophilic reactions in water because the coordinated amine will not form a highly basic amide ion.[25]

The lanthanide-promoted reaction of adenosine with MBP gives preferential monobenzoylation of the secondary hydroxyl groups (2′ and 3′) while avoiding reaction of the 5′-OH and the normally more reactive amino group based on reaction patterns with related compounds. This reaction selectivity of MBP and lanthanides with adenosine and its analogues is a clear indication that the combination of lanthanide, acyl phosphate monoester, and RNA will lead to the desired biomimetic acylation. We are currently extending our studies to the reactions of nucleotides, dinucleotides, and more complex entities.

Acknowledgment. We thank the Natural Sciences and Engineering Research Council of Canada (NSERC) for support through a Discovery Grant. S.C.W. received an NSERC summer undergraduate research fellowship.

Supporting Information Available: Illustrative chromatograms and tabular summary for product analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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