Solid phase lanthanum catalysis of monoacylation of diols in water by acyl phosphate monoesters

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Dedicated to Professor Stan Brown, a pioneer of biomimetic metal ion chemistry

Abstract

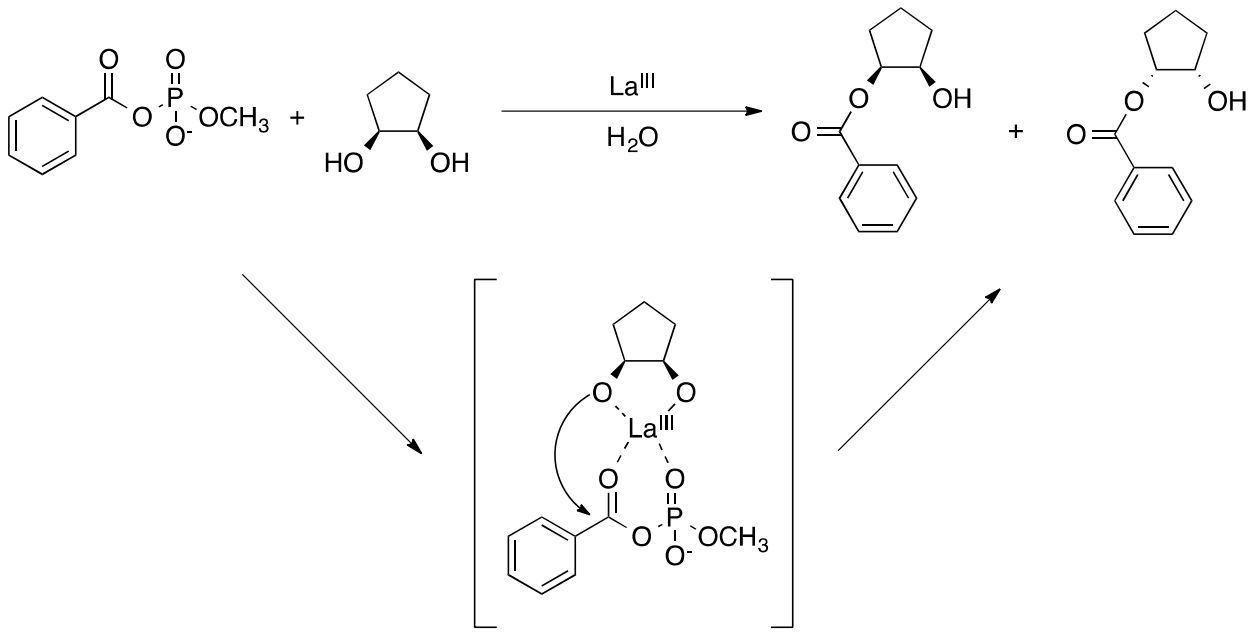
Lanthanide ions are readily bound to ion-exchange resins. The lanthanide-containing resins serve as immobilized catalysts for the biomimetic monoacylation of diols in water using acyl phosphate monoesters as acylation agents. This method provides an efficient route for recovering the catalyst in the process of modifying RNA derivatives and carbohydrates.

Keywords: Lanthanide catalysis, solid phase, biomimetic aminoacylation

Introduction

Lanthanum has been developed as a catalyst for biomimetic monoacylation of diols by acyl phosphate monoesters, presumably occurring via co-chelation followed by lanthanum promoted acylation. The general approach mimics the biological system that utilizes enzymically produced aminoacyl adenylates for aminoacylation of the 3'-terminus of tRNA. The method has been shown to work successfully on nucleosides, nucleotides, RNA[1] and monosaccharides. [2, 3] This approach is distinct from other diol acylation methods, as esters will be produced through bis-bidentate chelates of lanthanum as shown in Scheme 1. The method has also been extended by Duffy and Dougherty to aminoacylation of full-length suppressor tRNAs, using the resulting aminoacyl-tRNAs in protein synthesis for their studies of ion channels. [4]

The water-solubility of bio-molecules and the unique regiospecificity of lanthanum-promoted acylation have the potential for wide use in the selective acylation of biomolecules. However, the process has a low efficiency in terms of lanthanum. Duffy and Dougherty report that a very large excess of the lanthanide is needed to produce a detectable yield of product, making isolation of a stable product challenging. [4] In any case, even with less lanthanum, the metal ion must be completely removed from the product for further work as lanthanides promote the cleavage of RNA. As lanthanides are water-soluble, subsequent separation from the reaction mixture is a complex process requiring quenching of the lanthanide with DTPA followed by size-exclusion chromatography. Moreover, lanthanide salts are widely used in general as Lewis acids in water and can be recycled if recovered. [5] In the existing biomimetic acylation process, the lanthanide is difficult to separate from the product and is not readily recovered for re-use.



Scheme 1. Lanthanum-mediated acylation of a *cis*-diol: bis-bidentate coordination of BMP (benzoyl methyl phosphate) and a *cis*-diol about a lanthanide center followed by acyl transfer completes the reaction.

Immobilization of the lanthanide ion onto a polymer support is potentially an effective solution for convenient separation and re-use of the catalyst. Due to the established affinity of polyols for lanthanides, lanthanide-modified ion-exchange resins have been employed by Angyal for carbohydrate separations. [6] Angyal's procedure uses water as a solvent and depends on coordination of the hydroxyl groups of the sugar to the immobilized metal ion, which is similar to the basis of the affinity we would use for an immobilized catalyst for reactions of diols in water. In principle, the addition of an acyl phosphate monoester would then convert a solid support designed for separations into a recoverable reagent for biomimetic acylation. This approach should be practical as ion-exchange resins are readily available and are relatively inexpensive. The use of the immobilized catalyst would simplify the separation of the lanthanide and avoid the need for a chromatographic isolations. This approach also has the added benefit of recycling of the lanthanide, which reduces waste and is desirable in terms of environmental considerations. [7]

A similar approach to recovery and recycling of lanthanides for aqueous aldol reactions has been reported by Kobayashi. [8] However, that approach requires synthesis of the polymer support.

A related study by Yu *et. al.* describes the preparation and evaluation of lanthanides bound to ion exchange resins as catalysts for several reactions. [9] There examples in that report of the catalysts is not designed for use in water and is not tested for biomimetic acylation of diols. We note that there exist several methods for the monoacylation of diols utilizing approaches based on tin-containing reagents, borinic acids and organocatalysts. [10] These methods are very effective but none of these approaches utilizes a recyclable catalyst and are generally not useful in water. In the present report we note the successful application of resin-bound lanthanides for the biomimetic aminoacylation of nucleosides and nucleotides. The desired outcome in which the materials promote the reaction and are easily recovered by filtration for use in further reaction cycles has been successfully achieved.

Materials and Methods

Nuclear magnetic resonance analysis was performed at the CSICOMP Facility, University of Toronto. NMR spectra were recorded at 300 MHz for ^{1}H, 75 MHz for ^{13}C, 121.5 MHz for ^{31}P and 42.5 MHz for ^{139}La. Electrospray mass spectrometry analysis was performed at the AIMS facility at the University of Toronto. Ion exchange resins (DOWEX™ HCR-W2, Amberlyst™ 15, and Amberlite™ IR-120) and lanthanide salts were purchased from commercial suppliers. Metal ion resins were prepared by the literature procedure of Yu *et. al*. with the use of lanthanide and magnesium triflates. [9] N-*t*-boc-phenylalanine ethyl phosphate (BocPheEP), benzoyl methyl phosphate (BMP) and ethylene glycol monobenzoate were prepared by literature procedures. [1, 11, 12]

HPLC analysis for benzoylation reactions was performed on a C18 preparative column (Waters μBondapak™ 7.8 mm × 300 mm, 125 Å, 10 μm) using an isocratic gradient with a mobile phase of 10/90 (v/v) acetonitrile/water containing 0.1% trifluoroacetic acid (TFA) with a flow rate of 1.5 mL/min and effluent detection at 230 nm. HPLC analysis for N-*t*-boc aminoacylation reactions was performed on C18 reversed-phase preparative column (Phenomenex™ 10.00 mm x 250 mm, 90 Å, 10 μm) with 40/60 (v/v) acetonitrile/water mobile phase containing 0.1% TFA with a flow rate of 3.00 mL/min and effluent detection at 263 nm. All acylation and aminoacylation reactions were conducted at room temperature in freshly prepared HEPES buffer (0.1 M, pH = 8.0 at 23 °C).

Monobenzoylation of ethylene glycol: Ethylene glycol (0.42 mmol) and La^{III}-DOWEX™ resin (0.28 g resin, 0.42 mmol of La^{III}) were added to a vial containing 3.0 mL of HEPES buffer. BMP (0.42 mmol) was added to the vial and the mixture was stirred overnight. A 150 μL sample of the reaction mixture was then subjected to HPLC analysis. The same reaction was performed with La^{III}- Amberlite™ IR-120 resin in place of the La^{III}-DOWEX™ resin. Benzoic acid and glycol monobenzoate were identified in the reaction by comparison to authentic materials. For catalyst retrieval and recycling, the La^{III}-resin was isolated by filtration and washed with methanol and water. The resin was then dried in an oven for two hours at 100 °C, cooled to room temperature and weighed. The resin was added to another vial for another acylation reaction. The procedure was repeated two more times in order to establish the recyclability of the catalyst.

Control experiments to establish reaction on the solid phase: 5 mL of freshly prepared HEPES buffer was incubated with dried La^{III}-DOWEX™ resin and the mixture was stirred overnight. The resin was filtered; 3.0 mL of the buffer was added to another vial containing ethylene glycol and BMP (0.42 mmol each). The resulting mixture was stirred overnight (reaction A). A parallel reaction was set up using 3.0 mL of fresh buffer that had not been incubated with resin (reaction B). Each reaction was analyzed by HPLC. The filtered resin from buffer incubation was washed with water and oven dried for two hours. One equivalent of the resin was added to reaction A. The reaction was stirred overnight. As a control, La^{III}-DOWEX™ resin, which was not soaked in buffer, was added to reaction B. Each reaction was analyzed by HPLC after stirring overnight.

Aminoacylation of nucleosides and nucleotides with solid phase lanthanides: Stock solutions of nucleotides, nucleosides and BocPheEP, $Mg(OTf)_2$ (80 mM each) and $Ln(OTf)_3$ (160 mM) were prepared in HEPES buffer. 100 mg of resin was used for reactions that were performed with the solid phase catalyst. Reactions were conducted on a 1 mL scale. Substrate, lanthanide salt (or resin), Mg^{II} (for nucleotide reactions) were added to a vial and made up to 800 μL by the addition of buffer. The mixture was then stirred for five minutes. 200 μL of the BocPheEP stock solution was added to the vial in order to initiate the reaction. The concentration of each component in the reaction was 16 mmol except for the lanthanide salt, which was 32 mmol. The reactions were stirred for 2 hours. Then, a 100 μL sample of the reaction mixture was subjected to HPLC analysis.

^{139}La NMR Experiments: NMR experiments were performed on a Varian 400 MHz spectrometer. A standard solution was prepared by dissolving $LaCl_3$ in HEPES buffer. 350 µL of this solution was diluted with an equal volume of D_2O to give a final lanthanum concentration of 0.01 M. For analysis of the solid phase benzoylation reaction with La^{III}-DOWEX™ resin, 350 µL of the reaction solvent was transferred to an NMR tube and then diluted with an equal volume of D_2O.

Results and Discussion

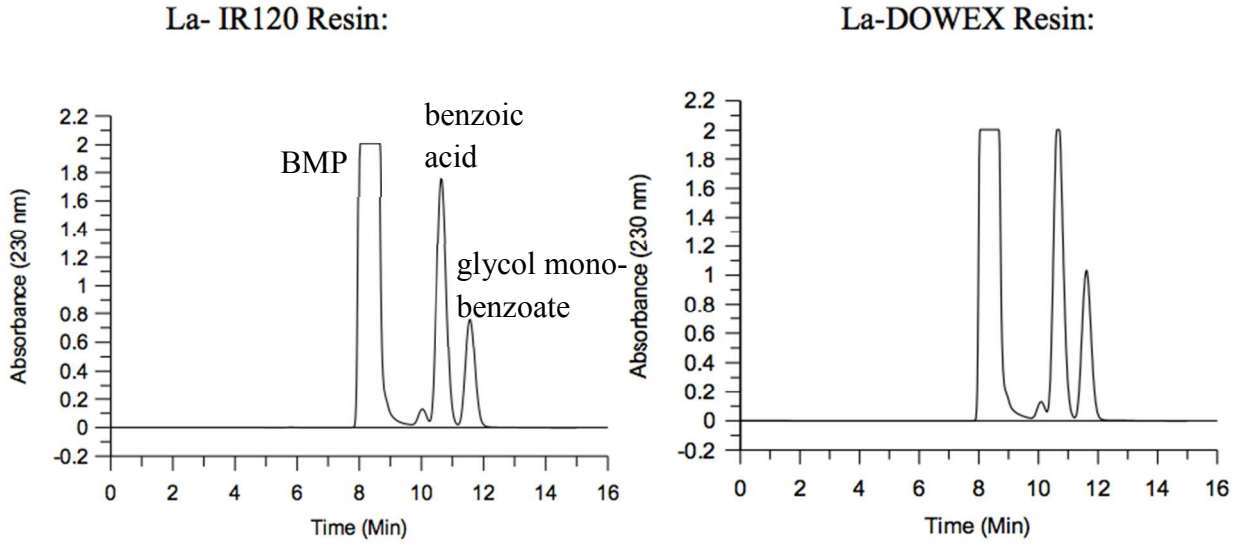


Figure 1. HPLC analysis of reactions that are based on lanthanide-immobilized resin.

Both La^{III}-DOWEX™ and La^{III}-Amberlite™ IR120 resins promote monoacylation of ethylene glycol (Figure 1). Based on the peak area of the glycol monobenzoate, we find that the La^{III}-DOWEX is the more effective catalyst as it provided a slightly better yield based on HPLC analysis. This finding is consistent with results reported by Yu *et. al.* where Yb^{III}-DOWEX™ was a more active catalyst compared to Yb^{III}-Amberlite™ IR120. [9] The surface area for the DOWEX™ resin is greater than that of the Amberlite™ IR120 resin and may account for the observed difference in yield. [13] Control reactions with Na^{I}-DOWEX™ in place of La^{III}-DOWEX™ provide no conversion of the acyl phosphate reagent into products (Figure 2). This demonstrates that the lanthanide ion is essential and that neither the resin nor the sodium cation alone promotes the reaction. The La^{III}-DOWEX™ resin from the acylation was isolated by filtration, washed with water and dried at 100 ^{o}C. The resin is then ready for further reactions.

This was done for two further acylation reactions. In each case the HPLC product analysis was identical to that with the initial catalyst.

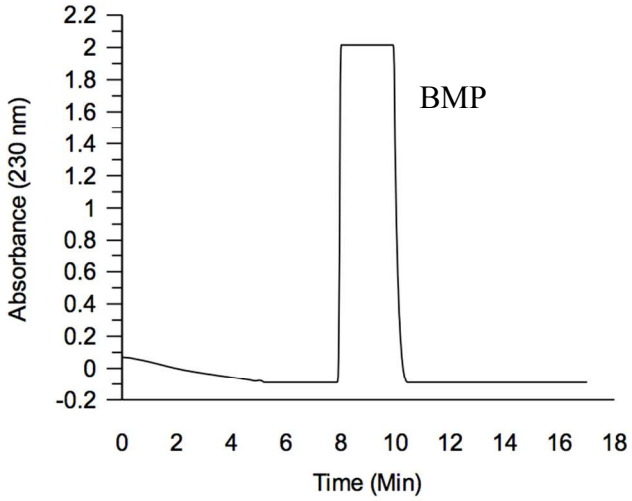


Figure 2. HPLC analysis of acylation reaction with Na-DOWEX resin after 72 hours of reaction time.

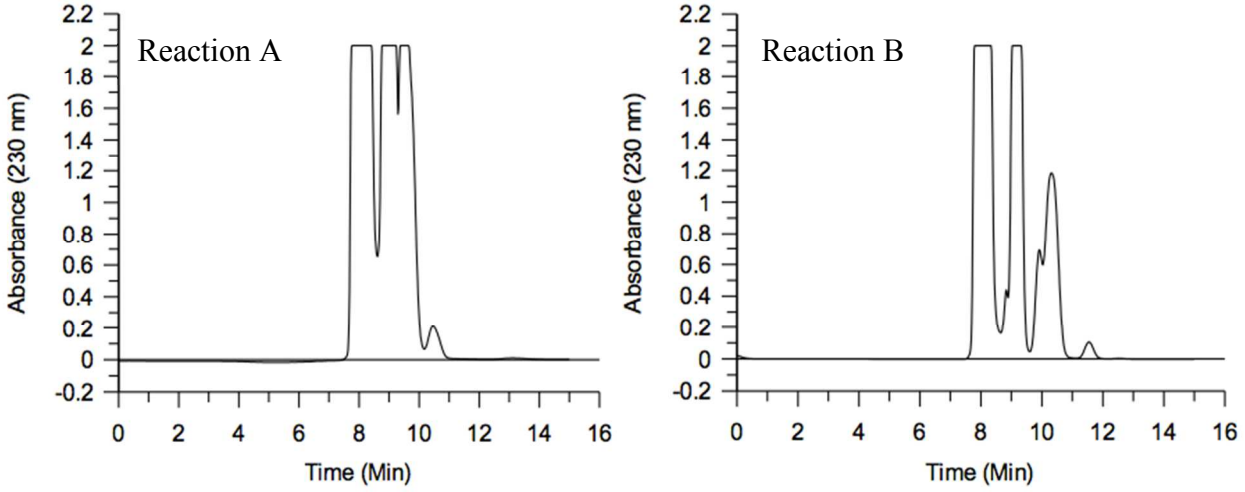


Figure 3. HPLC analysis for control experiments indicates no catalytic activity from leached metal ions in solution. Reaction A condition: HEPES buffer (La-resin incubation +), BMP, ethylene glycol; Reaction B condition: HEPES buffer (La-resin incubation –), BMP, and ethylene glycol.

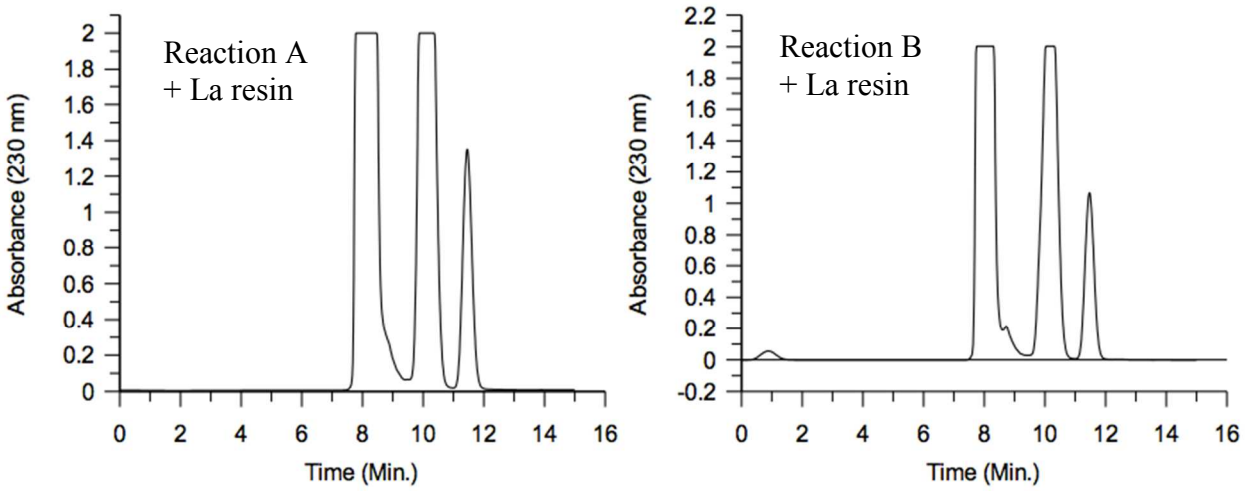


Figure 4. HPLC analysis shows the addition of immobilized lanthanide resin promoted acylation reaction in Reaction A and B. The chromatograms are comparable to those shown in Figure 1.

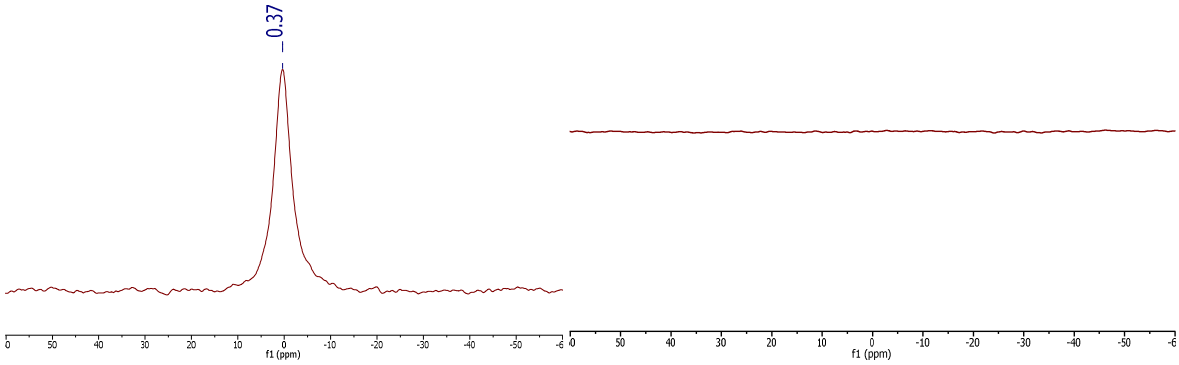
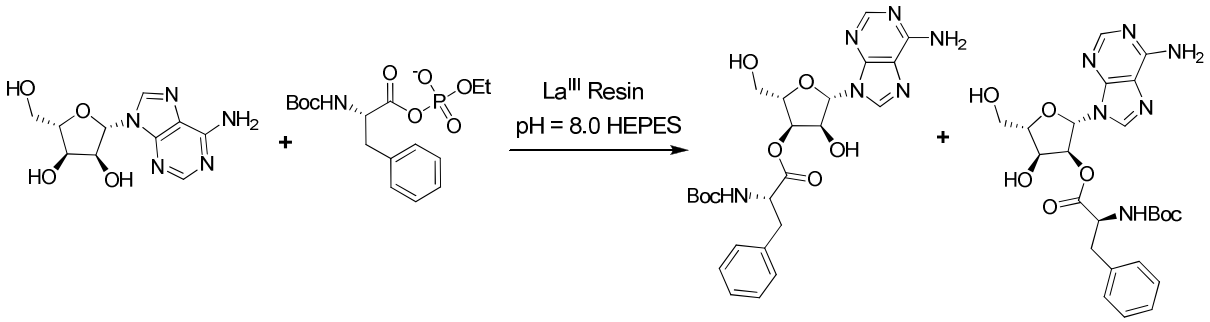


Figure 5. Left panel: ^{139}La NMR spectra of 0.01 M $LaCl_3$ standard in D_2O. (Right) Aqueous solvent from an acylation reaction after removal of the La^{III}-DOWEX™ resin.

Further control experiments were conducted in order to test whether the reactions occur with the solid phase catalyst or with leached metal ions in solution. [14] We incubated the reaction buffer with the catalytic resin overnight, removed the resin and tested the buffer for activity (Reaction A). We find that the solution was inactive in promoting acylation, as the product glycol benzoate peak was not observed in the chromatogram (Figure 3). Upon addition of the immobilized catalyst, the reaction proceeded effectively (Figure 4). We then performed ^{139}La NMR experiments to see if any significant amounts of the metal ion were present in any of the reaction mixtures after removal of the catalyst. There was no detectable signal in any of the

mixtures we analyzed (Figure 5). This observation, in combination with the recyclability of the catalyst, establishes that the reaction proceeds only in the presence of the solid phase catalyst.



Scheme 2. Aminoacylation of adenosine with BocPheEP using immobilized lanthanides.

In order to develop a solid phase method for tRNA acylation, it is necessary to demonstrate that immobilized lanthanides are able to promote aminoacylation reactions. The reaction of adenosine with BocPheEP was chosen as the starting point for solid-phase aminoacylation reactions (Scheme 2). As the acylating agent is subject to competitive hydrolysis in the presence of a lanthanide, an excess of the reagent can be used to ensure complete conversion of the substrate. To evaluate the effectiveness of the solid-phase catalysts, reactions were carried out with solid-phase resin (La-DOWEX, La-IR120, and Na-DOWEX as control). These reactions were compared to those carried out with $La(OTf)_3$. The reactions were then subject to HPLC analysis and the resultant yield is reported as the total area of ester products. The HPLC product peaks were isolated and their identity was confirmed as aminoacyl esters by mass analysis (ESI-MS(+) calculated m/z 514.2, found m/z 515.2 ($M+H^+$)). Consistent with previous work from our group, the lanthanide promoted reaction lead to formation of the 2'- and 3'-aminoacyl esters of adenosine with the 3'-ester being favored [15]. Product analysis revealed that each lanthanide resin was able to catalyze amino acylation of the nucleoside and the use of Na-DOWEX does to give ester products (Figure 6). We find that the activity of the solid-phase catalyst is reduced in comparison to the lanthanide salt $La(OTf)_3$ as indicated by the reduction in the peak areas of the ester products. This is common in the development of solid-phase methods and is attributed to the diffusion of the reactants to the active metal ion within the resin matrix. [16] However, the differences are minimal and can be addressed by extension of the reaction time. Both gel-type resins (DOWEX™ and Amberlite™ IR120) are able to promote acylation with DOWEX™ again being more effective. Amberlyst™ 15 is a macroporous resin that is specifically designed for

catalysis due to its high surface area. [17] We expected this resin to be the best choice for our reaction. However, we observed that the macroporous resin was susceptible to mechanical degradation when stirred. Therefore we chose DOWEX™ as the solid support for all subsequent method development.

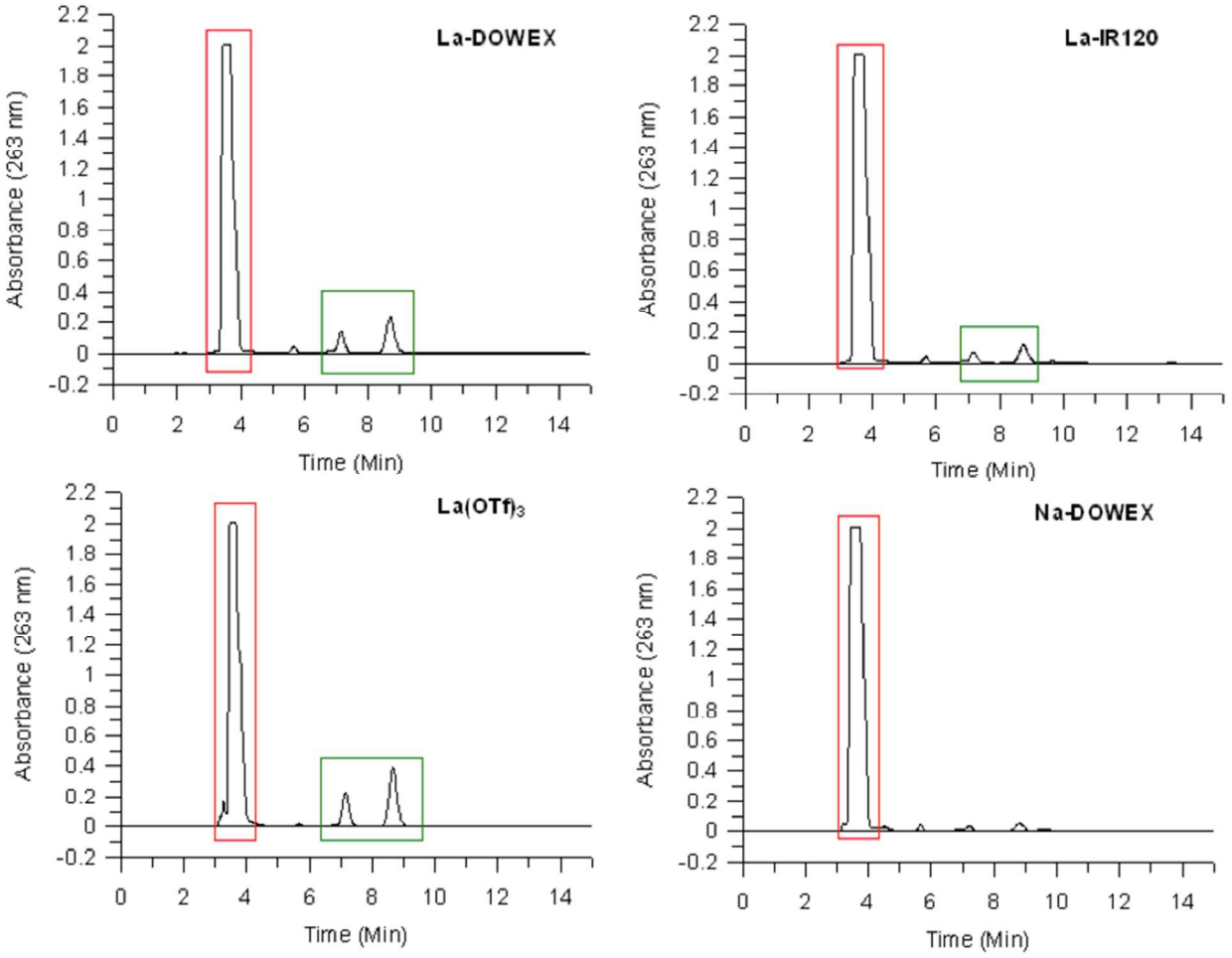


Figure 6. HPLC analysis for adenosine aminoacylation reactions. Adenosine is indicated in red (peak 1) and the ester products are indicated in green (peak 2 & 3). Ester products are eluted in the order of 2'- and 3'-aminoacyl esters. (The ester products were identified by comparison of retention times to previously reported results. [3])

We also assessed the effect of different immobilized lanthanide ions on the aminoacylation reaction. We tested La^{III}, Eu^{III} Yb^{III}, Nd^{III}, Dy^{III}, Tb^{III} forms of the DOWEX™ catalyst. We tested the Sc^{III} form of the resin for comparison and the Mg^{II} form of the resin as a control. When varying the lanthanide ion, highest yields were obtained with lanthanides with larger ionic radii

(Table 1). This observation is consistent with the expected result based on lanthanide contraction. [18] Smaller lanthanides have a greater affinity for phosphates due to their charge density and are not as efficient at coordination to adjacent hydroxyl groups in comparison to larger lanthanides. Therefore, the optimal combination for biomimetic acylation reactions is provided by La^{III}-DOWEX™.

Table 1. Yields for the aminoacylation of adenosine with BocPheEP using differing DOWEX™ catalysts.

Immobilized Metal Ion	**Ionic Radius (pm)** [18]	**Yield**
La^{III}	103.2	28%
Nd^{III}	98.3	26%
Eu^{III}	94.7	20%
Tb^{III}	92.3	20%
Dy^{III}	91.2	16%
Yb^{III}	86.8	8%
Sc^{III}	74.5	5%
Mg^{II}	72.0	No Conversion

With the optimized catalyst in hand, we then applied our optimized conditions to the aminoacylation of cytidine and observed similar results. As an additional control, we performed reactions with 2'-deoxy nucleosides as these substrates lack the required *cis*-diol functionality to coordinate effectively with the metal ion. HPLC analysis gave no indication of ester formation (Figure 7).

The aminoacyl esters of adenosine are formed in a 2:1 ratio with the 3'-isomer being favoured. This is consistent with equilibrium ratios of o-acyl adenosine derivatives reported by Griffin *et. al*. [19] We note that the selectivity of the aminoacylation reaction is not of great concern as we intend to apply this method to the direct aminoacylation of tRNA. Chemically modified tRNA can be used to incorporate un-natural amino acids into proteins through *in-vitro* translation methods. Peptide bond formation does not depend on the site of acylation on the terminal ribonucleotide of tRNA as the resultant ester is subject to rapid equilibration between

the 2' and 3' positions. [20] We have observed similar behaviour in previously reported work. [21] Selectivity is of greater importance in applications where the site of acylation needs to more precise *e.g.* regioselective protection of carbohydrates. [22]

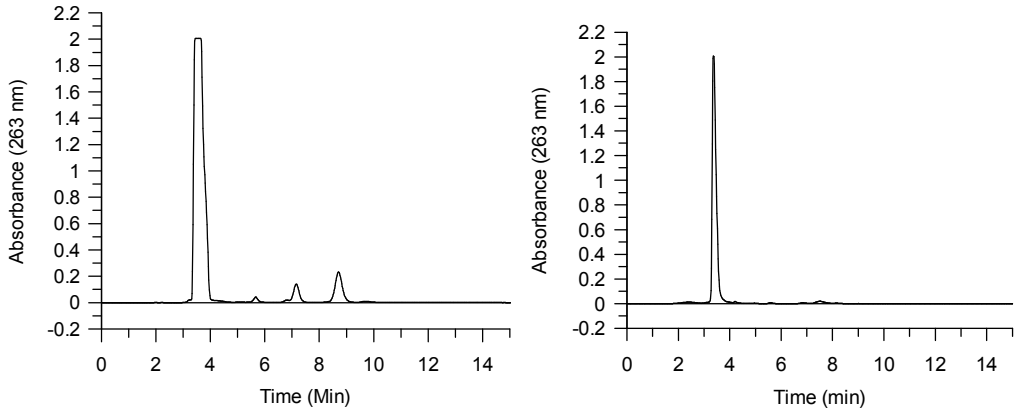


Figure 7. (Left) aminoacylation of adenosine with La^{III}-DOWEX™ resin. (Right) aminoacylation of 2'-deoxyadenosine under the same conditions, no esters formed.

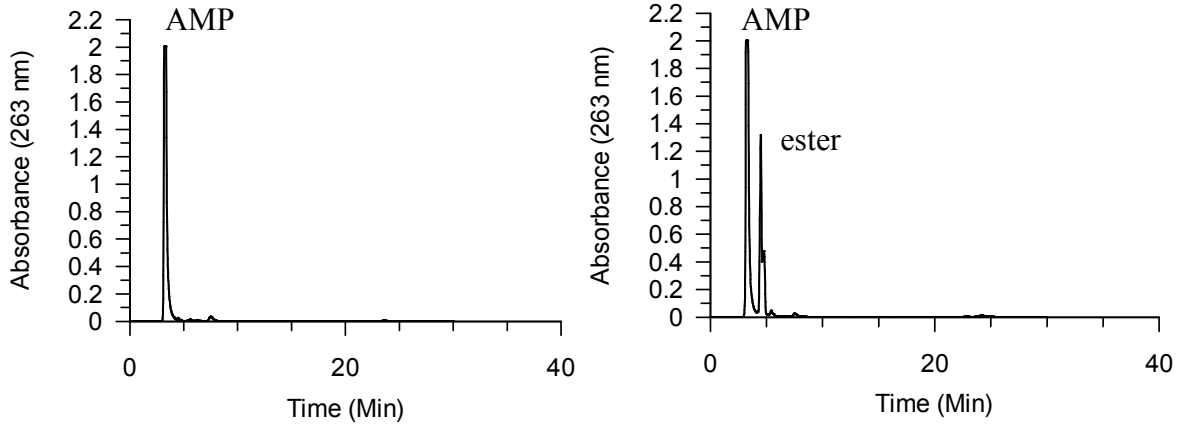


Figure 8. HPLC output for aminoacylation of 5'-AMP with use of La^{III}-DOWEX with (left) and without added Mg^{II} (right).

In contrast to reactions of nucleosides, additional complexity is provided by nucleotide substrates whose phosphate groups may bind and remove lanthanum from the solid support. It has been shown that Mg^{II} can be introduced into the reaction mixture in order to bind to phosphate groups, allowing the lanthanide to be available for catalysis. Therefore, the addition of Mg^{II} into the solid-phase reaction would be ideal for the reaction of nucleotide substrates. We

performed reactions with adenosine-5'-monophosphate (5'-AMP) and the immobilized catalyst. We noted ester formation takes place in the presence of added Mg^{II} (Figure 8).

The newly formed peak was isolated and analyzed by ESI-MS and we confirmed that the peak is an aminoacyl ester of the nucleotide (ESI-MS (-) calculated m/z 594.2, found m/z 593.2 ($M-H^+$)). Similar results were obtained in the acylation of cytidine 5'-monophosphate and uridine 5'-monophosphate.

Conclusions

Lanthanides immobilized onto ion-exchange resins effectively catalyze monoacylation of ethylene glycol in addition to the aminoacylation of nucleosides and nucleotides. The development of the catalysts greatly improves the efficiency of the reaction as the catalysts are easily recovered by filtration and reused for further reactions. With this development, we are able to reuse the lanthanide which was originally discarded when used as a homogenous catalyst. Finally, the addition of Mg^{II} to the reaction mixture improves the efficiency of acylation when a ribonucleotide serves as a substrate. Based on these results, we will be expanding the scope of this method to include carbohydrate acylation and the direct aminoacylation of tRNA.

Acknowledgements

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Graphical Abstract