JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

ARTICLE

pubs.acs.org/JACS

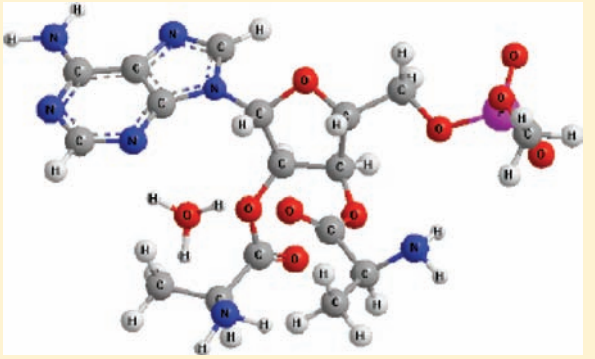
Structural Basis for the Exceptional Stability of Bisaminoacylated Nucleotides and Transfer RNAs

Maria Duca,[†] Carl O. Trindle, and Sidney M. Hecht*[,‡]

Departments of Chemistry and Biology, University of Virginia, Charlottesville, Virginia 22904, United States

Supporting Information

ABSTRACT: At least one bisaminoacyl-tRNA is synthesized in nature (by *Thermus thermophilus* phenylalanyl-tRNA synthetase), and many disubstituted tRNAs have been prepared in vitro. Such misacylated tRNAs are able to participate in protein synthesis, even though they lack the free 2′-OH group of the 3′-terminal adenosine moiety. Their ready participation in protein synthesis implies significant chemical reactivity. The basis for this reactivity has been documented previously. Surprisingly, the aminoacyl moieties of these tRNAs also exhibit exceptional chemical stability. In the present report, bisaminoacylated nucleotides are investigated computationally and experimentally to define the basis for the stability of such species. Molecular modeling of bisalanyl-AMP in the absence of solvent and in the presence of a limited number of water molecules revealed two common features among the low-energy structures. The first was the presence of H-bonding interactions between the two aminoacyl moieties. The second was the presence of a H-bonding interaction between the 2′-*O*-alanyl moiety and the N-3 atom of the adenine nucleobase, typically mediated through a water molecule. The prediction of an interaction between an aminoacyl moiety and the adenine nucleobase was confirmed experimentally by comparing the behavior of bisalanyl-AMP and bisalanyl-UMP in the presence of model nucleophiles. This study suggests a possible role for the adenosine moiety at the 3′-end of aminoanyl-tRNAs in controlling the stability and reactivity of the aminoacyl moiety and has important implications for the reactivity and stability of normal aminoacyl-tRNAs.



INTRODUCTION

Aminoacyl-tRNAs are essential to the process of translation of mRNA to form proteins, being responsible for the introduction of the appropriate amino acids into the growing peptide chain. This in turn requires activation of the aminoacyl moiety for the peptidyltransferase reaction.[1] Each tRNA usually bears an amino acid linked to the 2′- or 3′-hydroxyl groups on the 3′-terminal adenosine moiety; the 3′-regioisomer group participates in protein synthesis.[2] In the generally accepted mechanism, the 2′-OH group plays an essential role catalyzing peptide bond formation, probably by acting as a "proton shuttle" between the amino acid in the 3′-position and the growing peptide chain.[3] However, it has recently been shown that the 2′-OH group of adenosine is not essential for protein synthesis and it was suggested that a nucleophile located on 23S RNA close to the ribosomally bound tRNAs acts as the proton shuttle.[4]

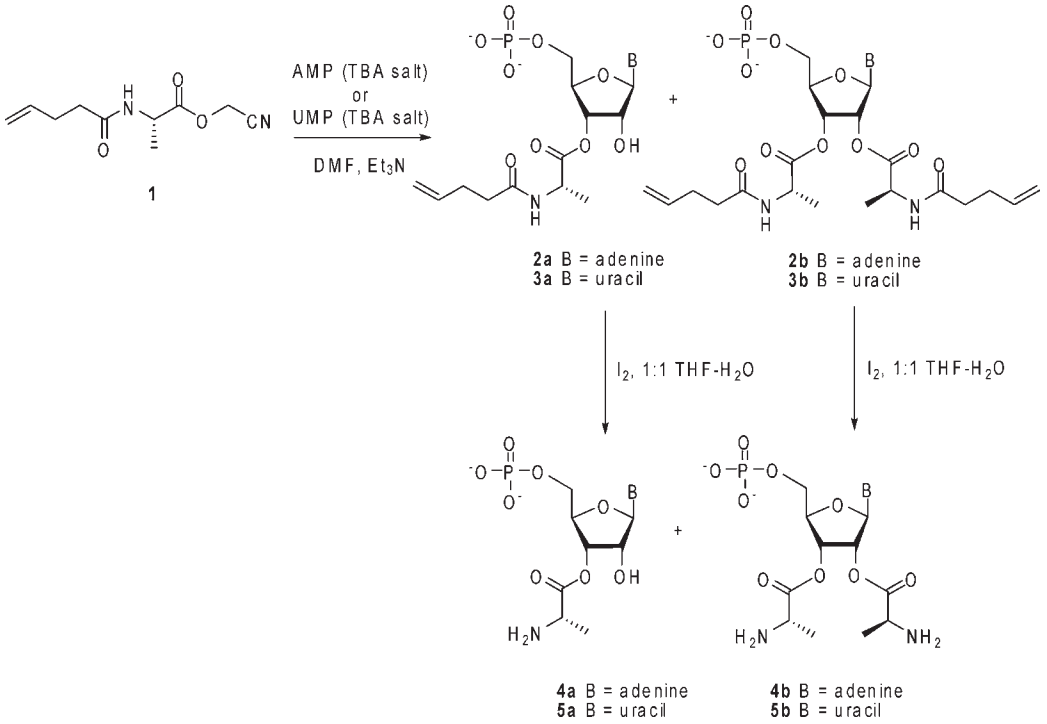
It has been shown that phenylalanyl-tRNA synthetase from *Thermus thermophilus* is able to form a tandemly activated tRNA, leading to the formation of bis-2′,3′-(*O*-phenylalanyl)-tRNAs using *T. thermophilus* $tRNA^{Phe}$ as a substrate.[5] *Escherichia coli* $tRNA^{Phe}$ and an RNA transcript identical in sequence with *E. coli* $tRNA^{Phe}$[5] were also shown to undergo bisaminoacylation with phenylalanine.

While bisphenylalanyl-tRNA is presently the only tandemly activated tRNA reported as a constituent of a biochemical system, it has been possible to prepare a number of bisaminoacylated tRNA transcripts.[6,7] This has been achieved by modification of a scheme used to produce misacylated tRNAs, in which a tRNA transcript lacking the 3′-terminal CpA sequence[8] is ligated enzymatically to an aminoacylated pdCpA that has been prepared by chemical synthesis.[9] By the use of chemically synthesized bisaminoacylated pdCpA derivatives, it is also possible to prepare bisaminoacylated tRNAs containing the same[6,7] or different[6a,10] amino acids on the 2′-and 3′-positions of adenosine (Scheme 1).

The bisaminoacylated tRNA transcripts prepared by the strategy outlined in Scheme 1 have been shown to participate quite efficiently in protein biosynthesis in in vitro systems.[6,7] By defining the structural elements in the bisaminoacylated tRNAs required to support their participation in protein synthesis and by studying the reactivity of modified bisaminoacylated pdCpA derivatives with model *N*-nucleophiles, we have been able to formulate a chemically plausible mechanism consistent with the observed participation of the bisaminoacylated tRNAs in ribosomally mediated protein synthesis.[7]

Received: April 30, 2011
Published: June 07, 2011

Scheme 1. Synthesis of Mono-2′(3′)-*O*-alanyl-AMP (4a), Mono-2′(3′)-*O*-alanyl-UMP (5a), Bis-2′,3′-*O*-alanyl-AMP (4b), and Bis-2′,3′-*O*-alanyl-UMP (5b)



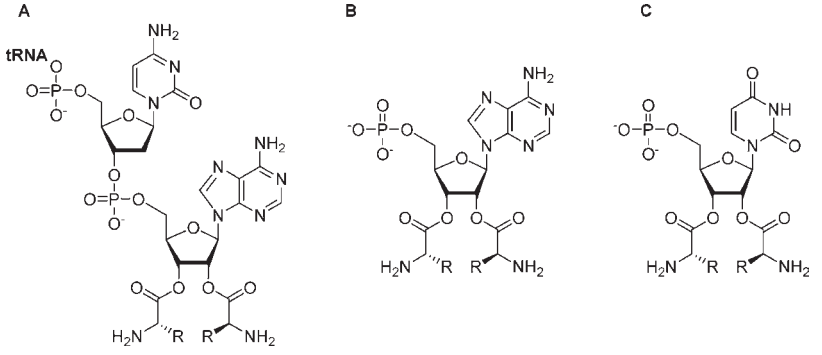


Figure 1. General structures of the bisaminoacylated derivatives studied: (A) tandemly activated tRNA, (B) bisaminoacyl-AMP, and (C) bisaminoacyl-UMP.

In addition to providing new insights into the mechanism of aminoacyl-tRNA reactivity in protein synthesis, the bisaminoacylated tRNAs hold the promise of permitting significantly increased amounts of proteins to be elaborated in vitro. This is a consequence of the findings that both of the amino acid moieties of bisaminoacylated tRNAs are incorporated into protein and, especially, that the bisacylated species have much greater chemical and biochemical stability than normal monoaminoacylated tRNAs. The latter feature permits in vitro protein synthesis to proceed over an extended time period.

One facet of the behavior of bisaminoacylated tRNAs that is not well understood is their exceptional stability. Presently, we report a computational and experimental study of bisaminoacylated nucleotides that is focused on the stability issue. The results of this study identify H-bonding between the aminoacyl moieties as one likely source of the observed stability of the bisaminoacylated nucleotides and tRNAs. A second, and more surprising, source of stability is a H-bonding interaction between the 2′-*O*-aminoacyl moiety and N-3 of the adenine nucleobase at the 3′-end of the tRNA. The present findings have important implications for the sources of reactivity and stability of normal aminoacyl-tRNAs during protein synthesis.

RESULTS

The chemical and biochemical data reported previously[6,7] permitted the formulation of a molecular mechanism for protein synthesis when bisaminoacyl-tRNAs are employed.[6a,10] However, these data provided no insight concerning the molecular basis for the chemical stability of bisaminoacyl-tRNAs and the corresponding pdCpAs. Thus, a molecular modeling study was undertaken initially in order to identify the possible interactions responsible for the chemical stability of these species.

Computational Study of the Stability of Bisacyl-AMP and Bisacyl-UMP Derivatives. Bisaminoacyl-AMP (Figure 1B)

dx.doi.org/10.1021/ja203994e |*J. Am. Chem. Soc.* 2011, 133, 11368–11377

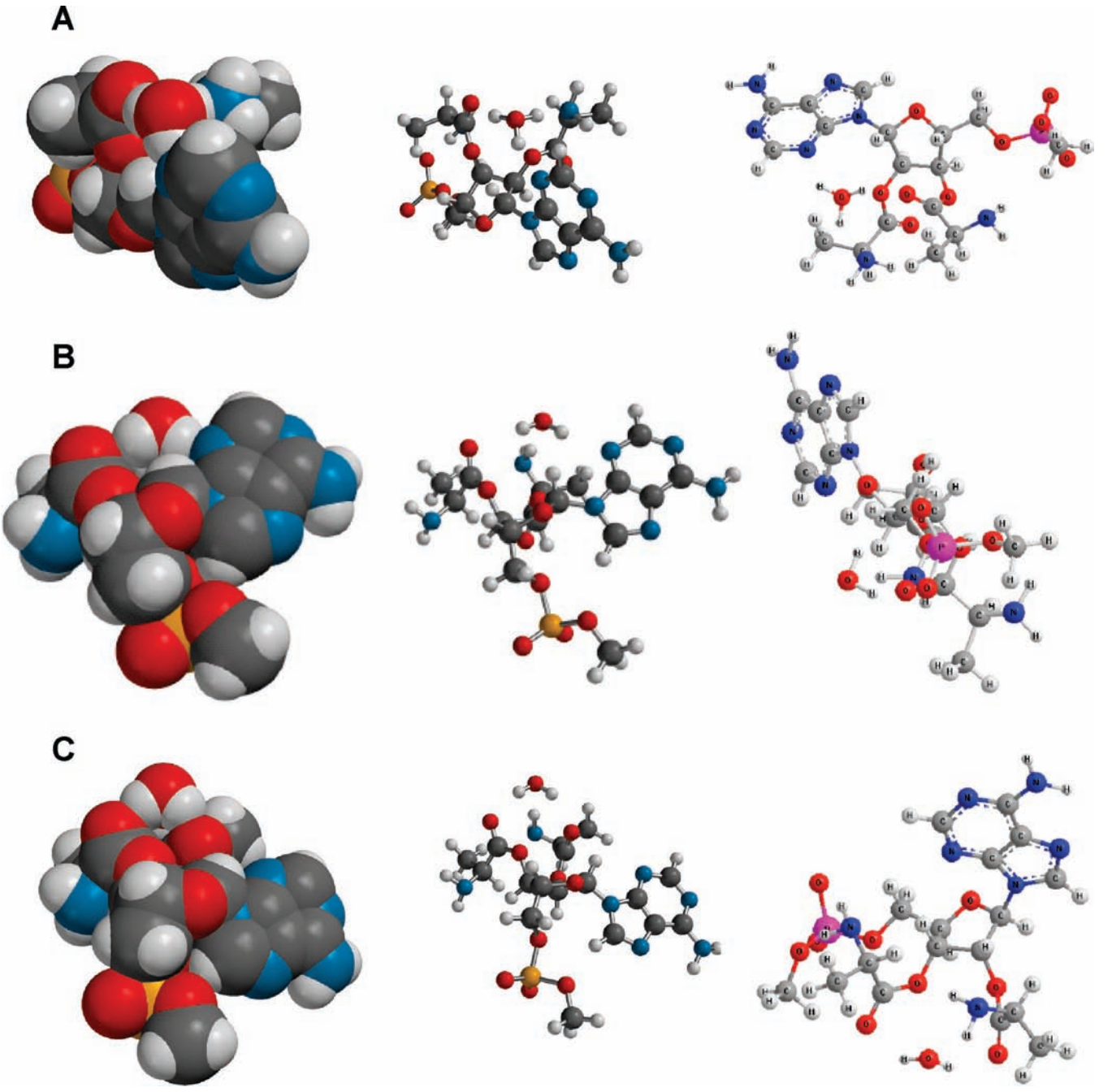


Figure 2. Computationally determined energetically favorable structures of a (phosphate-methylated) 5′-AMP derivative containing alanyl esters at the 2′ and 3′-positions. (A) Representative structure illustrating the strong interaction of a hydronium ion with N-3 of the adenine nucleobase as well as both alanyl moieties. Hydronium O−nucleobase N-3 distance, 2.77 Å; hydronium O−N^{α} (2′-alanyl) distance, 2.60 Å; hydronium O−carboxyl O (3′-alanyl) distance, 2.63 Å. (B) Representative structure illustrating a weaker interaction of water with N-3 of the adenine nucleobase as well as both alanyl moieties. Water O−nucleobase N-3 distance, 3.14 Å; water O−N^{α} (2′-alanyl) distance, 3.06 Å; water O−carboxyl O (3′-alanyl) distance, 3.24 Å. (C) Representative structure illustrating the interaction of water with both alanyl esters. Water O−carboxyl O (2′-alanyl) distance, 3.04 Å; water O−carboxyl O (3′-alanyl) distance, 2.90 Å. The diagrams to the extreme right illustrate potential H-bonding interactions.

was chosen as a simplified model to study possible three-dimensional structures of bisaminoacylated pdCpA derivatives and the interactions that could be realized to confer the observed chemical stability to the molecule. Using the software Spartan, a series of torsional isomer scans was conducted beginning with molecular mechanics and PM3 models, followed by HF/STO-3G and finally B3LYP/6-31G(d) calculations. This procedure has been applied to (i) the neutral structure, (ii) structures with one of the amino groups of the aminoacyl chains protonated, (iii) neutral structures including one water, as well as (iv) structures containing a protonated water molecule. In each case, about 20 structures survived the screening and were judged by B3LYP/6-31G(d) to lie within 5 kcal/mol of the most stable form. The most stable molecules contain certain common features: hydrogen bonds link

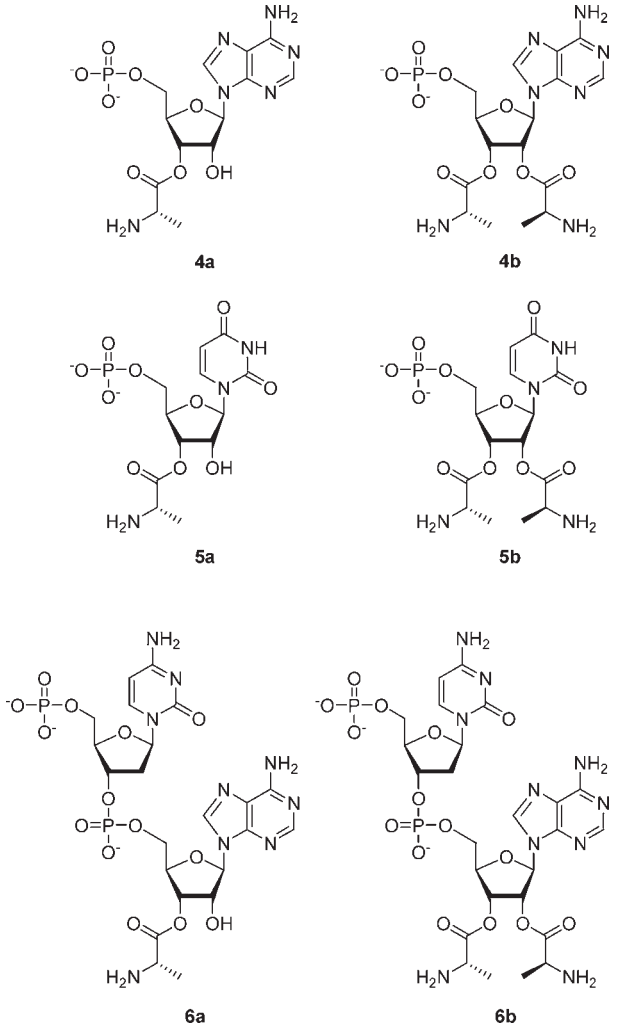


Figure 3. Compounds synthesized and studied for their reactivity toward nucleophiles. Mono-2′(3′)-*O*-alanyl-AMP (**4a**), bis-2′,3′-*O*-alanyl-AMP (**4b**), mono-2′(3′)-*O*-alanyl-UMP (**5a**), bis-2′,3′-*O*-alanyl-UMP (**5b**), mono-2′(3′)-*O*-alanyl-pdCpA (**6a**), and bis-2′,3′-*O*-alanyl-pdCpA (**6b**).

the aminoacyl moieties, and also link the 2′-aminoacyl moiety with the N-3 ring atom of the adenine nucleobase. Figure 2 provides examples of structures in which a hydronium ion exhibits a strong interaction with N-3 of the adenine nucleobase and both aminoacyl moieties (A), water interacts with the nucleobase and two aminoacyl moieties (B), and water interacts with the two aminoacyl moieties (C). Parallel studies of models of bis-acyl UMP showed no H-bonding of types A or B, as is to be expected from the structural differences between bisacyl UMP and bisacyl AMP.

Synthesis of New Monoaminoacylated and Bisaminoacylated Derivatives of AMP and UMP. The computational study of AMP derivatives strongly suggested that the two NH_2 groups of amino acids in the 2′- and 3′-positions and the N-3 atom of adenosine could interact via the formation of hydrogen bonds and were thus involved in the observed chemical stabilization[6,7] of bisaminoacyl-pdCpAs and tRNAs. The chemical synthesis of appropriate bisaminoacylated derivatives was carried out in order to permit experimental study of the interactions suggested by the computational data. Thus, bis-2′,3′-*O*-alanyl-AMP (**4b**) and bis-2′,3′-*O*-alanyl-UMP (**5b**) (Figure 3) were synthesized as well as their corresponding monoaminoacylated analogues, **4a** and **5a**. In analogy with the method used previously for the preparation of bisaminoacyl-pdCpAs,[6,7] the tetra-*n*-butylammonium (TBA) salts of AMP and UMP were prepared in order to render these compounds soluble in organic solvents. As outlined in Scheme 1, the TBA salt of AMP was treated with *N*-(4-pentenoyl)-*S*-alanine cyanomethyl ester (**1**) in anhydrous DMF containing Et_3N. After 3 days of stirring at room temperature, the reaction mixture was first analyzed and then purified by HPLC using a C_{18} reversed-phase column with a gradient of 0% → 63% CH_3CN in 50 mM NH_4OAc, pH 4.5, over a period of 35 min. Mono-2′(3′)-*O*-[*N*-(4-pentenoyl)-*S*-alanyl]-AMP (**2a**) and bis-2′,3′-*O*-[*N*-(4-pentenoyl)-*S*-alanyl]-AMP (**2b**) were obtained in 40% and 60% yields, respectively. Each of the purified compounds (**2a** and **2b**) was separately treated with iodine in a mixture 1:1 THF−H_2O for 50 min. HPLC purification using the same conditions described above afforded 2′(3′)-*S*-alanyl-AMP (**4a**) and bis-2′,3′-*O*-alanyl-AMP (**4b**) in 80% and 75% yields, respectively.

Analogously, the TBA salt of UMP was treated with *N*-(4-pentenoyl)-*S*-alanine cyanomethyl ester (**1**)[11] in anhydrous DMF containing Et_3N. After 3 days of stirring at room temperature, the reaction mixture was analyzed and purified by HPLC using a C_{18} reversed-phase column and a gradient of 0% → 63% CH_3CN in 50 mM NH_4OAc, pH 4.5, over a period of 35 min. Mono-2′(3′)-*O*-[*N*-(4-pentenoyl)-*S*-alanyl]-UMP (**3a**) and bis-2′,3′-*O*-[*N*-(4-pentenoyl)-*S*-alanyl]-UMP (**3b**) were obtained in 25% and 75% yields, respectively. Each compound was then treated with iodine in a mixture 1:1 THF−H_2O for 50 min. HPLC purification using the same conditions described above afforded mono-2′(3′)-*O*-alanyl-UMP (**5a**) and bis-2′,3′-*O*-alanyl-UMP (**5b**) in 98% and 57% yields, respectively.

Chemical Study of the Stability of Bisacylated AMP Compared to pdCpA Derivatives. The treatment of the synthesized molecules in the presence of nucleophiles was performed in order to model the chemical conditions during acyl transfer and peptide bond formation and, more generally, to understand the stability of the bisaminoacylated nucleotides. Compounds **4a**, **4b**, **5a**, and **5b** were treated with aqueous *n*-butylamine or imidazole in order to characterize their chemical behavior and permit comparison to the analogous pdCpA derivatives **6a** and **6b** studied previously (Figure 3).[7] Each compound was incubated for 2 h in the presence of 2 mol equiv of aqueous *n*-butylamine or imidazole, and aliquots were taken periodically. The reaction mixtures were then analyzed by C_{18} reversed-phase HPLC after 15, 30, 60, and 120 min. AMP derivatives **4a** and **4b** were first studied and compared to the respective pdCpA analogues. The use of AMP in comparison to pdCpA was intended to improve our understanding of the possible role of the dC moiety of pdCpA in conferring stability to the aminoacylated nucleotides. Concerning the monoacylated derivatives, it was found that **4a** and **6a** were completely deacylated within 2 h in the presence both of *n*-butylamine and imidazole (Figure 4A). There was no difference in the kinetics of deacylation between the pdCpA and AMP derivative. The bisacylated derivatives, **4b** and **6b**, were much more stable than the corresponding monoalanine derivatives, as expected (Figure 4B).[6,7] However, the kinetic profile indicated that deacylation of the AMP derivative **4b** was slightly faster than that of the pdCpA derivative **6b** in the presence both of *n*-butylamine and imidazole.

Although a slight difference in the reaction rates between AMP and pdCpA derivatives was observed for the bisaminoacylated species, the data lead to the conclusion that the chemical behaviors of the AMP and pdCpA derivatives are quite similar and that the

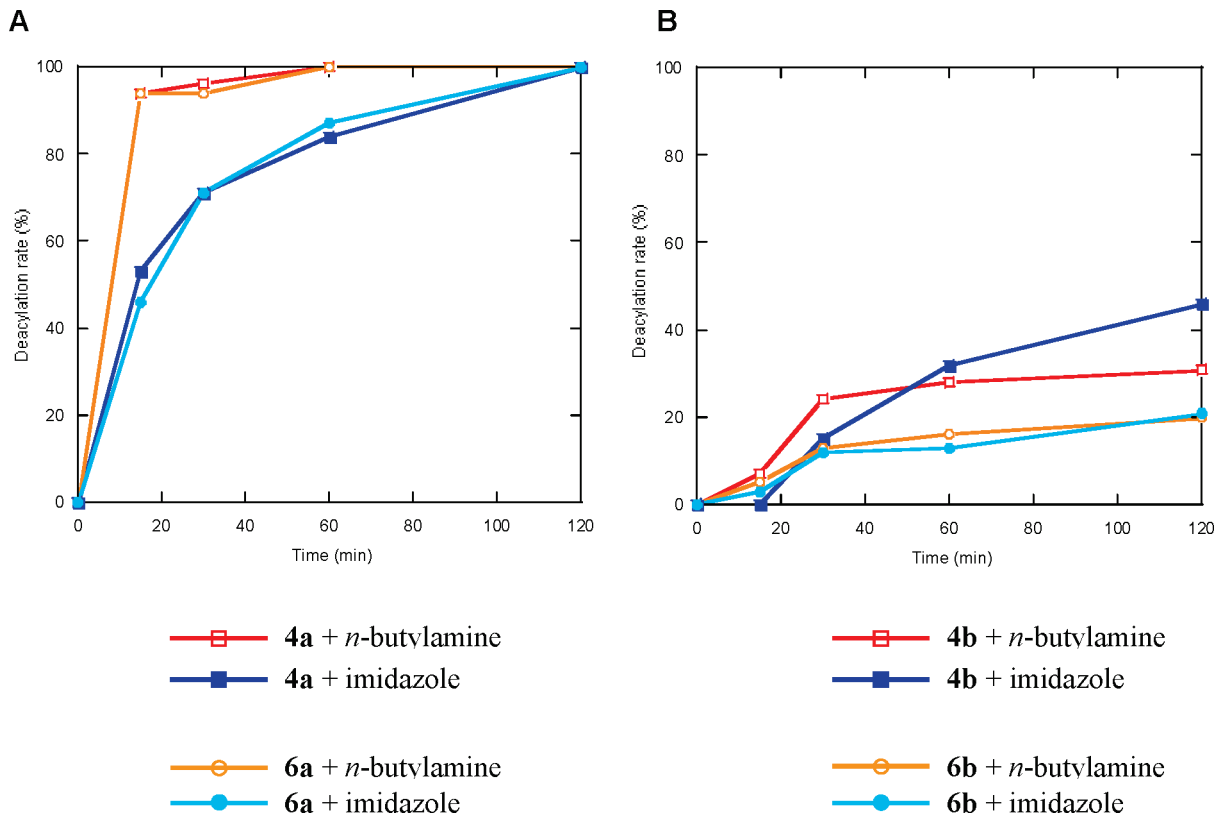


Figure 4. (A) Kinetic analysis of the deacylation of mono-2′(3′)-*O*-alanyl-AMP (**4a**) and mono-2′(3′)-*O*-alanyl-pdCpA (**6a**) in the presence of 2 equiv of *n*-butylamine (red and orange, respectively) and imidazole (dark blue and light blue, respectively). (B) Kinetic analysis of the deacylation of bis-2′,3′-*O*-alanyl-AMP (**4b**) and bis-2′,3′-*O*-alanyl-pdCpA (**6b**) in the presence of 2 equiv of *n*-butylamine (red and orange, respectively) and imidazole (dark blue and light blue, respectively). The percent formation of AMP and pdCpA after treatment is shown over a period of 120 min as analyzed by reversed-phase HPLC (250 × 4.6 mm, 5 μm column). The column was washed with 0% → 63% CH_3CN in 50 mM NH_4OAc, pH 4.5, over a period of 35 min at a flow rate of 1 mL/min (monitoring at 260 nm). Aliquots were analyzed after 15, 30, 60, and 120 min.

aminoacylated AMP can be used experimentally as a simplified model for pdCpA.

Chemical Study of the Stability of Bisacylated UMP Compared to AMP and pdCpA Derivatives. Both the molecular modeling study described above and previously published data on the reactivity of mono- and bis-aminoacyl-pdCpAs suggested the possible participation of the adenine nucleobase in the stabilization of the bisaminoacylated nucleotides. The use of UMP analogues precludes possible hydrogen-bond formation in direct analogy with AMP and could potentially confirm the hypothesis regarding the stabilizing participation of the adenosine N-3 atom. At first, mono-2′(3′)-*O*-alanyl-AMP (**4a**) and mono-2′(3′)-*O*-alanyl-UMP (**5a**) were treated in the presence of *n*-butylamine and imidazole. As shown in Figure 5A, both compounds were sensitive to nucleophilic deacylation, leading to 90%−100% deacylated product after 2 h in the presence of *n*-butylamine or imidazole. The AMP derivative was slightly more reactive than the UMP derivative in both cases. Figure 5B shows the comparable kinetic profiles for bis-2′,3′-*O*-alanyl-AMP (**4b**) and bis-2′,3′-*O*-alanyl-UMP (**5b**). While **4b** was deacylated to the extent of ∼40% after 2 h, **5b** was completely deacylated at the same reaction time in the presence of *n*-butylamine and >80% deacylated when treated with imidazole.

This result confirmed what had been suggested by the computational study. The absence of hydrogen bonding between the *N*-3 of adenosine and the aminoacyl moiety at the 2′-position of ribose was probably responsible for the greatly increased reactivity of the bisalanyl-UMP derivative. The data obtained for compounds **5a** and **5b** in panels A and B of Figure 5 are replotted as Figure 6. As is clear from the latter figure, in complete contrast to the mono- and bisaminoacylated derivatives of AMP and pdCpA, bisalanyl-UMP (**5b**) was actually deacylated at a rate comparable to that of monoalanyl-UMP (**5a**) in the presence of *n*-butylamine and imidazole.

■ DISCUSSION

The 2′,3′ *cis*-diol moiety at the 3′-terminus (A_{76}) of tRNA has important functions at a number of steps in the overall process of protein translation, in addition to providing the attachment site for the activated amino acid or peptide.[12] In solution, the amino acid or peptide ester attached to the A_{76} ribose is in rapid equilibrium between the 2′- and the 3′-hydroxyl groups,[13] but the 3′-linked ester is the regioisomeric form of the substrate that participates in the peptidyltransferase reaction at both the A- and P-sites.[2] Thus, the 2′-aminoacyl (peptidyl) ester is not required to form any essential covalent linkage. While the detailed molecular mechanism of protein synthesis catalyzed by the ribosome is not yet completely elucidated, a "proton shuttle" model has been suggested, which would involve the 2′-OH group in the peptidyltransferase reaction.[12,14] In fact, this hydroxyl group may participate as a H-bond acceptor from the amino group of the aminoacyl moiety and also donate a H-bond to the 3′-oxygen of

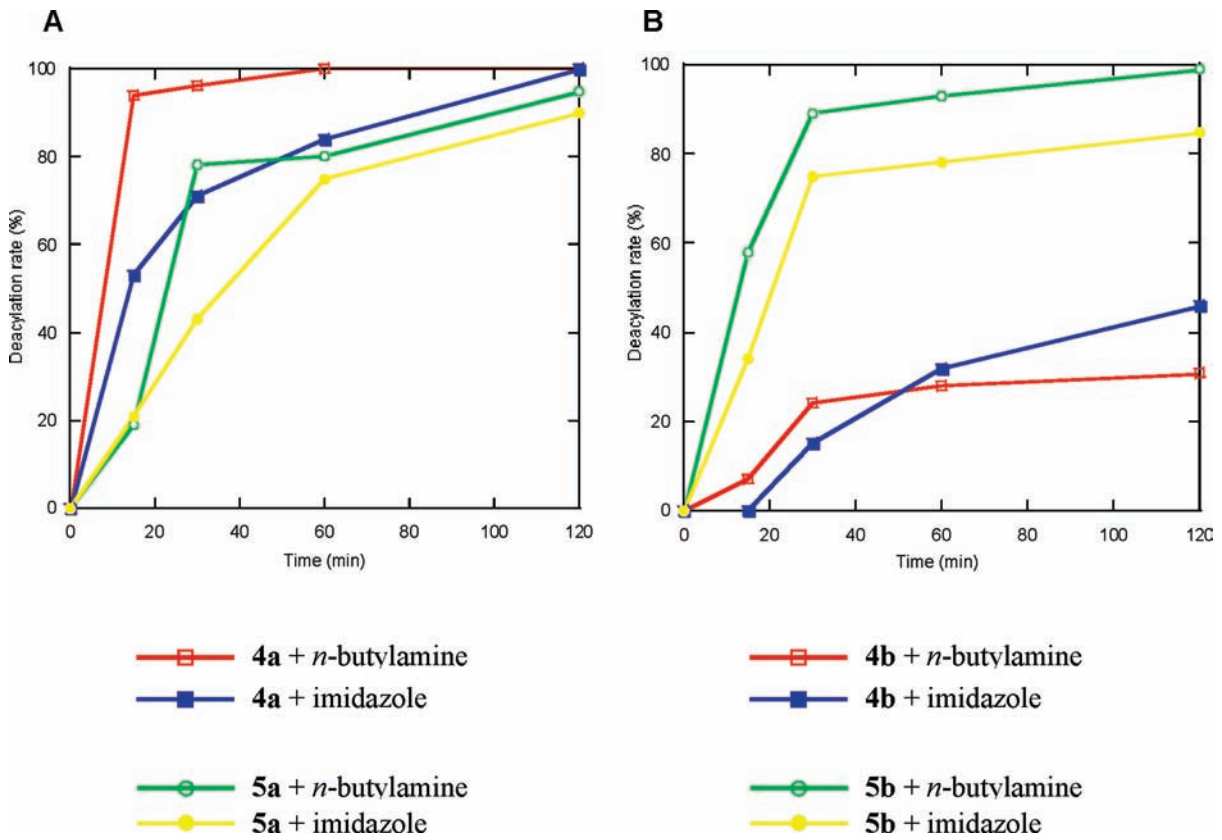


Figure 5. (A) Kinetic analysis of the deacylation of mono-2′(3′)-*O*-alanyl-AMP (**4a**) and mono-2′(3′)-*O*-alanyl-UMP (**5a**) in the presence of 2 equiv of *n*-butylamine (red and green, respectively) and imidazole (blue and yellow, respectively). (B) Kinetic analysis of the deacylation of bis-2′,3′-*O*-alanyl-AMP (**4b**) and bis-2′,3′-*O*-alanyl-UMP (**5b**) in the presence of 2 equiv of *n*-butylamine (red and green, respectively) and imidazole (blue and yellow, respectively). The percent formation of AMP and UMP after treatment is shown over a period of 120 min as analyzed by reversed-phase HPLC (250 × 4.6 mm, 5 μm column). The column was washed with 0% → 63% CH_3CN in 50 mM NH_4OAc, pH 4.5, over a period of 35 min at a flow rate of 1 mL/min (monitoring at 260 nm). Aliquots were analyzed after 15, 30, 60, and 120 min.

ribose, leading to the activation of both groups involved in the peptidyltransferase reaction. However, a recent study suggested that the proton shuttling from the incoming aminoacyl-tRNA to the peptidyl-tRNA during peptide bond formation need not involve the 2′-OH group of the peptidyl-tRNA; it seems possible that some other nucleophile spatially close to this tRNA acts as the proton shuttle.[4]

Despite the importance of the discovery that bisaminoacylated tRNAs are synthesized in nature by *Thermus thermophilus* phenyalanyl-tRNA synthetase, such tandemly activated tRNAs have not been studied in detail, probably because of the difficulty of obtaining them. The techniques developed for synthesizing misacylated tRNAs in vitro has facilitated the preparation and the detailed study of tandemly activated tRNAs.[6,7,10] While the substitution of the 2′-hydroxyl group with different functional groups (such as 2′-OCH_3 or 2′-H) led to tRNAs with diminished facility of function in protein synthesis,[15−17] its substitution with a second aminoacyl moiety results in complete maintenance of activity in protein synthesis and also confers a surprising chemical stability to the molecule.[6,7]

The ability of bisaminoacylated tRNAs to participate in protein synthesis has been analyzed by biochemical investigation of suitably modified bisaminoacyl-pdCpAs and tRNAs bearing free or protected NH_2 groups on the aminoacyl moieties.[6a,7] It has been demonstrated that the NH_2 functional group of the 2′-aminoacyl residue can serve a role analogous to that of the 2′-OH group of corresponding monoaminoacyl-pdCpAs in activating the aminoacyl moiety for participation in peptide bond formation.[7] However, the molecular basis for the distinctive chemical stability of the bisaminoacylated pdCpA and tRNA derivatives remains to be explored and superficially seems at odds with the ability of these species to participate in protein synthesis. In order to understand the molecular basis for the observed stability of bisaminoacylated pdCpA derivatives and their corresponding tRNAs, a molecular modeling study of simplified bisaminoacylated analogues was undertaken. As described above, low-energy conformations of bisaminoacyl-AMP (Figure 1B) were investigated. Water and hydronium establish a variety of types of hydrogen-bonding interactions between aminoacyl chains and between the aminoacyl moieties and the adenine nucleobase. The latter finding is unprecedented and prompted us to investigate the structure of bisaminoacyl-UMP (Figure 1C), which should be incapable of analogous stabilization.

In order to confirm experimentally the role of the adenine nucleobase in the chemical stability of bisaminoacyl-pdCpAs, bisalanyl-AMP and UMP have been prepared. The synthesis of these compounds was carried out in analogy to the synthetic procedure reported for the preparation of bisaminoacyl-pdCpAs.[6] The use of AMP derivatives allowed us to determine whether the cytidine residue adjacent to the adenosine bearing the aminoacyl moieties could influence the reactivity of the aminoacyl moiety,

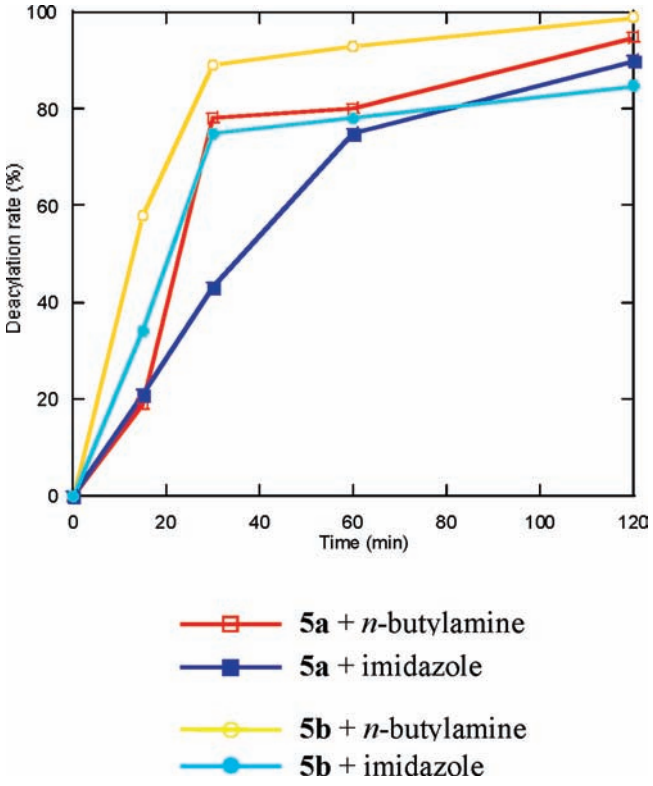


Figure 6. Kinetic analysis of the deacylation of mono-2′(3′)-*O*-alanyl-UMP (**5a**) and bis-2′,3′-*O*-alanyl-UMP (**5b**) in the presence of 2 equiv of *n*-butylamine (red and yellow, respectively) and imidazole (dark blue and light blue, respectively). The percent formation of UMP after treatment is shown over a period of 120 min as analyzed by reversed-phase HPLC (250 × 4.6 mm, 5 μm column). The column was washed with 0% → 63% CH_3CN in 50 mM NH_4OAc, pH 4.5, over a period of 35 min at a flow rate of 1 mL/min (monitoring at 260 nm). Aliquots were analyzed after 15, 30, 60, and 120 min.

while the use of activated UMP derivatives led to important insights concerning the role of the adenine nucleobase.

Mono-2′(3′)-*O*-alanyl-AMP (**4a**) proved to be very unstable in the presence of the nucleophiles *n*-butylamine and imidazole; its behavior was thus comparable to that of 2′(3′)-*O*-alanyl-pdCpA (**6a**) (Figure 4A) and the corresponding activated tRNA. In contrast, bis-2′,3′-*O*-alanyl-AMP (**4b**) was quite stable in the presence of *n*-butylamine and imidazole, although a slightly faster deacylation rate was observed compared to that of bis-2′,3′-*O*-alanyl-pdCpA (**6b**) (Figure 4B). These results indicated that the cytidine residue adjacent to the bisaminoacylated adenosine moiety was only marginally involved in the chemical stability of the aminoacyl moiety in the bisaminoacyl-pdCpAs and confirmed that mononucleotides can be used as a simplified model system to study bisaminoacylated pdCpA and tRNA chemistry.

Regarding the UMP derivatives, it has been shown that both 2′(3′)-*O*-alanyl-UMP (**5a**) and bis-2′,3′-*O*-alanyl-UMP (**5b**) were very sensitive to nucleophilic attack, since after 2 h these compounds were converted to free UMP (Figure 6). Comparison with the corresponding AMP analogs **4a** and **4b** (Figure 5) underscores the ready deacylation of all of the monoaminoacylated nucleotide derivatives by nucleophiles. In the case of UMP, the bisaminoacylated derivative also proved to be sensitive to nucleophilic attack, in complete contrast to the stability of bisacylated AMP (**4b**). Thus, the adenine nucleobase of **4b** must play a key role in the chemical stability of such compounds. Monoaminoacyl- and bisaminoacyl-UMP have essentially the same sensitivity to nucleophiles (Figure 6), while the AMP derivatives clearly do not (cf. Figures 4 and 5).

It has recently been shown that the transesterification rate of aminoacylated adenosine derivatives depends strongly on the presence both of free vicinal 2′,3′-OH groups and the nature of the attached nucleobase.[18] This supports the hypothesis that the nucleobase in tRNA position A_{76} can help to define the reactivity of the aminoacyl moiety of an activated tRNA.

On the basis of these theoretical and experimental results, we suggest that in the presence of water, three hydrogen-bonding interactions are present in bisaminoacylated adenosines, as shown in Figure 2. These interactions must contribute to the chemical stability observed for this class of molecules. Further, the possibility of hydrogen bonding between the NH_2 group of 2′-aminoacyl moiety and the carbonyl oxygen of the 3′-aminoacyl moiety in bisaminoacyl-tRNAs likely activates the aminoacyl moiety for the peptidyltransferase reaction during protein synthesis.[7]

CONCLUSIONS

The present findings provide strong computational and experimental support for the participation of the N-3 atom in the adenine nucleobase as a determinant of the stability and reactivity of the activated amino acid added to the growing polypeptide chain from aminoacyl-tRNAs during protein synthesis. It seems unlikely that the presence of adenosine as the 3′-terminal nucleotide in every tRNA in all organisms has occurred by chance, but rather helps to define the stability and reactivity of (mono)aminoacylated tRNAs.[19] There is also direct evidence for the involvement of the 3′-terminal adenosine moiety of *T. thermophilus* $tRNA^{Phe}$ in activation by its cognate aminoacyl-tRNA synthtease.[20] Bisaminoacylated tRNAs have also been shown to bind to elongation factor Tu,[6a] and it would be of great interest to assess the roles of both amino acids, as well as the 3′-terminal adenosine moiety, in modulating interaction with this protein factor.[21] These findings contribute to our understanding of the chemical behavior of bisaminoacylated tRNA, provide novel insights into the protein synthesis mechanism involving such bisaminoacylated tRNAs, and suggest a more general role for the 3′-terminal adenosine moiety of tRNA in protein synthesis.

MATERIALS AND METHODS

General Methods. All chemicals reagents were purchased from Aldrich Chemical Co. (Milwaukee, WI) or Sigma Chemical Co. (St. Louis, MO) and used without further purification. Anhydrous grade DMF and acetonitrile were purchased from VWR scientific. Moisture sensitive reactions were conducted under argon in oven-dried glassware. Analytical thin-layer chromatography (TLC) was performed on 60 F_{254} (E. Merck) plates that were visualized by irradiation (254 nm) or by staining with Hanessian's stain (cerium molybdate). Flash chromatography was performed using Silicycle 40−60 mesh silica gel. High-resolution mass spectra were recorded at the Mitchigan State University−NIH Mass Spectrometry Facility. ^1H and ^{13}C NMR spectra were recorded on a 300 MHz Varian instrument. Chemical shifts are reported in parts per million (ppm, δ) referenced to the residual ^1H resonance of the solvent ($CDCl_3$, δ 7.26; CD_3OD, δ 3.31). ^{13}C spectra were referenced to the residual ^{13}C resonance of the solvent ($CDCl_3$, δ 77.3; DMSO-d_6, δ 39.5). Splitting patterns are designated as follows: s, singlet; br, broad; d, doublet; dd, doublet of doublets; t, triplet; q, quartet; m, multiplet. HPLC was performed using a Varian 9012 pump

coupled with a Varian 2050 UV detector. Analytical HPLC was performed using an Alltech Alltima RPC$_{18}$ column (250 × 4.6 mm, 5 μm), while semipreparative HPLC was performed using an Alltech Alltima RPC$_{18}$ column (250 × 10 mm, 5 μm).

Molecular Modeling. All modeling results were obtained with Spartan 04 and 08 software; complementary results were obtained for simpler systems in Gaussian 03. Spartan's automated conformational energy search feature was central to the systematic generation of candidate structures. The conformation generator was applied to (a) the neutral model of bis-acyl AMP we call model A, (b) model A with aminoprotonation, (c) model A with a single water variously sited in the initial structure, and (d) model A with a hydronium ion. To begin this process, torsional angles in the aminoacyl chains, including the angle defined by their attachment to the furan ring, were stepped systematically in 30° increments through full 360° rotations. More than 20 000 structures were produced. Each unique conformation was then optimized by molecular mechanics (MM2). This produced many replicants, which were excluded from the candidate list. Of unique structures, those within 20 kcal/mol of the minimum energy structures were retained for the next screening step. Roughly 1000 retained structures were reoptimized with a semiempirical method, PM3. The 100 lowest-energy forms were retained for further study. Those predicted by the simplest ab initio method HF/STO-3G to lie within 10 kcal/mol of the lowest energy form were reoptimized with the well-tested and reliable density functional method B3LYP/6-31G(d). About 20 isomers of each type (a–d) survived this screening. These low-energy forms all displayed multiple hydrogen bonding established by water or hydronium ion and bridging aminoacyl chain to chain and often chain to base. Details of the low-energy structures are available in the Supporting Information.

Synthesis of Aminoacylated pdCpA Derivatives. Mono-2′(3′)-*N*-(4-pentenoyl)-*S*-alanyl-AMP (2a) and Bis-2′,3′-[*N*-(4-pentenoyl)-*S*-alanyl]-AMP (2b). To a conical vial containing 12.6 mg (60.2 μmol) of *N*-(4-pentenoyl)-*S*-alanine cyanomethyl ester (**1**)[11] was added a solution of 5.00 mg (6.02 μmol) of the tetra-*n*-butylammonium salt of AMP in 100 μL of DMF, followed by 20 μL of Et$_3$N. The reaction mixture was stirred at room temperature. After 24 h a 5-μL aliquot of the reaction mixture was diluted with 100 μL of 1:2 CH$_3$CN–50 mM NH$_4$OAc, pH 4.5, and was analyzed by HPLC using a semipreparative C$_{18}$ reversed-phase column (250 × 10 mm). The column was washed with 0% → 63% acetonitrile in 50 mM NH$_4$OAc, pH 4.5, over a period of 35 min at a flow rate of 3.5 mL/min (monitoring at 260 nm). The remaining reaction mixture was diluted to a total volume of 400 μL with 1:2 CH$_3$CN–50 mM NH$_4$OAc, pH 4.5, and purified using the same C$_{18}$ reversed-phase column. After lyophilization of the appropriate fractions both compounds were obtained as colorless solids: mono-2′(3′)-*N*-(4-pentenoyl)-*S*-alanyl-AMP (**2a**) (retention times 16.6 and 16.9 min for the two positional (2′,3′) isomers): yield 1.2 mg (40%); ^{1}H NMR (D$_2$O) δ 1.30 and 1.43 (2 d, J = 7.5 and 7.0 Hz, together integrating for 3H), 2.45–2.65 (m, 4H), 3.90–4.10 (m, 2H), 4.25–4.35 (m, 1H), 4.36 and 4.49 (t, J = 7.0 and 7.5 Hz, together integrating for 1H), 4.38–4.42 (m, 1H), 4.95–5.15 (m, 2H), 5.55 and 5.44 (t, J = 5.5 and 5.5 Hz, together integrating for 1H), 5.59–5.65 and 5.78–5.83 (m, together integrating for 1H), 6.07 and 6.21 (d, J = 7.5 and 7 Hz, together integrating for 1H), 8.11 and 8.13 (s, together integrating for 1H), and 8.42 and 8.45 (s, together integrating for 1H); ^{13}C NMR (D$_2$O) δ 16.4, 29.4, 34.8, 49.0, 64.5, 69.3, 73.3, 82.8, 86.2, 115.9, 118.7, 137.1, 140.0, 152.9, 155.8, 173.7, 176.5, and 181.6; mass spectrum (ESI), m/z 501.1492 (M + H)$^+$ (C$_{18}$H$_{26}$N$_6$O$_9$P requires 501.1493). Bis-2′,3′-[*N*-(4-pentenoyl)-*S*-alanyl]-AMP (**2b**) (retention time 23.6 min): yield 2.3 mg (60%); ^{1}H NMR (D$_2$O) δ 1.22 (d, 3H, J = 7.0 Hz), 1.43 (d, 3H, J = 7.5 Hz), 2.00–2.15 (m, 4H), 2.25–2.40 (m, 4H), 4.10–4.15 (m, 2H), 4.20–4.25 (m, 1H), 4.45–4.50 (m, 1H), 4.55–4.60 (m, 1H), 4.85–4.90 (m, 2H), 4.98–5.10 (m, 2H), 5.50–5.60 (m, 1H), 5.65–5.70 (m, 1H), 5.75–5.85 (m, 2H), 6.30 (d, 1H, J = 7.0 Hz), 8.22 (s, 1H), and 8.49 (s, 1H); ^{13}C NMR (D$_2$O) δ 15.6, 16.4, 29.4, 29.5, 34.5, 34.8, 48.3, 48.8, 73.0, 75.0, 82.7, 84.9, 115.6, 115.8, 118.7, 136.8, 137.1, 141.0, 149.1, 156.0, 161.0, 171.3, 173.1, 173.3, 175.8, and 176.3; mass spectrum (ESI), m/z 654.2284 (M + H)$^+$ (C$_{26}$H$_{37}$N$_7$O$_{11}$P requires 654.2283).

Mono-2′(3′)-*N*-(4-pentenoyl)-*S*-alanyl-UMP (3a) and Bis-2′,3′-[*N*-(4-pentenoyl)-*S*-alanyl]-UMP (3b). To a conical vial containing 13 mg (62 μmol) of *N*-4-pentenoyl-*S*-alanine cyanomethyl ester (**1**)[11] was added a solution of 5.0 mg (6.2 μmol) of the tetra-*n*-butylammonium salt of UMP in 100 μL of DMF, followed by 20 μL of Et$_3$N. The reaction mixture was stirred at room temperature. After 24 h a 5-μL aliquot of the reaction mixture was diluted with 100 μL of 1:2 CH$_3$CN–50 mM NH$_4$OAc, pH 4.5, and was analyzed by HPLC using a semipreparative C$_{18}$ reversed-phase column (250 × 10 mm). The column was washed with 0% → 63% acetonitrile in 50 mM NH$_4$OAc, pH 4.5, over a period of 35 min at a flow rate of 3.5 mL/min (monitoring at 260 nm). The remaining reaction mixture was diluted to a total volume of 400 μL with 1:2 CH$_3$CN–50 mM NH$_4$OAc, pH 4.5, and purified using the same C$_{18}$ reversed-phase column. After lyophilization of the appropriate fractions, both compounds were obtained as colorless solids: mono-2′(3′)-*N*-(4-pentenoyl)-*S*-alanyl-UMP (**3a**) (retention times 18.3 and 18.7 min for the two positional (2′,3′) isomers): yield 0.7 mg (25%); ^{1}H NMR (D$_2$O) δ 1.34 and 1.38 (2 d, J = 7.0 and 7.0 Hz, together integrating for 3H), 2.20–2.35 (m, 4H), 3.90–4.00 (m, 2H), 4.25–4.35 (m, 1H), 4.38–4.45 (m, 1H), 4.48 and 4.54 (t, J = 7.0 and 7.5 Hz, together integrating for 1H), 4.95–5.10 (m, 2H), 5.25–5.30 (m, 1H), 5.70–5.80 (m, 1H), 5.85 and 5.90 (d, J = 8.0 and 8.5 Hz, together integrating for 1H), 6.00 (d, J = 7.0 Hz, 1H) and 7.87 and 7.91 (d, J = 8.0 and 8.0 Hz, together integrating for 1H); ^{13}C NMR (D$_2$O) δ 16.4, 29.5, 34.8, 49.0, 64.5, 68.6, 72.4, 82.4, 87.6, 103.4, 115.9, 137.1, 141.8, 152.2, 166.4, 173.6 and 176.5; mass spectrum (ESI), m/z 478.1230 (M + H)$^+$ (C$_{17}$H$_{25}$N$_3$O$_{11}$P requires 478.1223). Bis-[*N*-(4-pentenoyl)-*S*-alanyl]-UMP (**3b**) (retention time 23.6 min): yield 2.9 mg (75%); ^{1}H NMR (D$_2$O) δ 1.29 (d, 3H, J = 7.5 Hz), 1.37 (d, 3H, J = 7.5 Hz), 2.20–2.35 (m, 8H), 4.00–4.10 (m, 2H), 4.25–4.30 (m, 1H), 4.40–4.45 (m, 2H), 4.95–5.05 (m, 4H), 5.42 (t, 1H, J = 6.0 Hz), 5.50–5.55 (m, 1H), 5.65–5.80 (m, 2H), 5.88 (d, 1H, J = 8.5 Hz), 6.11 (d, 1H, J = 6.0 Hz), and 7.86 (d, 1H, J = 8.0 Hz); ^{13}C NMR (D$_2$O) δ 16.2, 16.3, 29.4, 29.5, 34.7, 34.8, 48.7, 48.8, 64.4, 72.2, 74.0, 82.0, 86.3, 103.4, 110.0, 115.8, 115.9, 137.1, 141.6, 151.7, 166.3, 173.1, 173.3, 176.2, and 176.3; mass spectrum (ESI), m/z 631.2009 (M + H)$^+$ (C$_{25}$H$_{36}$N$_4$O$_{13}$P requires 631.2011).

Mono-2′(3′)-*O*-alanyl-AMP (4a). To a conical vial containing 1.2 mg (2.4 μmol) of protected *N*-(4-pentenoyl)-(*S*)-alanyl-AMP (**2a**) was added 150 μL of a solution of I$_2$ in 15:85 THF–H$_2$O.[11,22] The reaction mixture was stirred at room temperature for 50 min. A 5-μL aliquot of the reaction mixture was diluted with 100 μL of 1:2 CH$_3$CN–50 mM NH$_4$OAc, pH 4.5, and was analyzed by HPLC using a semipreparative C$_{18}$ reversed-phase column (250 × 10 mm). The column was washed with 0% → 63% acetonitrile in 50 mM NH$_4$OAc, pH 4.5, over a period of 35 min at a flow rate of 3.5 mL/min (monitoring at 260 nm). The remaining reaction mixture was diluted to a total volume of 200 μL with 1:2 CH$_3$CN–50 mM NH$_4$OAc, pH 4.5, and purified using the same C$_{18}$ reversed-phase column. After lyophilization of the appropriate fractions, compound **4a** [retention times 14.7 and 15.3 min for the two positional (2′,3′) isomers] was obtained as a colorless solid: yield 0.8 mg (80%); ^{1}H NMR (D$_2$O) δ 1.38 (d, 3H, J = 7.5 Hz), 4.00–4.10 (m, 2H), 4.25–4.30 (m, 1H), 4.40–4.45 (m, 1H), 4.60–5.70 (m, 2H), 6.04 (t, 1H, J = 6.0 Hz), 8.15 (s, 1H), and 8.40 (s, 1H); ^{13}C NMR (D$_2$O) δ 16.4, 22.4, 64.6, 70.7, 74.7, 84.4, 87.3, 118.8, 140.3, 149.1, 152.1, 155.1, and 179.9; mass spectrum (ESI), m/z 419.1082 (M + H)$^+$ (C$_{13}$H$_{20}$N$_6$O$_8$P requires 419.1080).

Bis-2′,3′-*O*-alanyl-AMP (4b). To a conical vial containing 2.3 mg (4.7 μmol) of protected bis-2′,3′-[*N*-(4-pentenoyl)-(*S*)-alanyl]-AMP

(**2b**) was added 150 μL of a solution of I_2 in 15:85 THF−H_2O.[11,22] The reaction mixture was stirred at room temperature for 50 min. A 5-μL aliquot of the reaction mixture was diluted with 100 μL of 1:2 CH_3CN−50 mM NH_4OAc, pH 4.5, and was analyzed by HPLC using a semipreparative C_{18} reversed-phase column (250 × 10 mm). The column was washed with 0% → 63% acetonitrile in 50 mM NH_4OAc, pH 4.5, over a period of 35 min at a flow rate of 3.5 mL/min (monitoring at 260 nm). The remaining reaction mixture was diluted to a total volume of 200 μL with 1:2 CH_3CN−50 mM NH_4OAc, pH 4.5, and purified using the same C_{18} reversed-phase column. After lyophilization of the appropriate fractions, compound **4b** (retention time 18.6 min) was obtained as a colorless solid: yield 1.7 mg (75%); ^{1}H NMR (D_2O) δ 1.39 (d, 3H, J = 7.0 Hz), 1.37 (d, 3H, J = 7.0 Hz), 3.65−3.75 (m, 1H), 4.00−4.10 (m, 2H), 4.20−4.25 (m, 1H), 4.40−4.45 (m, 1H), 4.60−4.65 (m, 2H), 6.03 (d, 1H, J = 5.5 Hz), 8.13 (s, 1H), and 8.41 (s, 1H); ^{13}C NMR (D_2O) δ 16.4, 23.3, 49.0, 64.4, 70.7, 74.7, 84.5, 87.2, 110.0, 118.8, 140.1, 149.2, 152.9, 155.7, 176.1, and 181.5; mass spectrum (ESI), m/z 490.1453 $(M + H)^+$ ($C_{16}H_{25}N_7O_9P$ requires 490.1451).

Mono-2′(3′)-*O*-alanyl-UMP (5a). To a conical vial containing 0.8 mg (1.7 μmol) of protected mono-2′(3′)-*N*-(4-pentenoyl)-(*S*)-alanyl-UMP (**3a**) was added 150 μL of a solution of I_2 in 15:85 THF−H_2O.[11,22] The reaction mixture was stirred at room temperature for 50 min. A 5-μL aliquot of the reaction mixture was diluted with 100 μL of 1:2 CH_3CN−50 mM NH_4OAc, pH 4.5, and was analyzed by HPLC using a semipreparative C_{18} reversed-phase column (250 × 10 mm). The column was washed with 0% → 63% acetonitrile in 50 mM NH_4OAc, pH 4.5, over a period of 35 min at a flow rate of 3.5 mL/min (monitoring at 260 nm). The remaining reaction mixture was diluted to a total volume of 200 μL with 1:2 CH_3CN−50 mM NH_4OAc, pH 4.5, and purified using the same C_{18} reversed-phase column. After lyophilization of the appropriate fractions, compound **5a** [retention times 16.5 and 17.5 min for the two positional (2′,3′) isomers] was obtained as a colorless solid: yield 0.65 mg (98%); ^{1}H NMR (D_2O) δ 1.31 (d, 3H J = 7.0 Hz), 3.60−3.65 (m, 1H), 3.85−4.00 (m, 2H), 4.10−4.25 (m, 3H), 5.79 (d, 1H, J = 8.0 Hz), 5.84 (d, 1H, J = 5.0 Hz), and 7.88 (d, 1H, J = 8.0 Hz); ^{13}C NMR (D_2O) δ 16.3, 50.6, 70.0, 74.1, 88.5, 102.7, 141.9, 152.0, 163.2, 166.5, 176.0, and 185.5; mass spectrum (MALDI-TOF), m/z 396.5 $(M + H)^+$ (theoretical m/z 396.1).

Bis-2′,3′-*O*-alanyl-UMP (5b). To a conical vial containing 3.4 mg (5.4 μmol) of protected 2′,3′-[*N*-(4-pentenoyl)-(*S*)-alanyl]-UMP (**3b**) was added 150 μL of a solution of I_2 in 15:85 THF−H_2O.[11,22] The reaction mixture was stirred at room temperature for 50 min. A 5-μL aliquot of the reaction mixture was diluted with 100 μL of 1:2 CH_3CN−50 mM NH_4OAc, pH 4.5, and was analyzed by HPLC using a semipreparative C_{18} reversed-phase column (250 × 10 mm). The column was washed with 0% → 63% acetonitrile in 50 mM NH_4OAc, pH 4.5, over a period of 35 min at a flow rate of 3.5 mL/min (monitoring at 260 nm). The remaining reaction mixture was diluted to a total volume of 200 μL with 1:2 CH_3CN−50 mM NH_4OAc, pH 4.5, and purified using the same C_{18} reversed-phase column. After lyophilization of the appropriate fractions, compound **5b** (retention time 20.2 min) was obtained as a colorless solid: yield 1.43 mg (57%); ^{1}H NMR (D_2O) δ 1.29 (d, 3H, J = 7.5 Hz), 1.37 (d, 3H, J = 7.0 Hz), 3.55−3.65 (m, 1H), 3.85−4.00 (m, 2H), 4.10−4.15 (m, 1H), 4.15−4.25 (m, 3H), 5.77 (d, 1H, J = 8.0 Hz), 5.82 (d, 1H, J = 5.0 Hz), and 7.83 (d, 1H, J = 8.0 Hz); ^{13}C NMR (D_2O) δ 16.3, 22.8, 50.6, 64.1, 70.0, 74.1, 83.6, 88.6, 102.7, 141.8, 152.0, 166.5, 169.8, 176.0, and 180.7; mass spectrum (MALDI-TOF), m/z 467.2 $(M + H)^+$ (theoretical 467.1).

General Procedure for the Treatment of Aminoacylated AMP (4a, 4b) and UMP (5a, 5b) Derivatives and Aminoacylated Dinucleotides (6a, 6b) with Nucleophiles. Compounds **4a**, **4b**, **5a**, **5b**, **6a**, and **6b** (0.2 mg) were each dissolved in 30-μL aliquots of water. To these stirred solutions was added 30 μL of DMF containing 2 equiv of *n*-butylamine or imidazole, and the corresponding reaction mixture was stirred for 2 h. After 15, 30, 60, and 120 min, 15 μL of each solution was analyzed by HPLC using a reversed-phase column (Alltima RPC_{18} 4.6 × 250 mm, 5 μm). The column was washed with 0% → 63% CH_3CN in 50 mM NH_4OAc, pH 4.5, over a period of 35 min at a flow rate of 1 mL/min (monitoring at 260 nm).

■ ASSOCIATED CONTENT

Supporting Information. PDB files of the computationally determined energetically favorable structures of a (phosphate-methylated) 5′-AMP derivative containing alanyl esters at the 2′- and 3′-positions. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author
sid.hecht@asu.edu

Present Addresses
[†]CNRS UMR6001 LCMBA, Université de Nice-Sophia Antipolis, ParcValrose, 06108 Nice Cedex 2, France.
[‡]Center for BioEnergetics, Biodesign Institute and Department of Chemistry and Biochemistry, Arizona State University, Tempe, Arizona 85287, United States.

■ REFERENCES

(1) (a) Crick, F. H. C. *Nature* **1970**, *227*, 561. (b) Green, R.; Noller, H. F. *Annu. Rev. Biochem.* **1997**, *66*, 679. (c) Rodnina, M. V.; Wintermeyer, W. *Curr. Opin. Struct. Biol.* **2003**, *13*, 334.

(2) (a) Hecht, S. M.; Kozarich, J. W.; Schmidt, F. J. *Proc. Natl. Acad. Sci. U. S. A.* **1974**, *71*, 4317. (b) Hecht, S. M. *Acc. Chem. Res.* **1977**, *10*, 239. (c) Wagner, T.; Cramer, F.; Sprinzl, M. *Biochemistry* **1982**, *21*, 1521. (d) Hansen, J. L.; Schmeing, T. M.; Moore, P. B.; Steitz, T. A. *Proc. Natl. Acad. Sci. U. S. A.* **2002**, *99*, 11670. (e) Sprinzl, M. *J. Biosci.* **2006**, *31*, 489. (f) Huang, K. S.; Weinger, J. S.; Butler, E. B.; Strobel, S. A.; Steitz, T. A. *J. Am. Chem. Soc.* **2006**, *128*, 3108.

(3) (a) Schmeing, T. M.; Huang, K. S.; Kitchen, D. E.; Strobel, S. A. *Mol. Cell* **2005**, *20*, 437. (b) Trobro, S.; Aqvist, J. *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102*, 12395.

(4) Koch, M.; Huang, Y.; Sprinzl, M. *Angew. Chem., Int. Ed.* **2008**, *47*, 7242.

(5) (a) Stepanov, V. G.; Moor, N. A.; Ankilova, V. N.; Lavrik, O. I. *FEBS Lett.* **1992**, *311*, 192. (b) Stepanov, V. G.; Moor, N. A.; Ankilova, V. N.; Vasil'eva, I. A.; Sukhanova, M. V.; Lavrik, O. I. *Biochim. Biophys. Acta* **1998**, *1386*, 1.

(6) (a) Wang, B.; Zhou, J.; Lodder, M.; Anderson, R. D.; Hecht, S. M. *J. Biol. Chem.* **2006**, *281*, 13865. (b) Duca, M.; Maloney, D.; Lodder, M.; Wang, B.; Hecht, S. M. *Bioorg. Med. Chem.* **2007**, *15*, 4629. (c) Duca, M.; Chen, S.; Hecht, S. M. *Methods* **2008**, *44*, 87.

(7) Duca, M.; Chen, S.; Hecht, S. M. *Org. Biomol. Chem.* **2008**, *6*, 3292.

(8) Noren, C. J.; Anthony-Cahill, S. J.; Suich, D. J.; Noren, K. A.; Griffith, M. C.; Schultz, P. G. *Nucleic Acids Res.* **1990**, *18*, 83.

(9) (a) Noren, C. J.; Anthony-Cahill, S. J.; Griffith, M. C.; Schultz, P. G. *Science* **1989**, *244*, 182. (b) Bain, J. D.; Diala, E. S.; Glabe, C. G.; Dix, T. A.; Chamberlin, A. R. *J. Am. Chem. Soc.* **1989**, *111*, 8013. (c) Hecht, S. M. *Acc. Chem. Res.* **1992**, *25*, 545. (d) Cornish, V. W.; Mendel, D.; Shultz, P. G. *Angew. Chem., Int. Ed.* **1995**, *34*, 621. (e) Mamaev, S. V.; Laikhter, A. L.; Arslan, T.; Hecht, S. M. *J. Am. Chem. Soc.* **1996**, *118*, 7243.

(10) Maloney, D. J.; Ghanem, N.; Zhou, J.; Hecht, S. M. *Org. Biomol. Chem.* **2007**, *5*, 3135.

(11) Lodder, M.; Golovine, S.; Laikhter, A. L.; Karginov, V. A.; Hecht, S. M. *J. Org. Chem.* **1998**, *63*, 794.

(12) Weinger, J. S.; Strobel, S. A. *Biochemistry* **2006**, *45*, 5939.

(13) Griffin, B. E.; Jarman, M.; Reese, C. B.; Sulston, J. E.; Trentham, D. R. *Biochemistry* **1966**, *5*, 3838.
(14) (a) Dorner, S.; Polacek, N.; Schulmeister, U.; Panuschka, C.; Barta, A. *Biochem. Soc. Trans.* **2002**, *30*, 1131. (b) Weinger, J. S.; Strobel, S. A. *Blood Cells Mol. Dis.* **2007**, *38*, 110.
(15) (a) Sprinzl, M.; Cramer, F. *Proc. Natl. Acad. Sci. U. S. A.* **1975**, *72*, 3049. (b) Chinault, A. C.; Tan, K. H.; Hassur, S. M.; Hecht, S. M. *Biochemistry* **1977**, *16*, 766.
(16) Weinger, J. S.; Parnell, K. M.; Dorner, S.; Green, R.; Strobel, S. A. *Nat. Struct. Mol. Biol.* **2004**, *11*, 1101.
(17) (a) Hecht, S. M.; Hawrelak, S. D.; Kozarich, J. W.; Schmidt, F. J.; Bock, R. M. *Biochem. Biophys. Res. Commun.* **1973**, *52*, 1341. (b) Hecht, S. M.; Chinault, A. C. *Proc. Natl. Acad. Sci. U. S. A.* **1976**, *73*, 405.
(18) Changalov, M. M.; Ivanova, G. D.; Rangelov, M. A.; Acharya, P.; Acharya, S.; Minakawa, N.; Foldesi, A.; Stoineva, I. B.; Yomtova, V. M.; Roussev, C. D.; Matsuda, A.; Chattopadhyaya, J.; Petkov, D. D. *ChemBioChem* **2005**, *6*, 992.
(19) Limmer, S.; Hofmann, H.-P.; Ott, G.; Sprinzl, M. *Proc. Natl. Acad. Sci. U. S. A.* **1993**, *90*, 6199.
(20) (a) Moor, N. A.; Stepanov, V. G.; Ankilova, V. N.; Favre, A.; Lavrik, O. I. *Biochemistry (Moscow)* **1998**, *63*, 1044. (b) Vasil'eva, I. A.; Ankilova, V. N.; Lavrik, O. I.; Moor, N. A. *Biochemistry (Moscow)* **2000**, *65*, 1157. (c) Vasil'eva, I. A.; Favre, A.; Lavrik, O. I.; Moor, N. A. *Biochemistry (Moscow)* **2004**, *69*, 192.
(21) LaRiviere, F. J.; Wolfson, A. D.; Uhlenbeck, O. C. *Science* **2001**, *294*, 165.
(22) Lodder, M.; Golovine, S.; Hecht, S. M. *J. Org. Chem.* **1997**, *62*, 778.