Synthesis and Properties of Aminoacylamido-AMP: Chemical Optimization for the Construction of an *N*-Acyl Phosphoramidate Linkage

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This paper describes the design and synthesis of a new type of aminoacyl-adenylate analogue (aa-AMPN) having an *N*-acyl phosphoramidate linkage where the oxygen atom of the mixed anhydride bond of aminoacyl-adenylate (aa-AMP) is replaced by an amino group. This new type of aa-AMP analogue is expected to be useful as material for studies on the recognition mechanism of the aminoacylation of tRNA and other biochemical reactions. The condensation of phosphoramidite derivatives of carboxamides with nucleoside derivatives failed, because the activated phosphoramidite derivatives reacted with not only the hydroxyl groups but also another reactive species. An alternative approach was examined by the reaction of 5′-*O*-phosphoramidite adenosine derivatives with carboxamide derivatives. The TBTr and TSE groups were chosen for protection of the amino group of amino acid amides and the phosphate group, respectively. Detailed studies revealed that the use of 5-(3,5-dinitrophenyl)-1*H*-tetrazole as an activating catalyst of phosphoramidites resulted in rapid condensation within 10 min to give fully protected aa-AMPN derivatives. No side reaction occurred. Deprotection of these products via a two-step procedure gave aa-AMPN derivatives in good yields. It also turned out that aa-AMPNs thus obtained are stable under both acidic and basic conditions, such as 0.1 M HCl (pH 1.0) and 0.1 M NaOH (pH 13.0).

Introduction

Some nucleotide antibiotics bearing a P−N bond at the 5′-hydroxyl of the adenosine derivatives have been found to date.[1−3] Phosmidosine is a new type of antifungal antibiotic, which was isolated from a culture filtrate of a newly isolated storeptomycete identified as *Streptomyces* sp.[1] This unique antibiotic exhibits specific inhibitory activity against spore formation of *Botytis cinerea*, a pathogenic fungus that causes a gray mold disease in a variety of fruits and vegetables. It was found by mass spectrometry and NMR spectroscopy that phosmidosine has a novel proline-containing nucleotide type antibiotic.[4] Moreover, it has an *N*-acyl phosphoramidate linkage that connects a nucleoside analogue, 8-oxoadenosine, with an L-proline residue (Figure 1).

On the other hand, Agrocin 84 is an adenine nucleotide antibiotic that controls crown gall disease biologically.[2] Its structure was determined by sequential degradation that showed an *N*-acyl phosphoramidate linkage at the 5′-hydroxyl moiety of the adenosine analogue. Dinogunellin is a toxic phospholipid found in the northern blenny roe, which also has an *N*-acyl phosphoramidate linkage at the 5′-hydroxyl of adenosine.[4] To synthesize such natural products having *N*-acyl phosphoramidate linkage,[5,6] the construction of the *N*-acyl phosphoramidate linkages seems to be a key step. We have previously reported the attempt for the synthesis of the natural products.[5,6]

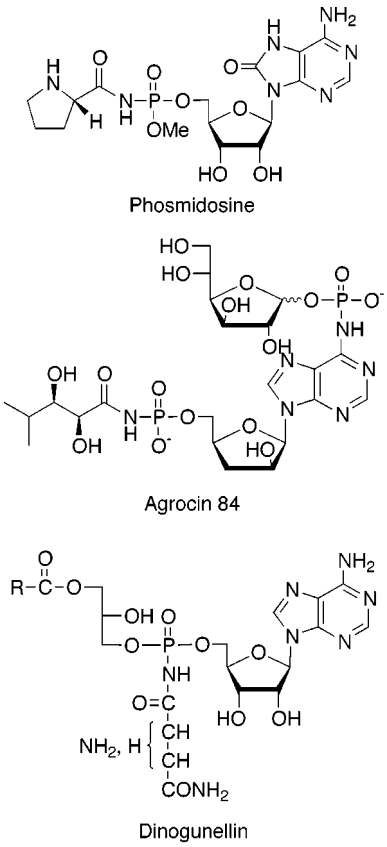

Figure 1. Natural products having *N*-acyl phosphoramidate linkages.

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It is well-known that aminoacyl-adenylates are very important intermediates in protein biosynthesis. The natural products have a mixed anhydride bond formed by condensation between the phosphate group of 5′-AMP and the carboxylic group of an amino acid. It was also found that the mixed anhydride bond is extremely unstable under aqueous conditions.[7] From a different point of view, natural products bearing the *N*-acyl phosphoramidate linkage described above are considered as the analogues of aminoacyl-adenylates (aa-AMP). Since the displacement of the oxygen atom of the mixed anhydride bond of aa-AMP with an NH group gives the *N*-acyl phosphoramidate derivatives, the synthesis of aa-AMP analogues having an *N*-acyl phosphoramidate linkage is important to understand the recognition mechanism of an amino acid of aa-AMP by the cognate aminoacyl-tRNA synthetase (ARS). The presence of a hydrogen bond network between the aa-AMP and the cognate ARS has been suggested by site-directed mutagenesis or crystal structure analysis.[8,9] In all cases, the oxygen atom of the mixed anhydride bond is not recognized by the polypeptide residue of the cognate ARS. Therefore, it is expected that substitution of this unrecognizable atom would not affect the enzyme recognition ability and would show highly specific enzyme inhibitory activity.

Various aa-AMP analogues have been reported as stable mimics of aa-AMP to date. Some of them were designed as inhibitors of the aminoacylation reaction of tRNA by ARSs.[10−12] Others were used for cocrystallization with ARSs or complexes of ARS-tRNA to elucidate the recognition mechanism of tRNAs and aa-AMPs by the cognate ARSs.[13,14] In all cases, however, the amino acid core structure or the mixed acid anhydride bond was changed to the stable but obviously structurally different substrates having aminoalkyl phosphoryl[10,11] and pyrophosphate[12] linkages. Since it is likely that the P−N bond of the *N*-acyl phosphoramidate linkage is stable under physiological conditions, the aa-AMP analogue bearing an *N*-acyl phosphoramidate linkage can be expected to have activity as a potential specific inhibitor in aminoacylation of tRNA.

To achieve the synthesis of such aa-AMP analogue, the construction of the *N*-acyl phosphoramidate linkage is a key step. Therefore, a general method for the synthesis of the *N*-acyl phosphoramidate linkage should be explored. In this paper, we report the synthesis and properties of a new type of aminoacyl-adenylate analogues which have an *N*-acyl phosphoramidate linkage.

Results and Discussion

Construction of *N*-Acylphosphoramidate Linkages via Carboxamide Phosphoramidite Derivatives. Synthesis of *N*-acyl phosphoramidate derivatives has been reported previously.[15−19] The synthetic strategies are classified into three types: The first involves C−N bond forming reaction by the N-acylation of phosphoramidates.[15,16] The second involves construction of the P−N bond by the reaction of a carboxamide anion species with phosphorochloridates.[17,18] The last example involves the Arbzov-type reaction of trialkyl phosphites with *N*-haloamides.[19] However, these methods require the use of strong bases, such as butyllithium, and are limited to the synthesis of only a few substrates. Recently, Grandas et al. have reported the synthesis of DNA-peptide hybrids connected through the *N*-acyl phosphoramidate linkage.[20] The naturally occurring DNA-peptide hybrid, which had been synthesized by several groups,[21−25] has a base-labile phosphodiester linkage, therefore the several problems happen to synthesize this hybrid. According to their paper, the construction of the *N*-acyl phosphoramidate linkage was achieved by the phosphoramidite method via condensation of carboxamides with nucleoside derivatives. Two synthetic strategies are available to construct the *N*-acylphosphoramidate linkage. One strategy is the condensation of phosphoramidite derivatives of carboxamide derivatives with nucleoside derivatives followed by oxidation. The other method is the condensation of nucleoside phosphoramidite derivatives with carboxamide derivatives.

To obtain the key intermediates **4a** and **4d** in one of the two approaches, the N-protected phenylalaninamide derivatives **3a** and **3d** were synthesized as starting materials (Scheme 1). The *N*-Fmoc phenylalaninamide **3a**[21] was synthesized by the amidation of the corresponding carboxylic acid derivative with NH_4HCO_3 in the presence of EEDQ. An attempt to synthesize the *N*-Tr

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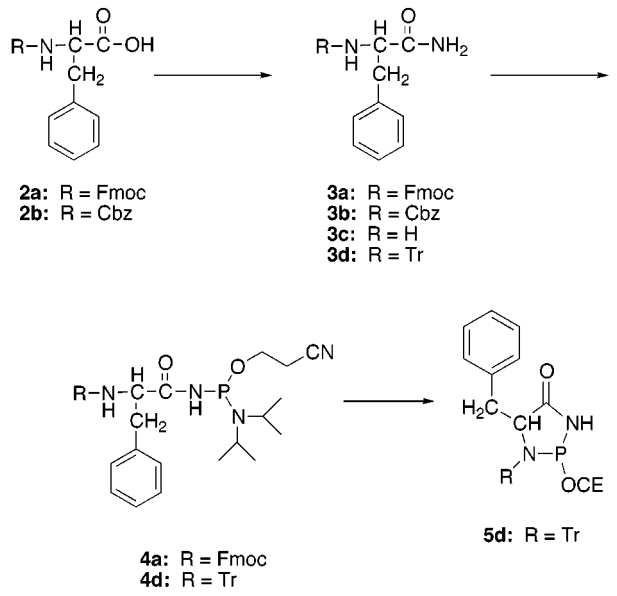
Scheme 1



derivative **3d** in a similar manner failed. In this reaction, the ethoxycarbonylated derivative was obtained as the main product. This amidation reaction requires an electron-withdrawing group for the amino protecting group.[26] Therefore, compound **3d** was prepared by hydrogenation of the *N*-Cbz phenylalaninamide **3b** followed by N-tritylation.[27,28]

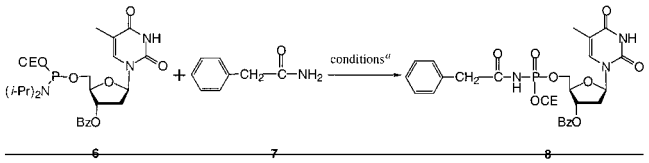
The *N*-carbamate-type derivative **3a** was phosphitylated by the reaction with 2-cyanoethyl *N*,*N*-diisopropylaminophosphorochloridite to give the phosphorodiamidite derivative **4a**. The formation of the phosphorodiamidite **4a** was confirmed by ^{1}H NMR and ^{31}P NMR (δ_P 118.2 ppm) after simple purification using reprecipitation from hexane. Next, reaction of compound **4a** was carried out with *N*-benzoyl-2′,3′-di-*O*-benzoyladenosine **9** in the presence of 1*H*-tetrazole. However, many products were found as suggested by ^{31}P NMR spectra.

The reaction of the *N*-Tr derivative **4d** with **9** in the presence of 1*H*-tetrazole gave a cyclized derivative **5d**. The structure of **5d** was determined by ^{1}H, ^{13}C, and ^{31}P NMR and FAB mass spectra. This compound resulted from the reaction of the activated phosphoramidite with the NH group of the *N*-Tr part of the same compound.

Recently, van Boom et al. have reported the synthesis of a partial structure of Agrocin 84, which has an *N*-acyl phosphoramidate linkage. They used the reaction of a carboxamide phosphoramidite derivative with 5′-OH of the adenosine derivative.[29] In this case, no intramolecular side-reaction occurred because an α-amino group was not present in the *N*-acyl phosphoramidate derivative.

Construction of *N*-Acylphosphoramidate Linkages via Nucleosides 5′-Phosphoramidite Derivatives. Next, we attempted to construct the *N*-acyl phosphoramidate linkage *by the reaction of carboxamides with nucleoside phosphoramidite derivatives*. The synthesis of the DNA-peptide hybrids having the *N*-acyl phosphoramidate linkage was previously.[20] In the paper, the *N*-acyl phosphoramidate linkage was constructed by the reaction of the nucleoside 3′-phosphoramidate derivatives with carboxamide derivatives in the presence of 1*H*-tetrazole. Previously, we reported the synthesis of nucleoside *N*-acylphosphoramidate derivatives by use of 5-(4-nitrophenyl)-1*H*-tetrazole[30] in place of 1*H*-tetrazole as an activator of nucleoside 5′-*O*-phosphoramidite derivatives.[31] However, the yields of the coupling reactions were around 40%. Therefore, to optimize the coupling reaction of phosphoramidite derivatives and carboxamide derivatives, a model reaction **6** with phenylacetamide (**7**) was studied in the presence of various types of activators. The reaction was monitored by ^{31}P NMR.

Table 1. Preparation of *N*-Acyl Phosphoramidate Linkage in the Presence of Various Activators



6 7 8

entry	activators	time (min)	yield of **8**[a] (%)
1	1*H*-tetrazole	30	17
2	5-(4-nitrophenyl)-1*H*-tetrazole	30	46
3	5-(3,5-dinitrophenyl)-1*H*-tetrazole	5	quant.
4	*N*-methylimidazolium triflate	30	78
5	*N*-methylimidazolium perchlorate	30	72
6	*o*-sulfobenzimide	30	22

[a] Conditions: (a) 0.15 mmol of **6** (82 mg), 0.1 mmol of **7** (14 mg), 0.3 mmol of activators in CH_3CN (2 mL) at room temperature; (b) 0.5 mmol of *t*-BuOOH (94 μL) for 5 min at room temperature. [b] All yields were estimated by ^{31}P NMR chemical shifts.

These results are summarized in Table 1. The activators tested have been used for the activation of less reactive phosphoramidite derivatives, such as phosphorothioamidite derivatives.[32] Most of these activators were unsatisfactory because the reaction did not complete. While 1*H*-tetrazole (entry 1), which is commonly used for the current oligonucleotides synthesis by the phosphoramidite method, required long reaction time, it was found that other tetrazole derivatives with an electron-withdrawing group at C-5 of tetrazole showed better yields. It seems that the once generated phosphorotetrazolide intermediates should have more efficient leaving ability on the tetrazole residues. 1*H*-Tetrazole has sufficient acidity ($pK_a = 4.8$)[33] for the activation of a phosphoramidite derivative. However, it is likely that the phosphorotetrazolide intermediate derived from **6** dose not have sufficient reactivity to effect new P−N bond formation. A more acidic tetrazole derivative, 5-(4-nitrophenyl)-1*H*-tetrazole ($pK_a = 3.8$)[34] resulted in an improved yield (46%). A more satisfactory result was obtained in the case of 5-(3,5-dinitrophenyl)-1*H*-tetrazole (entry 3).[35] The reaction proceeded within only 10 min to give the desired product quantitatively. Since the phosphite intermediate

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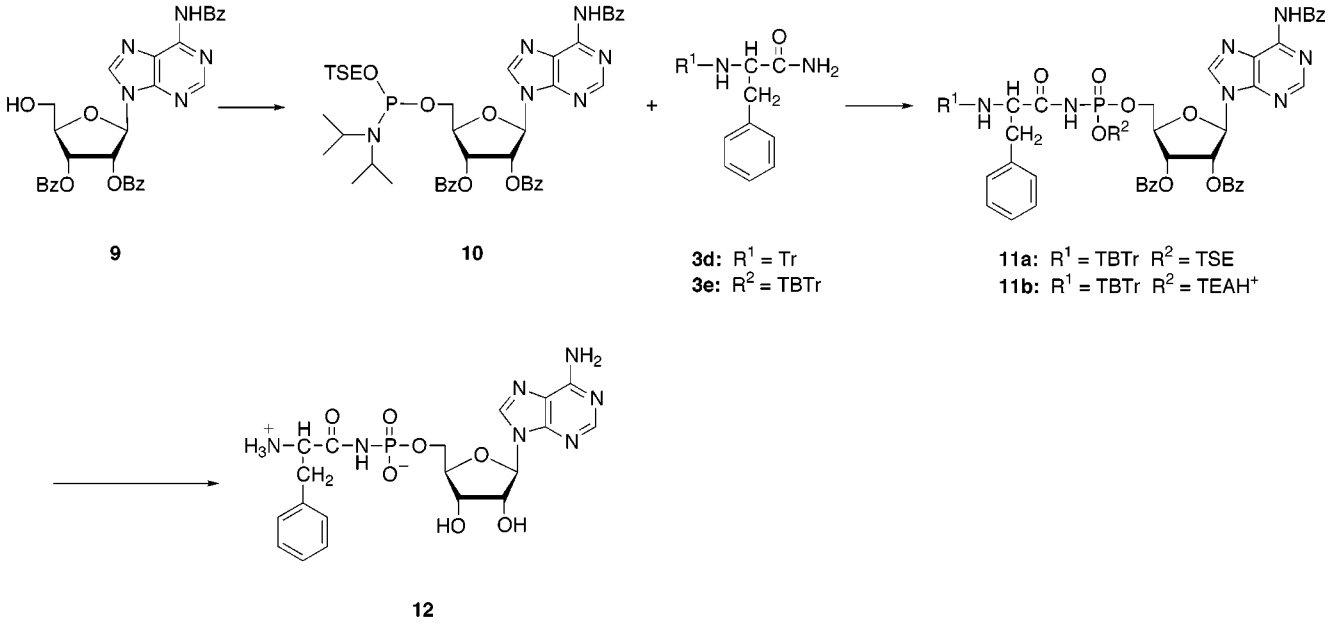
Scheme 2



is found to be also very unstable, prolonged reaction time promotes the hydrolysis of this intermediate. In contrast, *N*-methylimidazolium derivatives[36] (entries 4 and 5) reacted with the 5′-phosphoramidite derivative to form the phosphorimidazolide intermediate indicated in ^{31}P NMR (δ_P 130 ppm), and gave the desired product in a moderate yield. The other acid activators, such as *N*-methylanilinium salts or carboxylic acids, showed unsatisfactory results. Formation of the *N*-acyl phosphoramidate linkage requires sufficiently reactive azolide intermediates capable of substitution with poor nucleophilic carboxamide derivatives.

As far as side reactions are concerned, only the coupling efficiency was poor in the case of 1*H*-tetrazole and the reagents tested in this study except for 5-(3,5-dinitrophenyl)-1*H*-tetrazole and the competitive hydrolysis of the phosphoramidite derivatives was the main unavoidable side reaction. In summary, 5-(3,5-dinitrophenyl)-1*H*-tetrazole satisfies this requirement for the construction of the *N*-acyl phosphoramidate linkage.

Synthesis of Aminoacylamido-Adenylates (aa-AMPNs). Fully esterified neutral *N*-acyl phosphoramidate derivatives $R^1C(O)NH-P(O)(OR^2)OR^3$, such as compound **11a** in Scheme 2, are unstable and could not be purified by silica gel column chromatography. It was found that dissociated *N*-acyl phosphoramidates are more stable than the protected ones. Therefore, 2-(trimethylsilyl)ethyl (TSE)[37] was used as the phosphate protecting group, which can be removed selectively by treatment with Bu_4NF after construction of the P−N bond.

The 5′-*O*-phosphoramidite derivative **10** was prepared by the reaction of *N*-benzoyl-2′,3′-di-*O*-benzoyladenosine **9** with 2-(trimethylsilyl)ethyl *N*,*N*,*N*′,*N*′-tetraisopropyl phosphorodiamidite in the presence of diisopropylammonium tetrazolide.

The amino groups of the amino acid amide derivatives needed to be protected with a lipophilic group (conferring higher solubility in organic solvents), yet which could be easily removed under basic conditions. Accordingly, 4,4′,4″-tris(benzoyloxy)trityl (TBTr)[38,39] was selected to meet these criteria. The TBTr group has been previously used for protection of the primary hydroxyl group of deoxynucleosides[38] and for *exo*-amino protecting group of deoxyadenosine, deoxycytidine and deoxyguanosine in DNA[39] and RNA[40] synthesis.

The starting material, *N*-TBTr-L-phenylalaninamide was synthesized in 95% yield by the reaction of L-phenylalaninamide with TBTrBr in the presence of triethylamine at 60 °C. This *N*-TBTr derivative **3e** was allowed to react with the 5′-*O*-phosphoramidite derivative **10** in the presence of 1*H*-5-(3,5-dinitrophenyl)tetrazole. After oxidation of the phosphite intermediate with *tert*-butyl hydroperoxide, the selective removal of the TSE group by treatment with an equimolar mixture of TBAF·H_2O and acetic acid gave the desired *N*-acyl phosphoramidate derivative **11b** in 62% yield. Finally, all the protecting groups were removed by treatment with aqueous ammonia to give the desired product **12**. The aa-AMPN derivative **12** was purified by C-18 silica gel column chromatography, and its structure was determined by means of ^{1}H, ^{13}C, and ^{31}P NMR and MALDI-TOF mass.

Another amino acid derivative was also synthesized by a similar procedure. The *N*-TBTr amino acid amide derivatives were synthesized by the reaction of the corresponding *N*-unprotected amino acid amide derivatives, which were synthesized in 68% yield by amidation of *N*-Cbz amino acids followed by successive treatments with H_2 on Pd/C and TBTr. In the case of L-isoleucine and L-valine derivatives, the condensation of the *N*-TBTr derivatives **14i** and **14v** with **10**, followed by oxidation with *t*-BuOOH and removal of the TSE group gave the

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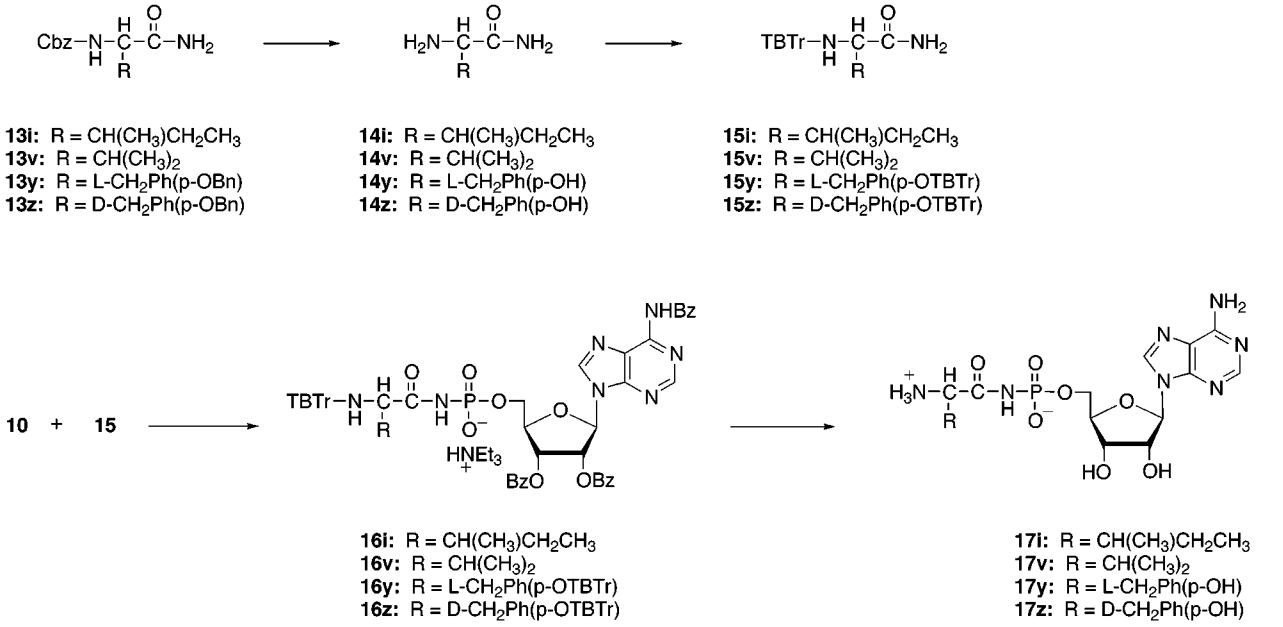
Scheme 3

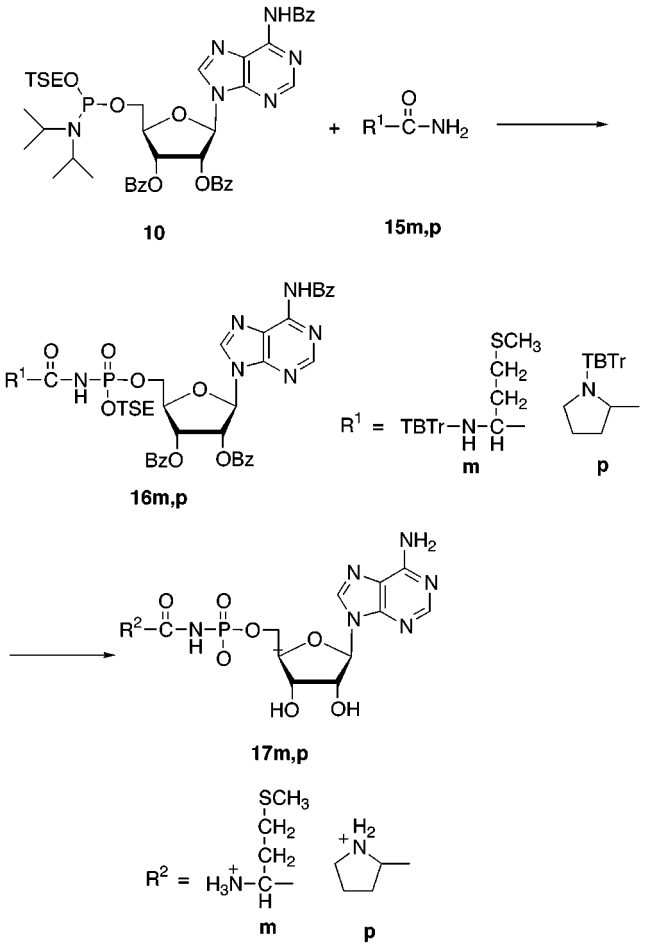


condensed products **16i** and **16v** in 57 and 83% yields, respectively (Scheme 3). All the protecting groups were removed by treatment with aqueous ammonia to give the desired products **17i** and **17v** in 48 and 77% yields, respectively. In the case of the L-methionine derivative, the Cbz group which protects the amino group could not be removed by the catalytic hydrogenolysis. Therefore, 9-(fluorenyl)methoxycarbonyl (Fmoc) was used to protect the amino group instead of the Cbz group. The N-unprotected carboxamide derivative **14m** was obtained by treatment with morpholine−DMF (1:1, v/v) and was crystallized by addition of an equimolar amount of acetic acid to give a white crystalline acetate salt. The final product **17m** was obtained by the same procedure as described in the case of the L-phenylalanine derivative.

The L-proline derivative, an analogue of phosmidosine, was also synthesized by the same procedure. The coupling reaction of **10** with *N*-TBTr prolinamide (**15p**) gave the fully protected derivative **16p** in 76% yield. The following deprotection successfully gave the L-proline derivative in 65% yield (Scheme 4).

The functional groups on the side chain of amino acids should be protected at the same time. For the synthesis of tyrosine derivatives, both the L- and D-forms of which are expected to have interesting properties, the phenolic OH group of the tyrosine side chain must be protected with an appropriate protecting group. Thus, the fully unprotected tyrosinamide derivatives **14y** and **14z** were synthesized from N,O-protected tyrosine derivatives. Simultaneous protection of the amino and phenolic groups of the unprotected tyrosinamide derivatives **14y** and **14z** were attempted by the reaction with 2.5 equiv of TBTrBr. However, these reactions gave predominantly the mono-TBTr derivatives. Satisfactory results were obtained by the use of 4.0 equiv of TBTrBr and prolonged reaction time. Under these conditions, the bis-TBTr derivatives **15y** and **15z** were obtained as the main products. The TBTr acetate ester derivative was formed when the *acetate salts* of amino acid amide derivatives were used as the starting materials. This acetate ester species did not react with the phenolic OH group of the tyrosine side chain residue, but reacted with the amino

Scheme 4



group. Therefore, an excess amount of TBTrBr to fully protect the side chain of tyrosine derivatives is required. Condensation of the *N*,*O*-bis-TBTr derivatives **15y** and **15z** with **10** followed by the oxidation and the selective removal of the TSE group gave the coupled products **16y** and **16z** in 60 and 61% yields, respectively. All the protecting groups were removed by treatment with

aqueous ammonia to give the desired products **17y** and **17z** in 55 and 35% yields, respectively.

It seems possible to synthesize aa-AMPNs of all amino acid derivatives despite natural or non-natural amino acids, if their side chain functional groups can be masked with appropriate protecting groups that can be removed without damage the aminoacylamido linkage.

Chemical Properties of aa-AMPN Derivatives. It is known that the phosphoric acid derivatives (RC(O)-NH−P(O)$(OH)_2$) which have *N*-acyl phosphoramidate linkages are extremely unstable even at 0 °C and are difficult to store even for a few days.[18] Because the carboxyl amide residues act as good leaving groups, these compounds decompose via a monomeric metaphosphate derivative, which rapidly reacts with water to give orthophosphate, even during storage at 0 °C. Moreover, *N*-acyl phosphoramidate diester derivatives ($R^1C(O)NH-P(O)(OR^2)(OR^3)$) such as phosmidosine[1] are stable under weakly acidic conditions. However, it was also reported that the basic conditions cause decrease of the antifugal activity of phosmidosine. Grandas et al. also reported that 2-cyanoethyl 5′-*O*-(dimethoxytrityl)thymidine-3′-yl *N*-(phenylacetyl)phosphoramidate having the structure of $R^1C(O)NH-P(O)(OR^2)(OR^3)$ is fairly unstable. On the other hand, it was previously reported that *N*-acyl phosphoramidate monoester derivatives ($R^1C(O)NH-P(O)(OR^2)$ (OH)) are stable under the basic conditions prescribed for removal of the 2-cyanoethyl and acyl protecting groups, or under acidic conditions using 80% AcOH prescribed for removal of the DMTr group.[20] Therefore, the stability of aa-AMPN derivative **12** under acidic and basic conditions was reexamined. As the result, this phenylalanine derivative **12** was found to be stable to acidic conditions of 0.1 M HCl (pH 1.0) for 24 h. Moreover, treatment of **12** with 0.1 M NaOH (pH 13) did not affect the *N*-acyl phosphoramidate monoester derivative for 24 h.

Conclusion

Aminoacyl-adenylates (aa-AMPs) are very important intermediates in protein biosynthesis, and the recognition mechanism between an amino acid and the cognate aminoacyl-tRNA synthetase (ARS) is not clear in this field. The lability of aa-AMPs in aqueous solution causes difficulty in analysis of the aminoacyl transfer reaction. The results above-mentioned show that replacement of the oxygen atom of aa-AMP by the amino group greatly stabilized the extremely unstable mixed anhydride bond between the phosphate of 5′-AMP and the carboxylate of an amino acid. The above-mentioned specific properties of this type aa-AMP analogue would be useful for studies of the mechanism of the aminoacylation reaction of tRNA as well as the amino acid specific inhibitory activity in the process of the protein biosynthesis in an amino acid-specific manner. Therefore, the most simplified analogues which we proposed here would provide a powerful tool for the search of diverse biological reactions. The attempt for the chemical synthesis of the natural products having the *N*-acyl phosphoramidate linkages is now under investigation.[5,6]

Experimental Section

General Procedures. CH_2Cl_2 and MeCN were distilled from CaH_2 after being refluxed for several hours, and stored over molecular sieves 4A. Triethylamine was distilled from CaH_2 after being refluxed for several hours, and stored over CaH_2. Pyridine was distilled after being refluxed over *p*-toluenesulfonyl chloride for several hours, redistilled from CaH_2, and stored over molecular sieves 4A. 2-(Trimethylsilyl)-ethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite was synthesized by the method reported previously.[37] 4,4′,4″-Tris-(benzoyloxy)trityl bromide (TBTrBr) was synthesized by the method reported previously.[38] ^{1}H NMR spectra were obtained at 270 MHz with tetramethylsilane (TMS) as an internal standard in $CDCl_3$ and with sodium 3-(trimethylsilyl)propionesulfonate (DSS) as an external standard in D_2O. ^{13}C NMR spectra were obtained at 67.8 MHz with TMS as an internal standard and with DSS as an external standard in D_2O. ^{31}P NMR spectra were obtained at 109.25 MHz using 85% H_3PO_4 as an external standard. MALDI-TOF mass spectra were obtained in the positive ion mode. The MALDI matrix used was α-cyano-4-hydroxycinnamic acid (α-CHCA, 10 mg/mL, 1:1, H_2O−MeCN, (v/v)). The calibration was performed with α-CHCA ([M + H] = 190.050) and its dimer ([M + H] = 379.093) as internal standards.

***N*-(9-Fluorenylmethoxycarbonyl)-L-phenylalaninamide (3a).** To a solution of *N*-(9-fluorenylmethoxycarbonyl)-L-phenylalanine (6.5 g, 20.0 mmol) in MeCN (200 mL) were added EEDQ (4.6 g, 22.0 mmol) and NH_4HCO_3 (4.0 g, 60.0 mmol), and the mixture was stirred at room temperature for 18 h. The precipitate was removed by filtration, and was washed successively by H_2O, hexane, and $CHCl_3$. The filtrate and washings were combined and washed twice with 5% $NaHCO_3$. The organic layer was collected, dried over Na_2SO_4, filtered, and concentrated to dryness under reduced pressure. The residue was applied to a silica gel column with hexane−$CHCl_3$ (1:9, v/v) to give **3a** (3.34 g, 51%) as a white solid: ^{1}H NMR (DMSO-d_6) δ 2.79 (1H, dd), 3.03 (1H, dd), 4.15 (4H, m), 7.05−7.88 (14H, m); ^{13}C NMR (DMSO-d_6) δ 37.43, 46.51, 55.95, 65.52, 119.91, 125.14, 126.90, 127.89, 129.09, 138.20, 140.56, 143.65, 155.67, 173.30. Anal. Calcd for $C_{24}H_{22}N_2O_3$: C, 74.59; H, 5.74; N, 7.25. Found: C, 75.20; H, 5.70; N, 7.23.

L-Phenylalaninamide (3c). To a solution of *N*-benzyloxycarbonyl-L-phenylalaninamide (1.82 g, 1.7 mmol) in MeOH (200 mL) was added 10% Pd/C (700 mg), and the suspension was vigorously stirred under hydrogen atmosphere at room temperature for 3 h. The reaction mixture was filtered by use of Celite, and the filtrate was concentrated to dryness under reduced pressure. Recrystalization of the residue from ethyl acetate−EtOH (1:1, v/v) gave **3c** (1.0 g, quant) as white crystals: ^{1}H NMR (DMSO-d_6) δ 2.82 (1H, dd, J = 7.6 Hz, 13.5 Hz), 3.02 (1H, dd, J = 5.9 Hz, 13.5 Hz), 3.66 (1H, dd, J = 5.9 Hz, 7.6 Hz), 7.19−7.32 (6H, m), 7.73 (1H, br); ^{13}C NMR (DMSO-d_6) δ 39.02, 54.86, 126.56, 128.29, 129.49, 137.16, 173.41.

***N*-Trityl-L-phenylalaninamide (3d).** To a solution of *N*-Benzyloxycarbonyl-L-phenylalaninamide (1.82 g, 1.7 mmol) in MeOH (200 mL) was added 10% Pd/C (700 mg), and the suspension was vigorously stirred under hydrogen atmosphere at room temperature for 3 h. The reaction mixture was filtered by use of Celite, and the filtrate was concentrated to a small volume under reduced pressure. The residue was dried by repeated coevaporation with dry pyridine and dry toluene and finally dissolved in CH_2Cl_2 (60 mL). To the mixture were added trityl chloride (1.8 g, 6.6 mmol) and triethylamine (1.0 mL, 6.6 mmol), and the mixture was stirred at room temperature for 6 h. The reaction mixture was concentrated to a small volume under reduced pressure. The residue was diluted with $CHCl_3$ and washed twice with 5% $NaHCO_3$. The organic layer was dried over Na_2SO_4, filtered, and concentrated to dryness under reduced pressure. The residue was applied to a silica gel column with hexane−ethyl acetate (7:3, v/v) to give **3d** (2.4 g, 99%) as a colorless foam: ^{1}H NMR ($CDCl_3$) δ 2.32 (1H, dd, J = 13.5 Hz), 2.93 (1H, dd, J = 13.5 Hz), 3.44 (1H, m), 5.03 (1H, br), 6.05 (1H, br), 7.05−7.38 (20H, m); ^{13}C NMR ($CDCl_3$) δ 41.06, 59.03, 71.61, 126.77, 126.88, 128.01, 128.54, 128.9, 128.9, 129.9, 136.87,145.68, 177.30. Anal. Calcd for $C_{28}H_{26}N_2O$: C, 82.73; H, 6.45; N, 6.89. Found: C, 82.94; H, 6.66; N, 6.86.

***N*-[4,4′,4″-Tris(benzoyloxy)trityl]-L-phenylalaninamide (3e).** L-Phenylalaninamide (**3c**) (96 mg, 0.60 mmol) was dried by repeated coevaporation with dry pyridine and finally

dissolved in dry pyridine (6 mL). To this solution were added 4,4′,4″-tris(benzoyloxy)trityl bromide (492 mg, 0.72 mmol) and triethylamine (201 μL, 1.44 mmol), and the mixture was stirred at 60 °C for 2 h. The reaction mixture was concentrated to a small volume under reduced pressure, diluted with $CHCl_3$ and washed twice with 5% $NaHCO_3$. The organic layer was dried over Na_2SO_4, filtered, and concentrated to dryness under reduced pressure. The residue was applied to a silica gel column with hexane−ethyl acetate (7:3, v/v) to give **3e** (428 mg, 95%) as a yellow foam: ^{1}H NMR ($CDCl_3$) δ 2.78 (1H, dd, J = 13.3 Hz), 3.05 (1H, m), 3.41 (1H, m), 5.10 (1H, br), 5.46 (1H, br), 7.11−8.21 (32H, m); ^{13}C NMR ($CDCl_3$) δ 41.40, 59.41, 70.53, 121.24, 126.88, 128.52, 128.60, 129.31, 129.52, 129.83, 130.01, 130.09, 133.59, 137.29, 143.05, 149.61, 165.09, 177.80. Anal. Calcd for $C_{49}H_{38}N_2O_7$: C, 76.75; H, 4.99; N, 3.65. Found: C, 76.30; H, 5.10; N, 3.46.

(*N*,*N*-Diisopropylamino)(2-cyanoethyl)[*N*-(9-fluorenylmethoxycarbonyl)-L-phenylalaninamide]phosphine (4a). *N*-(9-fluorenylmethoxycarbonyl)-L-phenylalaninamide (**3a**) (369 mg, 0.96 mmol) was dried by repeated coevaporation with dry pyridine and dry toluene, and finally dissolved in CH_2Cl_2 (4 mL). To the mixture were added 2-cyanoethyl *N*,*N*-diisopropylphosphorochloridite (415 μL, 2.0 mmol) and *N*,*N*-diisopropylethylamine (522 μL, 3.0 mmol), and the mixture was stirred at room temperature for 45 min. The reaction mixture was diluted with $CHCl_3$ and washed three times with 5% $NaHCO_3$. The organic layer was dried over Na_2SO_4, filtered, and concentrated to dryness under reduced pressure. The residue was purified by reprecipitation of its $CHCl_3$ solution from hexane−ether (85:15, v/v) to give **4a** (241 mg, 43%) as a white powder: ^{31}P NMR ($CDCl_3$) δ 118.18; ^{1}H NMR ($CDCl_3$) δ 1.16 (6H, s), 1.19 (6H, s), 2.59 (2H, t), 3.09 (2H, m), 3.45 (2H, m), 3.86 (2H, m), 4.18−4.41 (4H, m), 7.05−7.88 (15H, m); MS-(FAB+) *m*/*z* calcd for $C_{33}H_{39}N_4O_4P$ 586, obsd 587 (M + H).

(*N*,*N*-Diisopropylamino)(2-cyanoethyl)(*N*-trityl-L-phenylalaninamide)phosphine (4d). *N*-Trityl-L-phenylalaninamide (**3d**) (121 mg, 0.30 mmol) was dried by repeated coevaporation with dry pyridine and dry toluene, and finally dissolved in CH_2Cl_2 (3 mL). To the mixture were added 2-cyanoethyl *N*,*N*-diisopropylphosphorochloridite (120 μL, 0.6 mmol) and *N*,*N*-diisopropylethylamine (150 μL, 0.9 mmol), and the mixture was stirred at room temperature for 5 min. The reaction mixture was diluted with $CHCl_3$ and washed three times with 5% $NaHCO_3$. The organic layer was dried over Na_2SO_4, filtered, and concentrated to dryness. The residue was dissolved in hexane, and washed three times with pyridine−H_2O (20 mL, 1:1, (v/v)) to give **4d** (174 mg, 97%) as a white powder: ^{31}P NMR ($CDCl_3$) δ 117.41, 119.23; ^{1}H NMR ($CDCl_3$) δ 1.04−1.21 (12H, 2s), 1.97, 2.26 (1H, dd), 2.58 (2H, m), 2.93 (1H, m), 3.30 (1H, m), 3.47 (2H, m), 3.88 (2H, m), 7.03−7.35 (20H, m); ^{13}C NMR ($CDCl_3$) δ 20.40, 20.50, 24.4, 39.10, 39.91, 44.57, 44.67, 44.76, 44.85, 58.91, 59.48, 60.06, 60.49, 71.68, 117.73, 123.63, 125.87, 126.76, 126.81, 127.03, 127.08, 127.13, 127.48, 127.51, 127.66, 127.78, 127.94, 128.03, 128.18, 128.23, 128.46, 128.53, 128.63, 128.72, 129.78, 129.94, 130.10, 136.17, 136.53, 145.50, 177.84, 178.00.

Cyclized product (5): ^{31}P NMR ($CDCl_3$) δ 126.42; ^{1}H NMR ($CDCl_3$) δ 2.05 (1H, dd), 2.19 (2H, t), 2.92 (1H, dd, J = 13.8 Hz), 3.30 (2H, m), 4.10 (1H, m), 6.96−7.45 (20H, m); ^{13}C NMR ($CDCl_3$) δ 20.45, 39.28, 59.87, 60.27 (d, J = 26.9 Hz), 60.29, 60.40, 71.59, 117.61, 126.11, 126.76, 126.84, 127.60, 127.81, 127.94, 127.99, 128.57, 128.79, 129.88, 130.53, 130.62, 160.67, 130.67, 130.97, 137.23, 143.20, 143.25, 145.66, 180.20, 180.30.

^{31}P NMR Study of the Reaction of Thymidine-5′-phosphoramidite Derivative (6) with Phenylacetamide (7). 3′-*O*-Benzoylthymidine-5′-*O*-(2-cyanoethyl *N*,*N*-diisopropylphosphoramidite) (**6**) (82 mg, 0.15 mmol) and phenylacetamide (**7**) (14 mg, 0.10 mmol) were dried by repeated coevaporation three times with dry pyridine and twice with dry toluene and dissolved in dry CH_3CN (2.0 mL). To this solution was added a condensing reagent (0.3 mmol) which was separately dried by repeated coevaporation three times with dry pyridine and twice with dry toluene, and these solutions were vigorously stirred at room temperature. Next, *tert*-butyl hydroperoxide was added to the reaction mixture, and this mixture was stirred for 5 min. The mixture was diluted with $CHCl_3$, and washed three times with 5% $NaHCO_3$. The organic layer was dried over Na_2SO_4, filtered, and concentrated to dryness under reduced pressure. The residue was dissolved with $CDCl_3$ (500 μL). The products were determined by ^{31}P NMR, and the yield of the product **8** was estimated on the basis of the integration of the signals.

***N*-Benzoyl-2′,3′-di-*O*-benzoyladenosine-5′-*O*-[2-(trimethylsilyl)ethyl *N*,*N*-diisopropylphosphoramidite] (10).** *N*-Benzoyl-2′,3′-di-*O*-benzoyladenosine (**9**) (2.89 g, 5.0 mmol) was dried by repeated coevaporation with dry pyridine and dry toluene and finally dissolved in dry CH_2Cl_2 (50 mL). To this solution were added 2-(trimethylsilyl)ethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite (2.0 mL, 6.0 mmol) and diisopropylammonium tetrazolide (420 mg, 2.5 mmol), and the mixture was stirred at room temperature for 8 h. The reaction mixture was concentrated to a small volume, diluted with $CHCl_3$, and washed three times with 5% $NaHCO_3$. The organic layer was dried over Na_2SO_4, filtered, and concentrated to dryness under reduced pressure. The residue was applied to a silica gel column, and elution was performed with hexane−ethyl acetate (5:3, v/v) containing 0.5% triethylamine. The fractions containing **10** were combined and concentrated to give **10** (3.3 g, 80%) as a colorless foam: ^{31}P NMR ($CDCl_3$) δ 147.27, 147.60; ^{1}H NMR ($CDCl_3$) δ 0.02 (9H, br), 1.04−1.26 (14H, m), 3.63−4.04 (6H, m), 4.65 (1H, m), 5.95−6.00 (1H, m), 6.10−6.21 (1H, m), 6.70−6.75 (1H, m), 7.15−8.06 (15H, m), 8.74 (1H, s), 8.83 (1H, br), 9.07 (1H, br); ^{13}C NMR ($CDCl_3$) δ 19.7, 24.3, 42.5, 60.72, 61.03, 61.33, 62.23, 62.46, 63.55, 62.80, 72.58, 72.80, 74.47, 74.61, 83.38, 83.52, 83.60, 83.72, 84.99, 85.14, 122.92, 122.97, 127.47, 127.79, 127.92, 128.12, 128.35, 128.50, 128.55, 128.59, 128.75, 129.00, 129.29, 129.61, 132.06, 133.12, 133.15, 133.29, 141.22, 141.44, 149.24, 149.40, 151.66, 151.84, 152.26, 164.31, 164.37, 164.49, 164.69, 164.89. Anal. Calcd for $C_{42}H_{51}N_6O_8PSi$: C, 61.00; H, 6.22; N, 10.16. Found: C, 60.62; H, 6.27; N, 9.85.

Triethylammonium *O*-(*N*-Benzoyl-2′,3′-di-*O*-benzoyladenosine-5′-*O*-yl)-*N*-[*N*-[4,4′,4″-Tris(benzoyloxy)trityl]-L-phenylalanine]phosphoramidate (11b). *N*-(4,4′,4″-Tris(benzoyloxy)trityl)-L-phenylalaninamide (**3e**) (153 mg, 0.2 mmol) and **10** (248 g, 0.3 mmol) were dried by repeated coevaporation with dry pyridine and dry toluene, and this mixture was dissolved in dry MeCN (4 mL). The solution was added to 5-(3,5-dinitrophenyl)-1*H*-tetrazole (142 mg, 0.6 mmol), which was dried by repeated coevaporation with dry pyridine and dry toluene. After being stirred at room temperature for 30 min, the mixture was added *tert*-butyl hydroperoxide (125 μL, 1.0 mmol) and stirred at room temperature for 10 min. The reaction mixture was diluted with $CHCl_3$ and washed three times with 5% $NaHCO_3$. The organic layer was dried over Na_2SO_4, filtered and concentrated to dryness under reduced pressure. The residue was dissolved in dry THF (10 mL), and tetrabutylammonium fluoride monohydrate (157 mg, 0.6 mmol) and AcOH (34 μL, 0.6 mmol) were added to this mixture. After being stirred at room temperature for 20 h, the reaction mixture was concentrated to a small volume and diluted with $CHCl_3$. The $CHCl_3$ solution was washed three times with 0.5 M triethylammonium hydrogen carbonate, dried over Na_2SO_4, filtered, and concentrated to dryness under reduced pressure. The residue was applied to a silica gel column, and elution was performed with $CHCl_3$−MeOH (99:1−98:2, v/v) containing 1% triethylamine. The fractions containing **11b** were combined and concentrated under reduces pressure to give **11b** (232 mg, 77%) as a yellowish foam: ^{31}P NMR ($CDCl_3$) δ −7.10; ^{1}H NMR ($CDCl_3$) δ 1.28 (9H, t, J = 7.3 Hz), 2.08 (1H, m), 2.54 (1H, m), 3.05 (6H, q, J = 7.3 Hz), 3.59 (1H, m), 4.25 (2H, m), 4.72 (1H, m), 6.07 (1H, m), 6.16 (1H, dd), 6.71 (1H, d J = 6.6 Hz), 7.06−8.16 (47H, m), 8.78 (1H, s), 9.11 (1H, s); ^{13}C NMR ($CDCl_3$) δ 8.63, 39.05, 45.50, 59.25, 65.04 (d, J = 4.7 Hz), 71.03, 72.80, 75.14, 83.28 (d, J = 7.6 Hz), 85.12, 120.81, 122.62, 125.14, 126.85, 127.73, 127.94, 128.07, 128.20, 128.27, 128.39, 128.57, 128.68, 128.87, 129.25, 129.47, 129.63, 129.67, 129.76, 129.97, 130.35, 130.55, 132.53, 133.44, 133.69, 135.94, 142.71, 142.84, 149.20, 149.52, 151.90, 152.49, 164.61, 164.69, 164.82, 165.12, 176.53. Anal. Calcd for $C_{86}H_{77}N_8O_{16}P\cdot H_2O$: C, 67.62; H, 5.21; N, 7.34. Found: C, 67.66; H, 5.42; N,

7.24; MS(FAB) *m*/*z* calcd for $C_{86}H_{77}N_8O_7P$ 1508.52, obsd 1406 (M − TEA − H), 1409 (M − TEA + H).

***O*-(Adenosine-5′-*O*-yl) *N*-(L-phenylalanyl)phosphoramidate (12).** Triethylammonium *O*-(*N*-benzoyl-2′,3′-di-*O*-benzoyladenosine-5′-*O*-yl)-*N*-[*N*-(4,4′,4″-tris(benzoyloxy)trityl)-L-phenylalanyl] phosphoramidate (**11b**) (2.24 g, 1.48 mmol) was treated with concentrated NH_3−dioxane (24 mL, 1:1, (v/v)) at room temperature for 8 h. The mixture was evaporated under reduced pressure, and residue was dissolved in water. The aqueous solution was washed five times with ether and concentrated to a small volume. The residue was applied to a C18 reversed-phase column, and elution was performed with water, applying a gradient of MeCN (0−10%). The fractions containing **12** were combined and lyophilized to give **12** (52%) as a white powder: ^{31}P NMR (D_2O) δ −5.37; ^{1}H NMR (D_2O) δ 2.74−2.82 (1H, m), 2.92 (1H, dd, J = 6.3 Hz, J = 13.9 Hz), 3.96 (1H, br). 4.04 (2H, m), 4.32 (1H, m), 4.39 (1H dd, J = 5.3 Hz, 4.0 Hz), 4.69 (1H, dd, J = 5.6 Hz, 5.3 Hz), 6.03 (1H, d, J = 5.6 Hz), 7.02−7.16 (5H, m), 8.11 (1H, s), 8.35 (1H, s); ^{13}C NMR (D_2O) δ 38.95, 57.33 (d, J = 9.6 Hz), 67.85, 72.82, 76.73, 86.12 (d, J = 9.8 Hz), 89.43 120.98, 129.90, 130.19, 131.62, 142.44, 151.37, 155.27, 157.88, 183.99; MALDI−TOF mass *m*/*z* calcd for $C_{19}H_{25}N_7O_7P$ 494.2, obsd (M + H) 494.2.

General Procedure for the Amidation of Amino Acid Derivatives (13). The reaction was carried out according to the procedure previously reported by Nozaki et al.[18]

To a solution of N-protected amino acid (10 mmol) in MeCN (50 mL) were added EEDQ (2.72 g, 11 mmol) and NH_4HCO_3 (2.37 g, 30 mmol), and the mixture was stirred at room temperature for 15 h. The precipitate was filtered, and this precipitate was washed by H_2O and Et_2O to give **13** as a white solid.

***N*-Benzyloxycarbonyl-L-isoleucinamide (13i).** Compound **13i** was synthesized by the general procedure to give 2.46 g (89% yield) as a white solid.

***N*-(9-Fluorenylmethoxycarbonyl)-L-methioninamide (13m).** Compound **13m** was synthesized by the general procedure to give 6.35 g (86% yield) as a white solid.

***N*-(9-Fluorenylmethoxycarbonyl)-L-prolinamide (13p).** Compound **13p** was synthesized by the general procedure to give 4.40 g (88% yield) as a white solid.

***O*-Benzyl-*N*-benzyloxycarbonyl-L-tyrosinamide (13y).** Compound **13y** was synthesized by the general procedure to give 4.22 g (85% yield) as a white solid.

***O*-Benzyl-*N*-benzyloxycarbonyl-D-tyrosinamide (13z).** Compound **13z** was synthesized by the general procedure to give 4.22 g (85% yield) as a white solid.

General Procedure for the Synthesis of N-Unprotected Amino Acid Amide (14). Procedure A. To a solution of *N*-benzyloxycarbonyl amino acid amide (**13**) in AcOH was added 10% Pd/C. The mixture was stirred under hydrogen atmosphere at room temperature for 1.5 h. The mixture was filtered by use of Celite, concentrated to dryness, coevaporated by toluene, and recrystallized from ethyl acetate to give **14** as white crystals.

Procedure B. *N*-(9-Fluorenylmethoxycarbonyl) amino acid amide (**13**) was treated with morpholine−DMF (1:1, (v/v)) for 1 h. To this mixture was added H_2O. The resulting precipitate was filtered, and washed with H_2O. This filtrate was diluted with H_2O, and washed three times with ether, and back-extracted with H_2O. The aqueous layers were combined, and concetrated to dryness. The residue was crysrallized by the addition of a small amount of AcOH, and recrystallized from ethyl acetate to give **14** as white crystals.

L-Isoleucinamide Acetate (14i). Compound **14i** was synthesized by the general procedure A to give 1.15 g (85% yield) as a white.

L-Methioninamide Acetate (14m). Compound **14m** was synthesized by the general procedure B to give 1.57 g (70% yield) as a white.

L-Prolinamide (14p). Compound **14p** was synthesized by the general procedure B to give 991 mg (73% yield) as a white.

L-Valinamide Acetate (14v). Compound **14v** was synthesized by the general procedure A to give 1.0 g (quant) as white crystals.

L-Tyrosinamide Acetate (14y). Compound **14y** was synthesized by the general procedure A to give 457 mg (63%) as white crystals.

D-Tyrosinamide Acetate (14z). Compound **14z** was synthesized by the general procedure A to give 457 mg (63%) as white crystals.

General Procedure for the Synthesis of *N*-TBTr Amino Acid Amide Derivatives (15). The amino acid amide derivative **14** (2 mmol) was dried by repeated coevaporation with dry pyridine and finally dissolved in dry pyridine (20 mL). To this solution were added 4,4′,4″-tris(benzoyloxy)trityl bromide (1.64 g, 2.4 mmol) and triethylamine (697 μL, 5.0 mmol), and the mixture was concentrated to a small volume, diluted with $CHCl_3$ and washed twice with 5% $NaHCO_3$. The organic layer was dried over Na_2SO_4, filtered, and concentrated to dryness under reduced pressure. The residue was applied to a silica gel column, and elution was performed with hexane−ethyl acetate. The fractions containing **15** were combined and concentrated under reduced pressure to give **15** as a yellow foam.

***N*-[4,4′,4″-Tris(benzoyloxy)trityl]-L-isoleucinamide (15i).** The reaction was carried out according to the general procedure as described in the case of **14i** (189 mg, 1.0 mmol), column chromatography was performed with hexane−ethyl acetate (1:1, v/v) to give **15i** (625 mg, 79%) as a yellow foam: ^{1}H NMR ($CDCl_3$) δ 0.86 (3H, dd, J = 7.3 Hz, 7.6 Hz), 1.00 (3H, d, J = 6.9 Hz), 1.25 (1H, m), 1.42−1.44 (1H, m), 1.69 (1H, m), 2.99 (1H, d, J = 7.2 Hz), 3.15 (1H, br), 5.17 (1H, br), 5.42 (1H, br), 7.15 (6H, d, J = 7.9 Hz), 7.47−7.61 (15H, m), 8.19 (6H, d, J = 7.3 Hz); ^{13}C NMR ($CDCl_3$) δ 12.11, 14.09, 26.83, 40.54, 60.24, 70.66, 120.95, 128.32, 129.15, 129.88, 133.39, 143.25, 149.38, 164.92, 175.02. Anal. Calcd for $C_{46}H_{40}N_2O_7 \cdot 0.5H_2O$: C, 74.48; H, 5.57; N, 3.78. Found: C, 74.60; H, 5.48; N, 3.60.

***N*-[4,4′,4″-Tris(benzoyloxy)trityl]-L-methioninamide (15m).** The reaction was carried out according to the general procedure as described in the case of **14m** (416 mg, 2.0 mmol), column chromatography was performed with hexane−ethyl acetate (3:2, v/v) to give **15m** (1.32 g, 88%) as a yellow foam: ^{1}H NMR ($CDCl_3$) δ 1.84−1.91 (1H, m), 1.98−2.05 (1H, m), 2.06 (3H, s), 2.34−2.47 (1H, m), 2.60−2.70 (1H, m), 3.21 (1H, br), 3.34 (1H, m), 5.33 (1H, br), 5.59 (1H, br), 7.16 (6H, d, J = 8.6 Hz), 7.47−7.65 (15H, m), 8.17 (6H, m); ^{13}C NMR ($CDCl_3$) δ 15.08, 29.99, 34.02, 56.05, 70.59, 121.15, 128.45, 129.24, 129.92, 129.99, 133.53, 143.34, 149.52, 165.05, 176.41. Anal. Calcd for $C_{45}H_{38}N_2O_7S$: C, 71.98; H, 5.10; N, 3.73; S, 4.17. Found: C, 71.56; H, 5.17; N, 3.52; S, 4.01.

***N*-[4,4′,4″-Tris(benzoyloxy)trityl]-L-prolinamide (15p).** The reaction was carried out according to the general procedure as described in the case of **14p** (228 mg, 2.0 mmol) column chromatography was performed with hexane−ethyl acetate (6:4−3:7, v/v) to give **15p** (996 mg, 74%) as a yellow foam: ^{1}H NMR ($CDCl_3$) δ 1.02−1.18 (2H, m), 1.54−1.64 (1H, m), 1.73−1.81 (1H, m), 3.03−3.13 (1H, m), 3.33−3.43 (1H, m), 3.95 (1H, d, J = 8.3 Hz), 5.92 (1H, d, J = 4.0 Hz), 7.18 (7H, d, J = 8.6 Hz), 7.51 (6H, dd, J = 7.3 Hz, 8.0 Hz), 7.61−7.66 (9H, m), 8.19 (6H, m); ^{13}C NMR ($CDCl_3$) δ 24.28, 31.36, 50.59, 64.94, 121.22, 128.55, 129.39, 130.14, 130.21, 133.62, 141.90, 149.54, 164.98, 178.72. Anal. Calcd for $C_{45}H_{36}N_2O_7 \cdot 1.5H_2O$: C, 72.67; H, 5.28; N, 3.77. Found: C, 72.15; H, 5.00; N, 3.73.

***N*-[4,4′,4″-Tris(benzoyloxy)trityl]-L-valinamide (15v).** The reaction was carried out according to the general procedure as described in the case of **14p** (269 mg, 1.53 mmol) column chromatography was performed with hexane−ethyl acetate (7:3, v/v) to give **15p** (751 mg, 68%) as a yellow foam: ^{1}H NMR ($CDCl_3$) δ 1.01 (3H, d, J = 6.9 Hz), 1.05 (3H, d, J = 6.9 Hz), 2.05 (1H, m), 3.04 (1H, d, J = 4.0 Hz), 5.11 (2H, br), 7.14−7.19 (6H, m), 7.49−7.68 (15H, m), 8.18−8.22 (6H, m); ^{13}C NMR ($CDCl_3$) δ 17.49, 20.25, 33.52, 61.94, 70.73, 121.20, 128.57, 129.45, 130.15, 130.21, 133.62, 143.50, 149.69, 165.21, 175.26. Anal. Calcd for $C_{45}H_{38}N_2O_7 \cdot 0.5H_2O$: C, 74.26; H, 5.40; N, 3.85. Found: C, 74.07; H, 5.33; N, 3.69.

***N,O*-Bis[4,4′,4″-tris(benzoyloxy)trityl]-L-tyrosinamide (15y).** The reaction was carried out according to the general procedure as described in the case of **14y** (240 mg, 1.0 mmol) column chromatography was performed with hexane−ethyl acetate (6:4, v/v) to give **15y** was given in yield

(944 mg, 68%) as a yellow foam: ^{1}H NMR ($CDCl_3$) δ 2.39 (1H, dd, J = 7.9 Hz, J = 13.2 Hz), 2.86 (1H, d, J = 7.3 Hz), 2.98 (1H, dd, J = 5.3 Hz, 13.2 Hz), 3.29 (1H, dd, J = 7.9 Hz, 5.3 Hz), 5.15 (1H, br), 5.35 (1H, br), 6.71 (2H, d, J = 8.6 Hz), 6.84 (2H, d, J = 8.6 Hz), 7.15 (12H, t, J = 8.6 Hz), 7.45−7.66 (30H, m), 8.15−8.19 (12H, m); ^{13}C NMR ($CDCl_3$) δ 59.12, 70.64, 89.97, 120.95, 121.22, 121.96, 128.48, 129.25, 129.33, 129.92, 130.08, 133.53, 141.42, 143.25, 149.58, 149.96, 154.82, 164.94, 165.01, 176.51. Anal. Calcd for $C_{89}H_{64}N_2O_{14}\cdot 2H_2O$: C, 75.31; H, 4.69; N, 1.97. Found: C, 75.36; H, 4.67; N, 2.13.

***N*,*O*-Bis[4,4′,4″-tris(benzoyloxy)trityl]-D-tyrosinamide (15z).** The reaction was carried out according to the general procedure as described in the case of **14z** (240 mg, 1.0 mmol) column chromatography was performed with hexane−ethyl acetate (6:4, v/v) to give **15z** (1.07 g, 77%) as a yellow foam: ^{1}H NMR ($CDCl_3$) δ 2.37 (1H, dd, J = 7.9 Hz, 13.2 Hz), 2.83 (1H, d, J = 6.9 Hz), 2.96 (1H, dd, J = 5.0 Hz, 13.2 Hz), 3.28 (1H, dd, J = 5.0 Hz, 7.9 Hz), 5.13 (1H, br), 5.34 (1H, br), 6.69 (2H, d, J = 8.6 Hz), 6.82 (2H, d, J = 8.6 Hz), 7.10−7.16 (12H, m), 7.43−7.65 (30H, m), 8.12−8.17 (12H, m); ^{13}C NMR ($CDCl_3$) δ 39.98, 59.09, 70.60, 89.96, 120.94, 121.20, 121.94, 128.46, 129.22, 129.31, 129.88, 130.05, 130.66, 133.55, 141.38, 143.23, 149.56, 149.94, 154.81, 164.91, 164.98, 176.46. Anal. Calcd for $C_{89}H_{64}N_2O_{14}\cdot H_2O$: C, 76.16; H, 4.74; N, 2.00. Found: C, 76.17; H, 4.92; N, 2.00.

General Procedure for the Synthesis of the Fully Protected aa-AMPNs (16). *N*-TBTr Amino acid amide (**15**) (0.20 mmol) and **10** (143 mg, 0.30 mmol) were dried by repeated coevaporation with dry pyridine and dry toluene, and this mixture was dissolved in dry MeCN (4 mL). The solution was added to 5-(3,5-dinitrophenyl)-1*H*-tetrazole (142 mg, 0.6 mmol), which was dried by repeated coevaporation with dry pyridine and dry toluene. The mixture was stirred at room temperature for 30 min. To the mixture was added *tert*-butyl hydroperoxide (125 μL, 1.0 mmol). After being stirred at room temperature for 10 min, the mixture was diluted with $CHCl_3$ and washed three times with 5% $NaHCO_3$. The organic layer was dried over Na_2SO_4, filtered, and concentrated to dryness under reduced pressure.

The residue was dissolved in dry THF (10 mL), and tetrabutylammonium fluoride monohydrate (157 mg, 0.6 mmol) and AcOH (34 μL, 0.6 mmol) were added to this mixture. After being stirred at room temperature for 20 h, the reaction mixture was concentrated under reduced pressure to a small volume, diluted with $CHCl_3$, and washed three times with 0.5 M triethylammonium hydrogen carbonate. The organic layer was dried over Na_2SO_4, filtered, and concentrated to dryness under reduced pressure. The residue was applied to a silica gel column, and elution was performed with $CHCl_3$−MeOH containing 1% triethylamine. The fractions containing **16** were combined and concentrated to give **16** as a yellow foam.

Triethylammonium *O*-(*N*-Benzoyl-2′,3′-di-*O*-benzoyladenosine-5′-*O*-yl)-*N*-[*N*-(4,4′,4″-tris(benzoyloxy)trityl)-L-isoleucyl]phosphoramidate (16i). The reaction was carried out according to the general procedure as described in the case of **15i** (248 mg, 0.20 mmol), column chromatography was performed with $CHCl_3$−MeOH (99.5:0.5−98:2, v/v) containing 1% triethylamine to give **16i** (167 mg, 57%) as a yellow foam: ^{31}P NMR ($CDCl_3$) δ 7.05; ^{1}H NMR ($CDCl_3$) δ 0.64 (3H, t, J = 7.3 Hz), 0.85 (4H, m), 1.23−1.30 (2H, m), 1.27 (9H, t, J = 7.3 Hz), 2.44 (1H, d, J = 5.3 Hz), 3.07 (6H, q, J = 7.3 Hz), 3.24 (1H, d, J = 5.0 Hz), 4.39 (2H, m), 4.73 (1H, m), 6.08 (1H, dd, J = 2.0 Hz, 5.3 Hz), 6.21 (1H, dd, J = 5.3 Hz, 6.9 Hz), 6.68 (1H, d, J = 6.9 Hz), 7.17−7.61 (32H, m), 7.77−8.16 (12H, m), 8.71 (1H, s), 8.71 (1H, s); ^{13}C NMR ($CDCl_3$) δ 8.32, 11.90, 13.77, 27.19, 40.47, 45.27, 61.65 (d, J = 6.1 Hz), 64.87 (d, J = 4.8 Hz), 71.34, 72.56, 74.90, 83.05 (d, J = 8.5 Hz), 85.12, 121.01, 122.64, 124.98, 127.51, 127.69, 127.89, 128.00, 129.16, 128.21, 128.52, 128.70, 128.77, 129.15, 129.47, 129.63, 129.81, 132.35, 133.15, 133.24, 133.55, 142.59, 142.91, 149.02, 149.36, 151.77, 152.26, 164.46, 164.64, 164.89, 174.68. Anal. Calcd for $C_{83}H_{79}N_8O_{16}P$: C, 67.56; H, 5.40; N, 7.59. Found: C, 65.19; H, 5.64; N, 8.05.

Triethylammonium *O*-(6-*N*-,2′,3′-*O*-Tribenzoyladenosine-5′-*O*-yl)-*N*-[*N*-(4,4′,4″-tris(benzoyloxy)trityl)-L-methionyl]phosphoramidate (16m). The reaction was carried out according to the general procedure as described in the case of **15m** (150 mg, 0.20 mmol), column chromatography was performed with $CHCl_3$−MeOH (99:1−98:2, v/v) containing 1% triethylamine to give **16m** (185 mg, 62%) as a yellow foam: ^{31}P NMR ($CDCl_3$) δ 7.28; ^{1}H NMR ($CDCl_3$) δ 1.19 (9H, t, J = 7.3 Hz), 1.92 (3H, s), 2.32 (1H, m), 2.47 (1H, m), 2.87 (6H, q, J = 7.3 Hz), 2.99 (1H, d, J = 5.9 Hz), 3.39 (1H, m), 4.33 (1H, m), 4.41 (1H, m), 4.72 (1H, m), 6.07 (1H, d, J = 4.9 Hz), 6.18 (1H, dd, J = 7.3 Hz, 4.9 Hz), 6.69 (1H, d, J = 7.3 Hz), 7.18 (6H, d, J = 8.9 Hz), 7.23−7.29 (2H, m), 7.37−7.63 (22H, m), 7.78 (2H, d, J = 7.6 Hz), 7.97−8.01 (4H, m), 8.15 (6H, d, J = 7.3 Hz), 8.72 (1H, s), 9.09 (1H, s); ^{13}C NMR ($CDCl_3$) δ 8.41, 15.19, 29.54, 33.21, 45.52, 58.06 (d, J = 4.9 Hz), 65.18, 71.11, 72.80, 75.13, 83.37 (d, J = 8.6 Hz), 85.18, 121.20, 122.65, 127.82, 128.03, 128.18, 128.30, 128.37, 128.57, 128.84, 129.22, 129.61, 129.67, 129.92, 132.44, 133.64, 142.52, 143.00, 149.34, 149.51, 151.91. 152.42, 164.69, 164.78, 164.96, 165.10, 176.14. Anal. Calcd for $C_{82}H_{77}N_8O_{16}PS$: C, 65.15; H, 6.33; N, 7.41; S, 2.12. Found: C, 65.05; H, 5.67; N, 7.04; S, 2.46.

Triethylammonium *O*-(*N*-Benzoyl-2′,3′-di-*O*-benzoyladenosine-5′-*O*-yl)-*N*-[*N*-(4,4′,4″-tris(benzoyloxy)trityl)-L-prolyl]phosphoramidate (16p). The reaction was carried out according to the general procedure as described in the case of **15p** (248 mg, 0.20 mmol), column chromatography was performed with $CHCl_3$−MeOH (99.5:0.5−98:2, v/v) containing 1% triethylamine to give **16p** (221 mg, 76%) as a yellow foam: ^{31}P NMR ($CDCl_3$) δ −7.46; ^{1}H NMR ($CDCl_3$) δ 1.08−1.16 (2H, m), 1.23 (9H, t, J = 7.3 Hz), 1.58−1.61 (1H, m), 1.73−1.85 (1H, m), 3.00 (6H, q, J = 7.3 Hz), 3.02 (1H, m), 3.48 (1H, m), 3.96 (1H, d, J = 8.6 Hz), 4.51 (2H, m), 4.76 (1H, m), 6.08 (1H, d, J = 5.3 Hz), 6.22 (1H, dd, J = 5.3 Hz, 6.9 Hz), 6.72 (1H, d, J = 6.9 Hz), 7.17 (6H, d, J = 8.6 Hz), 7.24−7.69 (21H, m), 7.79−7.81 (3H, m), 8.00−8.03 (6H, m), 8.14 (6H, d, J = 8.6 Hz), 8.43 (1H, d, J = 10.9 Hz), 8.68 (1H, s), 9.09 (1H, s); ^{13}C NMR ($CDCl_3$) δ 8.97, 24.23, 31.61, 45.59, 50.46, 65.20, 65.86, 72.87, 75.06), 83.34 (d, J = 9.8 Hz), 85.19, 121.08, 122.71, 127.75, 127.85, 128.19, 128.32, 128.37, 128.43, 128.66, 128.91, 129.67, 129.97, 130.19, 132.49, 133.35, 133.48, 133.66, 133.76, 142.52, 149.25, 149.36, 151.90, 152.45, 164.65, 164.78, 165.16, 177.90. Anal. Calcd for $C_{82}H_{75}N_8O_{16}P\cdot H_2O$: C, 66.66; H, 5.25; N, 7.58. Found: C, 66.37; H, 5.45; N, 7.27.

Triethylammonium *O*-(*N*-Benzoyl-2′,3′-di-*O*-benzoyladenosine-5′-*O*-yl)-*N*-[*N*-[4,4′,4″-tris(benzoyloxy)trityl]-L-valyl]phosphoramidate (16v). The reaction was carried out according to the general procedure as described in the case of **15v** (144 mg, 0.2 mmol), column chromatography was performed with $CHCl_3$−MeOH (99:1−98:2, v/v) containing 1% triethylamine to give **16v** (292 mg, 83%) as a yellow foam: ^{31}P NMR ($CDCl_3$) δ −7.05; ^{1}H NMR ($CDCl_3$) δ 0.86 (6H, d, J = 6.3 Hz), 1.23 (9H, t, J = 7.3 Hz), 2.45 (1H, m), 2.94 (6H, q, J = 7.3 Hz), 3.16 (1H, m), 4.24−4.37 (2H, m), 4.71 (1H, m), 6.06 (1H, dd, J = 5.3 Hz, 3.6 Hz), 6.16 (1H, dd, J = 6.6 Hz, 5.3 Hz), 6.71 (1H, d J = 6.6 Hz), 7.18 (6H, d, J = 8.6 Hz), 7.26−7.59 (21H, m), 7.76−7.81 (3H, m), 7.95−8.03 (6H, m), 8.15 (6H, d, J = 8.6 Hz), 8.72 (1H, s), 9.15 (1H, s); ^{13}C NMR ($CDCl_3$) δ 8.70, 17.06, 20.24, 33.66, 45.54, 63.02, 65.09, 71.32, 72.83, 75.16, 83.47, 85.23, 121.15, 122.68, 127.78, 127.92, 128.03, 128.19, 128.32, 128.39, 128.63, 128.90, 128.99, 129.27, 129.40, 129.63, 129.83, 129.96, 130.19, 130.46, 131.11, 132.45, 133.37, 133.42, 133.71, 143.07, 149.52, 151.88, 152.43, 164.67, 164.83, 165.09, 175.09. Anal. Calcd for $C_{82}H_{77}N_8O_{16}P\cdot 1.5H_2O$: C, 66.16; H, 5.42; N, 7.53. Found: C, 66.04; H, 5.67; N, 7.70.

Triethylammonium *O*-(*N*-Benzoyl-2′,3′-di-*O*-benzoyladenosine-5′-*O*-yl)-*N*-[Bis-*N*,*O*-[4,4′,4″-tris(benzoyloxy)trityl]-L-tyrosyl]phosphoramidate (16y). The reaction was carried out according to the general procedure as described in the case of **15y** (1.38 g, 1.0 mmol), column chromatography was performed with $CHCl_3$−MeOH (99.5:0.5−98:2, v/v) containing 1% triethylamine to give **16y** (2.11 g, 60%) as a yellow foam: ^{31}P NMR ($CDCl_3$) δ −7.46; ^{1}H NMR ($CDCl_3$) δ 1.29 (9H, t, J = 7.3 Hz), 2.92 (1H, m), 3.05 (6H, q, J = 7.3 Hz), 3.07 (1H, m), 3.51 (1H, m), 4.23 (1H, m), 4.32 (1H, m), 4.69 (1H, m), 6.05 (1H, m), 6.15 (1H, dd, J = 6.3 Hz, 5.6 Hz), 6.61−6.71 (5H, m), 7.08−7.64 (45H, m), 7.76−8.15 (22H, m), 8.71 (1H, s), 9.18 (1H, s); ^{13}C NMR ($CDCl_3$) δ 8.34, 33.17, 45.43, 62.11, 67.73, 70.94, 71.11, 72.76, 74.65, 87.58, 89.34, 89.97, 111.14,

120.85, 120.97, 121.17, 121.44, 121.69, 121.96, 127.71, 127.82, 127.98, 128.09, 128.16, 128.28, 128.62, 128.79, 128.86, 128.93, 129.13, 129.56, 129.68, 129.92, 130.24, 130.40, 130, 64, 131.45, 132.38, 132.72, 133.32, 141.08, 142.53, 142.75, 143.04, 149.04, 149.42, 149.79, 150.93, 152.36, 164.56, 164.71, 164.91, 165.03, 178.53. Anal. Calcd for $C_{126}H_{103}N_8O_{23}P$: C, 71.11; H, 4.88; N, 5.27. Found: C, 71.54; H, 4.42; N, 5.88.

Triethylammonium *O*-(*N*-Benzoyl-2′,3′-di-*O*-benzoyladenosine-5′-*O*-yl)-*N*-[*N*,*O*-bis[4,4′,4″-tris(benzoyloxy)trityl]-D-tyrosyl]phosphoramidate (16z). The reaction was carried out according to the general procedure as described in the case of **15z** (277 mg, 0.20 mmol), column chromatography was performed with $CHCl_3$−MeOH (99.5:0.5−98:2, v/v) containing 1% triethylamine to give **16z** (258 mg, 61%) as a yellow foam: ^{31}P NMR ($CDCl_3$) δ −7.37; ^{1}H NMR ($CDCl_3$) δ 1.29 (9H, t, J = 7.3 Hz), 2.91 (1H, m), 3.09 (6H, q, J = 7.3 Hz), 3.10 (1H, m), 3.55 (1H, m), 4.22 (1H, m), 4.36 (1H, m), 4.53 (1H, m), 6.11 (2H, m), 6.64 (5H, m), 7.11−7.60 (45H, m), 7.73−8.24 (22H, m), 8.68 (1H, s), 9.12 (1H, s); ^{13}C NMR ($CDCl_3$) δ 8.93, 34.25, 45.55, 62.25, 65.03, 70.92, 72.74, 73.33, 74.00, 75.17, 85.00, 87.73, 89.70, 111.14, 120.68, 120.88, 121.18, 121.69, 121.76, 122.07, 122.70, 122.97, 123.97, 127.73, 127.84, 127.89, 128.16, 128.21, 128.37, 128.59, 128.93, 128.97, 129.25, 129.58, 128.93, 128.96, 129.25, 129.58, 129.63, 129.70, 129.97, 130.39, 132.41, 132.56, 132.94, 133.37, 133.44, 133.71, 141.22, 142.50, 142.70, 142.93, 149.24, 149.47, 149.90, 150.11, 151.90, 152.20, 152.40, 154.72, 164.65, 164.73, 164.76, 165.07, 165.25, 176.60. Anal. Calcd for $C_{126}H_{103}N_8O_{23}P$: C, 71.11; H, 4.88; N, 5.27. Found: C, 70.62; H, 4.92; N, 5.25.

General Procedure for the Synthesis of aa-AMPN (17). Fully protected aa-AMPN (**16**) was treated with concentrated NH_3-dioxane (1:1, (v/v)) at room temperature for 8 h. The mixture was evaporated under reduced pressure, and residue was dissolved in water. The aqueous solution was washed five times with ether and concentrated to a small volume. The residue was applied to a C18 reversed-phase column, and elution was performed with water, applying a gradient of MeCN. The fractions containing **17** were combined and lyophilized to give **17** as a white powder.

***O*-(Adenosine-5′-*O*-yl) *N*-(L-isoleucyl)phosphoramidate (17i).** The reaction was carried out according to the general procedure as described in the case of **16i** (197 mg, 0.134 mmol). C-18 Reversed-phase column chromatography was performed with water, applying a gradient of MeCN (0−5%) to give **17i** (29 mg, 48%) as a white powder: ^{31}P NMR (D_2O) δ −5.26; ^{1}H NMR (D_2O) δ 0.58 (3H, d, J = 6.6 Hz, 7.3 Hz), 0.70 (3H, d, J = 6.9 Hz), 0.81−0.92 (1H, m), 1.11−1.21 (1H, m), 1.62−1.68 (1H, m), 3.67 (1H, d, J = 5.0 Hz), 3.89−4.04 (2H, m), 4.19 (1H, m), 4.31 (1H, dd, J = 5.3 Hz, 3.6 Hz), 4.59 (1H, dd, J = 5.9 Hz, 5.3 Hz), 5.93 (1H, d, J = 5.9 Hz), 8.06 (1H, s), 8.32 (1H, s); ^{13}C NMR (D_2O) δ 13.12, 16.82, 26.09, 38.76, 61.29 (d, J = 9.8 Hz), 67.74 (d, J = 4.9 Hz), 72.92, 76.67, 86.25 (d, J = 9.8 Hz), 89.31, 121.19, 142.64, 151.73, 155.52, 158.20, 173.74; MALDI-TOF mass m/z calcd for $C_{16}H_{27}N_7O_7P$ 460.2, obsd (M + H) 460.2.

***O*-(Adenosine-5′-*O*-yl) *N*-(L-methionyl)phosphoramidate (17m).** The reaction was carried out according to the general procedure as described in the case of **16m** (228 mg, 0.153 mmol). C-18 Reversed-phase column chromatography was performed with water, applying a gradient of MeCN (0−10%) to give **17m** (53 mg, 73%) as a white powder: ^{31}P NMR (D_2O) δ −5.36; ^{1}H NMR (D_2O) δ 1.91 (3H, s), 1.97−2.06 (2H, m), 2.39 (1H, dd, J = 7.6 Hz, 7.9 Hz), 4.07−4.14 (3H, m), 4.32 (1H, m), 4.44 (1H, dd, J = 5.0 Hz, 4.0 Hz), 4.70 (1H, dd, J = 5.9 Hz, 4.0 Hz), 6.08 (1H, d, J = 5.9 Hz), 8.23 (1H, s), 8.47 (1H, s); ^{13}C NMR (D_2O) δ 0.16.48, 30.53, 32.43, 55.73 (d, J = 11.0 Hz), 67.69 (d, J = 4.9 Hz), 72.83, 76.80, 86.24 (d, J = 9.7 Hz), 89.47, 121.24, 142.98, 151.66, 154.28, 157.39, 173.39; MALDI-TOF mass m/z calcd for $C_{15}H_{25}N_7O_7PS$ 478.1, obsd (M + H) 478.2.

***O*-(Adenosine-5′-*O*-yl) *N*-(L-prolyl)phosphoramidate (17p).** The reaction was carried out according to the general procedure as described in the case of **16p** (215 mg, 0.015 mmol). C-18 Reversed-phase column chromatography was performed with water, applying a gradient of MeCN (0−10%) to give **17p** (42 mg, 65%) as a white powder: ^{31}P NMR (D_2O) δ −5.26; ^{1}H NMR (D_2O) δ 1.72−1.92 (3H, m), 2.27−2.36 (1H, m), 3.23−3.28 (2H, m), 4.08 (2H, br), 4.28−4.42 (3H, m), 4.65 (1H, d, J = 5.6 Hz, 5.3 Hz), 5.97 (1H, d, J = 5.3 Hz), 8.03 (1H, s), 8.33 (1H, s); ^{13}C NMR (D_2O) δ 26.11, 32.22, 48.98, 63.07 (d, J = 11.0 Hz), 67.60 (d, J = 4.9 Hz), 72.87, 76.75, 86.22 (d, J = 8.5 Hz), 89.58, 121.17, 142.89, 151.61, 154.46, 157.46, 173.56; MALDI-TOF mass m/z calcd for $C_{15}H_{23}N_7O_7P$ 444.1, obsd (M + H) 444.1.

***O*-(Adenosine-5′-*O*-yl) *N*-(L-valyl)phosphoramidate (17v).** The reaction was carried out according to the general procedure as described in the case of **16v** (299 mg, 0.205 mmol). C-18 Reversed-phase column chromatography was performed with water, applying a gradient of MeCN (0−10%) to give **17v** (70 mg, 77%) as a white powder: ^{31}P NMR (D_2O) δ −5.21; ^{1}H NMR (D_2O) δ 0.85 (3H, d, J = 6.9 Hz), 0.92 (3H, d, J = 6.9 Hz), 2.08−2.15 (1H, m), 3.79 (1H, d, J = 5.0 Hz), 4.15 (2H, m), 4.35 (1H, m), 4.47 (1H, m), 4.72 (1H, m), 6.06 (1H, d, J = 5.9 Hz), 8.14 (1H, s), 8.44 (1H, s); ^{13}C NMR (D_2O) δ 18.71, 20.34, 32.22, 61.69 (d, J = 9.8 Hz), 67.69 (d, J = 6.2 Hz), 72.92, 76.80, 86.27 (d, J = 9.8 Hz), 89.52, 121.17, 142.73, 151.62, 155.02, 157.84, 173.53: MS(FAB) m/z calcd for $C_{15}H_{24}N_7O_7P$ 445.1475, obsd (M + H) 446.1560.

***O*-(Adenosine-5′-*O*-yl) *N*-(L-tyrosyl)phosphoramidate (17y).** The reaction was carried out according to the general procedure as described in the case of **16y** (1.27 g, 0.60 mmol). C-18 Reversed-phase column chromatography was performed with water, applying a gradient of MeCN (0−10%) to give **17y** (168 mg, 55%) as a white powder: ^{31}P NMR (D_2O) δ −5.01; ^{1}H NMR (D_2O) δ 2.65−2.73 (2H, m), 3.82−3.96 (3H, m), 4.18 (1H, m), 4.25 (1H, dd, J = 5.3 Hz, 3.6 Hz), 4.53 (1H, dd, J = 5.6 Hz, 5.3 Hz), 5.89 (1H, d, J = 5.6 Hz), 6.42 (2H, d, J = 8.6 Hz), 6.74 (2H, d, J = 8.6 Hz), 7.95 (1H, s), 8.16 (1H, s); ^{13}C NMR (D_2O) δ 39.26, 57.89 (d, J = 8.6 Hz), 67.86 (d, J = 6.1 Hz), 72.90, 76.91, 86.20 (d, J = 9.8 Hz), 117.91, 121.04, 128.43, 133.21, 142.46, 151.41, 155.27, 157.27, 157.95, 176.10; MALDI-TOF mass m/z calcd for $C_{19}H_{25}N_7O_8P$ 510.2, obsd (M + H) 510.1.

***O*-(Adenosine-5′-*O*-yl) *N*-(D-tyrosyl)phosphoramidate (17z).** The reaction was carried out according to the general procedure as described in the case of **16z** (167 mg, 0.079 mmol). C-18 Reversed-phase column chromatography was performed with water, applying a gradient of MeCN (0−10%) to give **17z** (14 mg, 35%) as a white powder: ^{31}P NMR (D_2O) δ −5.27; ^{1}H NMR (D_2O) δ 2.46−2.60 (2H, m), 3.82−3.95 (2H, m), 4.02−4.07 (1H, m), 4.15 (1H, m), 4.31 (1H, dd, J = 4.3 Hz, 4.6 Hz), 4.59 (1H, dd, J = 5.0 Hz, 4.3 Hz), 5.91 (1H, d, J = 5.0 Hz), 6.48 (2H, d, J = 7.6 Hz), 6.66 (2H, d, J = 7.9 Hz), 7.94 (1H, s), 8.19 (1H, s); ^{13}C NMR (D_2O) δ 38.63, 57.53 (d, J = 13.3 Hz,), 68.09 (d, J = 3.7 Hz), 72.96, 76.39, 86.19 (d, J = 8.6 Hz), 89.54, 118.04, 121.15, 127.73, 133.10, 142.17, 151.53, 155.33, 157.39, 157.95, 174.83; MALDI-TOF mass m/z calcd for $C_{19}H_{25}N_7O_8P$ 510.2, obsd (M + H) 510.3.

Stability of compound 12. A solution of **12** (1 mL, 1 OD unit/mL) were treated with the following reagents: (i) 0.1 M HCl, rt; (ii) 0.1 M NH_4OAc, rt; (iii) 0.1 M NaOH, rt. The products were monitored by reversed-phase HPLC.

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Supporting Information Available: Characterization data for all compounds obtained in Experimental Section; ^{1}H, ^{13}C, and ^{31}P NMR spectra and MALDI-TOF mass spectra of compounds **12** and **17i**,**m**,**p**,**v**,**y**,**z**. This material is available free of charge via the Internet at http://pubs.acs.org.

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