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Synthesis of New Aminoacyl-Adenylate Analogs Having an *N*-Acyl Phosphoramidate Linkage

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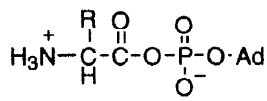
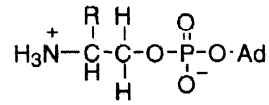
Abstract: New aminoacyl-adenylate analogs (aa-AMPNs), in which the oxygen atom of the mixed anhydride bond of aminoacyl-adenylates is replaced by an NH group, were synthesized by the reaction of an adenosine 5'-phosphoramidite derivative with appropriately protected amino acid amides. Among various reagents studied for activation of the 5'-phosphoramidite derivative, 5-(4-nitrophenyl)-1*H*-tetrazole was found to give *N*-acyl phosphoramidate derivatives in good yields.

In protein biosynthesis, the aminoacyl-tRNA synthesis is a very important step. This reaction is performed *via* two steps catalyzed by cognate aminoacyl-tRNA synthetases (ARSs). The first stage is the ATP-dependent activation of amino acids, giving rise to aminoacyl-adenylates (aa-AMPs), which exist as complexes with cognate ARSs.[1] The second involves the 2'- or 3'-aminoacylation of the 3'-terminal CCA end of tRNAs.[2] However, aa-AMPs are extremely unstable under aqueous conditions. To clarify the three-dimensional structures of tRNA-ARSs or ARSs in the presence or absence of aa-AMPs, more stable aminoacyl-adenylate analogs are required. Such stable analogs are also useful for inhibitory studies of the peptide synthesis. Therefore, some modified aa-AMP analogs have been reported to date.[3-7] In these cases, however, the amino acid residue or the mixed acid anhydride bond was changed to a stable structure such as an aminoalkylphosphoryl[3,4] or aminomethylphosphonyl[5] group, and an *N*-acylsulfonamide linkage. The structures of these analogs deviate far from those of aa-AMPs since they lack the C=O double bond, gain an additional minus charge, or lose a phosphate charge.



aminoacyl-AMP



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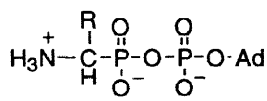

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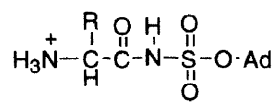
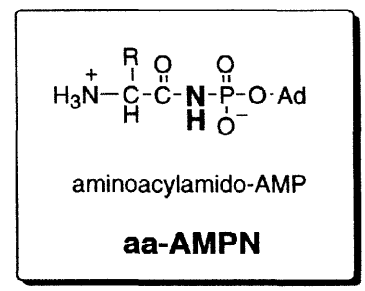


aminoacylamido-AMS



aminoacylamido-AMP

aa-AMPN

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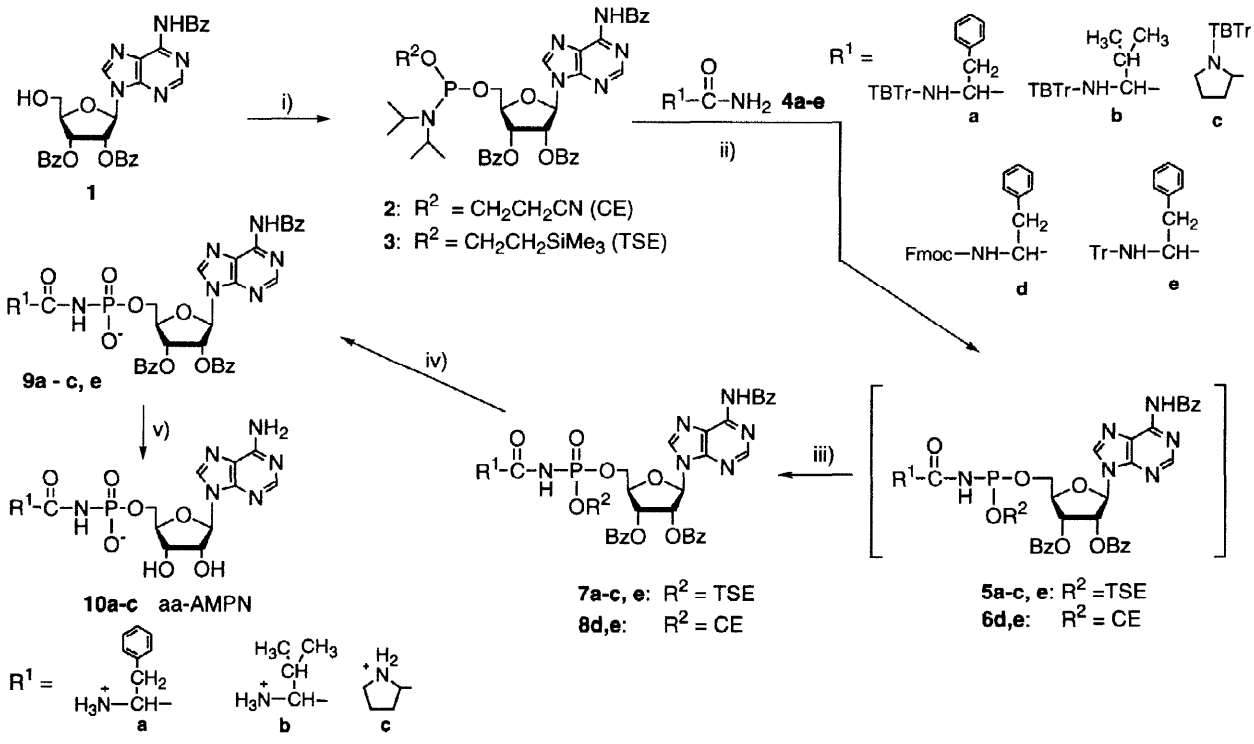
In this paper, we report the synthesis of new aa-AMP analogs which have an *N*-acyl phosphoramidate linkage capable of preserving the original zwitter ion structure and almost the same distance between the phosphorus and amino groups. These simplest aa-AMP analogs are of great importance to elucidate the recognition mechanism of amino acids by cognate ARSs.

Moreover, natural products such as Phosmidosine[8] and Agrocin 84,[9] which have an *N*-acyl phosphoramidate linkage at the 5'-position of adenosine derivatives, have been reported. Therefore, exploration of new methods for the construction of *N*-acyl phosphoramidate linkages is important also for the synthesis of these natural products. Synthesis of *N*-acyl phosphoramidate derivatives have previously been reported.[10-14] Recently, Grandas *et al.* reported the synthesis of DNA-peptide hybrids having an *N*-acyl phosphoramidate linkage[15] by condensation of *N*-phosphitylated carboxamides with alcohols, or carboxamides with *O*-phosphitylated alcohols in the presence of 1*H*-tetrazole.

First, we attempted to synthesize an *N*-acyl phosphoramidate derivative **10a** by the 1*H*-tetrazole promoted reaction of the carboxamide **4e** with an adenosine 5'-phosphoramidite derivative **2**, which was prepared by the reaction of *N*-benzoyl-2',3'-di-*O*-benzoyladenosine (**1**) with 2-cyanoethyl *N*,*N*,*N'*,*N'*-tetraisopropylphosphorodiamidite in the presence of diisopropylammonium tetrazolide. However, the fully protected *N*-acyl phosphoramidate derivative **8e**, which was obtained by oxidation of the condensation product **6e** with *t*-BuOOH, was extremely unstable to decompose with the cleavage of the P-N bond during silica gel column chromatography. Grandas *et al.* reported that removal of the phosphate protecting group from this type of compound led to considerable stabilization of the P-N bond.[15] Therefore, the 2-(trimethylsilyl)ethyl (TSE) group[16] was used as the phosphate protecting group that can be removed selectively by treatment with Bu_4NF. The starting material **3**, obtained from **1** in a similar manner, was allowed to react with *N*-Fmoc-L-phenylalaninamide (**4d**) in the presence of 3 equiv of 1*H*-tetrazole or pyridinium chloride[17] in CH_2Cl_2 or acetonitrile. However, the condensation sluggishly proceeded because of the poor solubility of **4d** in these solvents. Accordingly, a more soluble *N*-Tr derivative **4e** in the place of **4d** was used. This reaction gave the *N*-acyl phosphoramidite intermediate **5e** (133.2 ppm in ^{31}P NMR spectrum) in ca. 10% yield. The successive oxidation of this intermediate with *t*-BuOOH followed by treatment of the oxidation product **7e** with $Bu_4NF{\bullet}H_2O$ gave the desired *N*-acyl phosphoramidate derivative **9e** in a low yield.

When the condensation was carried out in the presence of 5-(4-nitrophenyl)-1*H*-tetrazole,[18] the reaction proceeded very fast, and the yield of **9e** was improved up to an overall yield of 55% from **3**. Since it was expected that the acidic treatment for detritylation caused the competitive P-N bond cleavage,[15] another type of liphophilic protecting group that can be removed without acidic conditions was required. For this purpose, the base-labile 4,4',4"-tris(benzoyloxy)trityl (TBTr) group[19,20] was chosen to meet these criteria. This TBTr group was used for protection of the primary hydroxyl group of deoxynucleosides[19] and the *exo*-amino group of deoxyadenosine, deoxycytidine and deoxyguanosine in DNA synthesis.[20]

The starting material, *N*-TBTr-L-phenylalaninamide, was synthesized by the reaction of L-phenylalaninamide with TBTrBr in the presence of triethylamine at 60 °C in 95% yield.[20] This *N*-TBTr derivative **4a** was allowed to react with the 5'-*O*-phosphoramidite derivative **3** in the presence of 5-(4-nitrophenyl)-1*H*-tetrazole. After oxidation of the resulting *N*-acyl phosphoramidite intermediate **5a** with *t*-BuOOH and removal of the TSE group from the product **7a**, the desired *N*-acyl phosphoramidate derivative **9a** was obtained in 62% yield by silica gel



Scheme 1

i) TSEOP(N(*i*-Pr)$_2$)$_2$ (1.2 eq), diisopropylammonium tetrazolide (0.5 eq), CH_2Cl_2, rt, 2 h; ii) **2** or **3** (1.5 eq), 5-(4-nitrophenyl)-1*H*-tetrazole (3 eq), CH_3CN, rt, 10 min; iii) *t*-BuOOH (5 eq), CH_3CN, rt, 5 min; iv) TBAF•H_2O (3 eq), AcOH (3 eq), THF, rt, 12 h; v) conc.NH_3 - dioxane (1:1, v/v), rt, 8 h.

column chromatography. Finally, all the protecting groups were removed by treatment with aqueous ammonia to give the desired product **10a** in 52% yield.[21]

L-Valine and L-proline aminoacyl adenylate derivatives **10b** and **10c** were also synthesized in a similar manner. The *N*-TBTr derivatives **4b** and **4c** were synthesized in 68% and 90% yields, respectively, from the corresponding *N*-unprotected amino acid amides. In the case of the L-valine derivative, the condensation with **4b**, followed by oxidation of the product **5b** and removal of the TSE group from **7b**, gave the condensed product **9b** in an overall yield of 55% yield from **3**.[22] All the protecting groups were removed by treatment with aqueous ammonia to give the desired product **10b** in 77% yield. Similarly, the L-prolinamide derivative **10c** was obtained in 65% yield from **7c**, which was prepared in an overall yield of 57%.[23]

In summary, we successfully synthesized new aminoacyl-adenylate analogs, which have an *N*-acyl phosphoramidate linkage. The present method would provide a clue for the total synthesis of some natural products which have the *N*-acyl phosphoramidate linkage, such as Phosmidosine and Agrocin 84. Studies toward the total synthesis of these natural products are now in progress.

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References and Notes

1. (a) Hoagland, M. B. *Biochim. Biophys. Acta* **1955**, *16*, 288. (b) Hoagland, M. B.; Zamecnik, P. C.; Keller, E. B. *J. Biol. Chem.* **1956**, *218*, 345.
2. Friest, W. *Biochemistry* **1989**, *28*, 6787.
3. Cassio, D.; Lemoine, F.; Waller, J.-P.; Sandrin, E.; Boissonnas, R. *Biochemistry* **1967**, *6*, 827.
4. Dprion, C.; Chenevert, R.; Lacosta, L.; Lapointe, J.; *BioMed. Chem. Lett.* **1993**, *3*, 2699.
5. Biryukov, A. I.; Ishmuratov, B. K.; Khomutov, R. M. *FEBS Lett.* **1978**, *91*, 249.
6. Ueda, H.; Shoku, Y.; Hayashi, N.; Mitsunaga, J.; In, Y.; Doi, M.; Inoue, M.; Ishida, T. *Biochim. Biophys. Acta* **1991**, *1080*, 126.
7. Biou, V.; Yaremchuk, A.; Tukalo, M.; Cusack, S. *Science* **1994**, *263*, 1404.
8. (a) Uramoto, M.; Kim, C. J.; Shin-ya, K.; Kusakabe, H.; Isono, K. *J. Antibiot.* **1991**, *44*, 375. (b) Phillips, D. R.; Uramoto, M.; Isono, K.; McCloskey, J. A. *J. Org. Chem.* **1993**, *58*, 854.
9. (a) Roberts, W. P.; Tate, M. E.; Kerr, A. *Nature* **1977**, *265*,379. (b) Tate, M. E.; Murphy, P. J.; Kerr, A. *Nature* **1979**, *280*, 697.
10. Challis, B. C.; Iley, J. N. *J. Chem. Soc., Perkin Trans. 2* **1987**, 1489.
11. Henddrickse, T. F.; Mizrahi, V.; Modro, T. A. *Phosphorus Sulfur* **1984**, *20*, 93.
12. Mizrahi, V.; Modro, T. A. *J. Org. Chem.* **1982**, *47*, 3533.
13. Chakravarty, P. K.; Greenlee, W. J.; Parsons, W. H.; Patchett, A. A.; Combs, P.; Roth, A.; Busch, R. D.; Mellin, T. N. *J. Med. Chem.* **1989**, *32*, 1886.
14. Desmarchelier, J. M.; Fukuto, T. R. *J. Org. Chem.* **1972**, *37*, 4218.
15. Robles, J.; Pedroso, E.; Grandas, A. *J. Org. Chem.* **1995**, *60*, 4856.
16. Wada, T.; Sekine, M. *Tetrahedron Lett.* **1994**, *35*, 757.
17. Gryaznov, S. M.; Letsinger, R. L. *Nucleic Acid Res.* **1992**, *20*. 1879.
18. Froehler, B. C.; Matteucci, M. D. *Tetrahedron Lett.* **1983**, *24*, 3171.
19. Sekine, M.; Hata, T. *J. Org. Chem.* **1983**, *26*, 3011.
20. (a) Sekine, M.; Masuda, N.; Hata, T. *Tetrahedron* **1985**, *41*, 5445. (b) Sekine, M.; Hata, T. *Bull. Chem. Soc. Jpn.* **1985**, *58*, 336.
21. **10a**: ^{31}P NMR (D_2O) δ -5.37; FAB MS Calcd for *m/z* $C_{19}H_{25}N_7O_7P$ $(M+H)^+$ 494.1553. Observed for *m/z* 494.1552.
22. **10b**: ^{31}P NMR (D_2O) δ -5.21; FAB MS Calcd for *m/z* $C_{15}H_{25}N_7O_7P$ $(M+H)^+$ 446.1553. Observed for *m/z* 446.1560.
23. **10c**: ^{31}P NMR (D_2O) δ -5.26; FAB MS Calcd for *m/z* $C_{15}H_{23}N_7O_7P$ $(M+H)^+$ 444.1397. Observed for *m/z* 444.1402.