Chemical Deprotection Strategy for the Elaboration of Misacylated Transfer RNA's

Michiel Lodder, Serguei Golovine, and Sidney M. Hecht*

Departments of Chemistry and Biology, University of Virginia, Charlottesville, Virginia 22901

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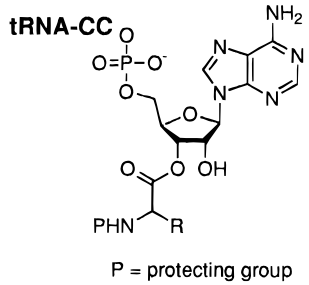
In recent years, the elaboration of proteins containing synthetic amino acids at predetermined sites has become technically feasible; the strategy employed involves readthrough of nonsense codons[1] with misacylated suppressor tRNA's.[2] As first shown by Hecht and coworkers,[2b,c] misacylated tRNA's (Scheme 1) are accessible by T4 RNA ligase-mediated coupling of aminoacylated pCpA or pdCpA derivatives with tRNA's from which the 3′-terminal dinucleotide has been removed.

While T4 RNA ligase-mediated ligation has been effected using unprotected aminoacyl-pCpA derivatives,[2d] the lability of the aminoacyl moiety has led most workers to employ amino acid protecting groups that impart chemical stability. Relatively few amine protecting groups can be removed under conditions compatible with the integrity of the derived aminoacyl-tRNA's. The most satisfactory ones reported to date have been the pyroglutamyl[2f] and nitroveratryloxycarbonyl (NVOC)[2i] groups, removable by enzymatic proteolysis and photolytic cleavage, respectively.[3] No protecting group removable with facility by a simple chemical treatment has been reported.[4]

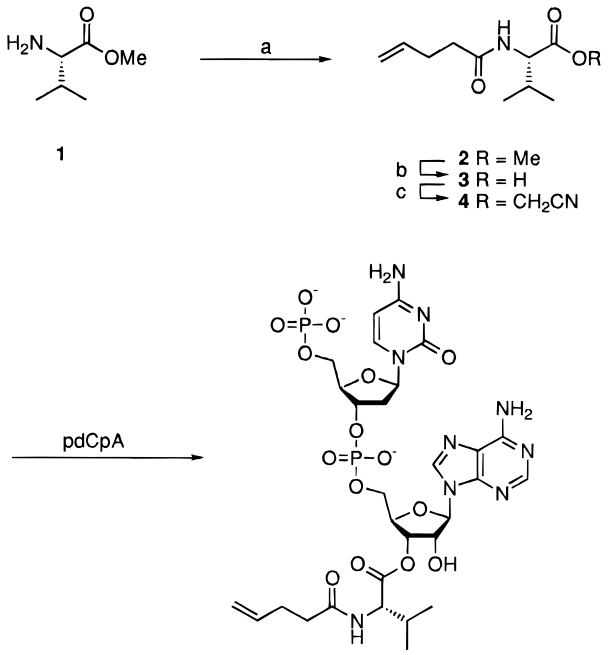
Presently, we describe the use of the 4-pentenoyl group for the elaboration of misacylated suppressor tRNA's.[5] This group forms a stable amide bond with N^{α} of amino acids and should stabilize the derived aminoacyl-pdCpA derivatives during T4 RNA ligase-mediated aminoacyl-tRNA synthesis. The use of aqueous iodine for deblocking of the pentenoyl group[5] seemed likely to be compatible with aminoacyl-tRNA structure since the same reagent is employed as an oxidant during the chemical synthesis of nucleic acids.[6]

Scheme 1. Misacylated Suppressor tRNA's



P = protecting group

Scheme 2. Synthesis of *N*-(4-Pentenoyl)-(*S*)-valyl-pdCpA[a]



[a] Pentenoic acid, BOP chloride, Et_3N, 0 °C, 2 h; (b) LiOH, 25 °C, 12 h; (c) $ClCH_2CN$, Et_3N, 25 °C, 28 h.

N-(4-Pentenoyl)valyl-pdCpA (**5**) was prepared as shown in Scheme 2. Treatment of *S*-valine methyl ester (**1**) with 4-pentenoic anhydride[5] afforded the respective amide **2** as a colorless oil in 73% yield. Following hydrolysis of the ester moiety (LiOH, aqueous THF), the derived intermediate **3** was converted to the respective cyanomethyl ester **4**.[7] Admixture of **4** and pdCpA[2g] in freshly distilled DMF afforded *N*-(4-pentenoyl)valyl-pdCpA (**5**) as a colorless solid in 94% yield. A sample of *N*-(6-nitroveratryloxycarbonyl)valyl-pdCpA (**6**) was prepared as described[2i] for comparative purposes.

Deblocking of protected valyl-pdCpA derivatives **5** and **6** was effected by treatment with iodine and *hν* irradiation, respectively (Scheme 3). HPLC analysis of the

(1) (a) Noren, C. J.; Anthony-Cahill, S. J.; Griffith, M. C.; Schultz, P. G. *Science* **1989**, *244*, 182. (b) Ellman, J.; Mendel, D.; Anthony-Cahill, S.; Noren, C. J.; Schultz, P. G. *Methods Enzymol.* **1991**, *202*, 301. (c) Bain, J. D.; Wacker, D. A.; Kuo, E. E.; Chamberlin, A. R. *Tetrahedron* **1991**, *47*, 2389. (d) Bain, J. D.; Diala, E. S.; Glabe, C. G.; Wacker, D. A.; Lyttle, M. H.; Dix, T. A.; Chamberlin, A. R. *Biochemistry* **1991**, *30*, 5411. (e) Bain, J. D.; Switzer, C.; Chamberlin, A. R.; Benner, S. A. *Nature* **1992**, *356*, 537. (f) Cornish, V. W.; Mendel, D.; Schultz, P. G. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 621. (g) Mamaev, S. V.; Laikhter, A. L.; Arslan, T.; Hecht, S. M. *J. Am. Chem. Soc.* **1996**, *118*, 7243.

(2) (a) Hecht, S. M.; Alford, B. L.; Kuroda, Y.; Kitano, S. *J. Biol. Chem.* **1978**, *253*, 4517. (b) Heckler, T. G.; Zama, Y.; Naka, T.; Hecht, S. M. *J. Biol. Chem.* **1983**, *258*, 4492. (c) Heckler, T. G.; Chang, L.-H.; Zama, Y.; Naka, T.; Chorghade, M. S.; Hecht, S. M. *Biochemistry* **1984**, *23*, 1468. (d) Baldini, G.; Martoglio, B.; Schachenmann, A.; Zugliani, C.; Brunner, J. *Biochemistry* **1988**, *27*, 7951. (e) Bain, J. D.; Glabe, C. G.; Dix, T. A.; Chamberlin, A. R.; Diala, E. S. *J. Am. Chem. Soc.* **1989**, *111*, 8013. (f) Roesser, J. R.; Xu, C.; Payne, R. C.; Surratt, C. K.; Hecht, S. M. *Biochemistry* **1989**, *28*, 5185. (g) Robertson, S. A.; Noren C. J.; Anthony-Cahill, S. J.; Griffith, M. C.; Schultz, P. G. *Nucleic Acids Res.* **1989**, *17*, 9649. (h) 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. (i) Robertson, S. A.; Ellman, J. A.; Schultz, P. G. *J. Am. Chem. Soc.* **1991**, *113*, 2722. (j) Hecht, S. M. *Acc. Chem. Res.* **1992**, *25*, 545.

(3) While both of these protecting groups are fairly versatile, the utility of the pyroglutamate group is limited by the substrate specificity of pyroglutamate aminopeptidase, while removal of the NVOC group requires a 500 W mercury−xenon lamp and conditions that are poorly compatible with some functionalized amino acids.[2d]

(4) See, however, refs 1a, 2a and Mendel, D.; Ellman, J. A.; Schultz, P. G. *J. Am. Chem. Soc.* **1991**, *113*, 2758.

(5) This group has been developed by Fraser-Reid and co-workers for the protection of simple organic amines. See (a) Debenham, J. S.; Madsen, R.; Roberts, C.; Fraser-Reid, B. *J. Am. Chem. Soc.* **1995**, *117*, 3302. (b) Madsen, R.; Roberts, C.; Fraser-Reid, B. *J. Org. Chem.* **1995**, *60*, 7920.

(6) Letsinger, R. L.; Lunsford, W. B. *J. Am. Chem. Soc.* **1976**, *98*, 3655.

(7) Bodanszky, M.; Bodanszky, A. In *The Practice of Peptide Synthesis*, 2nd rev. ed.; Springer-Verlag: Berlin, Heidelberg, New York, 1994; p 96.

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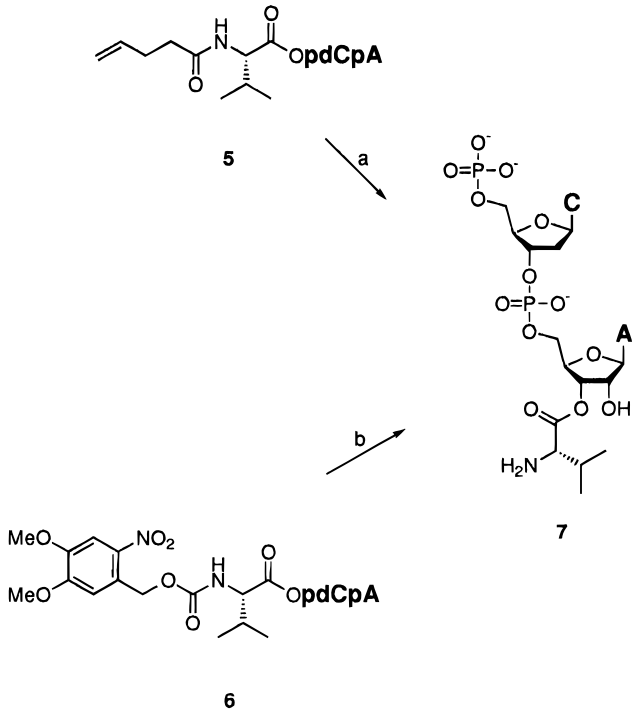
Scheme 3. Deprotection of *N*-(4-Pentenoyl)-(*S*)-valyl-pdCpA and *N*-(6-nitroveratryloxycarbonyl)-(*S*)-valyl-pdCpA[a]



[a] Iodine, 25 °C, 5 min; (b) *hν*, 2 °C, 5 min.

reaction mixtures indicated complete deblocking of both compounds within 5 min to afford putative valyl-pdCpA (**7**), although fewer byproducts detectable by UV were apparent in the reaction mixture derived from **5** (Figure 1, Supporting Information).

Protected valyl-pdCpA's **5** and **6** were each ligated to a suppressor tRNA transcript lacking the 3′-terminal pCpA moiety.[2] *N*-(4-Pentenoyl)valyl-tRNA was deprotected in aqueous THF containing 5 mM iodine; aliquots of the reaction mixture were employed in an *in vitro* protein-biosynthesizing system in direct comparison with valyl-tRNA prepared by photodeprotection of *N*-(6-nitroveratryloxycarbonyl)valyl-tRNA. As shown in Figure 1, the synthesis of dihydrofolate reductase (DHFR) from a mRNA containing a nonsense codon at position 10 proceeded to the same extent from deblocked *N*-(4-pentenoyl)valyl-tRNA and (NVOC)valyl-tRNA;[8] no DHFR was obtained in the presence of the *N*-(4-pentenoyl)valyl-tRNA if iodine treatment was omitted. The use of aliquots of *N*-(4-pentenoyl)valyl-tRNA treated with iodine for varying lengths of time suggested that deprotection was complete within 5−10 min (Figure 2). That the extent of DHFR production did not diminish when the activated tRNA was treated with iodine for up to 60 min is consistent with the lack of effect of iodine on the derived valyl-tRNA.[9]

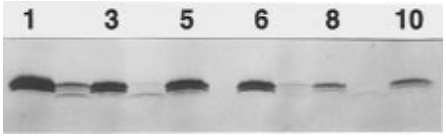


Figure 1. Effect of suppressor tRNA's on the synthesis of dihydrofolate reductase from a mRNA containing a nonsense codon. *In vitro* protein synthesis was carried out using [^{35}S]-methionine at 1000 Ci/mmol (lanes 1−5) or 20 Ci/mmol (lanes 6−10). Incubations employed (NVOC)valyl-tRNA either without (lanes 2 and 7) or with (lanes 3 and 8) deprotection; or *N*-(4-pentenoyl)valyl-tRNA either without (lanes 4 and 9) or with (lanes 5 and 10) deprotection. Lanes 1 and 6 illustrate DHFR synthesis from wild-type mRNA.

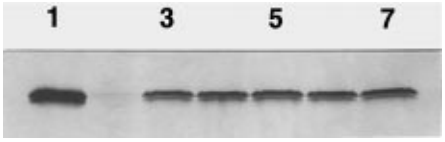


Figure 2. Effect of time of deprotection of *N*-(4-pentenoyl)-valyl-tRNA on the synthesis of DHFR. Aliquots of *N*-(4-pentenoyl)valyl-tRNA taken at predetermined times after treatment with iodine were added to *in vitro* protein synthesis reactions programmed with a mRNA containing a nonsense codon. Lanes 2−7, protected tRNA treated with iodine for 0, 5, 10, 20, 40, and 60 min. Lane 1, DHFR synthesis from wild-type mRNA. The relative suppression efficiencies in lanes 2−7 were 0.6, 16.3, 18.6, 19.2, 19.0, and 19.0%, respectively.

The foregoing experiments establish the feasibility of using the pentenoyl group for protection of the α-amino group during the synthesis of aminoacylated tRNA's. The availability of a chemically removable protecting group for N^{α} of the amino acid should also permit the elaboration of aminoacyl-tRNA's in which side chain functional groups of amino acids are protected differentially, such that deblocking can be effected subsequent to protein synthesis. This may, for example, facilitate the incorporation of charged amino acids such as aspartic acid, which has proved to be problematic.[10]

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Supporting Information Available: Reverse-phase HPLC analysis of deblocked samples of **7**; experimental details (7 pages).

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(8) The presence of valine at position 10 was verified by degradation of the formed DHFR with Glu-C endopeptidase, affording a peptide that encompassed amino acids 1−17. This peptide was identical with an authentic standard prepared by chemical synthesis.

(9) Also supporting this interpretation was the lack of any degradation observed when the iodine-treated tRNA was analyzed by polyacrylamide gel electrophoresis at pH 5.0.

(10) Preliminary experiments with aspartic acid derivatives having protected side chain carboxylates suggests that substantially improved yields may be realized in this fashion. See also ref 4.