

Expanding the Genetic Code: Synthesis of Unnatural Amino Acids and Misacylated Suppressor tRNA

Jin Ho Ahn¹, Taek Jin Kang², Kwan Hong Min², Dal Ho Huh², Tae Wan Kim²,
Nam Young Kim², In Seok Oh², Young Gyu Kim², Yeo Joon Yoon³, Cha Yong Choi^{1, 2, *}

Interdisciplinary Program for Biochemical Engineering and Biotechnology

College of Engineering, Seoul National University¹;

School of Chemical Engineering, College of Engineering, Seoul National University²;

School of Chemical Engineering and Bioengineering, University of Ulsan³;

TEL : 02-880-7528, FAX : 02-874-1206

We describe here synthetic advances in the preparation of chemically misacylated suppressor tRNA which can serve as the decoder of amber stop codon. A general and facile procedure for the synthesis of two unnatural amino acids, *D,L*-(2-nitrophenyl)glycine (NPG) and *L*-S-(2-nitrobenzyl)cysteine (NBC), and dinucleotide 5'-O-phosphoryl-2'-deoxycytidylyl-(3'→5')adenosine (pdCpA) has been developed. Both NPG and NBC were protected at the N^d site using 4-pentenoyl anhydride and were activated on carboxylate as cyanomethyl esters. These active esters were used to aminoacylate pdCpA. T4 ligase-mediated ligation of these pdCpA esters to tRNA lacking the 3'-end CA yielded full-length amber suppressor tRNA. Following the deprotection by iodine, the (2-nitrophenyl)glycyl tRNA and S-(2-nitrobenzyl)cysteinyl tRNA were shown to be competent in an *in vitro* protein translation system. These procedure greatly simplify the preparation of chemically misacylated tRNA in the synthesis of proteins containing unnatural amino acid.

References

1. Lodder M, Golovine S, Hecht S. M. (1997), Chemical deprotection strategy for the elaboration of misacylated transfer RNA's, *J. ORG. CHEM.* **62**(4), 778-779.
2. Taek Jin Kang, Ji Hyoung Woo, Hui Kyoung Song, Jin Ho Ahn, Jae Wook Kum, Jin Han, Cha Yong Choi, Hyun Joo (2002), A cell-free protein synthesis system as an investigational tool for the translation stop processes, *FEBS Lett.* **24**, 517(1-3):211-4.