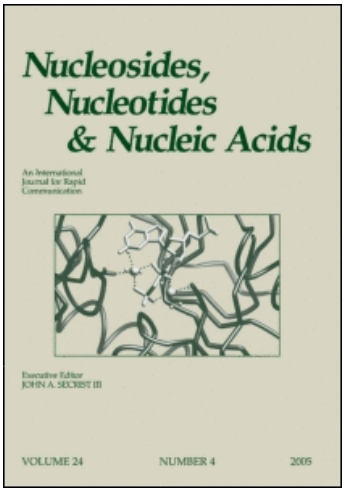

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An Attempt to Design and Chemical Synthesis of a Eukaryotic Messenger RNA

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AN ATTEMPT TO DESIGN AND CHEMICAL SYNTHESIS OF A EUKARYOTIC MESSENGER RNA

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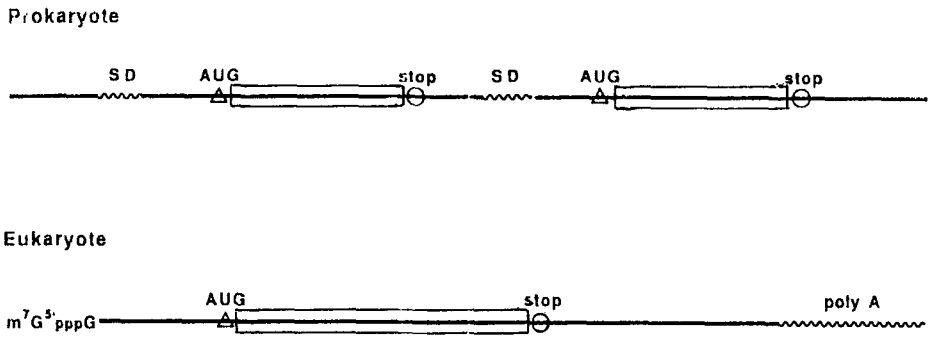
Abstract: RNA (42 mer) bearing cap structure was synthesized by combination use of the chemical and enzymatic methods.

Chemical synthesis of nucleic acids has considerably contributed to biosciences, especially, DNA synthesis including the gene synthesis has been contributed to genetic engineering as well as protein engineering. Genetic engineering has developed very rapidly and enable us to produce the desired protein in living cells. However, there still remain several technical problems, such as how the folding on the amino acid sequences is controlled and how separation of the desired protein from the harvest proteins is performed. Therefore, the successful results has discovered empirically and case-by-case. Under these circumstances, the protein synthesis starting from messenger RNA (mRNA) in vitro system seems to be highlighted. Consequently, much attention is being denoted to the synthesis of RNAs.

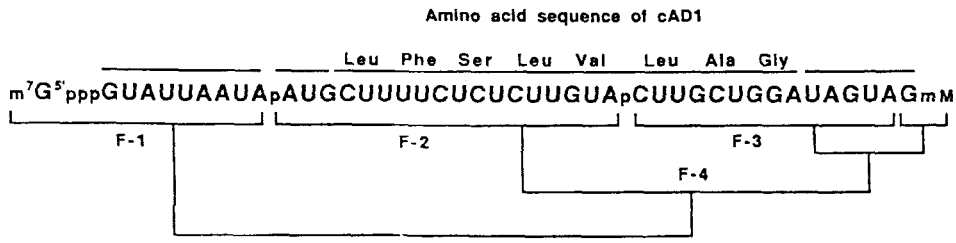
Prokaryotic mRNAs are poly-cistronic and each protein synthesis is controlled by Shine-Dalgarno sequence (SD), whereas eukaryotic mRNAs are mono-cistronic and they have

so-called "cap structure" at the 5'-terminus. Eukaryotic mRNAs consist of the cap structure, leader sequence, initiation codon (AUG), a single cistron, stop codon (UAG, UAA or UGA), 3'-noncoding region, and poly A.



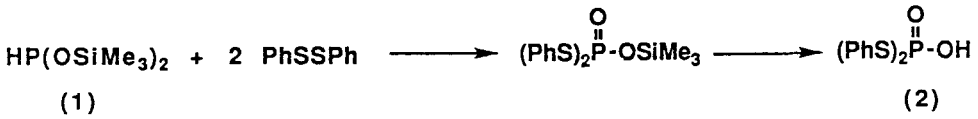
In this paper, we wish to report the design and the synthesis of a man-made eukaryotic mRNA.

Among various leader sequences of eukaryotic mRNAs, we have chosen the sequence from the mRNA of brome mosaic virus (BMV) No. 4[1] coding its coat protein synthesized very efficiently. Streptoccus feacalis pheromone, cAD1 consists of eight amino acids.[2] It can be detected at very low concentrations. The corresponding cistron consists of 24 nucleotides and the codon of each amino acid was selected by the codon usage of BMV. Up to date, little is known about the 3'-noncoding region, and the poly A tail is said to act as a role of protection against exonucleases.[3] There are many factors to be taken into consideration, such as the secondary structure of mRNAs which have to be investigated calmly. However, considering the latest knowledge regarding molecular biology, the RNA can be designed as follows:



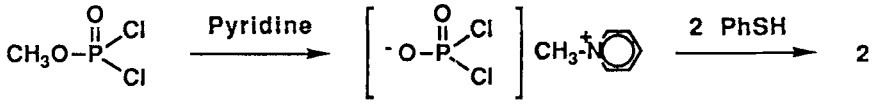
The total synthesis of the above RNA has been done by the use of the chemical and enzymatic methods.

Synthesis of the leader sequence (9 mer) was performed by the phosphotriester method by using phenylthio group as a protecting group of the phosphate residues.[4] S,S-Diphenyl phosphorodithioyl group (PSS) was introduced to the appropriately protected ribonucleosides by use of S,S-diphenyl phosphorodithioate (**2**) which was prepared by the reaction of bis(trimethylsilyl) phosphinate (**1**) with two equiv. of diphenyl disulfide.[5]



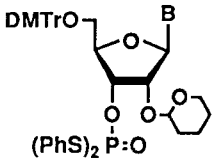
(1) (2)

The same compound **2** can be more easily prepared in large scale starting from methyl phosphorodichloridate (**3**) and two equiv. of benzenethiol in dry pyridine[6] by the modification of the procedure of Smrt.[7]

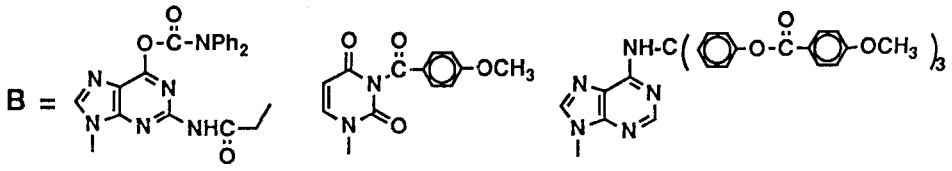


(3)

Fully protected building blocks (**4**)[8] are as follows:

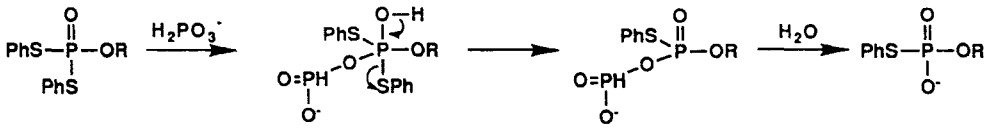


(4)

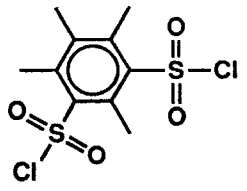


(Gu Dpc-Pro) (Ur Ac) (Ad TanTr)

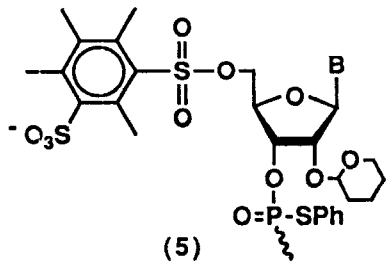
One of two phenylthio groups of PSS was removed selectively from **4** by use of triethylammonium phosphinate.[9]



The fully protected 9 mer was synthesized by means of the fragment condensation using isodurenedisulufonyl dichloride (DDS) which has a separation-handle, namely, when it reacts unexpectedly with 5'-hydroxyl group of a oligomer during the condensation reaction, the 5'-sulfonylated oligomer (**5**) is more polar than the expected oligomer having 5'-DMTr group. Therefore, the side product **5** can be easily removed.

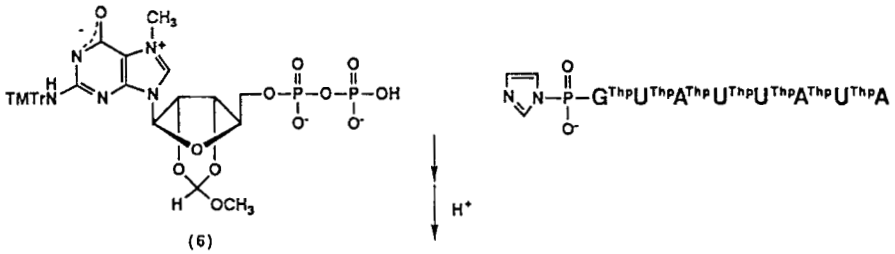


(DDS)



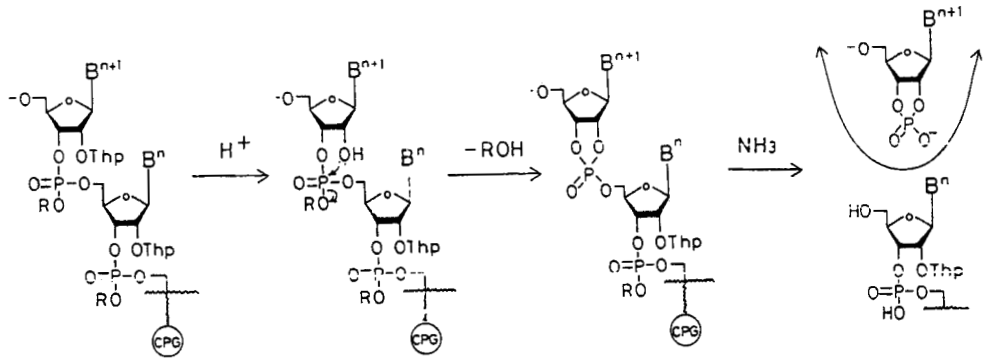
(5)

The 5'-DMTr group of the fully protected 9 mer was removed and then PSS group was introduced to the 9 mer by using DDS to give the corresponding 5'-phosphorylated 9 mer. After deprotection of all phenylthio groups and the base protecting groups from the 5'-phosphorylated 9 mer, it was allowed to react in situ with carbonyldiimidazole to give the 5'-phosphorimidazolide and followed by treatment with a capping agent, 2',3'-O-methoxymethylene N^2-(4,4',4"-trimethoxytrityl)-7-methyl-guanosine 5'-diphosphate (**6**). After removal of trimethoxytrityl, methoxymethylene, and tetrahydropyranyl groups, the capped 9 mer (**F-1**) was obtaind. **F-1** may be generally used as a cassette of leader sequence for protein synthesis in vitro. Therefore, the phosphotriester method was suitable for relatively large scale synthesis of **F-1**.

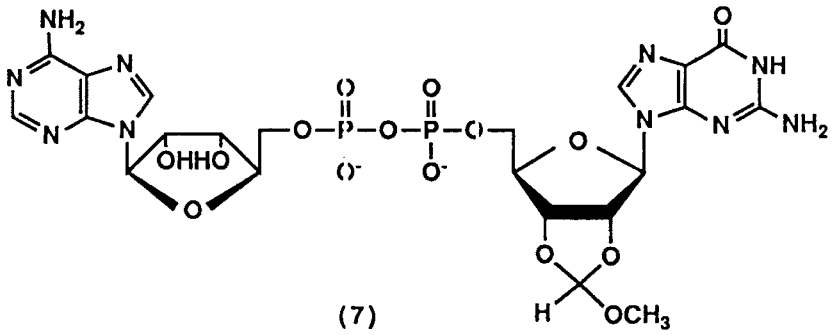


$m^7G^{5'}pppG\,U\,A\,U\,U\,A\,A\,U\,A$ (F-1)

RNA fragments, **F-2** and **F-3** were synthesized by the phosphoramidite method similar to the DNA synthesis. Synthesis of the building blocks and their application to the synthesis of RNA fragments (10-20 mer) has been described previously.[10] By use of the solid phase method for RNA fragment synthesis, we have investigated the mechamism of the 3'⟶2' phosphoryl migration and elucidated no formation of 2'-5' internucleotidic bonds but cleavage of the RNA linkages took place under the deprotection (acidic) conditions.[11]



The ligation by use of T4 RNA ligase proceeds through a 5'-adenylated intermediate. According to the results described by McLaughlin,[12] the 3'-terminal nucleotide of the man-made mRNA, 2',3'-O-methoxymethylene guanosine (pGmM) was introduced effectively to **F-3** by use of P^1-2',3'-O-methoxymethylene guanosine-5'-yl P^2-adenosine-5-yl diphosphate (**7**).



(7)

Kination of **F-3**-GmM was performed by use of ATP with polynucleotide kinase to afford p**F-3**-GmM (15 mer). The ligation was carried out first between **F-2** and p**F-3**-GmM by use of T4 RNA ligase and then the product was further kinated and separated by polyacrylamide gel electrophoresis. The kinated 33 mer (p**F-4**) was further ligated with **F-1** in a similar manner to afford the final 42 mer having the cap structure.

We have only marked the first step in our studies in this particular field. Further investigation is now in progress.

Acknowledgements

This work has been done by collaborations with Professor Mitsuo Sekine, Department of Life Science, Tokyo Institute of Technology, and Kin-ichiro Miura, Department of Industrial Chemistry, The University of Tokyo.

This paper is dedicated to Daniell Professor Colin Bernard Reese on the occasion of his 60th birthday.

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