Pergamon

Tetrahedron Letters 42 (2001) 8853–8856

TETRAHEDRON LETTERS

Solid-phase synthesis of a 5′-terminal TMG-capped trinucleotide block of U1 snRNA

Michinori Kadokura,[a] Takeshi Wada,[a] Kohji Seio,[a] Tomohisa Moriguchi,[a] Jochen Huber,[b] Reinhard Lührmann[b] and Mitsuo Sekine[a,*]

[a]*Department of Life Science, Tokyo Institute of Technology, Nagatsuta, Midoriku, Yokohama 226-8501, Japan*
[b]*Institut für Molekularbiologie und Tumorforschung, Philipps-Universität Marburg, Emil-Mannkopff-Straße 2, D-3550 Marburg, Germany*

Received 31 May 2001; revised 10 October 2001; accepted 12 October 2001

Abstract—A 5′-terminal 2,2,7-trimethylguanosine-capped trinucleotide block ($m_3^{2,2,7}G^{5'}$pppAmpUmpA) was successfully synthesized on a highly cross-linked polystyrene resin by a new method for introduction of the first nucleoside onto the resin and a newly developed pyrophosphorylating agent having a benzotriazolyloxy substitutent as the leaving group. © 2001 Published by Elsevier Science Ltd.

In the current oligonucleotide synthesis, RNA fragments have been successfully obtained by extensive application of the phosphoramidite approach to the solid-phase synthesis.[1] Now, a variety of base-modified RNA fragments have also been synthesized by the solid-phase strategy.[2] However, this straightforward approach has never been applied to the synthesis of extremely base-labile 7-methylguanosine (MMG)-capped mRNAs and 2,2,7-trimethylguanosine (TMG)-capped U snRNAs.[3] This is a major problem to be solved in the organic synthesis of nucleic acids. Here, we report the first successful solid-phase synthesis of $m_3^{2,2,7}G^{5'}$pppAmUmA[4] **1**, i.e. the 5′-terminal site of U1 snRNA (Fig. 1)[5] by exploring a new method for pyrophosphorylation.

To realize the solid-phase synthesis of **1**, we considered that a TMG-capping reaction should be carried out at a later stage than the deprotection of all base-labile protecting groups, since the cap structure is extremely fragile under basic conditions.[6] As a possible choice to allow the release of capped RNAs from the solid support without damage of the TMG-cap structure, we used the following phosphoramidate linker between the 3′-terminal phosphate of the RNA chain and the amino group on solid supports. Previously, Letsinger reported that the P–N bond of ROP(O)(OR′)(NHR″) is sufficiently stable under conditions prescribed for removal of the DMTr group but becomes acid-labile once the R′ group is eliminated.[7] The nature of the inherent dramatic change in this linker allows us to carry out successively: (1) elongation of the RNA chain; (2) 5′-terminal phosphorylation; (3) deprotection of all base-labile protecting groups; (4) 5′-terminal pyrophosphorylation; (5) addition of a cap structure to the terminal site of the pyrophosphate group; (6) release of the synthetic RNA oligomer derivative by the action of acids, and (7) dephosphorylation of the 3′-terminal phosphate group. Our strategy for the synthesis of **1** on a highly cross-linked polystyrene resin is outlined, as shown in Schemes 1 and 2.

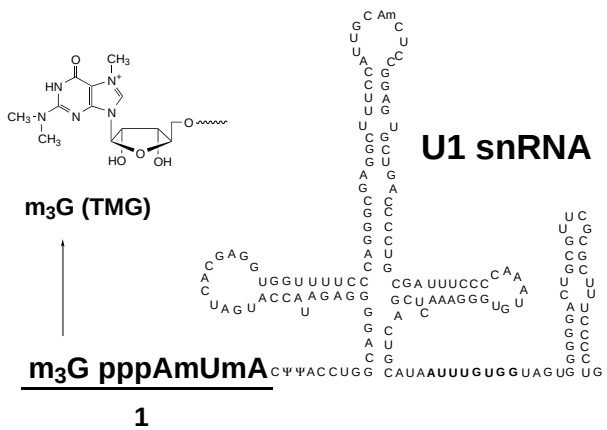
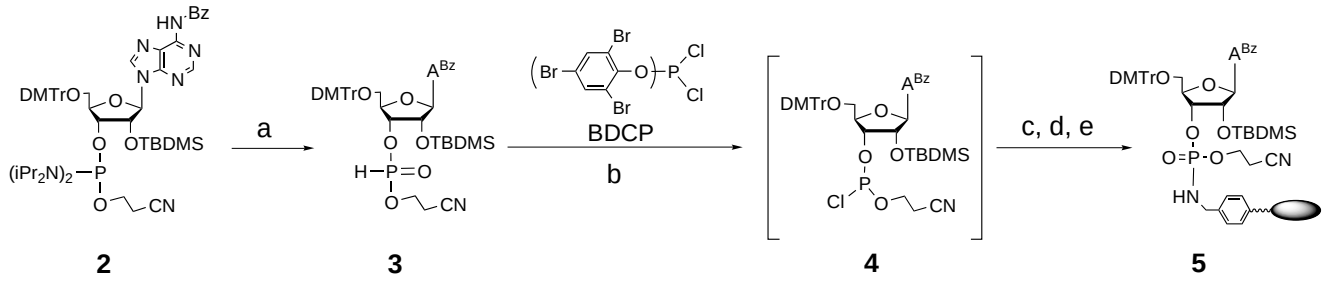


Figure 1. The secondary structure of U1 snRNA with a 2,2,7-trimethylguanosine cap.

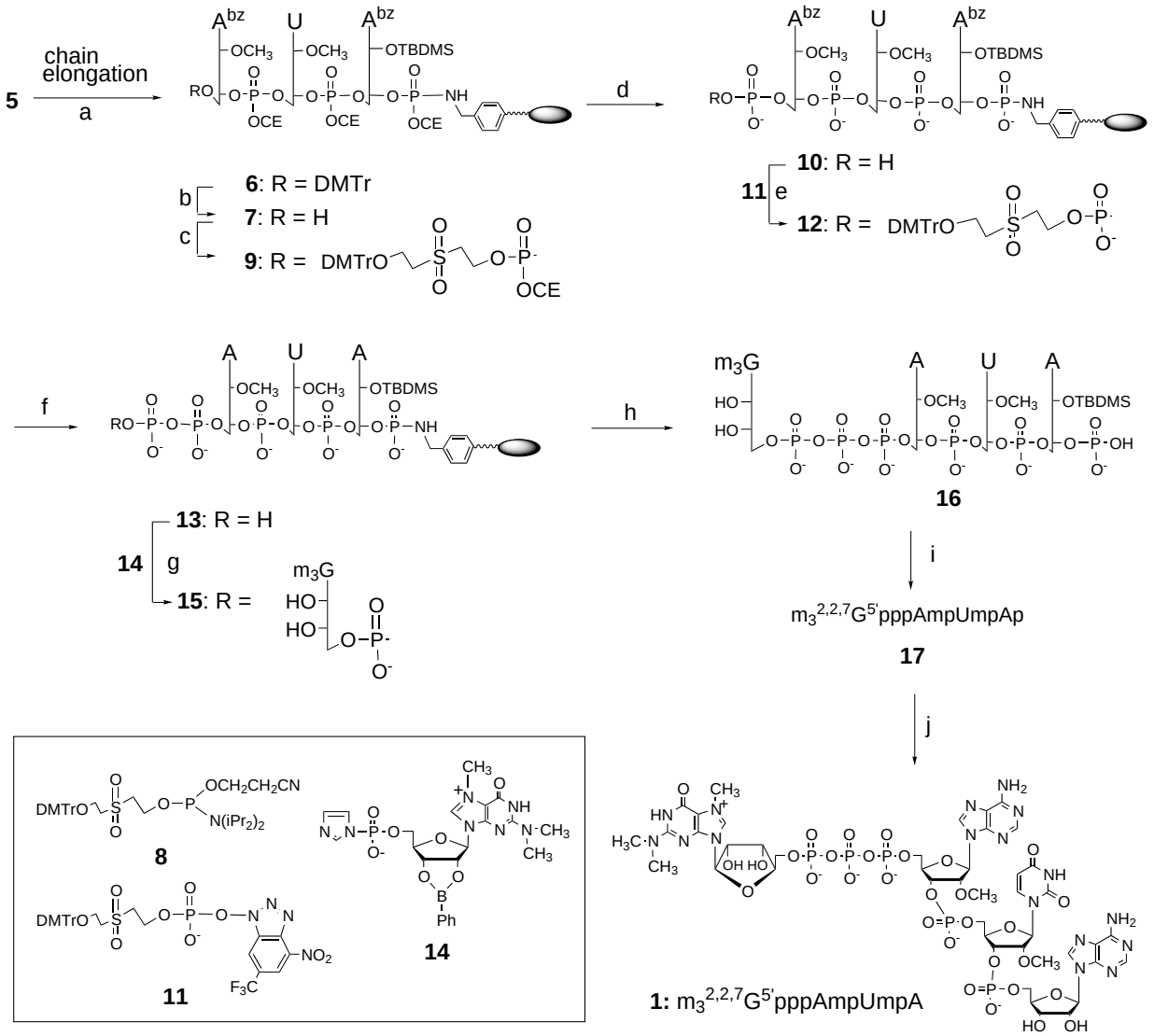
Keywords: TMG-cap; solid-phase synthesis of RNA; BDCP; U1 snRNA.

* Corresponding author. Tel.: +81-45-924-5706; fax: +81-45-924-5772; e-mail: msekine@bio.titech.ac.jp

0040-4039/01/$ - see front matter © 2001 Published by Elsevier Science Ltd.
PII: S0040-4039(01)01941-4



Scheme 1. Synthesis of an adenosine-loaded resin by use of BDCP. (a) 1 equiv. of 1*H*-tetrazole, H_2O–pyridine (1:20, v/v), rt, 30 min; (b) 3 equiv. of BDCP (30 mmol), pyridine, rt, 5 min; (c) compound **4** (30 mmol), highly cross-linked aminopropyl-polystyrene resin (1 mmol, 35 μmol/g of NH_2 group), rt, 5 min; (d) 17 mM of I_2, 50 mM pyridine, H_2O–THF (9:91, v/v), 15 s×3; (e) Ac_2O–pyridine (1:9, v/v), rt, 6 h.



Scheme 2. Solid-phase synthesis of $m_3^{2,2,7}G^{5'}$pppAmpUmpA. (a) (1) Detritylation: 2.5% dichloroacetic acid in CH_2Cl_2, 15 s×3, (2) washing: CH_2Cl_2, CH_3CN, (3) condensation: 0.05 M amidite, 0.5 M 1*H*-tetrazole, CH_3CN, 10 min, (4) washing: pyridine, (5) capping: 10% Ac_2O, 0.1 M DMAP, pyridine, 2 min, (6) washing: pyridine, CH_2Cl_2, (7) oxidation: 17 mM I_2, 50 mM pyridine, H_2O–THF (9:91, v/v); (b) detritylation: 2.5% dichloroacetic acid in CH_2Cl_2; (c) compound **8**, 1*H*-tetrazole, CH_3CN, 5 min; (d) (1) 10% DBU/pyridine–BSA (1:1, v/v), rt, 10 min, (2) Et_3N–MeOH (1:9, v/v); (e) 0.1 M of **11**, pyridine, 2 h; (f) conc. NH_3–EtOH (3:1, v/v), 55°C, 4 h; (g) 0.1 M of **14**, pyridine, 24 h; (h) 80% AcOH, rt, 24 h; (i) HCl (pH 2.0), rt, 12 h; (j) calf intestinal alkaline phosphatase, Tris–HCl buffer (pH 7.0), 10 mM $MgCl_2$, 37°C, 1 h.

First, we encountered much difficulty in synthesizing an adenosine-linked polymer **5** by the reaction of an adenosine 3′-phosphoramidite derivative **2** with a highly cross-linked aminomethylpolystyrene support[8] (35 μmol/g) in the presence of 1*H*-tetrazole. The loading amount of the adenosine unit was only 7 μmol/g, unlike that of the deoxy counterpart.[7] This low efficiency might be due to the steric hindrance arising from the 2′-*O*-TBDMS group and neutralization of the amino group with the activator.

Therefore, we developed a new method for the synthesis of **5**. Reaction of the *H*-phosphonate derivative **3** derived from **2** with tris(2,4,6-tribromophenoxy)dichlorophosphorane (BDCP)[9,10] gave a highly reactive chlorophosphite intermediate **4**, which, in turn, was allowed to react with the same resin to give **5** with a loading amount of 31.5 μmol/g (Scheme 1).

A 5′-phosphosphorylated trimer block **9** was synthesized with the average coupling yield of 99% by the standard phosphoramidite approach[11] using the 2′-*O*-methyluridine and *N*-benzoyl-2′-*O*-methyladenosine phosphoramidite units and the phosphalink agent **8**[12] via the intermediates **6**–**7**, as shown in Scheme 2. All the cyanoethyl and sulfonylethyl groups were promptly and simultaneously deprotected by using DBU–bis(trimethylsilyl)acetamide (BSA)[13] to give the 5′-terminal free product **10**. Pyrophosphorylation of **10** with a new reagent **11**[14] in pyridine gave the product **12** in 80% yield. Removal of the remaining *N*-benzoyl groups from **12** was successively performed by treatment with ammonia–EtOH[15] to afford the product **13**. For triphosphate bond formation, coupling reaction of **13** with the boranylated 2,2,7-trimethylguanosine 5′-phosphorimidazolide derivative **14**,[16] which was synthesized to improve the solubility via a one-pot reaction from *N*,*N*-dimethylguanosine 5′-phosphate, gave the capped product **15**.

At the final stage, treatment of **15** with 80% acetic acid resulted in release of a mixture containing the TMG-capped trimer block **16**. This mixture was further treated with a diluted HCl solution (pH 2.0)[17] to remove the last remaining TBDMS group from **16**. Finally, the resulting product **17** was dephosphorylated by calf intestinal alkaline phosphatase to give the desired product **1**, which appeared as the main peak in HPLC (Fig. 2) and was isolated in an overall yield of 20% from **12**. The structure of **1** was confirmed by MALDI-TOF mass (calcd, 1478.24; found 1478.44) and enzymatic analysis using nuclease P1.

This strategy could be applied to the chemical synthesis of other oligonucleotides having a pyro- or triphosphate bond bridge. It should be also emphasized that our present method would provide a powerful tool to clarify the detailed mechanisms of complex splicing reaction[18] and RNA transport[19] in which the TMG cap structure has been proven to play an important role[20] since the TMG-capped trimer block could be the smallest substrate[21] for RNA ligase that enables us to prepare freely longer TMG-capped RNAs.[22] The second phosphorylation and capping steps should be improved in the future.

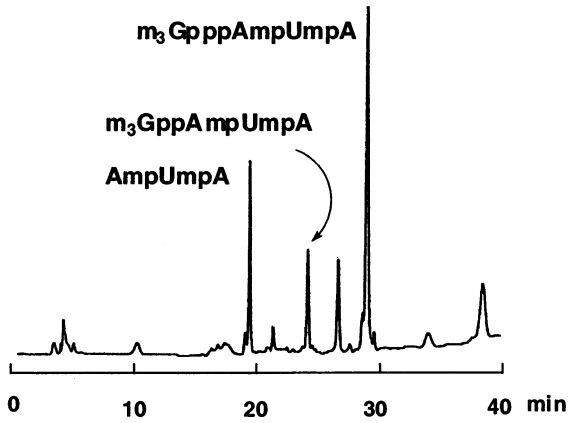


Figure 2. Anion-exchange HPLC profile of the mixture obtained by acid treatment of **17**.

Acknowledgements

This work was supported by a Grant from 'Research for the Future' Program of the Japan Society for the Promotion of Science (JSPS-RFTF97I00301) and a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

References

1. Beaucage, S. L.; Caruthers, M. H. In *Current Protocols in Nucleic Acid Chemistry*; Beaucage, S. L.; Bergstrom, D. E.; Glick, G. D.; Jones, R. A., Eds. Solid-phase supports for oligonucleotide synthesis; John Wiley & Sons: New York, 2000; pp. 3.3.1–3.3.20.
2. For recent studies on the chemical synthesis of modified RNAs, see: (a) Kadokura, M.; Wada, T.; Seio, K.; Sekine, M. *J. Org. Chem.* **2000**, *65*, 5104–5113; (b) Sundaram, M.; Crain, P. F.; Davis, D. R. *J. Org. Chem.* **2000**, *65*, 5609–5114; (c) Sochacka, E.; Czerwinska, G.; Guenther, R.; Cain, R.; Agris, P. F.; Malkiewicz, A. *Nucleosides Nucleotides Nucleic Acids* **2000**, *19*, 515–531.
3. Sekine, M.; Hata, T. *Curr. Org. Chem.* **1998**, *3*, 25–66.
4. The previous liquid-phase synthesis of **1**: Sekine, M.; Kadokura, M.; Satoh, T.; Seio, K.; Wada, T.; Fischer, U.; Sumper, V.; Lührmann, R. *J. Org. Chem.* **1996**, *61*, 4412–4422.
5. *Structure and Function of Major and Minor Small Nuclear Ribonucleoprotein Particles*; Birnstiel, M. L., Ed.; Springer: Berlin, 1988.
6. Hecht, S. M.; Benjamin, L. A.; Kozarich, J. W. *J. Org. Chem.* **1976**, *41*, 2303–2311.
7. Gryaznov, S.; Letsinger, R. *Tetrahedron Lett.* **1992**, *33*, 4127–4128.
8. (a) McCollum, C.; Andrus, A. *Tetrahedron Lett.* **1991**, *32*, 41069–41072; (b) Wada, T.; Sato, Y.; Honda, F.; Kawahara, S.; Sekine, M. *J. Am. Chem. Soc.* **1997**, *119*, 12710–12721.

9. Hotoda, H.; Wada, T.; Sekine, M.; Hata, T. *Tetrahedron Lett*. **1987**, *28*, 1681–1684.
10. Wada, T.; Kato, R.; Hata, T. *J. Org. Chem*. **1991**, *56*, 1243–1250.
11. Damha, M. J.; Ogilvie, K. K. In *Protocols for Oligonucleotides and Analogs*; Agrawal, S., Ed.; Humana Press: Totowa, 1993; pp. 81–114.
12. Horn, T.; Urdea, S. *Tetrahedron Lett*. **1986**, *27*, 4705–4708.
13. Sekine, M.; Tsuruoka, H.; Iimura, S.; Wada, T. *Nat. Prod. Lett*. **1994**, *5*, 41–46.
14. This reagent [^{31}P NMR (109.4 MHz, pyridine-d_5, 25°C) δ 0.84)] was synthesized almost quantitatively via a three-step reaction from 2-(2′-*O*-4,4′-dimethoxytrityloxysulfonyl)-ethanol, which involves phosphorylation using *S*,*S*-diphenyl phosphorodithioate,[3] the removal of one of the two PhS groups,[3] and activation of the PhS group by I_2 or NCS in the presence of the hydroxylbenzotriazole derivative.
15. Mullah, B.; Andrus, A. *Nucleosides Nucleotides* **1996**, *15*, 419–430.
16. Compound **14** (^{31}P NMR (pyridine-d_5) δ −8.68) was obtained almost quantitatively by a three-step reaction, which involves the 7-*N*-methylation of *N*,*N*-dimethylguanosine 5′-phosphate with methyl iodide in DMF, the boranylation of the residue with phenylboronic acid, and the reaction with 1,1′-carboyldiimidazole.
17. Kawahara, S.; Wada, T.; Sekine, M. *J. Am. Chem. Soc*. **1996**, *118*, 9461–9468.
18. Padgett, R. A.; Mount, S. M.; Steitz, J. A.; Sharp, P. A. *Cell* **1983**, *35*, 101–107.
19. Massenet, S.; Mougin, A.; Branlant, C. In *Posttranscriptional modifications in the U small nuclear RNAs*; Grosjean, H.; Benne, R., Eds. Modification and Editing of RNA; ASM Press: Washington, DC, 1988; pp. 201–227.
20. Huber, J.; Cronshagen, U.; Kadokura, M.; Marshallsay, C.; Wada, T.; Sekine, M.; Lührmann, R. *EMBO J*. **1998**, *17*, 4114–4126 and references cited therein.
21. England, T. E.; Uhlenbeck, O. C. *Biochemistry* **1978**, *17*, 2069–2076.
22. Iwase, R.; Maeda, M.; Fujiwara, T.; Sekine, M.; Hata, T. *Nucleic Acids Res*. **1992**, *20*, 1643–1648.