Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 14 (2004) 4431–4434

Synthesis of 2′,5′-oligoadenylate analogs containing an adenine acyclonucleoside and their ability to activate human RNase L

Yoshihito Ueno, Shinji Ishihara, Yasutomo Ito and Yukio Kitade*

Department of Biomolecular Science, Faculty of Engineering, Gifu University, Yanagido, Gifu 501-1193, Japan

Received 10 May 2004; accepted 17 June 2004

Abstract—This paper described synthesis of 2′,5′-oligoadenylate (2–5A) analogs containing the purine acyclonucleoside, 9-[(2′*S*,3′*R*)-2′,3′,4′-trihydroxybutyl]adenine (**2**). The ability of the analogs to activate recombinant human RNase L was evaluated using 5′-^{32}P-r($C_{11}U_2C_7$)-3′ as a substrate. The EC_{50} value (the concentration of the 2–5A required to cleave half of the RNA) of the parent 2–5A tetramer **13** was 1.0 nM, whereas those of the analog **14** incorporating **2** at the second position from the 5′-end and the analog **15** incorporating **2** at the third position from the 5′-end were 9.0 and 1.7 nM, respectively. The analogs **14** and **15** were only 9- and 1.7-fold less potent than the parent 2–5A **13** itself, in RNase L activation ability. Furthermore, the oligodeoxynucleotide containing **2** was more resistant to nucleolytic hydrolysis by snake venom phosphodiesterase (a 3′-exonuclease) than the unmodified oligodeoxynucleotide. Thus, incorporation of an acyclonucleoside into 2–5A may be useful for developing an antiviral agent based on the 2–5A system.

Interferons mediate diverse and important cellular responses such as the induction of an antiviral state, proliferation, and various immunological processes.[1,2] One extensively characterized mechanism of interferon action is the 2–5A system.[1–3] Oligoadenylates that linked 2′–5′ (2–5A) are formed from ATP by 2–5A synthetase in response to interferon and double-stranded RNA. These 2–5As bind to and allosterically activate a latent endoribonuclease (RNase L). The activated RNase L cleaves viral and cellular RNAs on the 3′-sides of UpNp sequences.[4] The RNA degradation results in inhibition of protein synthesis and thereby inhibition of viral replication. Another member in the 2–5A system is the 2′–5′ phosphodiesterase that rapidly degrades the 2–5As.

During the past two decades, various 2–5A analogs have been synthesized to study the biological function of 2–5A and its structure–activity relationship and to explore the potential for deriving an antiviral agent based on the 2–5A system.[5] On the other hand, various acyclonucleosides in which the carbohydrate moieties are acyclic chains mimicking the sugar portion of naturally occurring nucleosides have also been synthesized to evaluate their antiviral activities.[6–8] It is expected that incorporation of acyclonucleosides into 2–5As instead of adenosine (**1**) (Fig. 1) would enhance their stability against nucleolytic degradation by nucleases found inside cells. However, the biological property of a 2–5A analog incorporating an acyclonucleoside has not been reported.[9] In this paper, we wish to report the synthesis of the 2–5A analogs containing the purine acyclonucleoside, 9-[(2′*S*,3′*R*)-2′,3′,4′-trihydroxybutyl]adenine (**2**), and their ability to activate recombinant human RNase L. The resistance of the oligodeoxynucleotide containing **2** to nucleolytic hydrolysis by snake venom phosphodiesterase was also examined.

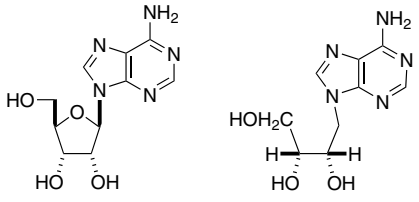


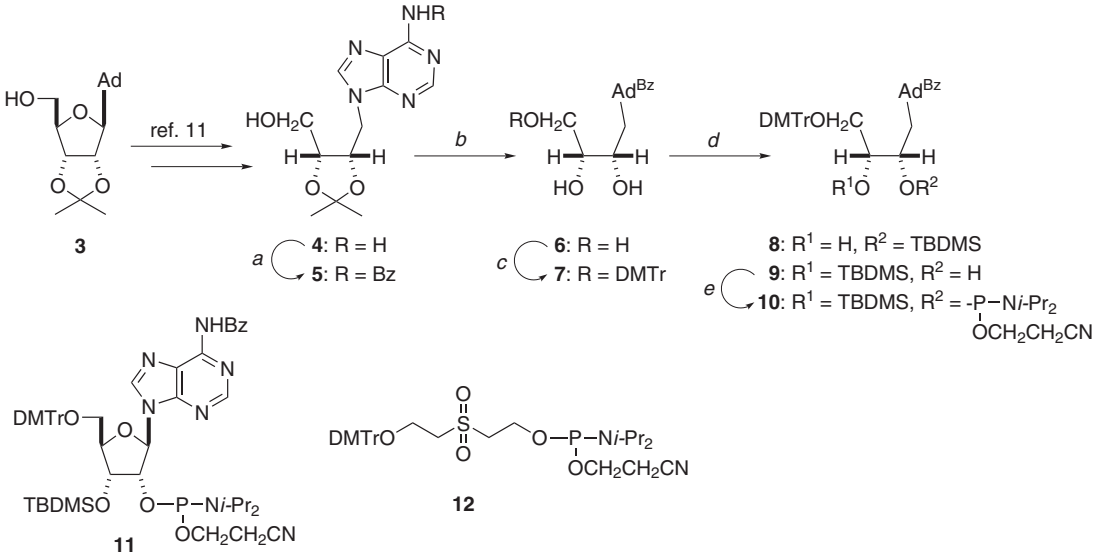
Figure 1. Structures of nucleosides.

Keywords: Adenine; Acyclonucleoside; 2–5A; RNA; RNase L.
* Corresponding author. Tel./fax: +81-58-293-2640; e-mail: kitade@biomol.gifu-u.ac.jp

0960-894X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2004.06.071

9-[(2′*S*,3′*R*)-4′-Hydroxy-2′,3′-isopropylidenedioxybutyl] adenine (**4**), which was prepared by the reported method,[10,11] was treated with TMSCl in pyridine and then reacted with benzoyl chloride (BzCl) to produce the N^6-Bz derivative **5** (Scheme 1). Treatment of **5** with 80% CH_3CO_2H afforded the triol **6** in 44% yield from **4**. The primary hydroxyl group of **6** was protected with a 4,4′-dimethoxytrityl (DMTr) group to give **7** in 94% yield. *O*-DMTr derivative **7** was treated with *tert*-butyldimethylsilyl chloride (TBDMSCl) to afford 2′-*O*-TBDMS and 3′-*O*-TBDMS derivatives, **8**[12] and **9**,[13] in 7% and 35% yields, respectively. 3′-*O*-TBDMS derivative **9** was phosphitylated by a standard procedure to give the corresponding phosphoramidite **10**[14] in 65% yield.

The 2–5A analogs **14** and **15** were synthesized using the phosphoramidites **10**, **11**, and **12**[15] with a DNA/RNA synthesizer (Fig. 2). The analog **14** has **2** at the second position from the 5′-end, whereas the analog **15** has **2** at the third position from the 5′-end. To examine the resistance of the oligodeoxynucleotide containing **2** to nucleolytic hydrolysis by snake venom phosphodiesterase, 5′-d($A_2G_2A_3$**2**GAG_2A_3GA)-3′ (**16**)[18] was also synthesized. The fully protected 2–5As (1 μmol each) linked to the solid support were treated with concentrated NH_4OH/EtOH (3:1, v/v) at 55 °C for 12 h and then 1 M TBAF/THF at room temperature for 12 h. The released 2–5As were purified by reversed phase HPLC to give deprotected 2–5As **14** and **15** in 1.8 and 2.4 OD_{260} units, respectively. These 2–5As were ana-



Scheme 1. (a) (1) TMSCl, pyridine, rt; (2) BzCl, pyridine, rt; (b) 80% CH_3CO_2H, 60 °C, 44% from **4**; (c) DMTrCl, pyridine, rt, 94%; (d) TBDMSCl, imidazole, DMF, rt, 7% (**8**), 35% (**9**); (e) 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite, *N*,*N*-diisopropylethylamine, THF, rt, 65%.

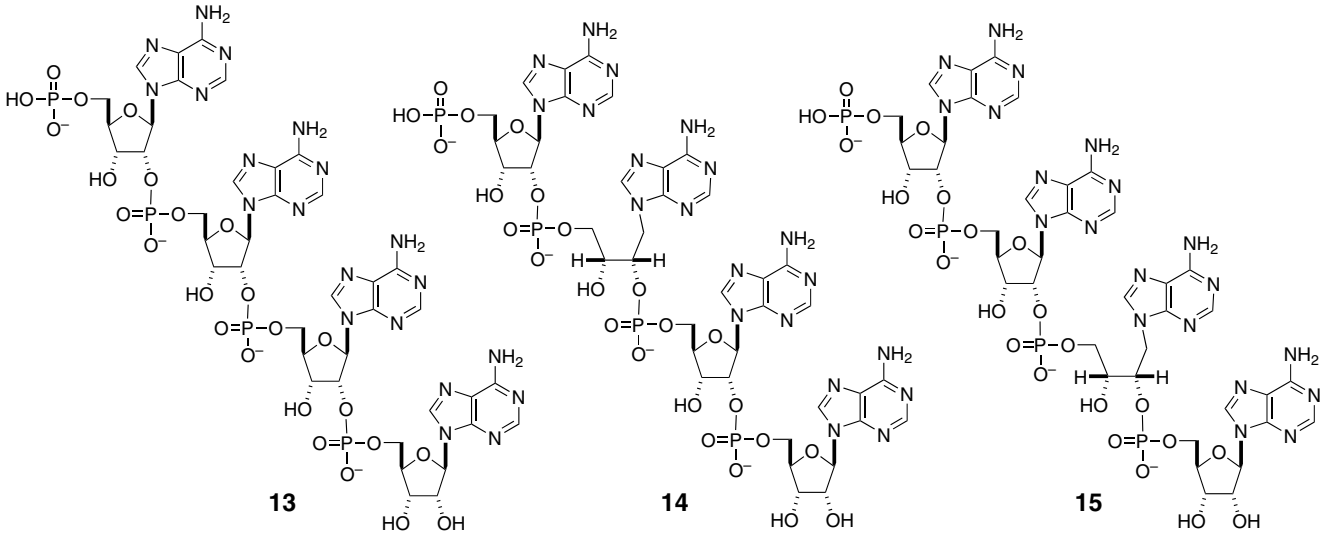


Figure 2. Structures of 2–5As.

lyzed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/MS), and the observed molecular weights supported their structures.[18]

The ability of 2–5A analogs **14** and **15** to activate RNase L was estimated by monitoring the cleavage of a synthetic RNA by the activated RNase L. In this study, 5′-r($C_{11}U_2C_7$)-3′ was used as a substrate. Carroll et al. reported that initial cleavage of RNA by RNase L occurs on the 3′-side of r($C_{11}U_2$) to yield r(C_{11}UpUp), and second cleavage occurs on the 3′-side of r(C_{11}U) to give r(C_{11}Up) with a higher enzyme concentration or longer incubation time.[4] The abilities of 2–5A analogs **14** and **15** to activate the enzyme were represented in terms of the concentrations of the analogs required to cleave half of the RNA (EC_{50}). Recombinant human RNase L was expressed in *E. coli* and purified according to the reported procedure.[19] The RNA labeled at the 5′-end with ^{32}P was incubated with the enzyme (60 nM) that had been pre-incubated with the 2–5A analogs **14** and **15**.[20] The reactions were analyzed by polyacrylamide gel electrophoresis under denaturing conditions (Fig. 3a). The densities of radioactive bands on the gel were determined with a bio-imaging analyzer (Fig. 3b). The EC_{50} value of the 2–5A tetramer **13** was 1.0 nM, whereas those of the analogs **14** and **15** were 9.0 and 1.7 nM, respectively. Although the abilities of **14** and **15** to activate RNase L were weaker than that of the parent 2–5A **13**, the EC_{50} values of **14** and **15** were only 9- and 1.7-fold higher than that of **13**, respectively.

We next examined the stability of the oligodeoxynucleotide **16** containing **2** to nucleolytic hydrolysis by snake venom phosphodiesterase (a 3′-exonuclease). The oligodeoxynucleotide **16** labeled at the 5′-end with ^{32}P was incubated with the enzyme, and the reactions were analyzed by polyacrylamide gel electrophoresis under denaturing conditions (Fig. 4). The control oligodeoxynucleotide, 5′-d($A_2G_2A_4GAG_2A_3GA$)-3′, was hydrolyzed randomly by the enzyme after 60 min of incubation. In contrast, it turned out that the phosphodiester linkage at the 3′-side of dG at the 3′-side of the analog **2** was highly resistant to the enzyme.

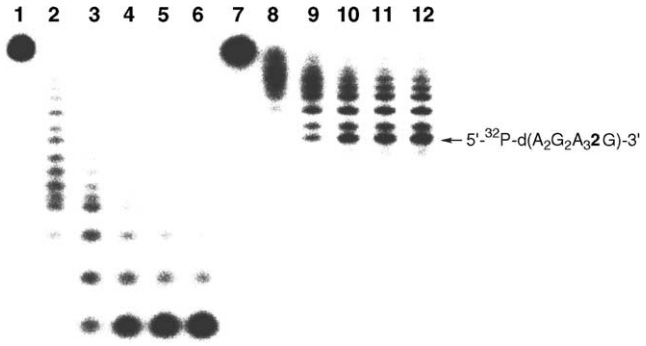


Figure 4. Polyacrylamide gel electrophoresis of 5′-^{32}P-labeled oligodeoxynucleotides hydrolyzed by snake venom phosphodiesterase: (lanes 1–6) 5′-^{32}P-d($A_2G_2A_4GAG_2A_3GA$)-3′; (lanes 7–12) 5′-^{32}P-d($A_2G_2A_3$**2**-GAG_2A_3GA)-3′. The oligodeoxynucleotides were incubated with snake venom phosphodiesterase for 0 min (lanes 1 and 7), 5 min (lanes 2 and 8), 10 min (lanes 3 and 9), 20 min (lanes 4 and 10), 30 min (lanes 5 and 11), and 60 min (lanes 6 and 12).

In conclusion, we have demonstrated the synthesis of the 2–5A analogs containing the purine acyclonucleoside, 9-[(2′*S*,3′*R*)-2′,3′,4′-trihydroxybutyl]adenine (**2**). The abilities of the analogs to activate recombinant human RNase L were evaluated using 5′-^{32}P-r($C_{11}U_2C_7$)-3′ as a substrate. It was found that the analog **14** containing **2** at the second position from the 5′-end and the analog **15** containing **2** at the third position from the 5′-end were only 9- and 1.7-fold less potent than the parent 2–5A tetramer itself, in RNase L activation ability. Furthermore, the oligodeoxynucleotide containing **2** was more resistant to nucleolytic hydrolysis by snake venom phosphodiesterase than the unmodified oligodeoxynucleotide. Thus, incorporation of an acyclonucleoside into 2–5A may be useful for developing a novel antiviral agent based on the 2–5A system.

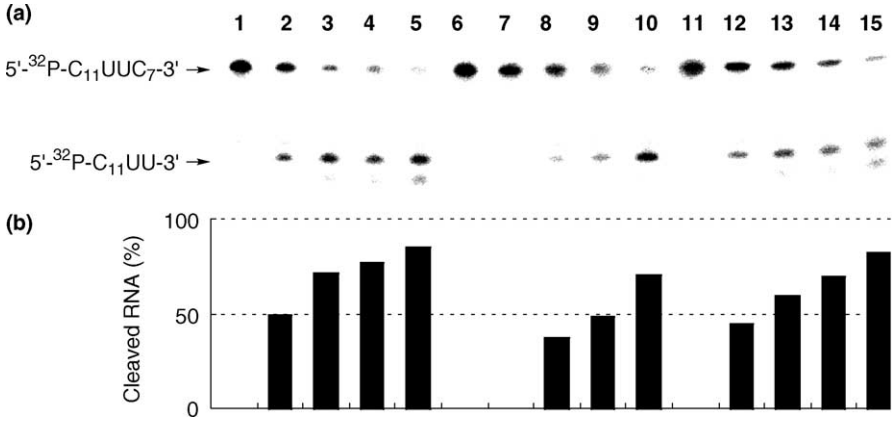


Figure 3. (a) Polyacrylamide gel electrophoresis of 5′-^{32}P-$C_{11}U_2C_7$-3′ hydrolyzed by recombinant human RNase L activated with 2–5A **13** (lanes 1–5), 2–5A analog **14** (lanes 6–10), or 2–5A analog **15** (lanes 11–15). Concentrations of 2–5As: 0 nM (lanes 1, 6, and 11), 1 nM (lanes 2, 7, and 12), 5 nM (lanes 3, 8, and 13), 10 nM (lanes 4, 9, and 14), and 20 nM (lanes 5, 10, and 15). Concentration of the RNA substrate: 100 nM. Concentration of recombinant human RNase L: 60 nM. (b) The graph represents percent RNA cleavage determined with a bio-imaging analyzer.

Acknowledgements

This research was in part supported by a Grant-in-Aid for Scientific Research (B) (KAKENHI 15390036) from Japan Society for the Promotion of Science (JSPS).

References and notes

1. Lengyel, P. *Annu. Rev. Biochem.* **1982**, *51*, 251.
2. Pestka, S.; Langer, J. A.; Zoon, K. C.; Samuel, C. E. *Annu. Rev. Biochem.* **1987**, *56*, 727.
3. Torrence, P. F.; Xiao, W.; Li, G.; Khamnei, S. *Curr. Med. Chem.* **1994**, *1*, 176.
4. Carroll, S. S.; Chen, E.; Viscount, T.; Geib, J.; Sardana, M. K.; Gehman, J.; Kuo, L. C. *J. Biol. Chem.* **1996**, *271*, 4988.
5. Player, M. R.; Torrence, P. F. *Pharmacol. Ther.* **1998**, *78*, 55.
6. Lerner, L. M. *Carbohydr. Res.* **1984**, *127*, 141.
7. Bessodes, M.; Antonakis, K. *Tetrahedron Lett.* **1985**, *26*, 1305.
8. Chu, C. K.; Cutler, S. J. *J. Heterocycl. Chem.* **1986**, *23*, 289.
9. Synthesis of the 2–5A analogs consisting of 9-[(2′*R*,4′*S*)-1′,5′-dihydroxy-4′-hydroxymethyl-3′-oxapent-2-yl]adenine has been reported: Mikhailov, S. N.; Pfleiderer, W. *Tetrahedron Lett.* **1985**, *26*, 2059.
10. Kitade, Y.; Hirota, K.; Maki, Y. *Tetrahedron Lett.* **1993**, *34*, 4835.
11. Hirota, K.; Monguchi, Y.; Sajiki, H.; Sako, M.; Kitade, Y. *J. Chem. Soc., Perkin. Trans. 1* **1998**, 941.
12. Physical data of **8**: ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.13 (1H, s, 6-NH), 8.70 (1H, s, 8- or 2-H), 8.33 (1H, s, 2- or 8-H), 8.06–6.86 (18H, m, Bz and DMTr), 5.44 (1H, d, J = 5.2 Hz, 3′-OH), 4.37 (2H, m, 1′-H), 4.21 (1H, m, 2′-H), 3.75–3.69 (7H, m, 3′-H and OMe×2), 3.10 (1H, dd, J = 11.3 and 5.0 Hz, 4′-H), 3.03 (1H, dd, J = 11.3 and 6.4 Hz, 4′-H), 0.57 (9H, s, Si*t*-Bu), −0.30 (3H, s, SiMe), −0.54 (3H, s, SiMe), the assignments were in agreement with COSY spectrum; FAB-HRMS calcd for $C_{43}H_{50}N_5O_6Si$ (MH^+), 760.3530; found, 760.3535.
13. Physical data of **9**: ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.12 (1H, s, 6-NH), 8.70 (1H, s, 8- or 2-H), 8.31 (1H, s, 2- or 8-H), 8.05–6.86 (18H, m, Bz and DMTr), 5.21 (1H, d, J = 5.2 Hz, 2′-OH), 4.48 (1H, d, J = 10.0 Hz, 1′-H), 4.09–4.05 (2H, m, 1′-H and 2′-H), 3.82 (1H, m, 3′-H), 3.72 (6H, s, OMe×2), 3.19 (1H, dd, J = 9.9 and 5.2 Hz, 4′-H), 3.05 (1H, dd, J = 9.9 and 5.4 Hz, 4′-H), 0.85 (9H, s, Si*t*-Bu), 0.08 (3H, s, SiMe), −0.06 (3H, s, SiMe), the assignments were in agreement with COSY spectrum; FAB-HRMS calcd for $C_{43}H_{50}N_5O_6Si$ (MH^+), 760.3530; found, 760.3535.
14. Physical data of **10**: ^{31}P NMR (162 MHz, $CDCl_3$): δ 149.78, 146.81; FAB-HRMS calcd for $C_{52}H_{67}Z_7O_7PSi$ (MH^+), 960.4609; found, 960.4612.
15. The phophoramidite **11** was synthesized as reported previously.[16] The phophoramidite **12**[17] was purchased from GLEN Research.
16. Ueno, Y.; Kato, Y.; Okatani, S.; Ishida, N.; Nakanishi, M.; Kitade, Y. *Bioconjugate Chem.* **2003**, *14*, 690.
17. Horn, T.; Urdea, M. S. *Tetrahedron. Lett.* **1986**, *27*, 4705.
18. Data of MALDI-TOF/MS. 2–5A **14**: calcd for $C_{39}H_{51}N_{20}O_{24}P_4$ (MH^+), 1307.2; found, 1307.1. 2–5A **15**: calcd for $C_{39}H_{51}N_{20}O_{24}P_4$ (MH^+), 1307.2; found, 1306.0. The oligodeoxynucleotide **16**: calcd for $C_{169}H_{205}N_{85}O_{89}P_{16}$ (M^+), 5346.5; found, 5346.2.
19. Yoshimura, A.; Nakanishi, M.; Yatome, C.; Kitade, Y. *J. Biochem.* **2002**, *132*, 643.
20. The extinction coefficients of the 2–5As at 260 nm were estimated as 4.16×10^4.