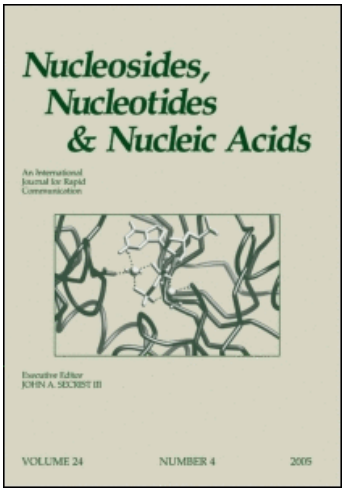


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

2-Bromoadenosine-Substituted 2',5'-Oligoadenylates Modulate Binding and Activation Abilities of Human Recombinant RNase L

Yukio Kitade[a]; Masaharu Wakana[a]; Shin-ichi Terai[a]; Takayuki Tsuboi[a]; Masayuki Nakanishi[a]; Chizuko Yatome[a]; Beihua Dong[b]; Robert H. Silverman[b]; Paul F. Torrence[c]
[a] Laboratory of Molecular Biochemistry, Department of Biomolecular Science, Faculty of Engineering, Gifu University, Gifu, Japan [b] Department of Cancer Biology, The Lemer Research Institute, The Cleveland Clinic Foundation, Cleveland, OH, USA [c] Section on Biomedical Chemistry, Laboratory of Medicinal Chemistry, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD, USA

To cite this Article Kitade, Yukio , Wakana, Masaharu , Terai, Shin-ichi , Tsuboi, Takayuki , Nakanishi, Masayuki , Yatome, Chizuko , Dong, Beihua , Silverman, Robert H. and Torrence, Paul F.(1998) '2-Bromoadenosine-Substituted 2',5'-Oligoadenylates Modulate Binding and Activation Abilities of Human Recombinant RNase L', Nucleosides, Nucleotides and Nucleic Acids, 17: 12, 2323 — 2333
To link to this Article: DOI: 10.1080/07328319808004320
URL: http://dx.doi.org/10.1080/07328319808004320

NUCLEOSIDES & NUCLEOTIDES, 17(12), 2323-2333 (1998)

2-BROMOADENOSINE-SUBSTITUTED 2′,5′-OLIGOADENYLATES MODULATE BINDING AND ACTIVATION ABILITIES OF HUMAN RECOMBINANT RNASE L

Yukio Kitade,*[a] Masaharu Wakana,[a] Shin-ichi Terai,[a] Takayuki Tsuboi,[a] Masayuki Nakanishi,[a] Chizuko Yatome,[a] Beihua Dong,[b] Robert H. Silverman,[b] and Paul F. Torrence[c]

[a]Laboratory of Molecular Biochemistry, Department of Biomolecular Science, Faculty of Engineering, Gifu University, Yanagido 1-1, Gifu 501-1193, Japan,
[b]Department of Cancer Biology, The Lerner Research Institute, The Cleveland Clinic Foundation, Cleveland, OH 44195, USA,
[c]Section on Biomedical Chemistry, Laboratory of Medicinal Chemistry, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892, USA

ABSTRACT: 2-Bromoadenosine-substituted analogues of 2-5A, p5′A2′p-5′A2′p5′(br^2A), p5′(br^2A)2′p5′A2′p5′A, and p5′(br^2A)2′p5′(br^2A)2′p-5′(br^2A), were prepared *via* a modification of a lead ion-catalyzed ligation reaction and were subsequently converted into the corresponding 5′-triphosphates. Both binding and activation of human recombinant RNase L by various 2-bromoadenosine-substituted 2-5A analogues were examined. Among the 2-bromoadenosine-substituted 2-5A analogues, the analogue with 2-bromoadenosine residing in the 2′-terminal position, p5′A2′p5′A2′p-5′(br^2A), showed the strongest binding affinity and was as effective as 2-5A itself as an activator of RNase L. The CD spectrum of p5′A2′p-5′A2′p5′(br^2A) was superimposable on that of p5′A2′p5′A2′p5′A, indicative of an *anti* orientation about the base-glycoside bonds as in naturally occurring 2-5A.

INTRODUCTION

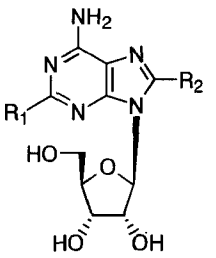
The unique 2′,5′-phosphodiester bond-linked oligoadenylate, 2-5A, acts as a potent inhibitor of translation in vertebrate cells through the activation of a constituent latent 2-5A-dependent endoribonuclease, RNase L. This 2-5A system plays a major role in the interferon natural defense mechanism against virus infection.[1] The nucleotide bases of 2-5A are recognized by RNase L. Previous studies reported that oligonucleotides of the

* Tel/Fax: +81-58-293-2640; E-mail: kitade@apchem.gifu-u.ac.jp

general formula, ppp5′A2′p5′A2′p5′N (N = any common nucleotide other than adenylyl), could not activate RNase L, but could antagonize the action of 2-5A.[2] Thus, it appeared that the specificity of base recognition for RNase L binding resided primarily in the either (or both) of the first two (from the 5′-terminus) bases of 2-5A.[3-6]

Dramatic variation in RNase L binding and activating abilities of 2′,5′-oligoadenylates can be achieved by replacement of the adenine 8-hydrogen in adenylate residues. For instance, complete substitution of br^{8}A for all three A's in pApApA gave dramatic reductions in activity; however, replacement by 8-bromoadenosine (br^{8}A, **2**) at the third adenosine of pp5′A2′p5′A2′p5′A to give pp5′A2′p5′A2′p5′(br^{8}A) provided a significant increase in RNase L activation.[7,8] Conformational studies of such 8-brominated 2′,5′-oligoadenylates led to the hypothesis that a *syn* base-sugar orientation about the glycosidic bond of the 5′-terminal adenosine nucleotide positively influenced activating activities for RNase L.[9]

In line with this, when 8-methyladenosine (me^{8}A, **3**) replaced adenosine in the 2′-terminal position of the nucleotide, the resultant oligonucleotide, ppp5′A2′p5′A2′p-5′(me^{8}A), was somewhat more active than parent 2-5A trimer.[10]



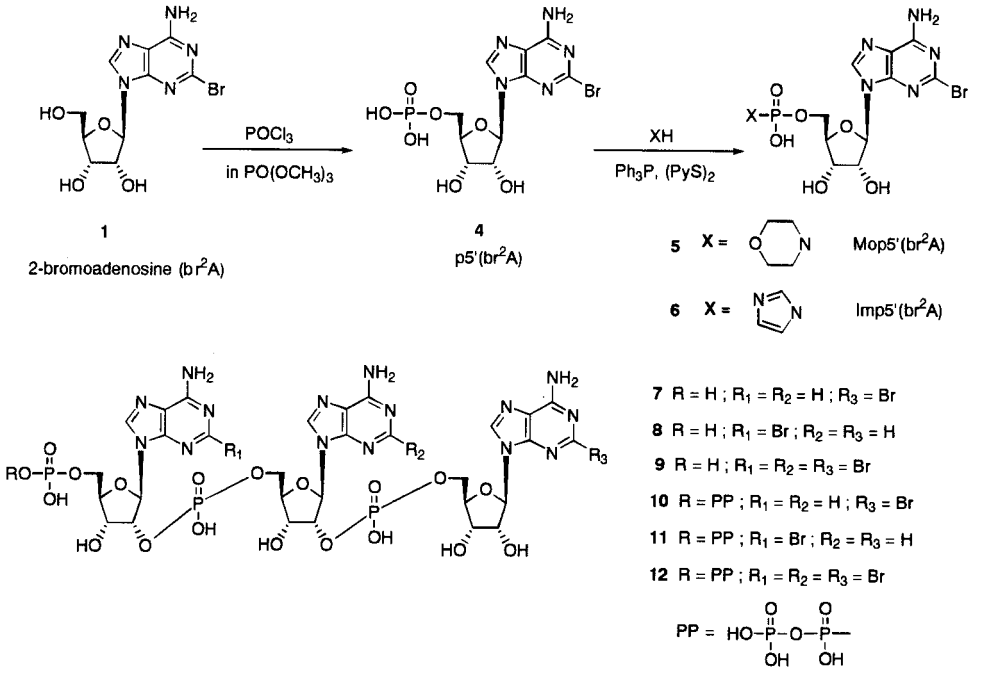
1 R_1 = Br ; R_2 = H
2 R_1 = H ; R_2 = Br
3 R_1 = H ; R_2 = CH_3

SCHEME 1

Herein we describe the synthesis of 2-bromoadenosine (**1**)-substituted 2-5A derivatives and their interaction with human recombinant 2-5A-dependent RNase. This 2-bromoadenosine (br^{2}A, **1**) introduction to the 2′-terminal position of the 2-5A molecule is of interest since it can force the nucleoside to adopt an *anti* orientation about the base-glycoside bond.[11] This modification with the same *anti* orientation as naturally occurring 2-5A reveals the importance base-sugar conformation in binding to and activation of RNase L.

RESULTS AND DISCUSSION

Using the previously published procedure,[6,10] three 2′,5′-linked oligoadenylates possessing 2-bromoadenosine could be generated; namely p5′A2′p5′A2′p5′(br^{2}A), p5′(br^{2}A)2′p5′A2′p5′A, and p5′(br^{2}A)2′p5′(br^{2}A)2′p5′(br^{2}A)(see SCHEME 2 and EXPERIMENTAL). The assigned structures were confirmed by NMR spectroscopy (see TABLE 1) as well as degradation methods (see TABLE 2 and 3 in EXPERIMENTAL). Three trimer 5′-monophosphates were subsequently converted into the corresponding 5′-triphosphates.



SCHEME 2

TABLE 1. Characteristic Proton NMR Signals of 2′,5′-Oligoadenylates

Oligomer	H-2 and H-8 ppm		H-1′ ppm
	8.01	7.98	5.98 (d, J = 3,18 Hz)
pApApA	7.85	7.81	5.80 (d, J = 3.66 Hz)
	7.79	7.76	5.68 (d, J = 4.39 Hz)
	7.96	7.83	5.91
pApAp(br^{2}A) (**7**)	7.78	7.76	5.78
	7.74		5.56 (d, J = 3.91 Hz)
	8.01	7.94	5.88
p(br^{2}A)pApA (**8**)	7.87	7.81	5.82
	7.77		5.70 (d, J = 3.42 Hz)
	7.96		5.79 (s, 2H)
p(br^{2}A)p(br^{2}A)p(br^{2}A) (**9**)	7.82		5.64
	7.67		

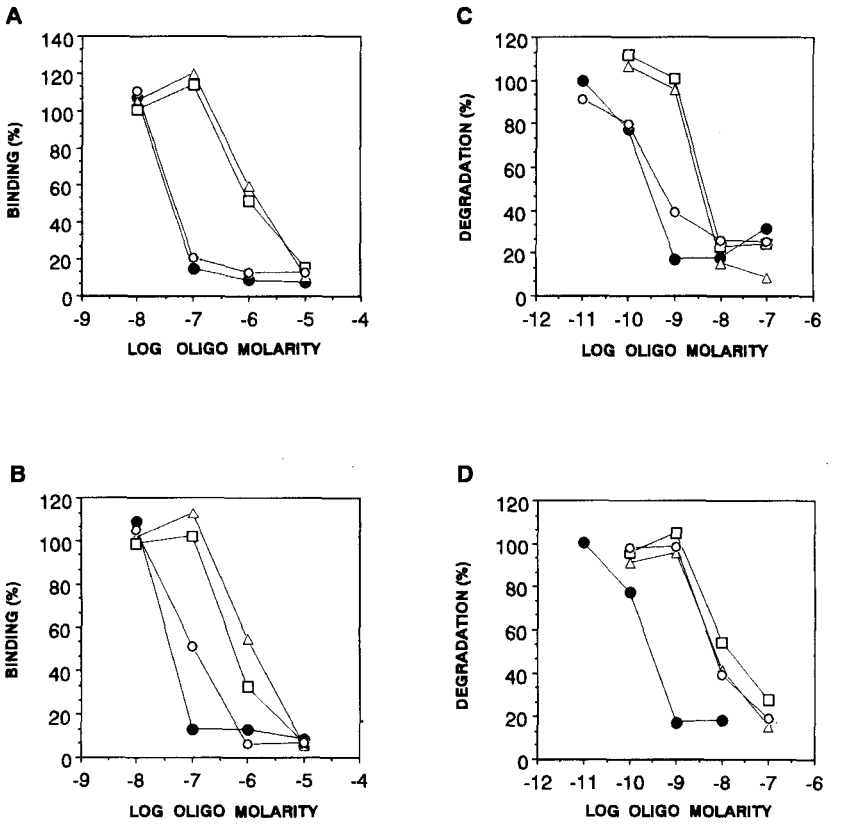


FIG. 1. Binding ability of 2′,5′-oligoadenylates to purified recombinant human RNase L as measured by displacement of the probe p(A2′p)2(br8A2′p)2A3′[32p]pCp. The assay was performed with 1 mg of the RNase L in a final assay volume of 20 mL. The results of two separate experiments are shown. A: ● pApApApA; ○ pApAp(br2A) (**7**); △ p(br2A)pApA (**8**); □ p(br2A)p(br2A)p(br2A) (**9**). B: ● pApApApA; ○ pppApAp(br2A) (**10**); △ ppp(br2A)pApA (**11**); □ ppp(br2A)p(br2A)p(br2A) (**12**). Activation of pure recombinant human RNase L as measured by the degradation of poly(U)[32p]p5′C3′p. Reaction mixtures contained 0.1 mg of RNase L in final volumes of 25 mL containing 12nM poly(U). Incubations were at 30℃ for 30min. C: ● pApApApA; ○ pApAp(br2A) (**7**); △ p(br2A)pApA (**8**); □ p(br2A)p(br2A)p(br2A) (**9**). D: ● pApApApA; ○ pppApAp(br2A) (**10**); △ ppp(br2A)pApA (**11**); □ ppp(br2A)p(br2A)p(br2A) (**12**).

Introduction of a 2-bromo substituent to the adenine ring of the 5′-terminal adenosine of 2-5A trimer caused a greater than tenfold decrease in binding affinity to RNase L as determined by ability to compete with radiolabeled $p(A2'p)_2(br^8A2'p)_2A3'[^{32}P]pCp$ in the assay of Knight et al.[14] (FIG. 1: A and B). This diminution in binding was true for both the 5′-monophosphate and 5′-triphosphate analogues, (pp)p5′(br^2A)2′p5′A2′p5′A (**8**, **11**). When evaluated for their abilities to activate the RNase L, as judged by ability to stimulate the degradation of labeled poly(U)

by RNase L, both the monophosphate and triphosphate, (pp)p5′(br^{2}A)2′p5′A2′p5′A (**8**, **11**), showed an approximate one log decrease in activation ability (FIG. 1: C and D). Thus, the loss of binding ability was directly related to the loss of RNase L activation capacity.

The 2-terminally-modified analogues, (pp)p5′A2′p5′A2′p5′(br^{2}A) (**7**, **10**), bound to the RNase L at least as well as parent 2-5A (FIG. 1: A and B). RNase L activation by the 5′-monophosphate congener, p5′A2′p5′A2′p5′(br^{2}A) (**7**), was just as effective as 2-5A tetramer, p5′A2′p5′A2′p5′A2′p5′A (FIG. 1: C); however, the corresponding 5′-triphosphate was an order of magnitude less active than 2-5A tetramer monophosphate (FIG. 1: D). This was an unexpected result. The human RNase L has been shown to require only a 5′-monophosphate-terminated 2′,5′-oligoadenylate trimer for full activation, in definitive contrast to its murine counterpart.[17] Moreover, no significant difference was noted in a comparison of 2-5A mono- and triphosphates using the pure recombinant human RNase L.[17] On the other hand, it has been demonstrated that the murine RNase L's requirement for a 5′-di- or 5′-triphosphate terminus can be eliminated by substituting 8-bromoadenosine for adenosine in the third (or 2′-terminus) position of 2-5A trimer to give an fully active 5′-monophosphorylated 2′,5′-oligoadenylate.[7,8] This has been related to a conformational change caused by introduction of a bromine at the adenosine 8 position. It may be hypothesized that if appropriate base modification can positively affect activation of RNase L by a normally inactive 5′-monophosphate, so too might base modification negatively influence the activity of a 5′-triphosphorylated 2′,5′-oligoadenylate.

Complete substitution of 2-5A trimer mono- or triphosphates with 2-bromoadenines brought about a decrease in binding affinity to the human RNase L (FIG. 1: A and B) and, concomitantly, a similar drop in ability to activate the nuclease (FIG. 1: C and D). Since the activity of (pp)p5′(br^{2}A)2′p5′(br^{2}A)2′p5′(br^{2}A) (**9**, **12**) was essentially equivalent to (pp)p5′(br^{2}A)2′p5′A2′p5′A, it may be concluded that the loss of activity of the tribrominated 2-5A was due largely to the 5′-terminal 2-bromoadenosine residue, shown above to be detrimental to both binding to and activation of RNase L.

Earlier studies[7-9] have provided much evidence that effective binding to and activation of RNase L are favored by an *anti* conformation of the 5′-terminal nucleotide of 2-5A. According to the CD results (FIG. 2), the analogue p5′(br^{2}A)2′p5′A2′p5′A (**8**) possesses a conformation different from parent p5′A2′p5′A2′p5′A. Thus, the decrease in RNase L binding and activation ability of p5′(br^{2}A)2′p5′A2′p5′A (**8**) probably may reflect an altered conformation. It is also possible that steric or electronic properties associated with 2-bromo substitution may play a role. For instance, the essential role of the adenine *N1* nitrogen and/or *N6* amino moiety for RNase L binding has been established in several studies.[5,10] Alternation of adenine *N1* basicity, or the pKa of the *N6* amino

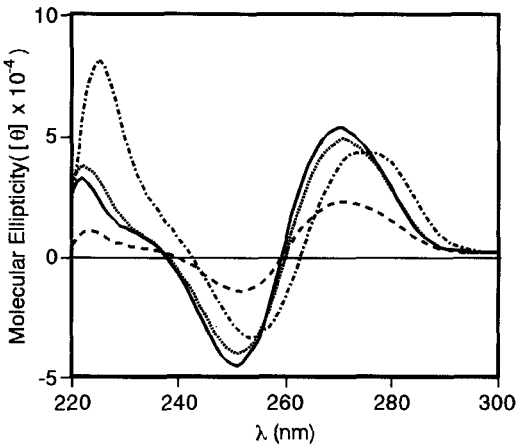


FIG. 2. CD spectra of 2',5'-oligoadenylates.

——— pApApA - - - - - - p(br^{2}A)pApA (**8**)
····· pApAp(br^{2}A) (**7**) ·····-····· p(br^{2}A)p(br^{2}A)p(br^{2}A) (**9**)

group thus might be expected to influence oligoadenylate binding to RNase L. On the other hand, these CD spectra of the 5′-triphosphate derivatives (**10**, **11**, and **12**) are similar to the spectra of the corresponding 5′-monophosphates (**7**, **8**, and **9**), respectively (data not shown).

Substitution of 2-bromoadenosine for adenosine in the 2′-terminal nucleotide position of 5′-monophosphorylated 2-5A trimer fully supported nuclease activation to the same extent as parent 2-5A. This result is similar to that obtained for the corresponding 8-bromoadenosine analogue, which actually was observed to possess an enhanced ability to activate the RNase L.[8,9] Since there exists a rigid requirement for the *N1* adenine base nitrogen and/or the *N6* amino group of the 2′-terminal adenosine of 2-5A trimer for activation of RNase L, the present results suggest that the RNase L enzyme may be able to adapt to either *syn* or *anti* conformation of the 2′-terminal nucleotide, perhaps through modulation of the nucleotide conformation itself.

EXPERIMENTAL

Snake venom phosphodiesterase was a product of Cooper Biomedical, alkaline phosphatase was from Pharmacia, and nuclease P1 was from Yamasa Shoyu Co., Ltd. The proton NMR spectra were recorded on a JEOL a-400 spectrometer and a Varian INOVA 500 using HDO as an internal standard. Mass spectra were taken on a JEOL JMS-D300 machine operating at 70 eV. All UV measurements were carried out on a HITACHI U-2001 spectrophotometer. The CD spectrum was measured by a JASCO J-

600 spectrophotometer. High-performance liquid chromatography was executed with a Jasco Gulliver PU-980 Intelligent HPLC Pump and a Jasco 807 IT Integrator. The detector was a Jasco UV-970 Intelligent UV/VIS spectrometric detector. The HPLC column employed was Mightysil RP-18 (5 mm) (Cica-Reagent). Buffer A (50 mM ammonium phosphate, pH 7.0) in 0 to 50% Buffer B (methanol: water, 1:1) over 30 minutes at 1 mL/min. Column chromatographic separation was accomplished using silica gel (Wakogel C-300).

Normal-pressure ion-exchange column chromatography on DEAE-Sephadex A-25 was carried on at 4 ℃, with various concentrations of triethylammonium bicarbonate, pH 7.6, as an elution buffer. Buffer was removed by repeated coevaporation with water. Triethlyammonium salts of oligonucleotides were usually exchanged into sodium salts by preparation from 1% acetone solution of sodium iodide. Purity of all obtained compounds was determined by means of HPLC.

Preparation of 2-Bromoadenosine 5′-Monophosphate [p5′(br^{2}A), 4]. A mixture of 2-bromoadenosine[12] (**1**: 35 mg, 0.1 mmol) in trimethyl phosphate (400 mL) was allowed to stand for 10 min. Phosphorus oxychloride (120 mL) was added dropwise to the mixture which was stored then at 4℃ overnight. The mixture was poured into ice-cold water (30 mL) and extracted with ether (30 mL x 3). The water-layer was diluted with water to 200 mL and applied to DEAE-Sephadex A-25 (16 x 20 cm) preequilibrated with water. Elution was with a linear gradient of 0.0 M (600 mL) to 0.3 M (600 mL) triethylammonium bicarbonate (pH 7.6). Appropriate fractions were collected and concentrated *in vacuo*, and water was added to and evaporated from the residue to remove residual triethylammonium bicarbonate. The desired product was obtained as the triethylammonium salt (1370 OD_{260}, 86 %). ^{1}H nmr (D_2O, 400 MHz) δ 8.20 (1H, d, J = 8.1 Hz, H-8), 5.79 (1H, d, J = 5.4 Hz, H-1′), 4.51 (1H, td, H-2′), 4.32 (1H, t, H-3′), 4.20 (1H, t, H-4′), 3.96 (2H, m, H-5′); UV (H_2O) λ_{max} 264 nm (**1**; λ_{max} 264 nm). The alkaline phosphatase digestion of **4** gave the dephophoylated **1**, which was identical with an authentic sample.

Preparation of 5′-Phosphoromorpholidates (5) and 5′-Phosphoro-imidazolidates (6). *2-Bromoadenosine 5′-phosphoromorpholidate (5).* Triphenylphosphine (32 mg, 0.12 mmol) and morpholine (26 mL, 0.3 mmol) were added to a mixture of 2-bromoadenosine 5′-monophosphate (0.044 mmol, 704 OD_{260}, triethylammonium salt) in DMF (800 mL). The mixture was stirred for 5 min and 2,2′-dipyridyl disulfide (28 mg, 0.13 mmol) was added to the mixture. The mixture was stirred for 2 h. The whole mixture was poured into 0.1 M sodium iodide solution in acetone (10 mL) with stirring. The resulting precipitate was collected by centrifugation and washed several times with fresh acetone until the yellow color was gone. After

drying at room temperature for 1 h under vacuum, the sodium salt was obtained [Mop5′(br^{2}A), 41 %]. ^{1}H nmr (DMSO-d_6, 400 MHz) δ 8.75 (1H, s, H-8), 7.87 (2H, brs, NH_2), 6.16 (1H, brs, OH), 6.03 (1H, brs, OH), 5.82 (1H, s, H-1′), 4.56 (1H, s, H-2′), 4.23 (1H, s, H-3′), 4.05 (1H, s, H-4′), 3.87 (2H, s, H-5′), 3.42 (4H, s, CH_2NCH_2), 2.89 (4H, s, CH_2OCH_2); UV (H_2O) λ_{max} 264 nm.

2-Bromoadenosine 5′-phosphoroimidazolidate (6).

The 5′-phosphoroimidazolidate was prepared in a similar manner. The sodium salt was obtained [Imp5′(br^{2}A), 72 %]. ^{1}H nmr (DMSO-d_6, 400 MHz) δ 8.39 (1H, s, H-8), 7.62 (2H, s, NH_2), 7.09 (1H, s, imidazole-H), 7.00 (1H, s, imidazole-H), 6.84 (1H, s, imidazole-H), 5.79 (1H, d, J = 6.4 Hz, H-1′), 5.8-5.2 (2H, br, 2 x OH), 4.50 (1H, dd, J = 5.0 and J = 6.4 Hz, H-2′), 3.98 (1H, dd, J = 2.8 and J = 5.0 Hz, H-3′), 3.94 (1H, dd, J = 3.0 and J = 6.4 Hz, H-4′), 3.72 (2H, dd, J = 6.2 and J = 10.5 Hz, H-5′); UV (H_2O) λ_{max} 265 nm.

Preparation of p5′A2′p5′A2′p5′(br^{2}A) (7). $Pb(NO_3)_2$ (0.25 M, 0.45 mL) was added to a mixture of Mop5′A2′p5′A^4 (2600 OD_{260}, 0.10 mmol) and Imp5′(br^{2}A) (720 OD_{260}, 0.045 mmol) in 1-methylimidazole buffer (0.2 M, 3.0 mL, pH7.5) at 4℃. After 24 h of stirring at 4℃, Chelex 100 (NH_4^+ form, 3 mL) was added to the turbid reaction mixture which gradually became clear. The Chelex was removed by filtration, and the filtrate was acidified with acetic acid. The mixture was incubated at 37℃ for 24 h and the solvent was removed under reduced pressure. The residue was dissolved in water (50 mL) and the pH of the solution was adjusted to 5.75 with acetic acid. Nuclease P1 (100 unit) was added to the mixture which was incubated at 37℃ for 24 h. The solution was treated with boiling water for 5 min and diluted with water to 300 mL and applied to a DEAE-Sephadex A-25 column (1.6×20 cm), preequilibrated with water. Elution was with a linear gradient of 0.0 M (600 mL) to 0.7 M (600 mL) triethylammonium bicarbonate (pH 7.6). Appropriate fractions were pooled and concentrated *in vacuo*, and water was added to and evaporated from the resulting residue to remove residual triethylammonium bicarbonate. The desired product, p5′A2′p5′A2′p5′(br^{2}A) (**7**), was isolated as the triethylammonium salt (152 OD_{260}, 10 %).

Preparation of p5′(br^{2}A)2′p5′A2′p5′A (8). $Pb(NO_3)_2$ (0.25 M, 0.5 mL) was added to a mixture of Mop5′(Br^{2}A) (656 OD_{260}, 0.041 mmol) and Imp5′A2′p5′A (2574 OD_{260}, 0.099 mmol) in 1-methylimidazole buffer (0.2 M, 5.0 mL, pH 7.5) at 4℃. The mixture was worked up as with p5′A2′p5′A2′p5′(br^{2}A). The desired product, p5′(br^{2}A)2′p5′A2′p5′A (**8**), was isolated as the triethylammonium salt (437 OD_{260}, 31 %).

Preparation of p5′(br^{2}A)2′p5′(br^{2}A)2′p5′(br^{2}A) (9). $Pb(NO_3)_2$ (0.25 M, 0.2 mL) was added to a mixture of Mop5′(br^{2}A) (655 OD_{260}, 0.041 mmol) and Imp5′(br^{2}A) (854 OD_{260}, 0.053 mmol) in 1-methylimidazole buffer (0.2 M, 2.0 mL, pH

TABLE 2. Enzymatic Characterization of 2′,5′-Oligoadenylates

Oligomer (Retention Time)	Alkaline Phosphatase (AP) Digest Product (Retention Time)	Snake Venom Phosphodiesterase (SVP) Digest (Molar Ratio)	AP/SVP Digest (Molar Ratio)
pApApA (17.3)	ApApA (26.8)	pA	A + pA (1.0 : 1.57)
pppApApA (14.2)	ApApA (26.8)	pA	A + pA (1.0 : 2.25)
pApAp(br^{2}A) (7) (20.1)	ApAp(br^{2}A) (32.7)	pA + p(br^{2}A) (2.03 : 1.0)	A + pA + p(br^{2}A) (0.81 : 1.15 : 1.0)
pppApAp(br^{2}A) (**10**) (17.7)	ApAp(br^{2}A) (32.7)	pA + p(br^{2}A) (2.04 : 1.0)	A + pA + p(br^{2}A) (0.86 : 1.36 : 1.0)
p(br^{2}A)pApA (**8**) (18.2)	(br^{2}A)pApA (33.6)	pA + p(br^{2}A) (1.98 : 1.0)	br^{2}A + pA (1.0 : 2.25)
ppp(br^{2}A)pApA (**11**) (15.8)	(br^{2}A)pApA (33.6)	pA + p(br^{2}A) (1.80 : 1.0)	br^{2}A + pA (1.0 : 1.94)
p(br^{2}A)p(br^{2}A)p(br^{2}A) (**9**) (24.7)	(br^{2}A)p(br^{2}A)p(br^{2}A) (38.9)	p(br^{2}A)	br^{2}A + p(br^{2}A) (1.0 : 1.99)
ppp(br^{2}A)p(br^{2}A)p(br^{2}A) (**12**) (24.3)	(br^{2}A)p(br^{2}A)p(br^{2}A) (38.9)	p(br^{2}A)	br^{2}A + p(br^{2}A) (1.0 : 2.06)

7.5) at 4℃. The mixture was worked up as with p5′A2′p5′A2′p5′(br^{2}A). The desired product, p5′(br^{2}A)2′p5′(br^{2}A)2′p5′(br^{2}A) (**9**), was isolated as the triethylammonium salt (46 OD_{260}, 5 %), along with p5′(br^{2}A)2′p5′(br^{2}A) (104 OD_{260}).

Preparation of 2-Bromoadenosine-substituted 2-5A 5′-Triphosphates (10, 11, and 12). All monophosphates were converted to triphosphates using the method described earlier.[10,13] For example, p5′A2′p5′A2′p5′(br^{2}A) (150 OD_{260}, 4.3 μmol) was converted to the imidazole derivative, Imp5′A2′p5′A2′p5′(br^{2}A), by reaction with triphenylphosphine (11.6 mg, 43 μmol), imidazole (2.9 mg, 43 μmol), and 2,2′-dipyridyl disulfide (9.5 mg, 43 μmol). It was isolated as the sodium salt, which was dissolved in tri-n-butylammonium pyrophosphate in DMF (1.5 mL, 0.4 M). The reaction mixture was kept at room temperature for 20 h. After dilution with H_2O, the solution was applied to DEAE-Sephadex A-25 (1 cm x 20 cm) and eluted with a linear gradient of 0.0 M (300 mL) to 0.7 M (300 mL) triethylammonium bicarbonate (pH 7.6). The final triphosphate products were isolated after addition and evaporation of water to remove volatile buffer from the column chromatography. Yields were as follows: ppp5′A2′p5′A2′p5′(br^{2}A) (**10**), 74%: ppp5′(br^{2}A)2′p5′A2′p5′A (**11**), 70%: ppp5′(br^{2}A)2′p5′(br^{2}A)2′p5′(br^{2}A) (**12**), 53%.

Enzymatic and Chemical Degradation. 5′-Dephosphorylation was effected *via* alkaline phosphatase exposure using 0.4 OD_{260} of substrate and 0.06 unit of enzyme in Tris-acetate (0.2 M, pH 8.8), $MgCl_2$ (0.001 M), and a total volume of 100 mL, with incubation at 37 ℃ for 1 h. The digested products were identified by comparison with authentic materials. Ratios of nucleotide and /or nucleoside products were determined via integration of the chromatogram. TABLE 2 provides the results of such experiments. Chemical characterization was performed with 0.3 OD_{260} of substrate in 0.1 N NaOH

TABLE 3. Chemical Characterization of 2′,5′-Oligoadenylates

Oligomer	1/10 NaOH Digest (Molar Ratio)
pApApA	pA2′(3′)p + A2′(3′)p + A (1.0 : 0.91 : 0.88)
pppApApA	pppA2′(3′)p + A2′(3′)p + A (1.0 : 0.98 : 0.97)
pApAp(br^{2}A) (**7**)	pA2′(3′)p + A2′(3′)p + (br^{2}A) (1.0 : 0.95 : 1.02)
pppApAp(br^{2}A) (**10**)	pppA2′(3′)p + A2′(3′)p + (br^{2}A) (1.0 : 0.71 : 1.0)
p(br^{2}A)pApA (**8**)	p(br^{2}A)2′(3′)p + A2′(3′)p + A (1.0 : 0.78 : 0.81)
ppp(br^{2}A)pApA (**11**)	ppp(br^{2}A)2′(3′)p + A2′(3′)p + A (1.0 : 0.95 : 1.28)
p(br^{2}A)p(br^{2}A) p(br^{2}A) (**9**)	p(br^{2}A)2′(3′)p + (br^{2}A)2′(3′)p + (br^{2}A) (1.0 : 0.78 : 0.79)
ppp(br^{2}A)p(br^{2}A) p(br^{2}A) (**12**)	ppp(br^{2}A)2′(3′)p + (br^{2}A)2′(3′)p + (br^{2}A) (1.0 : 0.68 : 1.16)

solution (100 mL), with incubation at 37℃ for 12 h. TABLE 3 shows the results of such experiments.

2-5A-Dependent RNase Activities. Binding of the synthetic analogues to the 2-5A-dependent RNase was examined using a modification of the radiobinding assay originally reported by Knight et al.[14] and further refined by Silverman and Krause.[15] The procedure employed the oligonucleotide p(A2′p)$_2$(br^{8}A2′p)$_2$A3′[^{32}P]pCp as a radioactive probe which would be displaced in a competitive manner by 2-5A or an analogue. Nitrocellulose filters were employed to trap the radioprobe-endonuclease complex. Analyses of the ability of the 2-bromoadenosine analogs to activate RNase L were performed using modifications of the procedure originally developed by Silverman.[16] In this methodology, the degradation of poly(U)[^{32}P]pCp was followed by monitoring the disappearance of trichloroacetic acid-insoluble radioactivity. The precipitated poly(U)[^{32}P]pCp was isolated, together with carrier yeast RNA, by filtration on glass filter discs. The 2-5A-dependent RNase was expressed in SF21 insect cells from a human cDNA subcloned in a baculovirus vector.[17,18] It was purified with the aid of three fast protein liquid chromatography (FPLC) columns (Pharmacia).

ACKNOWLEDGMENTS: This investigation was supported in part by the grant (to Y. K.) awarded by Kowa Life Science Foundation in Japan and the Grant-in-Aid for Scientific Research (to Y. K.) from the Ministry of Education of Japan, which are gratefully acknowledged. Research at NIH was supported in part by a cooperative research and development agreement with Atlantic Pharmaceuticals (Raleigh, NC) in USA.

REFERENCES AND NOTES

1. Kerr, I. M., Brown, R. E. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 256-260.
2. Drocourt, J.-L; Dieffenbach, C. W.; Ts'o, P. O. ; Justesen, J.; Thang, M. N. *Nucleic Acids Res.* **1982**, *10*, 2163-2174.
3. Torrence, P. F.: Imai, J.; Lesiak, K.; Jamoulle, J.-C.; Sawai, H. *J. Med. Chem.* **1984**, *27*, 726-733.
4. Imai, J., Torrence, P. F., *J. Org. Chem.*, **1985**, *50*, 1418-1426.
5. Imai, J., Lesiak, K., Torrence, P. F., *J. Biol. Chem.* **1985**, *260*, 1390-1394.
6. Kitade, Y., Alster, D. K., Pabuccuoglu, A., Torrence, P. F., *Bioorganic Chem.* **1991**, *19*, 283-299.
7. Lesiak, K.; Torrence, P. F. *J. Med. Chem.* **1986**, *29*, 1015-1022.
8. Lesiak, K.; Torrence, P. F. *J. Biol. Chem.* **1987**, *262*, 1961-1965.
9. Van den Hoogen, Y. T.; Hilgersom, C. M.; Brozda, D.; Lesiak, K.; Torrence, P. F.; Altona, C. *Eur. J. Biochem.* **1989**, *182*, 629-637.
10. Kitade, Y.; Nakata, Y.; Hirota, K.; Maki, Y.; Pabuccuoglu, A.; Torrence, P. F. *Nucleic .Acids Res.* **1991**, *19*, 4103-4108.
11. Kitade, Y., Wakana, M., Yatome, C. unpublished results: In fact, the chemical shift of the H2´-proton on 2-bromoadenosine (4.50 ppm) supports an anti- orientation the same as that of adenosine (4.60 ppm). On the other hand, the down field shift of H2´-proton of 8-bromoadenosine (5.08 ppm) and 8-methyladenosine (4.86 ppm) imply a *syn*-orientation as a result of the deshielding effect of purine ring.
12. Huang, M. C., Avery, T. L., Blakley, R. L., Secrist III, J. A., Montgomery, J. A. *J. Med. Chem.* **1984**, *27*, 800-802.
13. Sawai, H., Shibata, T., Ohno, M. *Tetrahedron Lett.*, **1979**, *47*, 4573-4576.
14. Knight, M., Wreschner, D. H., Silverman, R. H. and Kerr, I. M. (1981) *Methods in Enzymol.* **79**, 216-227.
15. Silverman, R. H., Krause, D. (1987) In Clemens, M. J., Morris, A. G. and Gearing, A. J. H., (eds) *Lymphokines and Interferons.* IRL Press, Oxford, pp149-193.
16. Silverman, R. H. *Anal. Biochem.* **1985**,*144*, 450-460.
17. Dong, B., Xu, L., Zhou, A., Hassel, B. A., Lee, X., Torrence, P. F., and Silverman, R. H. *J. Biol. Chem.* **1994**, *269*, 14153-14158.
18. Zhou, A., Hassel, B. A., Silverman, R. H. *Cell*, **1993**, *72*, 753-765.

Received 3/23/98
Accepted 5/29/98