0273-2289/97/6701-02—0033$11.00

The Solid-Phase Synthesis of 2′–5′-Linked Oligoriboadenylates Containing 8-Bromoadenine

KRYSTYNA B. LESIAK,[1] BOGDAN UZNANSKI,[2] AND PAUL F. TORRENCE*,[1]

[1]*Section on Biomedical Chemistry, Laboratory of Medicinal Chemistry, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892; and*
[2]*Center of Molecular and Macromolecular Studies, Polish Academy of Sciences, 90–363 Lodz, Poland*

Received July 8, 1996; Accepted December 7, 1996

ABSTRACT

To increase the accessibility of 8-bromo-2′,5′-oligoadenylates, we developed a synthesis of 2′–5′-linked oligoriboadenylates containing varying numbers of 8-bromoadenosine residues based on the use of a CPG-LCA solid support and the phosphoramidite approach. Although N^6-benzoyl protection was satisfactory for incorporation of nonmodified adenine residues into 2′,5′-oligonucleotides, the effective incorporation of 8-bromoadenine into such 2′,5′-linked oligomers required use of a non acyl protecting group. Amidine protection of the purine exocyclic amino function proved compatible with all aspects of the phophoramidite approach and with the hydroxyl protection groups employed.

Index Entries: Interferon; antisense; 2–5A; RNase L; *N,N*-dibutylformamidine protecting group; phosphoramidites; nucleotide conformation.

INTRODUCTION

8-Bromoadenosine and its nucleotides occupy an important place in nucleoside/nucleotide chemistry as substrates for synthesis of many other 8-substituted derivatives *(1–4)*. Their biological activity is often different

*Author to whom all correspondence and reprint requests should be addressed.

from that of adenosine, and this difference is most probably caused by a well-established *syn* conformation about the glycosidic bond *(5–7)*. For example, 8-bromoadenosine is 10^{15} times less efficient as a substrate for adenosine deaminase *(8)*, is inactive as a triphosphate at P_2 purinoreceptors *(9)*, but is phosphorylated by adenosine kinase *(10, 11)*.

We have studied extensively various 2′,5′-linked 8-bromoadenylate oligonucleotides as analogs of 2-5A, a series of 2′,5′-oligoadenylates of the general formula $p_m5'A2'(p5'A)_n$. This unique 2′,5′-linked nucleic acid is synthesized from ATP in interferon-treated cells by the 2′-5′-oligoadenylate synthetase after the latter is activated by dsRNA. 2-5A activates a latent endonuclease, 2-5A-dependent RNase or RNase L, which in turn degrades cellular, ribosomal, and/or viral RNA *(12–14)*. As compared to 2-5A, brominated analogs were more stable to degradation by 2′,5′-specific phosphodiesterase *(15,16)*, and analogs containing one or two unsubstituted adenine residues at the 5′ terminus of an oligobromoadenylate were bound to RNase L with the same efficiency as 2-5A itself and activated mouse cell RNase L as 5′-monophosphates *(17,18)*. This was in distinct contrast to the requirement of the mouse L-cell enzyme for a 2′,5′-oligoriboadenylate 5′-*di*-or *tri*phosphate for expression of nuclease activity *(19,20)*. The efficient binding and photoreactivity of 2′-5′-oligonucleotides containing 8-bromoadenine residues permitted their application as a highly sensitive photo-crosslinking probe for detecting RNase L *(21)*.

For some time, we used a method of synthesis for these sequence-specific 2′,5′-oligobromoadenylates based on controlled stepwise lead-ion-catalyzed addition of a corresponding nucleoside 5′-phosphoroimida- zolidate to the 2′-OH end of 5′-end protected (as phosphoromorpholidate) nucleoside or oligonucleotide *(17,22)*. Because a number of by products formed after each step, time-consuming product purification had to be performed at each step. During such studies, the need for a faster, more efficient approach to oligo(bromo)adenylate synthesis became mandatory; thus, we determined if the solid-phase method of oligoribonucleotide synthesis could be adapted to include 8-bromoadenylates.

Base modifications, such as 8-bromoadenine, have become of manifest importance with the development of 2-5A-antisense chimeras *(23,24)*, which target specific mRNAs for degradation. This new approach to the selective ablation of RNA depends on the RNase L activating ability of the 5′-terminal phosphate of the 2′,5′-oligoriboadenylate.

In initial considerations of the appropriate N^6 protection, we eliminated any acyl-protecting group. Although N^6-benzoyl protection of adenylyl residues has been used successfully in the solid-phase synthesis of both 2′,5′- and 3′,5′-linked *(25–27)* oligoribonucleotides, 8-bromosubstitution of the purine ring substantially increases the depurination rate during alkaline hydrolysis *(28)* and has a destabilizing effect on the glyco-

sidic bond under acidic conditions *(29)*. Both of these effects are further enhanced by acylation of the exocyclic amino function *(30)*. Recent reports report *(32–36)* on applications of dialkylformamidines as a depurination resistant amino group protection in the solid-phase synthesis of oligodeoxynucleotides and oligoribonucleotides *(37)* prompted us to evaluate this group for 8-bromoadenosine.

MATERIALS AND METHODS

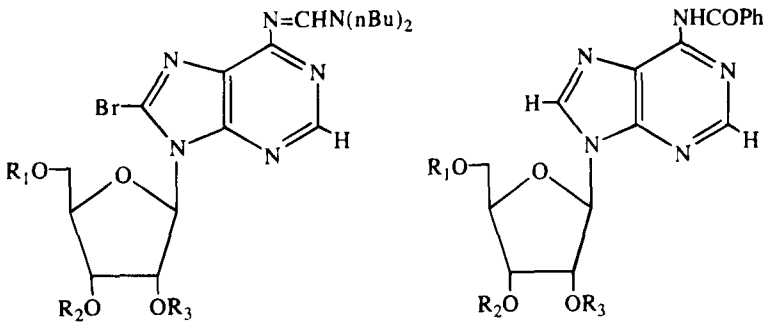
Sources of Reagents

Most reagents used in this study were from Aldrich (Milwaukee WI), including anhydrous solvents (SureSeal packing). Long-chain alkylamino controlled pore glass solid support (GPC-LCA, pore size 50 OA) was from Sigma (St. Louis, MO). 5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*-(*t*-butyldimethylsilyl)-N^6-benzoyl-adenosine **(4a)**, 5′-*O*-(4,4′-dimethoxytrityl)-3′-*O*-(*t*-butyldimethylsilyl)-N^6-benzoyladenosine **(4b)**, and 5′-*O*-(4,4′-dimethoxytrityl)-3′-*O*-(*t*-butyldimethylsilyl)N^6-benzoyladenosine 2′-(*N,N*- diisopropyl-2-cyanoethyl)-phosphoramidite **(6b)** were synthesized according to published procedures *(25,38)*.

Enzymes used for characterization of synthesized oligonucleotides were obtained from the following sources: snake venom phosphodiesterase (oligonucleotide 5′-nucleotidohydrolase, EC 3.1.4.1), Cooper Biomedical; bacterial alkaline phosphatase (phosphoric monoester phosphohydrolase, EC 3.1.3.1), US Biochemicals; nuclease P1 (from *Penicillium citrinum*, EC 3.1. 30.1), Pharmacia; nuclease T2 (from *Asergillus oryzae*, ribonucleate 3′-oligonucleotidohydrolase, EC 3.1.27.1), BRL.

HPLC Methods

Two HPLC systems were used throughout this study. The first was the Beckman System Gold software controlled with an IBM PS/2 computer, two 110B solvent delivery modules, and a 167 UV/VIS variable-wavelength detector (set to operate at 260 and 280 nm). The second system consisted of two Beckman 110B solvent delivery modules (controlled by an NEC computer) and a Beckman 153 UV detector operating at 254 nm. A semipreparative Ultrasphere ODS column (reversed-phase C_{18}, 10 × 250 mm, flow rate 2 mL/min, linear gradients of mobile phase B in buffer A, where A was 50 m*M* ammonium acetate, pH 7.0, and B consisted of 50% methanol in water) was used for purification of synthetic oligonucleotides. An analytical Ultrasphere ODS (reversed-phase C_{18}, 4.9 × 250 mm, flow rate 1 mL/min, linear gradients of B in 0.02*M* ammonium phosphate, pH 7.0) was used for analysis of purity and to determine enzymic digests of the products.



	R_1	R_2	R_3	
1	H	H	H	
2	DMTr	H	H	
3a	DMTr	H	tBDMSi	4a
3b	DMTr	tBDMSi	H	4b
5a	DMTr	DiPCEP	tBDMSi	6a
5b	DMTr	tBDMSi	DiPCEP	6b

DMTr=4,4'-dimethoxytrityl

tBDMSi=t-butyldimethylsilyl

DiPCEP=diisopropylamino-2-cyanoethoxy-phosphinyl

Fig. 1. Structures of 8-Bromoadensosine and Protected Intermediates.

Physical Measurements

Concentrations of oligonucleotides were measured spectrophotometrically using adenylates extinction coefficients reported in the literature *(39)*. For bromoadenylates, extinction coefficients were determined by means of HPLC as described earlier *(17)*. UV absorption spectra were recorded on a Varian DMS-200 spectrophotometer. ^{1}H-NMR and ^{31}P-NMR spectra were recorded on either Varian XL-300 or GE GN-300 instruments in solvents indicated below.

6-N-[(Di-n-butylamino)-methylene]-8-bromodenosine (1) (Fig. 1)

Method A (according to ref. [32]: A solution of 8-bromoadenosine (3.46 g, 10 mmol) and di-*n*-butylformamide dimethyl acetal (3.0 g, 15 mmol) in DMF (50 mL) was kept for 2 d, protected from light, at room temperature. The mixture then was evaporated to dryness, the residue dissolved in methylene chloride (50 mL), and the methylene chloride solution extracted first with 5% sodium bicarbonate (30 mL) and then with water. The organic layer was dried with sodium sulfate, the solvent was evaporated under

vacuum, and the product was obtained as a yellow oil that was used in the next step without further purification.

Method B (according to ref. *[35]*): 8-Bromoadenosine (3.46 g, 10 mmol) was dried by coevaporation with pyridine (2 × 70 mL). Methanol (25 mL) and *N,N*-di-*n*-butylformamide dimethyl acetal (3.0 g, 15 mmol) were then added, and the mixture was stirred for 6 h at room temperature. As the reaction progressed, 8-bromoadenosine slowly dissolved. The mixture was concentrated and separated by fast column chromatography (4.5 × 15 cm column, silicagel 230–400 mesh, 1–4% of MeOH, 0.2% of pyridine in ethyl acetate). TLC: Rf 0.34, ethyl acetate : methanol, 95:5. The yield was 2.66 g (71%) of white solid foam. Analysis calculated for $C_{19}H_{29}N_6O_4Br$: C 47.02, H 6.02, N 17.31, Br 16.46. Found: C 46.67, H 6.14, N 17.15, Br 16.32.

5′-O-(Di-p-methoxytrityl-6-N-[(di-n-butylamino)-methylene]-8-bromoadenosine (2) *(Fig. 1)*

The crude 6-*N*-[(di-*n*-butylamino)-methylene]-8-bromoadenosine (method A, 10 mmol) was dissolved in pyridine (50 mL), and 4,4′-dimethoxytrityl chloride (3.4 g, 10 mmol) was added. After 2 h of stirring at room temperature, the reaction was completed (TLC control). Methanol (0.5 mL) then was added to destroy any unreacted chloride, and the mixture was evaporated to dryness, dissolved in methylene chloride (50 mL), washed with 5% sodium bicarbonate, and then with brine. The organic layer was dried over anhydrous sodium sulfate and concentrated. The mixture was purified on a silicagel 4.5 × 15 cm column, which was eluted with petroleum ether-ethyl acetate-pyridine (50:50:0.2) until all fast-moving products were eluted. The solvent ratio was then changed stepwise to 25:75:0.2, and finally to ethyl acetate:pyridine (100:0.2). TLC: R_f 0.27, ethyl acetate : petroleum ether : pyridine, 50:50:0.2. Yield 4.16 g (53%, for two steps). ^{1}H-NMR ($CDCl_3$, 1% deuteriopyridine) δ[ppm]: 0.93 (m, 6H, CH_3-C), 1.35 (m, 4H, CH_2-C), 1.61 (m, 4H, CH_2-C), 3.36 (m, 4H, CH_2-N and H-5′), 3.65 (t, 7.5Hz, 2H, CH_2-N), 3.73 (s, 6H, CH_3-O), 4.22 (m, 1H, H-4′), 4.72 (m, 1H, H-3′), 5.52 (m, 1H, H-2′), 6.01 (d, 4.7Hz, 1H, H-1′), 6.71 (d, 4H), 7.1–7.4 (m), 8.18 and 8.84 (s, 1H). ^{13}C-NMR ($CDCl_3$, 1% deuteriopyridine) δ[ppm]: 63.4 (C-5′), 71.1 and 71.2 (C-3′ and C-2′), 83.7 (C-4′), 90.5 (C-1′), ribose carbons; 55.1 (CH_3-O); 13.7, 13.9, 19.7, 20.2, 29.2, 30.9, 45.3, 52.0, butyl carbons. MS: (M^+ + 1) 485 (^{79}Br) and 487 (^{81}Br).

5′-O-(Di-p-methoxytrityl)-6-N-[(di-n-butylamino)methylene]-2′-O-(t-butyldimethylsilyl)-8-bromoadenosine and 5′-O-(di-p-methoxytrity)-6-N-[(di-n-butylamino)methylene]-3′-O-(t-butyldimethylsily)-8-bromoadenosine (3a and 3b) (Fig. 1)

A solution of 5′-*O*-(di-*p* -methoxytrityl-6-*N*-[(di-*n*-butyl-amino)methylene]-8-bromoadenosine (1.57 g, 2 mmol), imidazole (408 mg, 6 mmol),

and *t*-butyldimethylsilyl chloride (622 mg, 4 mmol) in anhydrous DMF (20 mL) was stirred for 4 h at room temperature and left at –20°C overnight. When a TLC control (petroleum ether:ethyl acetate:pyridine, 75:25:0.2) showed that all of the substrate had disappeared, the reaction was terminated by the addition of 5% sodium bicarbonate (1 mL, at O°C), and the mixture was stirred for 15 min at temperature below 10°C. The solvent was evaporated under vacuum, the residue was dissolved in methylene chloride (50 mL), and the methylene chloride solution was extracted with water. The organic layer was dried over anhydrous magnesium sulfate, concentrated, and again dissolved in 15 mL of petroleum ether:ethyl acetate:pyridine (75:25:0.2). This solution was applied to a silica gel column (4.5 × 15 cm) and developed with the same solvent. The following fractions were separated: 2′-*O*-silylated isomer **3a,** R_f 0.47, 270 mg,15% and 3′-*O*-silylated isomer **3b,** R_f 0.35, 740 mg, 41%. **3a:** ^{1}H-NMR ($CDCl_3$, 1% deuteriopyridine) δ[ppm]: –0.25 (s, 3H, CH_3-Si), –0.08 (s, 3H, CH_3-Si), 0.81 (s, 9H, CH_3-C), 0.93 (m, 6H, CH_3-CH_2), 1.30 (m, 4H, CH_2-C), 1.61 (m, 4H, CH_2-C), 2.8 (d, 1H, 3′-OH), 3.36 (m, 4H, CH_2-N, H-5″), 3.46 (dd, 4.7 and 10.3Hz, 1H, H-5′), 3.71 (m, 2H, CH_2-N), 3.74 (s, 6H, CH_3-O), 4.20 (dd, 4.7 and 8.3Hz, 1H, H-4′), 4.47 (dd, 3.8 and 5.6Hz, 1H, H-3′), 5.63 (t, 5.6Hz, 1H, H-2′), 5.95 (d, 5.6Hz, 1H, H-1′), 6.74 (d, 8.8Hz, 4H), 7.1–7.4 (m), 8.23 (s, 1H), 8.87 (s, 1H). ^{13}C-NMR ($CDCl_3$, 1% deuteriopyridine) δ[ppm]: 63.3 (C-5′), 71.3 and 72.0 (C-3′ and C-2′), 84.1 (C-4′), 90.5 (C-1′), ribose carbons; 55.1 (CH_3-O); 25.5 (CH_3-C); 13.7, 13.9, 20.2, 29.2, 30.9, 45.1, 51.7, butyl carbons. MS (M^+ + 1): 901 (^{79}Br) and 903 (^{81}Br). **3b:** ^{1}H-NMR ($CDCl_3$, 1% deuteriopyridine) δ[ppm]: 0.04 (s, 3H, CH_3-Si), 0.14 (s, 3H, CH_3-Si), 0.90 (s, 9H, CH_3-C), 0.95 (m, 6H, $\underline{CH_3}$-CH_2), 1.37 (m, 4H, CH_2-C), 1.62 (m, 4H, CH_2-C), 3.12 (m, 2H, H-5″, 2′-OH), 3.37 (t, 7.3Hz, 2H, CH_2-N), 3.50 (dd, 3.6 and 10.7Hz, 1H, H-5′), 3.71 (m, 2H, CH_2-N), 3.76 (s, 6H, CH_3-O), 4.09 (m, 1H, H-4′), 5.15 (dd, 5.6 and 5.9Hz, 1H, H-3′), 5.32 (dd, 3.5 and 4.3Hz, 1H,H-2′), 6.05 (d, 3.1Hz, 1H, H-1′), 6.72 (d, 8.8Hz,4H), 7.1–7.4 (m), 8.41 (s, 1H), 8.87 (s, 1H). ^{13}C-NMR ($CDCl_3$, 1% deuteriopyridine) δ[ppm]: 62.2 (C-5′), 71.3 and 72.1 (C-3′ and C-2′), 83.0 (C-4′), 90.8 (C-1′), ribose carbons; 55.1 (CH_3-O); 25.7 (CH_3-C); 13.7, 13.9, 19.7, 20.2, 30.9, 45.1, 51.7, butyl carbons. MS (M^+ + 1): 901 (^{79}Br) and 903 (^{81}Br).

5′-O-(Di-p-methoxytrityl)-N6-[(di-n-butylamino)methylene]-2′-O-(t-butyl-dimethylsilyl)-8-bromoadenosine 3′-(N,N-diisopropyl-2-cyanoethyl)-phosphoramidite ***(5a)*** *(Fig. 1)*

To a solution of 5′-*O*-(di-*p*-methoxytrityl)-*N6*-[di-*n*-butylamino)-methylene]-2′-*O*-(*t*-butyl-dimethylsilyl)-8-bromoadenosine (**3a,** 0.13 mmol, 120 mg), *N*-ethyl-diisopropyl amine (65 mg, 0.5 mmol) and 4-dimethylaminopyridine (4 mg) in 5 mL of anhydrous methylene chloride, kept under nitrogen, 2-cyanoethyl *N,N*-diisopropyl-chlorophosphoramidite

(47 mg, 0.2 mmol) was added slowly with a gas-tight syringe. The mixture was stirred for 1 h at room temperature, concentrated quickly under vacuum, applied immediately to a silica gel column (2 × 10 cm), and eluted with petroleum ether : ethyl acetate : triethylamine, 30:10:0.5 (v/v). The product was obtained as white solid foam, and both P-enantiomers were not resolved on TLC (R_f 0.44, solvents as above). Yield 120 mg (82%). ^{31}P-NMR ($CDCl_3$, 1% deuteriopyridine) δ[ppm]: 151.2 and 148.6 (45:55 ratio). ^{1}H-NMR ($CDCl_3$, 1% deuteriopyridine, predominant isomer, selected signals) δ[ppm]: –0.30 and 0.08 (s, 6H, CH_3-Si), 0.75 (s, 9H, CH_3-C), 3.76 (s, 6H, CH_3-O), 3.95 (m, 1H, H-4′), 4.5 (m, 1H, H-3′), 5.7 (dd, 1H, H-2′), 5.99 (d, 3.7Hz, 1H, H-1′), 8.22 (s, 1H), 8.87 (s, 1H); minor isomer, selected signals: 0.32 and 0.12 (s, 6H, CH_3-Si), 0.75 (s, 9H, CH_3-C), 3.76 (s, 6H, CH_3-O), 3.95 (m, 1H, H-4′), 4.4 (m, 1H, H-3′), 5.6 (dd, 1H, H-2′), 6.01 (d, 4.1Hz, 1H, H-1′), 8.18 (s, 1H), 8.87 (s, 1H).

5′-O-(Di-p-methoxytrityl)-N6-[(di-n-butylamino)-methylene]-3′-O-(t-butyldimethylsilyl)-8-bromoadenosine 2′-(N,N-diisopropyl-2-cyanoethyl)phosphoramidite ***(5b)*** *(Fig. 1)*

To a solution of 5′-*O*-(di-*p*-methoxytrityl)-6-*N*-[(di-*n*-butylamino) methylene]-3′-*O*-(*t*-butyl-dimethylsilyl)-8-bromoadenosine (**3b,** 0.5 mmol, 450 mg), *N*-ethyl-diisopropyl amine (2 mmol, 258 mg), and 4-dimethylaminopyridine (10 mg) in 10 mL of anhydrous methylene chloride, kept under nitrogen, 2-cyanoethyl *N,N*-diisopropyl-chlorophosphoramidite (142 mg, 0.6 mmol) was added slowly with a gas-tight syringe. The mixture was stirred for 2 h at room temperature, concentrated quickly under vacuum, applied immediately to a silica gel column (2 × 12 cm), and eluted with petroleum ether : ethyl acetate : triethylamine, 20:10:0.5 (v/v). The product was obtained as a white glass (solid foam) and consisted of a mixture of two P-enantiomeric phosphoramidites, R_f = 0.69 and 0.81. Yield 410 mg (74%). ^{31}P-NMR ($CDCl_3$, 1% deuteriopyridine δ[ppm]: 150.9 and 149.9 (1:2 ratio). ^{1}H-NMR ($CDCl_3$, 1% deuteriopyridine, predominant isomer, selected signals) δ[ppm]: 0.08 and 0.16 (s, 6H, CH_3-Si), 0.85 (s, 9H, CH_3-C), 3.06 (dd, 3.9 and 10.6Hz, 1H, H-5″), 3.75 (s, 6H, CH_3-O), 4.16 (m, 1H, H-4′), 5.19 (dd, 4.6 and 6.7Hz, 1H, H-3′), 5,65 (2dd, 12.3, 2.6 and 4.4Hz, 1H, H-2′), 6.07 (d, 2.4Hz, 1H, H-1′), 8.44 (s, 1H), 8.87 (s, 1H); minor isomer, selected signals: 0.11 and 0.14 (s, 6H, CH_3-Si), 0.88 (s, 9H, CH_3-C), 3.20 (dd, 4.1 and 10.5Hz, 1H, H-5″), 3.76 (s, 6H, CH_3-O), 4.19 (m, 1H, H-4′), 4.96 (t, 2.9Hz, 1H, H-3′), 5.42 (dt, 10.2, 4.3 and 4.3Hz, 1H, H-2′), 6.18 (d, 3.1Hz, 1H, H-1′), 8.35 (s, 1H), 8.87 (s, 1H).

Synthesis of Oligonucleotides

Syntheses were carried out manually on DNA synthesis columns (1.5 cm, American Bionetics Inc.) loaded with approx 1 mmol of CPG-bound

Table 1
Solid-Phase Synthesis of Oligonucleotide–Coupling Cycle

Step	Solvents/reagents	Time	Volume
1. Detritylation	3% DCA in CH_2Cl_2	90 s	1 ml
2. Washing	2% Py in acetonitrile		1 ml
3. Washing	acetonitrile		3 ml
4. Drying	nitrogen	3 min	
5. Coupling	0.2M monomer in 0.5M tetrazole/acetonitrile	8 min for Ado,and br^{8}Ado, 3 min for 5'-end phosphitylation	0.15 ml
6. Washing	acetonitrile		3 ml
7. Drying	nitrogen	2 min	
8. Capping	A + B, 1:1 A: 30% Ac_2O in THF B: 0.6M DMAP in Py/ THF (3:2 v/v)	2 min	1 ml
9. Washing	acetonitrile		3 ml
10. Drying	nitrogen	2 min	
11. Oxidation	0.1 M I_2 in lutidine: THF:water, 20:80:1	45 s	1 ml
12. Washing	acetonitrile		3 ml
13 Drying	nitrogen	3 min	

3a or **4a** *(40)*, using adapters and gas-tight syringes *(41)*. The procedure was based on the phosphite triester method of DNA/RNA synthesis *(25–27,42,43)* using for chain elongation 2-cyanoethylphosphoramidite derivatives **5a, 5b,** or **6b** (Fig. 1). The applied synthesis cycle is presented in Table 1. Oligonucleotides were 5′-phosphorylated on the support with bis(2-cyanoethoxy)diisopropylamino-phosphine *(44)*. Syntheses were con-

trolled by quantitating spectrophotometrically the release of trityl cation, and the average coupling efficiency determined in this way was 90%. The synthesized oligonucleotides were cleaved from the support with a 3:1 mixture of 28% aq. ammonia and ethanol by 2 h incubation at room temperature. Then to remove N^6-protecting groups, 10% of ammonium acetate was added to the conc. ammonia solution and incubation continued for 3–4 h at 55°C. In the last step, *t*-butyldimethylsilyl groups were removed by treatment with 1*M* tetrabutylammonium fluoride in THF for 12 h at room temperature.

The oligonucleotides were purified by HPLC on semipreparative Ultrasphere ODS column. The collected samples were desalted on DEAE Sephadex A-25 anion-exchange column (HCO_3^-form) eluted with required linear gradients of triethylammonium bicarbonate. After evaporation of the buffer, oligonucleotides were converted into sodium salts by precipitation with a 2% solution of sodium iodide in acetone. The following oligonucleotides were synthesized: p5′A2′p5′A2′p5′(br^{8}A)2′p5′(br^8)A **(7)**, 47% yield; p5′A2′p5′A2′p5′(br^{8}A)3′p5′(br^8)A **(8)**, 38% yield; p5′A2′p5′A2′p5′(br^{8}A)-2′p5′(br^8)A2′p5′A **(9)**, 63% yield; p5′A(2′p5′A)$_9$ **(10)**, 26% yield.

Structures of the synthesized products were confirmed by HPLC analysis of the enzymic digestion products. All oligonucleotides were completely hydrolyzed with snake venom phosphodiesterase and gave the expected ratios of pA, pbr^{8}A. Compounds **7, 8, 9** and **10** were dephosphorylated at 5′-terminus with bacterial alkaline phosphatase and all but **8** were resistant to ribonuclease T_2. Oligonucleotides **7** and **9** were further analyzed by ^{1}H- and ^{3}P-NMR *(18)*.

RESULTS AND DISCUSSION

Several differently substituted amidine derivatives of deoxynucleosides (dAdo, dCyd, dGuo) have been synthesized and applied to the solid-phase oligodeoxynucleotide synthesis (34–36), and recently to the automated synthesis of oligoribonucleotides *(37)*. The advantage of amidine protection strategy was evident, since in stability studies performed under standard acidic deprotection conditions, enhanced resistance to depurination was observed; specifically, in 2% DCA/dichloromethane, the *N,N*-di-*n*-butylformamidine derivative of deoxyadenosine was 18–20 times more stable than the corresponding N^6-benzoyl derivative *(31,35)*.

We prepared the formamidine derivative of 8-bromoadenosine **(1)** (Fig. 1) using *N,N*-dibutylformamidine dimethyl acetal *(32)* and the reaction conditions described for 2-deoxyadenosine *(32,35)*. When N^6[(di-*n*-butylamino)-methylene]-8-bromoadenosine **(1)** was treated with concentrated (28%) aqueous ammonia/ethanol (3:1) solution containing 10%

ammonium acetate at 55°C, 95% removal of dibutylformamidine protecting group was observed after 1 h, and complete deprotection was achieved within 3 h. The resistance of **1** toward depurination by 2% dichloroacetic acid/dichloromethane was determined to be higher than that of 6-*N*-benzoyldeoxyadenosine. Only 20% glycosidic bond cleavage occurred during 2 h of incubation compared to $t_{1/2} = 1.7$ h reported for N^6-benzoyldeoxyadenosine *(35)*. The above results indicated that an application of N^6-[(di-*n*-butylamino)-methylene]-8-bromoadenosine **(1)** should be compatible with the standard phosphoramidite approach and usual protecting groups for the solid-phase synthesis of oligoribonucleotides. Conventional N^6-benzoyl protection was used for adenosine, since the benzoyl group can be completely removed under the above deprotection conditions.

5′-Dimethoxytritylation of **1** according to the routine procedure occurred smoothly giving 53% of 5′- and 6-*N*-protected 8-bromoadenosine, **2.** Mixtures of 2′- and 3′-hydroxyl-silylated 5′-*O*-dimethoxytrityl-N^6-[(di-*n*-butylamino)-methylene]-8-bromoadenosine (**3a** and **3b**) and 2′- and 3′-*O*-silylated 5′-*O*-dimethoxytrityl-N^6-benzoyl adenosine (**4a** and **4b**) derivatives were prepared by the reaction with *t*-butyldimethylsilyl chloride catalyzed by imidazole *(37)* (Fig. 1). The isomers were separated by silica gel column chromatography.

Structure assignment for the above 2′- and 3′-silylated isomers was based on ^{1}H- and ^{13}C-NMR. An empirical rule to assign structures to isomeric silylated ribonucleotides has been published by Ogilvie *(45)*. It states that silylation at a carbohydrate hydroxyl leads to a significant downfield movement of the chemical shift for the carbon to which this hydroxyl group is attached. Unfortunately, for 5′-dimethoxytrityl-protected nucleosides **3a** and **3b,** the difference in chemical shifts of C2′ and C3′ of both isomers was too small to provide reliable assignment. However, the same authors reported that for 5′-monomethoxytritylated silylated purine nucleosides, the methyl and *t*-butyl protons of the *t*-butyldimethyl-silyl group at the 2′-hydroxyl were shifted significantly upfield as compared to those of the 3′-isomer, and we based our assignment on this observation. Therefore, for the 8-brominated isomer with larger R_f the structure of **3a** was assigned, and the lower R_f isomer was assigned the structure **3b.** This assignment was later confirmed from the structures of oligonucleotides synthesized from phosphoramidites **5a** and **5b.**

The monosilylated nucleosides **3a, 3b,** and **4b** (Fig. 1) were phosphitylated with (2-cyanoethyl)-*N,N*-diisopropylphosphonamidic chloride according to a procedure reported first by Sinha et al. *(46)* for deoxynucleosides and by Usman et al. *(24)* for ribonucleosides. The reaction required strictly anhydrous conditions. Analysis by ^{31}P-NMR showed that the reactions were fully stereospecific. Phosphoramidites **5a** and **5b** were as reac-

tive as their nonbrominated analog **6b**, providing coupling efficiencies in a range of 84–95%.

In sum, amidine protection of the 8-bromoadenine ring permits the transfer of the standard phosphoramidite technology to the synthesis of 2′,5′-oligonucleotides containing 8-bromoadenosine. This, in turn, permits the facile application of the peculiar conformational properties of this nucleoside in understanding and modifying interactions of oligonucleotides with their target cellular receptors.

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