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Regiodefined Synthesis and Conformational Properties of Adenyldiyl Trimers with Unsymmetrical 2'–5' and 3'–5' Internucleotide Linkages

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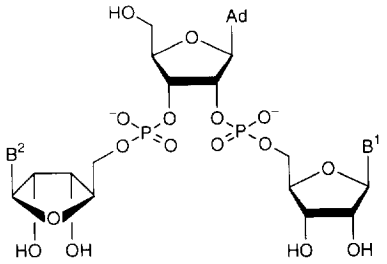
Abstract: Adenyldiyl trimers with different kinds of substituents on 2'-5' and 3'-5' phosphate linkages have been synthesized in a general, regiodefined manner. Examination of the 2D NMR spectra reveals that the trimers with adenylyl(2'–5')adenosine linkage make a syn–anti as well as syn–syn base stack between the two adenyl bases and exist as a mixture of the two conformers.

INTRODUCTION

Oligoribonucleotides with 2'–5',3'–5'-bis(internucleotide) linkages, generally called branched RNAs, are recognized for their integral role in eukaryotic splicing.[1] Particularly significant among them are the adenyldiyl derivatives such as adenyldiyl[(2'–5')guanosine](3'–5')cytidine (**4**) and adenyldiyl[(2'–5')guanosine](3'–5')uridine (**5**) which are the branch-point structures of the naturally occurring compounds. Immunochemical studies on 2'-5'-linked oligoadenylates (2–5A oligomers) have implied that the analogs with an internucleotide branch at the 3' position are chemotherapeutically attractive.[2] Although many syntheses of this class of compounds have been reported,[3,4] they have common drawbacks. One is the lack of regioselectivity in the formation of the unsymmetrical 2'- and 3'-internucleotide linkage. Development of a 2'- or 3'-O-selective protecting method is crucial for achieving the position-defined formation of internucleotide linkages. There are some bifunctional silicon compounds such as 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane,[5] di-*tert*-butyldichlorosilane,[6] or di-*tert*-butylsilylene bis(triflate),[6] which simultaneously protect the 3' and 5'-hydroxyl groups, but these are not suitable for the preparation of the targeted 2',3'-bis(phosphate) compounds, because (1) the required 3'-O-selective deprotection of the 2'-O-phosphorylation product is not easy and (2), even if it could be achieved, residual Si-OH in the 5'-hydroxyl protector would cause problems in the subsequent internucleotide-linkage formation. In addition, isolation of the target product requires tedious ion-exchange or reverse-phase silica gel chromatography. These problems

can be removed by using (1) the 3'-*O*-selective protection by a *tert*-butyldimethylsilyl (TBDMS) group[7] and (2) allyl protection deblocked by a Pd(0)-catalyzed reaction in the presence of a nucleophile such as diethylammonium hydrogencarbonate[8] which can be easily removed by evaporation from the target compound. This paper describes a regiospecific and operationally simple synthesis of the adenyldiyl trimers with 2',3'-unsymmetrical substituents. The conformation of the nucleotides has been determined by 2D NMR.



1: B^1 = Ad; B^2 = Cy
2: B^1 = Ad; B^2 = Gu
3: B^1 = Ad; B^2 = Ur
4: B^1 = Gu; B^2 = Cy
5: B^1 = Gu; B^2 = Ur

RESULTS AND DISCUSSION

Synthetic Strategy

As shown in Figure 1, the target structure was divided into three blocks I–III. The branched structure was to be constructed via (1) condensation of the 3'-O-protected adenosine (block I) and a nucleoside 5'-phosphoramidite (block II), forming a 2'–5' internucleotide linkage, (2) removal of the 3'-O-protector, and (3) the 3'-O-phosphorylation with another nucleoside 5'-phosphoramidite (block III). Since blocks II and III can be substituted for each other, it is sufficient to prepare only one type of nucleoside 5'-phosphoramidite as the building blocks in this approach.

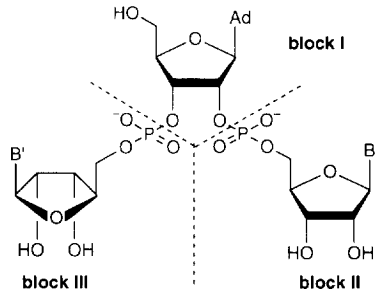
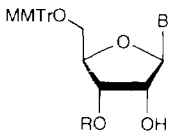


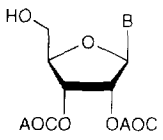
Fig. 1. Building blocks for the synthesis of branch-type adenyldiyl trimers.

Preparation of Building Blocks

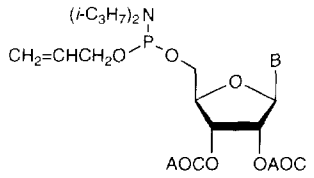
The N-protected 2'-O-free adenosine **8** was prepared in 86% overall yield from **7** by (1) transient trimethylsilylation of the 2' hydroxyl with *N*-trimethylsilylimidazole, (2) introduction of an allyloxycarbonyl (AOC) protector to the amino function with allyl benzotriazolyl carbonate (AOC–OBT)[8] assisted by *tert*-butylmagnesium chloride, and (3) selective removal of the transient 2'-*O*-trimethylsilyl protector by citric acid. During these transformations, the 3'-*O*-TBDMS protecting group did not migrate to the 2'-hydroxyl group. The nucleoside 5'-phosphoramidites **16**–**19** were constructed as follows. Reaction of **6** with AOC tetrazolide[9] followed by detritylation with dichloroacetic acid gave the tri-AOC product **12** in 85% overall yield without forming an undesired 2',3'-*O*-cyclic carbonate. In a similar manner, the 5'-*O*-protected cytidine **9** was converted to **13** in 87% yield and the uridine analogue **11** was transformed to **15** in 85% yield. By contrast, preparation of the tri-AOC guanosine **14** required slight modification. Thus, 2',3',5'-tri-*O*-*tert*-butyldimethylsilylguanosine was first N-allyloxycarbonylated using *tert*-butylmagnesium chloride and AOC chloride. Subsequent desilylation with tetrabutylammonium fluoride (TBAF) and 5'-O-protection with *p*-methoxytrityl chloride furnished the 2,3,-O-free product **10** (36% overall yield). This was then converted to **14** (77% overall yield) via allyloxycarbonylation with AOC tetrazolide and detritylation. These AOC protected products **12**–**15** were reacted with CH_2=$CHCH_2OP[N(i\text{-}C_3H_7)_2]_2$[9] assisted by of 1*H*-tetrazole and diisopropylamine[10] to afford **16**–**19** in 78–98% yields. The phosphoramidite structure was confirmed by ^{31}P NMR spectra which showed two singlets in the 150-ppm region due to the diastereomers.



6: B = Ad; R = H
7: B = Ad; R = TBDMS
8: B = Ad^{AOC}; R = TBDMS
9: B = Cy; R = H
10: B = Gu^{AOC}; R = H
11: B = Ur; R = H



12: B = Ad^{AOC}
13: B = Cy^{AOC}
14: B = Gu^{AOC}
15: B = Ur

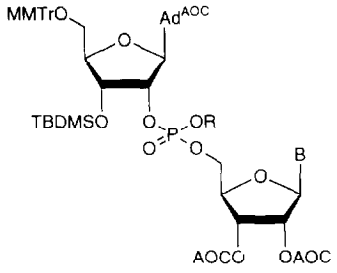


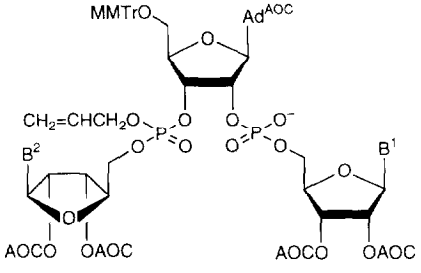
16: B = Ad^{AOC}
17: B = Cy^{AOC}
18: B = Gu^{AOC}
19: B = Ur

Preparation of the Branch-Type Adenylyl Trimers

The branched skeleton was constructed via successive formation of the 2'–5' and 3'–5' internucleotide linkages by condensation of the 2'-O-free adenosine **8** and the 5'-phosphoramidites, **16**–**19**. For instance, coupling of **8** and **16** by the aid of 1*H*-tetrazole[11] followed by oxidation with *tert*-butyl hydroperoxide (TBHP)[12] gave the protected A2'p5'A **20** as a mixture of diastereomers in 86% overall yield. In a similar way, the fully protected A2'p5'G **21** was produced in 88% yield. The homogeneous 2'-5' linked structure of **20** and **21** was verified by the ^{31}P–^{1}H 2D NMR spectra with cross peaks among the signals due to the phosphorus atom and 2' and 5' protons of the linked nucleosides. The next stage of the synthesis was formation of the 3'–5' internucleotide linkage, where the phosphotriester moiety was converted to the phosphodiester linkage before deblocking the 3'-*O*-silyl protection, because the 3'-O-free ribonucleoside 2'-phosphotriesters are extremely unstable and subjected to decomposition via the 2',3'-cyclic phosphate intermediate.[4b,13] Thus, the phosphate function of **20** was deallylated by exposure to sodium iodide in refluxing acetone to afford the phosphodiester

intermediate **22** in 86% yield, leaving the AOC protectors intact. The silyl protector of **22** was removed with TBAF, and the product was subjected to condensation with the 5'-phosphoramidite **17** in the presence of 1*H*-tetrazole and 4-dimethylaminopyridine[14] and then to TBHP oxidation to give the protected branch-type trimer **24** in 54% yield. Subsequent acid-catalyzed detritylation and Pd-catalyzed removal of the allyl and AOC protectors in the presence of diethylammonium hydrogencarbonate as a nucleophile[15] furnished the branch-type RNA **1** in 74% overall yield. This is a broad, general approach, allowing the preparation of a variety of unsymmetrically linked branch-type trimers simply by selecting the 5'-phosphoramidites for construction of the 2'–5' and 3'–5' internucleotide linkages. Actually, the 2',3'-adenyldiyl trimers **2**–**5** were synthesized via the corresponding 2'–5',3'–5'-linked adenyldiyl precursors, **25**–**28**, which were prepared from the 2'–5'-linked adenylyl derivative, **22** or **23**. Fortunately, all the target nucleotides **1**–**5** were obtained as solids after the usual extraction followed by trituration. Thus, the products could be isolated by simple filtration.





20: B = Ad^{AOC}; R = CH_2=$CHCH_2$
21: B = Gu^{AOC}; R = CH_2=$CHCH_2$
22: B = Ad^{AOC}; R = Na
23: B = Gu^{AOC}; R = Na

24: B^1 = Ad^{AOC}; B^2 = Cy^{AOC}
25: B^1 = Ad^{AOC}; B^2 = Gu^{AOC}
26: B^1 = Ad^{AOC}; B^2 = Ur
27: B^1 = Gu^{AOC}; B^2 = Cy^{AOC}
28: B^1 = Gu^{AOC}; B^2 = Ur

Structure Determination of the Products

The structures of adenosine 2',3'-bis(phosphate)s in **1**–**5** were confirmed by enzymatic experiments. For example, hydrolysis of **1** with snake venom phosphodiesterase formed a mixture of adenosine, 5'-AMP, and 5'-CMP in a reasonable mol ratio. By contrast, no digestion occurred in the reaction with RNase T_2 and spleen phosphodiesterase. The regiochemistry of the unsymmetrical 2'- and 3'-substituents on the branched point of adenosine was determined on the basis of Chattopadhyaya method[16] by the combined analysis of several 2D NMR experiments, in particular, ^{31}P–^1H correlated, homonuclear Harmann–Hahn (HOHAHA), and HMBC spectra. The structure determination of A(2'p5'A)3'p5'U (**3**) is representatively described below. First, on the basis of the ^{31}P–^1H correlated NMR spectrum (Fig. 2), we confirmed that the ^{31}P signal at δ –0.96 ppm was due to the phosphorus atoms forming 2'–5' internucleotide linkage, since the ^{31}P signal experiences a spin-spin coupling with the ^1H signal due to H-2' in the branch-point adenosine. At the same time, ^1H signals at δ 3.68 and 3.94 ppm could be assigned to 5' protons of the 2'-linked nucleoside, since cross coupling was observed with the above-mentioned ^{31}P signal. Subsequently, the signal due to the anomeric H-1' of the 2'-linked nucleoside could be determined based on the above-assigned 5' protons in the HOHAHA spectrum (Fig. 3). Finally, the presence of a long-range coupling between the signals due to the above-described H-1' and C-4 of the adenyl ring, observed in the HMBC spectrum (Fig. 4), revealed that the 2'-linked nucleoside is adenosine and, consequently, the 3'-linked nucleoside is uridine.[17] This regiochemical

relationship was also supported by the ROESY spectrum (Fig. 5) showing ROE's between H-1' and H-8 of the branch-point adenosine, and between H-1' or H-2' and H-2 of 2'-linked adenosine. The regiochemistry of the two internucleotide linkages of **1**, **2**, **4**, and **5** was elucidated in a similar manner. In addition, the protons of **1**–**5** could be fully assigned from these spectra. Table 1 summarizes the assignments.

Table 1. ^{31}P and ^{1}H NMR Signals of A(2'p5'X)3'p5'Y (1–5).

compd	A/X/Y	H-1'	H-2'	H-3'	H-4'	H-5'	H-5''	H-2	H-8	H-5	H-6	2'-p	3'-p
1	A	6.11	5.28	4.86	4.45	3.81	3.75	7.75	8.16			–0.91	–0.18
	A	5.80	4.45	4.29	4.04	3.96	3.72	8.00	8.14				
	C	5.86	4.32	4.31	4.22	4.17	4.14			5.89	7.80		
2	A	6.06	5.22	4.86	4.38	3.70	3.68	7.73	8.16			–0.87	–0.18
	A	5.78	4.30	4.18	4.00	3.89	3.65	7.95	8.14				
	G	5.81	4.73	4.78	4.27	4.17	4.14		7.96				
3	A	6.12	5.28	4.87	4.45	3.81	3.75	7.72	8.16			–0.96	–0.21
	A	5.79	4.27	4.23	4.02	3.94	3.68	7.98	8.14				
	U	5.87	4.32	4.31	4.24	4.18	4.14			5.80	7.81		
4	A	6.13	5.26	4.85	4.45	3.81	3.75	7.85	8.17			–0.82	–0.16
	G	5.58	4.40	4.22	3.97	3.88	3.63		7.67				
	C	5.86	4.27	4.24	4.20	4.16	4.13			5.92	7.79		
5	A	6.14	5.27	4.85	4.45	3.80	3.74	7.85	8.18			–0.89	–0.21
	G	5.58	4.40	4.18	3.95	3.87	3.58		7.67				
	U	5.86	4.31	4.29	4.21	4.16	4.12			5.80	7.81		

Conformational Analysis of the Branch-Type Nucleotides

Conformational analysis of the branch-type nucleotides also was done using NMR experiments by reference to Chattopadhyaya's works.[18] The ^{1}H- and ^{31}P-NMR data in Table 1 give information about the conformation. First, at elevated temperatures, a significant down-field shift is seen with the ^{1}H signals, due to H-2 of the branch-point adenosine, and H-2, H-8, and H-1' due to the 2'-linked adenosine (see Fig. 6), confirming that 2'–5' base-stacking occurs in **1**–**3** with adenylyl(2'–5')adenosine (see Fig. 7). A similar trend has already been reported for adenylyl(2'–5')guanosine structures such as **4** and **5**.[19] The 2'–5'-base stacking structure was also supported by the fact that: (1) all ^{1}H signals due to sugar protons of the 2'-linked adenosine appeared in a higher field compared to those due to the corresponding protons of the 3'-linked nucleoside; (2) chemical shift of the ^{31}P signal arising from the 2'-phosphoric moiety is higher than that of the signal due to the 3'-phosphate.

Of interest is that **1**–**3** with adenylyl(2'–5')adenosine linkage forms not only a syn–anti stack between the adenyl bases, which was previously observed in branch-type trimers such as **4** or **5** that have an

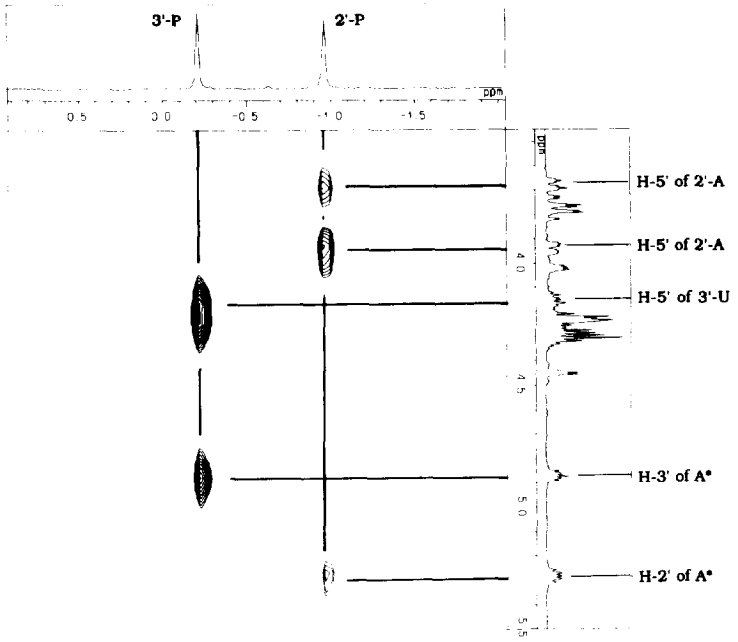


Fig. 2. The ^{31}P–^{1}H correlation spectrum of **3** in D_2O at 35 °C. A* = branch-point adenosine.

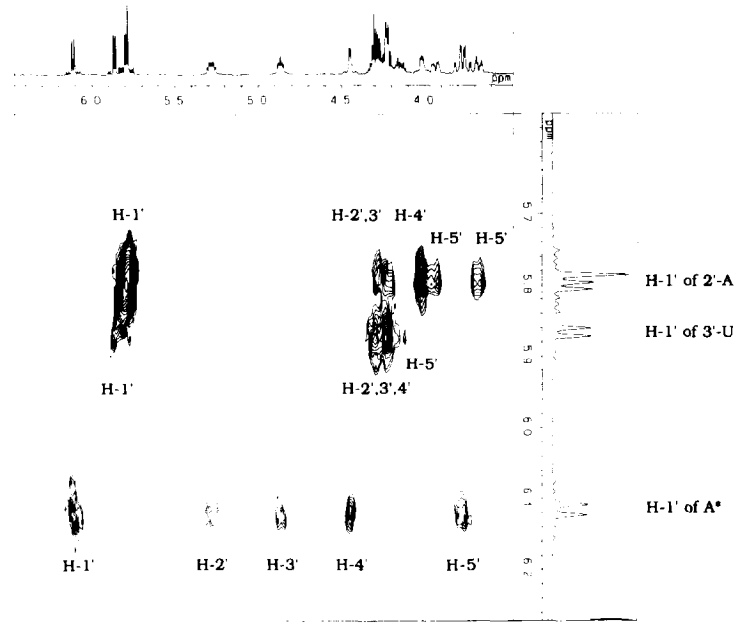


Fig. 3. The HOHAHA spectrum of **3** in D_2O at 35 °C. A* = branch-point adenosine.

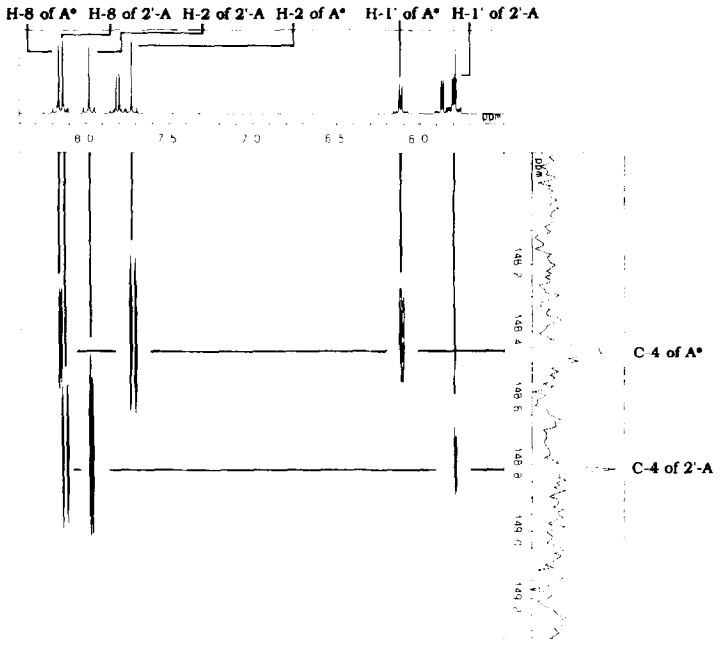


Fig. 4. The HMBC spectrum of **3** in D_2O at 35 °C. A* = branch-point adenosine.

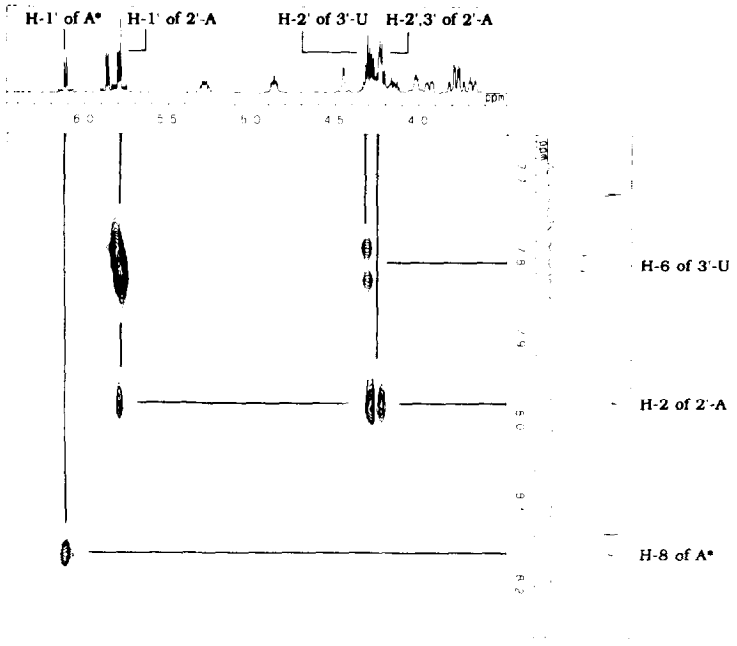


Fig. 5. The ROESY spectrum of **3** in D_2O at 35 °C. A* = branch-point adenosine.

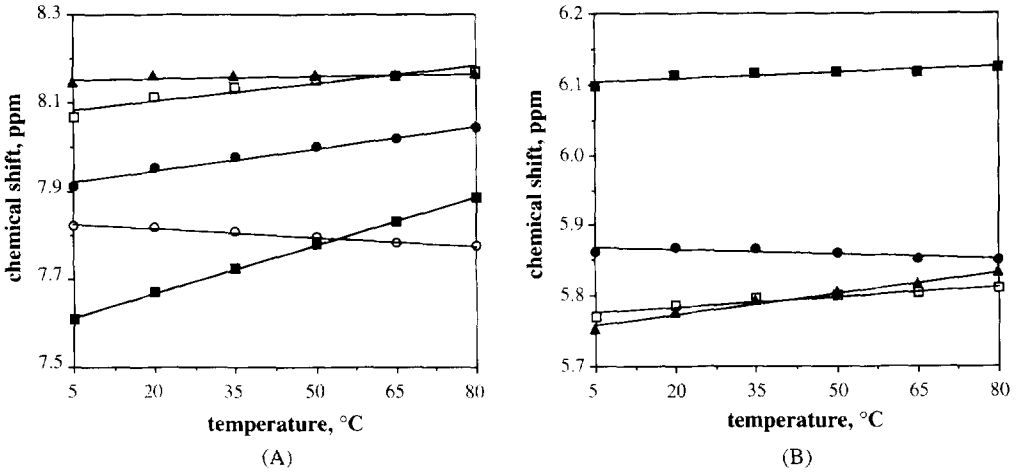


Fig. 6. Temperature-dependent variation of ^{1}H chemical shifts of aromatic and anomeric protons of **3** in D_2O. (A) The aromatic region. ▲ H-8 of A*; □ H-8 of 2'-A; ● H-2 of 2'-A; ○ H-6 of 3'-U; ■ H-2 of A*. (B) The anomeric region. ■ H-1' of A*; ● H-1' of 3'U; □ H-5 of 3'-U; ▲ H-1' of 2'-A. A* = branch-point adenosine.

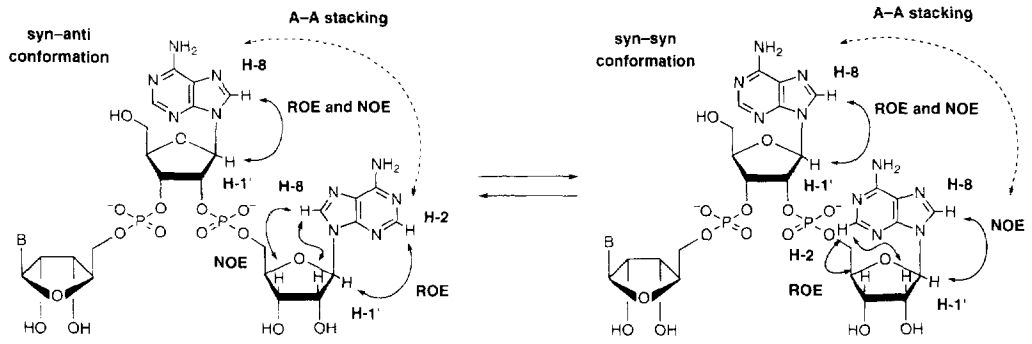


Fig. 7. Conformation of A(2'p5'A)3'p5'X **1**–**3**.

adenylyl(2'–5')guanosine linkage,[18,19] but also an unknown syn–syn stacking (Fig. 7). The analysis of **3** is used as an example. The nucleotide showed a strong ROE between H-8 and H-1' in the branch-point adenosine in the ROESY spectrum (Fig. 5). In the 1D NOE NMR examination, the NOE was detected only between H-8 and H-1'. These findings indicated that the adenyl base in the branch part has a syn conformation. While, in the 2'-linked adenosine moiety, ROEs were observed between H-2 and H-1', H-2 and H-2', and H-2 and H-3', and NOEs between H-8 and H-1' and H-8 and H-2', suggesting that the adenyl residue takes both syn and anti conformations. Thus, we concluded that 2' and 5'-adenyl residues in **3** make both syn–syn and syn–anti base stacks. Similar phenomena were observed in compounds **1** and **2**.

SUMMARY

We have realized a simple, regio-controlled synthesis of the adenyldiyl trimers with unsymmetrical 2'- and 3'-internucleotide linkages using a nucleoside 5'-phosphoramidite as a common building block for the construction of both 2'–5' and 3'–5' internucleotide linkages. Easy isolation of the target nucleotides without requiring time-consuming chromatography makes large-scale synthesis possible. The resulting branch-type trimers have a new type of syn–syn base stack between two adenyl residues in the derivatives containing adenylyl(2'–5')-adenosine linkage.

EXPERIMENTAL

IR spectra were measured with a JASCO IR-810 or JASCO-700 spectrometer. UV spectra were taken on a Hitachi 228 UV–visible light spectrometer. ^{1}H and ^{31}P NMR spectra in a stated solvent were obtained on a JEOL EX-270 or α-400 instrument. The chemical shifts were reported as δ values in ppm relative to $(CH_3)_4Si$ and 85% H_3PO_4 standard for ^{1}H and ^{31}P NMR spectra, respectively. ^{1}H NMR spectra in D_2O were recorded relative to CH_3CN. Elemental analysis was performed by the Faculty of Agriculture, Nagoya University. High-performance liquid chromatography (HPLC) using Develosil (Nomura, ODS-5 mm) was achieved on a JASCO 880-PU with a JASCO 870-UV detector. E. Merck Kieselgel 60 (70–230 mesh) deactivated by adding 6% of water or Nacalai Tesque silica gel 60 (230–400 mesh) was used for column chromatography. Unless otherwise stated, the reactions were carried out at ambient temperature. Enzymatic reactions were conducted in a 1.5-mL Eppendorf tube. 5'-*O*-Methoxytritylated nucleosides, **6**, **9**, and **11**,[20,21] 2',3',5'-tris-*O*-(*tert*-butyldimethylsilyl)guanosine,[22] diethylammonium hydrogencarbonate,[15] (allyloxy)-bis(diisopropylamino)phosphine,[9] and AOC-OBT[8] were prepared by literature methods. Tetrakis(triphenylphosphine)palladium (Aldrich), SVP (Worthington biochemical corporation), RNase T_2 (SIGMA), and spleen phosphodiesterase (BOEHRINGER MANNHEIM) were commercially supplied.

N^6-[(Allyloxy)carbonyl]-3'-O-(tert-*butyldimethylsilyl)-5'*-O-(p-*methoxy-trityl)adenosine (8).*

A mixture of 3'-*O*-(*tert*-butyldimethylsilyl)-5'-*O*-(*p*-methoxytrityl)-adenosine (**7**)[7] (2.30 g, 3.52 mmol), *N*-trimethylsilylimidazole (1.0 mL, 6.6 mmol), and 1*H*-tetrazole (42.4 mg, 0.61 mmol) in THF (20 mL) was stirred for 10 min. The reaction mixture was diluted with ethyl acetate (100 mL) and washed with water (100 mL) followed by brine (100 mL). The organic solution was concentrated to afford a colorless amorphous solid, which was dissolved in THF (30 mL). To the solution were added a 0.48 M solution of *tert*-butylmagnesium chloride (7.68 mmol) in THF (16 mL). After 10 min, a solution of AOC-OBT (1.23 g, 5.6 mmol) in THF (20 mL) was added over 15 min and stirring was continued for additional 15 min. The reaction mixture was poured into an aqueous ammonium chloride-saturated solution (100 mL) and extracted with ethyl acetate (100 mL). The organic extract was washed with brine (250 mL) and concentrated to give pale yellow solid. This material was treated with a 1.0 M solution of citric acid in methanol (100 mL) for 45 min. The reaction mixture was poured into ethyl acetate (250 mL) and washed with water (250 mL x 2). The aqueous layer was extracted with ethyl acetate (200 mL). The combined organic extracts were washed with brine (250 mL) and concentrated to give an oil. Column chromatography on silica gel (35 g) of the crude material with a 1:1 mixture of ethyl acetate and hexane as an eluent afforded **8** (2.23 g, 86%) as a foam: IR ($CHCl_3$) 3400, 1760, 1620, 1510 cm^{-1}; UV (CH_3OH) λmax 267 nm (ε 19,600); ^{1}H NMR ($CDCl_3$) δ 0.01 and 0.09 [two sets of s's, 6H, $(CH_3)_2Si$], 0.89 (s, 9H, *t*-C_4H_9Si), 3.21 (br d, 1H, J = 6.4 Hz, 2'-OH), 3.26 (dd, 1H, J = 10.9 and 3.6 Hz, H-5'), 3.52 (dd,

1H, J = 10.9 and 3.6 Hz, H-5'), 3.78 (s, 3H, CH_3O), 4.19 (dd, 1H, J = 7.3 and 3.6 Hz, H-4'), 4.59 (dd, 1H, J = 5.5 and 3.6 Hz, H-3'), 4.74–4.78 (m, 3H, CH_2=CHC*H*$_2$ and H-2'), 5.29 (ddd, 1H, J = 10.9, 2.7 and 1.3 Hz, *cis*-C*H*$_2$=CHCH$_2$), 5.42 (ddd, 1H, J = 16.4, 3.6 and 1.3 Hz, *trans*-C*H*$_2$=CHCH$_2$), 5.96–6.04 (m, 2H, CH_2=C*H*CH$_2$ and H-1'), 6.80 (d, 2H, J = 10.0 Hz, protons ortho to CH_3O of MMTr), 7.18–7.39 (m, 12H, aromatic protons of MMTr), 8.19 (s, 1H, H-2), 8.23 (br s, 1H, NHCO), 8.73 (s, 1H, H-8). Anal. Calcd for $C_{40}H_{47}N_5O_7Si$: C, 65.09; H, 6.43; N, 9.49. Found: C, 65.07; H, 6.49; N, 9.44.

N^2-*[(Allyloxy)carbonyl]-5'*-O-(p-*methoxytrityl)guanosine* (**10**).

To a solution of 2',3',5'-tris-*O*-(*tert*-butyldimethylsilyl)guanosine (1.00 g, 1.60 mmol) in a 5:1 mixture of THF and HMPA (12 mL) was added a 1.26 M solution of *tert*-butylmagnesium chloride in THF (3.90 mL, 4.9 mmol). After 15 min, a solution of AOC-Cl (0.26 mL, 2.45 mmol) in THF (4 mL) was added over 15 min and the mixture was stirred for 30 min. The reaction was quenched with methanol (5 mL) and the mixture was concentrated. The resulting residual oil was dissolved in ether (100 mL) and the solution was washed with an aqueous ammonium chloride (100 mL), a sodium hydrogencarbonate solution (100 mL), and brine (50 mL). Concentration of the organic solution gave a viscous oil, which was subjected to silica gel (50 g) column chromatography with a 1:2 mixture of ethyl acetate and hexane as an eluent to afford colorless foam (646 mg). This material was dissolved in THF (4 mL) and to the solution was added a 1.00 M solution of tetrabutylammonium fluoride in THF (4.6 mL, 4.6 mmol). The mixture was stirred for 15 min and the reaction was quenched by the addition of a 3:1:1 mixture of pyridine, methanol, and water (6.5 mL), and Dowex 50W x 8 pyridinium form (8 mL). The resulting suspension was stirred overnight. The resin was removed by filtration and the filtrate was concentrated to give a residual oil. After dryness by azeotropical evaporation with pyridine, the residue was dissolved in pyridine (6.5 mL). *p*-Methoxytrityl chloride (337 mg, 1.09 mmol) was added to the solution, which was stirred in the dark overnight. The mixture was concentrated and the residue was dissolved in dichloromethane (15 mL). The organic solution was washed with a saturated sodium hydrogencarbonate solution (15 mL x 2) and brine (10 mL). Evaporation of the organic solution gave a viscous oil, which was subjected to silica gel (15 g) column chromatography. Elution with a 3:1 mixture of ethyl acetate and hexane followed by a 10:1 mixture of dichloromethane and methanol gave **10** (367 mg, 36% overall) as a colorless foam: IR (KBr) 3239, 1688, 1611, 1566, 1510 cm^{-1}; UV (CH_3OH) λmax 260 (sh, ε 20,500), 251 (20,700) and 235 nm (22,200); ^1H NMR ($CDCl_3$) δ 3.30–3.45 (m, 2H, H_2-5'), 3.73 (s, 3H, CH_3O), 4.22 (d, 1H, J = 5.0 Hz, H-4'), 4.45 (t, 1H, J = 5.0 Hz, H-3'), 4.66–4.74 (m, 3H, CH_2=CHC*H*$_2$ and H-2'), 5.28 (d, 1H, J = 10.6 Hz, *cis*-C*H*$_2$=CHCH$_2$), 5.39 (d, 1H, J = 17.2 Hz, *trans*-C*H*$_2$=CHCH$_2$), 5.91–6.05 (m, 2H, CH_2=C*H*CH$_2$ and H-1'), 6.78 (d, 2H, J = 8.9 Hz, protons ortho to CH_3O of MMTr), 7.12–7.43 (m, 12H, aromatic protons of MMTr), 8.00 (s, 1H, H-8). Anal. Calcd for $C_{34}H_{33}N_5O_8$: C, 63.83; H, 5.21; N, 10.95. Found: C, 63.79; H, 5.21; N, 11.00.

A General Procedure for the Synthesis of 5'-O-Unprotected Ribonucleosides **12**–**15**. *Preparation of* N^6,*2',3'-O-Tri[(allyloxy)carbonyl]adenosine* (**12**).

To a stirred solution of 1*H*-tetrazole (967 mg, 13.8 mmol) and triethylamine (1.95 mL, 14.0 mmol) in THF (100 mL) was added a solution of AOC-Cl (1.50 mL, 14.14 mmol) in THF (50 mL) over 20 min at 0 °C and stirring was continued for 1.5 h. The resulting precipitates were removed by filtration and the filtrate was evaporated to give an oil. A solution of the residue in THF (30 mL) was added to a solution of **6** (1.57 g, 2.91

mmol) in DMF (10 mL). The resulting mixture was refluxed for 12 h and, after cooling to room temperature, poured into a 1:1 mixture of ethyl acetate and hexane (200 mL). The solution was washed with water (200 mL x 2), a sodium hydrogencarbonate solution (200 mL), and brine (100 mL). The organic layer was concentrated and the resulting residual material was chromatographed on a silica gel column (35 g). Elution with a 1:1 mixture of ethyl acetate and hexane afforded a pale yellow foam (2.21 g). To a solution of this product in dichloromethane (35 mL) was added dichloroacetic acid (5.00 mL, 60.7 mmol) and the mixture was stirred for 5 min. The reaction mixture was diluted with dichloromethane (300 mL) and washed with water (300 mL), a solution of sodium hydrogencarbonate (300 mL), and brine (300 mL). The organic solution was concentrated to give an oil. Silica gel (50 g) column chromatography of the product with a 3:1 mixture of ethyl acetate and hexane as an eluent afforded **12** (1.29 g, 85% overall) as colorless crystals, mp 60.0–61.5 °C: IR (KBr) 3400, 1760, 1620, 1590 cm^{-1}; UV (CH_3OH) λmax 266 nm (ε 21,200); ^1H NMR ($CDCl_3$) δ 3.86 (t, 1H, *J* = 12.8 Hz, H-5'), 4.00 (d, 1H, *J* = 12.8 Hz, H-5'), 4.47 (br s, 1H, H-4'), 4.49–4.78 (m, 6H, CH_2=CHC*H*$_2$ x 3), 5.23–5.43 (m, 6H, C*H*$_2$=CHCH_2 x 3), 5.62 (d, 1H, *J* = 5.2 Hz, H-3'), 5.80–6.03 (m, 4H, CH_2=C*H*CH_2 x 3 and H-2'), 6.07–6.11 (m, 2H, H-1' and 5'-OH), 7.99 (s, 1H, H-2), 8.46 (br s, 1H, NHCO), 8.75 (s, 1H, H-8). Anal. Calcd for $C_{22}H_{25}N_5O_{10}$: C, 50.86; H, 4.86; N, 13.48. Found: C, 50.67; H, 5.04; N, 13.29.

N^4,2',3'-O-*Tri[(allyloxy)carbonyl]cytidine* (**13**).

According to a similar procedure, **9** (1.35 g) was converted to **13** (1.14 g, 87%), mp. 110.0–111.5 °C: IR (KBr) 3314, 1756, 1651, 1558, 1502 cm^{-1}; UV (CH_3OH) λmax 292 (ε 8,030) and 240 nm (20,300); ^1H NMR ($CDCl_3$) δ 3.42–3.48 (br m, 1H, 5'-OH), 3.80–3.87 (br m, 1H, H-5'), 4.02 (br d, 1H, *J* = 12.8 Hz, H-5'), 4.36 (t, 1H, *J* = 2.5 Hz, H-4'), 4.57–4.71 (m, 6H, CH_2=CHC*H*$_2$ x 3), 5.25–5.41 (m, 6H, C*H*$_2$=CHCH_2 x 3), 5.68 (t, 1H, *J* = 4.9 Hz, H-3'), 5.74 (t, 1H, *J* = 4.9 Hz, H-2'), 5.86–5.98 (m, 3H, CH_2=C*H*CH_2 x 3), 6.15 (d, 1H, *J* = 4.9 Hz, H-1'), 7.28 (d, 1H, *J* = 7.3 Hz, H-5), 7.69 (br s, 1H, NHCO), 7.90 (d, 1H, *J* = 7.3 Hz, H-6). Anal. Calcd for $C_{21}H_{25}N_3O_{11}$: C, 50.90; H, 5.10; N, 8.48. Found: C, 50.06; H, 5.09; N, 8.55.

N^2,2',3'-O-*Tri[(allyloxy)carbonyl]guanosine* (**14**).

The nucleoside **10** (2.84 g) was converted to **14** (1.82 g, 77%), a colorless foam: IR (KBr) 3240, 1757, 1695, 1649, 1612, 1570, 1538 cm^{-1}; UV (CH_3OH) λmax 256 nm (ε 15,100); ^1H NMR ($CDCl_3$) δ 3.83 (dd, 1H, *J* = 12.2 and 2.3 Hz, H-5'), 4.01 (dd, 1H, *J* = 12.2 and 3.0 Hz, H-5'), 4.40 (d, 1H, *J* = 2.0 Hz, H-4'), 4.52–4.79 (m, 6H, CH_2=CHC*H*$_2$ x 3), 5.05 (br s, 1H, 5'-OH), 5.22–5.46 (m, 6H, C*H*$_2$=CHCH_2 x 3), 5.58 (dd, 1H, *J* = 5.0 and 2.0 Hz, H-3'), 5.77–6.19 (m, 5H, CH_2=C*H*CH_2 x 3, H-1', and H-2'), 7.27 (br s, 1H, NHCO), 7.76 (s, 1H, H-8), 11.5 (br s, 1H, NH). Anal. Calcd for $C_{22}H_{25}N_5O_{11}$: C, 49.34; H, 4.72; N, 13.08. Found: C, 49.18; H, 4.57; N, 13.24.

2',3'-O-*Di[(allyloxy)carbonyl]uridine* (**15**).

The nucleoside **15**, a colorless foam, was prepared in 85% yield from **11**. IR (KBr) 3452, 1757, 1695 cm^{-1}; UV (CH_3OH) λmax 259 nm (ε 10,800); ^1H NMR δ 3.24 (br s, 1H, 5'-OH), 3.85 (d, 1H, *J* = 12.2 Hz, H-5'), 3.95 (d, 1H, *J* = 12.2 Hz, H-5'), 4.33 (d, 1H, *J* = 3.7 Hz, H-4'), 4.57–4.69 (m, 4H, CH_2=CHC*H*$_2$ x 2), 5.25–5.40 (m, 4H, C*H*$_2$=CHCH_2 x 2), 5.44 (dd, 1H, *J* = 3.8 and 3.7 Hz, H-3'), 5.53 (t, 1H, *J* = 5.8 Hz, H-2'), 5.77 (d, 1H, *J* = 8.2 Hz, H-5), 5.85–5.97 (m, 3H, CH_2=C*H*CH_2 x 2 and H-1'), 7.62 (d, 1H, *J* = 8.2

Hz, H-6), 9.06 (br s, 1H, NHCO). Anal. Calcd for $C_{17}H_{20}N_2O_{10}$: C, 49.51; H, 4.90; N, 6.79. Found: C, 49.51; H, 5.14; N, 6.72.

*Synthesis of the Ribonucleoside 5'-O-Phosphoramidites (**16–19**). A Typical Procedure: Preparation of* N^6*,2',3'-O-Tri[(allyloxy)carbonyl]-adenosine 5'-(Allyl* N,N*-Diisopropylphosphoramidite) (**16**).*

A mixture of **12** (1.01 g, 1.94 mmol), diisopropylamine (0.15 mL, 108 mg, 1.07 mmol), and 1*H*-tetrazole (78.4 mg, 1.12 mmol) in acetonitrile (5.0 mL) was stirred for 10 min. To the mixture was added (allyloxy)bis(diisopropylamino)phosphine (1.07 g, 3.71 mmol) and stirring was continued for 2.5 h. The reaction mixture was diluted with dichloromethane (150 mL) and washed with a solution of sodium hydrogencarbonate (150 mL). The aqueous layer was extracted with dichloromethane (150 mL). The combined organic layers were washed with brine (150 mL) and concentrated. The residue was subjected to silica gel (40 g) column chromatography with a 1:1 mixture of ethyl acetate and hexane as an eluent to afford a diastereomeric mixture of **16** (1.34 g, 98%) as a viscous oil: IR ($CHCl_3$) 1760, 1614, 1591 cm^{-1}; UV (CH_3OH) λmax 267 nm (ε 22,700); ^1H NMR ($CDCl_3$) δ 1.17–1.27 [m, 12H, (CH_3)$_2$CH x 2], 3.53–3.67 [m, 2H, (CH_3)$_2$C*H* x 2], 3.87–4.03 (m, 2H, H_2-5'), 4.11–4.30 (m, 2H, CH_2=CHCH_2OP), 4.49–4.78 (m, 7H, CH_2=CHCH_2 x 3 and H-4'), 5.13–5.43 (m, 8H, CH_2=CHCH_2 x 4), 5.53 and 5.56 (two sets of dd's, 1H, J = 5.2 and 2.5 Hz and J = 7.6 and 2.8 Hz, H-3'), 5.74–5.87 (m, 1H, H-2'), 5.91–6.03 (m, 4H, CH_2=C*H*CH_2 x 4), 6.41 and 6.44 (two sets of d's, 1H, J = 6.7 Hz and J = 6.7 Hz, H-1'), 8.29 (br s, 1H, NHCO), 8.45 and 8.46 (two sets of s's, 1H, H-2'), 8.76 and 8.76 (two sets of s's, 1H, H-8); ^{31}P NMR ($CDCl_3$) δ 149.3 and 149.4. Anal. Calcd for $C_{31}H_{43}N_6O_{11}P$: C, 52.68; H, 6.14; N, 11.89. Found: C, 52.65; H, 6.30; N, 11.73.

N^4*,2',3'-O-Tri[(allyloxy)carbonyl]cytidine 5'-(Allyl* N,N*-Diisopropylphosphoramidite) (**17**).*

The nucleoside **13** (892 mg) was converted to the amidite **17** (1.13 g, 92%), a colorless viscous oil: IR ($CHCl_3$) 1757, 1671, 1631, 1553 cm^{-1}; UV (CH_3OH) lmax 293 (e 6,400) and 242 nm (13,900); ^1H NMR d 1.09–1.21 [m, 12H, (CH_3)$_2$CH x 2], 3.50–3.62 [m, 2H, (CH_3)$_2$C*H* x 2], 3.72–4.20 (m, 2H, H_2-5'), 4.34–4.64 (m, 9H, CH_2=CHCH_2 x 4 and H-4'), 5.03–5.34 (m, 10H, CH_2=CHCH_2 x 4, H-2', and H-3'), 5.78–5.95 (m, 4H, CH_2=C*H*CH_2 x 4), 6.22—6.27 (m, 1H, H-1'), 7.17 (br s, 1H, H-5), 8.25 (br s, 1H, H-6); ^{31}P NMR ($CDCl_3$) d 149.2 and 149.3. Anal. Calcd for $C_{30}H_{43}N_4O_{12}P$: C, 52.77; H, 6.36; N, 8.21. Found: C, 52.74; H, 6.58; N, 8.19.

N^2*,2',3'-O-Tri[(allyloxy)carbonyl]guanosine 5'-(Allyl* N,N*-Diisopropylphosphoramidite) (**18**).*

The amidite **18** (528 mg, 78%), a colorless foam, was prepared from the nucleoside **14** (501 mg): IR (KBr) 1763, 1695, 1615, 1568 cm^{-1}; UV (CH_3OH) λmax 258 nm (ε 11,600); ^1H NMR ($CDCl_3$) δ 1.12–1.26 [m, 12H, (CH_3)$_2$CH x 2], 3.53–3.74 [m, 2H, (CH_3)$_2$C*H* x 2], 3.75–4.03 (m, 2H, H_2-5'), 4.16–4.35 (m, 2H, CH_2=CHCH_2OP), 4.41–4.78 (m, 7H, CH_2=CHCH_2 x 3 and H-4'), 5.10–5.46 (m, 8H, CH_2=CHCH_2 x 4), 5.57–5.64 (m, 1H, H-3'), 5.78–6.07 (m, 6H, CH_2=C*H*CH_2 x 4, H-1', and H-2'), 7.92 and 7.99 (two sets of s's, 1H, H-8); ^{31}P NMR ($CDCl_3$) δ 148.2 and 149.6. Anal. Calcd for $C_{31}H_{43}N_6O_{12}P$: C, 51.51; H, 6.01; N, 11.61. Found: C, 51.47; H, 5.97; N, 11.51.

2',3'-O-Di[(allyloxy)carbonyl]uridine 5'-(Allyl N,N*-Diisopropylphosphoramidite) (**19**).*

The nucleoside **15** (614 mg) was converted to the amidite **19** (862 mg, 97%), a colorless viscous oil: IR ($CHCl_3$) 1757, 1695 cm^{-1}; UV (CH_3OH) λmax 259 nm (ε 8,260); ^1H NMR ($CDCl_3$) δ 1.18–1.27 [m, 12H, $(CH_3)_2CH$ x 2], 3.56–3.68 [m, 2H, $(CH_3)_2CH$ x 2], 3.79–4.02 (m, 2H, H_2-5'), 4.09–4.29 (m, 2H, CH_2=CHCH_2OP), 4.38 (d, 1H, J = 2.1 Hz, H-4'), 4.55–4.70 (m, 4H, CH_2=CHCH_2 x 2), 5.10–5.43 (m, 8H, CH_2=CHCH_2 x 3, H-2' and H-3'), 5.73 and 5.73 (two sets of d's, 1H, J = 8.2 Hz and J = 7.9 Hz, H-5), 5.83–6.00 (m, 3H, CH_2=CHCH_2 x 3), 6.29–6.34 (m, 1H, H-1'), 7.92 and 7.93 (two sets of d's, 1H, J = 8.2 Hz and J = 7.9 Hz, H-6); ^{31}P NMR ($CDCl_3$) δ 149.6 and 149.7. Anal. Calcd for $C_{26}H_{38}N_3O_{11}P$: C, 52.07; H, 6.40; N, 7.01. Found: C, 52.02; H, 6.57; N, 6.86.

Preparation of the Protected A2'p5'A ***20***.

A homogeneous mixture of **8** (929 mg, 1.26 mmol) and 1*H*-tetrazole (960 mg, 7.18 mmol) in acetonitrile (20 mL) was stirred for 5 min. To the mixture were successively added a solution of **16** (1.50 g, 2.13 mmol) in acetonitrile (20 mL) and, after 10 min, a 1.85 M solution of TBHP (5.25 mmol) in toluene (5 mL), and stirring was continued for an additional 1 h. The reaction mixture was poured into a solution of sodium hydrogencarbonate (100 mL) and extracted with dichloromethane (50 mL x 2). The combined organic extracts were washed with brine (50 mL) and concentrated. The crude material was purified by chromatography on silica gel (90 g) column eluted with a 3:1 mixture of ethyl acetate and hexane to afford a diastereomeric mixture of **20** (1.46 g, 86%) as a pale yellow foam: IR (KBr) 1759, 1612, 1588, 1510 cm^{-1}; UV (CH_3OH) λmax 267 (ε 35,200) and 237 nm (22,800); ^1H NMR ($CDCl_3$) δ 0.02, 0.06, 0.09 and 0.14 [four sets of s's, 6H, $(CH_3)_2Si$], 0.83 and 0.89 (two sets of s's, 9H, *t*-C_4H_9Si), 3.26–3.35 (m, 1H, H-5'), 3.52–3.59 (m, 1H, H-5'), 3.76 and 3.77 (two sets of s's, 3H, CH_3O), 4.19–4.38 (m, 4H, H-4' x 2 and H_2-5'), 4.53–4.80 (m, 10H, CH_2=CHCH_2 x 5), 5.02–5.45 (m, 11H, CH_2=CHCH_2 x 5 and H-3'), 5.53–6.06 (m, 8H, CH_2=CHCH_2 x 5, H-2' x 2, and H-3'), 6.19–6.13 (m, 2H, H-1' x 2), 6.77–6.82 (m, 2H, protons ortho to CH_3O of MMTr), 7.19–7.42 (m, 12H, aromatic protons of MMTr), 8.23 and 8.27 (two sets of s's, 1H, H-2), 8.68 and 8.74 (two sets of s's, 1H, H-2), 8.74 (s, 2H, H-8 x 2), 9.13, 9.20 and 9.28 (three sets of br s's, 2H, NHCO x 2); ^{31}P NMR ($CDCl_3$) δ –0.77 and 0.40.

Preparation of the Protected A2'p5'G ***21***.

In a way similar to that described for the preparation of **20**, a diastereomeric mixture of **21** (523 mg, 88%), a colorless foam, was prepared from **8** (320 mg) and **18** (471 mg): IR (KBr) 1763, 1709, 1613, 1510 cm^{-1}; UV (CH_3OH) λmax 266 (sh, ε 21,200), 258 (24,400), and 238 nm (sh, 21,200); ^1H NMR ($CDCl_3$) δ 0.12, 0.17, 0.22 and 0.25 [four sets of s's, 6H, $(CH_3)_2Si$], 0.91 and 0.99 (two sets of s's, 9H, *t*-C_4H_9Si), 3.17 and 3.43 (two sets of d's, 1H, J = 6.9 Hz and J = 6.6 Hz, H-5' of Ad), 3.57 and 3.68 (two sets of d's, 1H, J = 8.6 Hz and J = 6.9 Hz, H-5' of Ad), 3.86 and 3.89 (two s sets of s's, 3H, CH_3O), 4.20–4.88 (m, 15H, CH_2=CHCH_2 x 5, H-3' of Ad, H-4' x 2, and H-5' of Gu x 2), 5.02–5.78 (m, 12H, CH_2=CHCH_2 x 5, H-2' of Ad, and H-3' of Gu), 5.84–6.17 (m, 7H, CH_2=CHCH_2 x 5, H-1', and H-2' of Gu), 6.32 and 6.43 (two sets of d's, 1H, J = 5.3 Hz and J = 2.6 Hz, H-1'), 6.83 and 6.93 (two sets of d's, 2H, J = 8.9 Hz and J = 8.6 Hz, protons ortho to CH_3O of MMTr), 7.18–7.57 (m, 12H, aromatic protons of MMTr), 7.72 and 7.75 (two sets of s's, 1H, H-8 of Gu), 7.97 and 8.37 (two sets of s's, 1H, H-2 of Ad), 8.77 and 8.78 (two sets of s's, 1H, H-8 of Ad); ^{31}P NMR ($CDCl_3$) δ –2.05 and –1.95.

*Conversion of **20** to the Dinucleoside Phosphodiester **22**.*

A solution of **20** (205 mg, 0.15 mmol) in THF (5 mL) was mixed with a solution of sodium iodide (474 mg, 3.17 mmol) in acetone (3 mL) and refluxed for 30 min. The reaction mixture was cooled to room temperature and concentrated. The residue was treated with dichloromethane (20 mL) and the insoluble material was removed by filtration. The filtrate was washed with a 0.5 M solution of sodium thiosulfate (20 mL x 2) and brine (20 mL). Evaporation of the organic layer gave sodium salt of **22** (179 mg, 86%) as a pale yellow foam: IR (KBr) 3408, 1760, 1613, 1510 cm^{-1}; UV (CH_3OH) λmax 267 (ε 33,400) and 236 nm (22,900); ^1H NMR (DMSO-d_6) δ 0.05 and 0.10 [two sets of s's, 6H, $(CH_3)_2Si$], 0.79 (s, 9H, *t*-C_4H_9Si), 3.03–3.10 (m, 1H, H-5'), 3.71 (s, 3H, CH_3O), 3.76–4.11 (m, 3H, H-5' x 3), 4.30–4.73 (m, 10H, CH_2=CHCH_2 x 4 and H-4' x 2), 4.93 (br s, 1H, H-3'), 5.14–5.51 (m, 10 H, CH_2=CHCH_2 x 4, H-2', and H-3'), 5.72–6.09 (m, 5H, CH_2=C*H*CH_2 x 4 and H-2'), 6.25–6.40 (m, 2H, H-1' x 2), 6.77 (d, 2H, J = 8.6 Hz, protons ortho to CH_3O of MMTr), 7.12–7.29 (m, 12H, aromatic protons of MMTr), 8.54 (s, 1H, H-2), 8.60 (br s, 2H, H-2 and H-8), 8.93 (s, 1H, H-8); ^{31}P NMR (DMSO-d_6) δ –0.03.

*Preparation of the Dinucleoside Phosphodiester **23**.*

According to a way similar to that described above, **21** (400 mg, 0.29 mmol) was converted to sodium salt of **23** (315 mg, 79%): IR (KBr) 1761, 1703, 1613, 1510 cm^{-1}; UV (CH_3OH) λmax 270 (sh, ε 27,500), 258 (28,800), and 238 nm (sh, 23,700); ^1H NMR ($CD_3OD/CDCl_3$) δ 0.16 and 0.23 [two sets of s's, 6H, $(CH_3)_2Si$], 0.89 (s, 9H, *t*-C_4H_9Si), 3.14–3.55 (m, 2H, H_2-5' of Ad), 3.82 (s, 3H, CH_3O), 4.14–4.89 (m, 12H, CH_2=CHCH_2 x 4, H-4' x 2, and H_2-5' of Gu), 5.16–5.56 (m, 10H, CH_2=CHCH_2 x 4 and H-3' x 2), 5.72 (br s, 1H, H-2' of Ad), 5.82–6.15 (m, 6H, CH_2=C*H*CH_2 x 4, H-1' of Gu, and H-2' of Gu), 6.48 (br s, 1H, H-1' of Ad), 6.78 (d, 2H, J = 8.6 Hz, protons ortho to CH_3O of MMTr), 7.13–7.30 (m, 12H, aromatic protons of MMTr), 8.08 (s, 1H, H-8 of Gu), 8.17 (s, 1H, H-2), 8.74 (s, 1H, H-8 of Ad); ^{31}P NMR ($CD_3OD/CDCl_3$) δ –4.44.

*Preparation of the Protected A(2'p5'A)3'p5'C **24**: A General Procedure for Synthesis of the Protected Branch-Type Trimers.*

A solution of **22** (2.76 g, 1.19 mmol) in THF (35 mL) was mixed with a 1.0 M solution of TBAF (3.5 mmol) in THF (3.5 mL) and stirred for 10 h. The reaction mixture was diluted with ethyl acetate (200 mL) and washed with water (200 mL x 2) and brine (100 mL). Concentration of the organic solution gave a residual oil, which was subjected to silica gel column chromatography with a 15:1 mixture of dichloromethane and methanol as an eluent to give a colorless foam (2.53 g). The product was dissolved together with 1*H*-tetrazole (2.47 g, 35.2 mmol) and DMAP (0.92g, 7.50 mmol) in acetonitrile (20 mL) and to this solution was added a solution of **17** (5.10 g, 7.47 mmol). The resulting mixture was stirred for 1.5 h and to this was added allyl alcohol (5.0 mL, 73 mmol). After 1 h, the reaction mixture was treated with a 1.53 M solution of TBHP (14.90 mmol) in dichloromethane (9.4 mL) and stirred for an additional 1 h. The mixture was diluted with ethyl acetate (250 mL) and washed with a sodium hydrogencarbonate solution (250 mL x 2) and brine (150 mL x 2). The organic layer was evaporated to afford an oily residue. Purification of the crude product on a silica gel column using a 5:1 mixture of ethyl acetate and hexane followed by a 20:1 mixture of dichloromethane and methanol as eluents afforded sodium salt of **24** (2.09 g, 54% overall) as a colorless foam: IR (KBr) 3404, 1759, 1618, 1508 cm^{-1}; UV (CH_3OH) λmax 267 (ε 30,300) and 250 nm (29,100); ^1H NMR ($CD_3OD/CDCl_3$) δ 3.34–3.67 (m, 4H,

H-5' x 4), 3.75 and 3.76 (two sets of s's, 3H, CH_3O), 4.24–4.79 (m, 21H, CH_2=CHC*H*$_2$ x 8, H-4' x 3, and H_2-5' of Cy), 5.15–5.52 (m, 19H, C*H*$_2$=CHCH_2 x 8 and H-3' x 3), 5.58–6.08 (m, 12H, CH_2=C*H*CH_2 x 8, H-1' and H-2' x 3), 6.26–6.37 (m, 2H, H-1' x 2), 6.77 (d, 2H, J = 8.2 Hz, protons ortho to CH_3O of MMTr), 7.18–7.39 (m, 13H, aromatic protons of MMTr and H-5 of Cy), 7.71 and 7.92 (two sets of br s's, 1H, H-6 of Cy), 8.31 (s, 1H, H-2 of Ad), 8.50 and 8.54 (two sets of s's, 1H, H-2 of Ad), 8.62 and 8.64 (two sets of s's, 1H, H-8 of Ad), 8.64 (s, 1H, H-8 of Ad); ^{31}P NMR ($CD_3OD/CDCl_3$) δ –2.96, –2.61, and –1.41.

Preparation of the Protected A(2'p5'A)3'p5'G ***25***.

The nucleotide **25** was prepared in 60% yield from **18** and **22**: IR (KBr) 1759, 1615, 1510 cm^{-1}; UV (CH_3OH) λmax 266 nm (ε 71,800); ^{1}H NMR ($CD_3OD/CDCl_3$) δ 3.36–3.49 (m, 2H, H_2-5' of Ad at the branched point), 3.76 (s, 3H, CH_3O), 4.09–4.75 (m, 23H, CH_2=CHC*H*$_2$ x 8, H-4' x 3, and H-5' x 4), 5.07–6.04 (m, 31H, C*H*$_2$=CHCH_2 x 8, CH_2=C*H*CH_2 x 8, H-1', H-2' x 3, and H-3' x 3), 6.28–6.40 (m, 2H, H-1' x 2), 6.76 (d, 2H, J = 7.6 Hz, protons ortho to CH_3O of MMTr), 7.19–7.41 (m, 12H, aromatic protons of MMTr), 7.91 and 8.04 (two sets of s's, 1H, H-8 of Gu), 8.34 (s, 1H, H-2 of Ad), 8.52 and 8.53 (two sets of s's, 1H, H-2 of Ad), 8.63 (s, 2H, H-8 of Ad x 2); ^{31}P NMR ($CD_3OD/CDCl_3$) δ –3.19, –2.66, and –1.34.

Preparation of the Protected A(2'p5'A)3'p5'U ***26***.

The protected trimer **26** was prepared in 71% overall yield from **19** and **22**: IR (KBr) 1759, 1616, 1510 cm^{-1}; UV (CH_3OH) λmax 266 (ε 31,700) and 240 nm (sh, 21,200); ^{1}H NMR ($CD_3OD/CDCl_3$) δ 3.38–3.58 (m, 2H, H-5' x 2 of Ad at the branched point), 3.76 and 3.78 (two sets of s's, 3H, CH_3O), 3.94–4.78 (m, 21H, CH_2=CHC*H*$_2$ x 7, H-4' x 3, and H-5' x 4), 5.16–5.57 (m, 17H, C*H*$_2$=CHCH_2 x 7 and H-3' x 3), 5.60–6.08 (m, 12H, CH_2=C*H*CH_2 x 7, H-1', H-2' x 3, and H-5 of Ur), 6.31–6.42 (m, 2H, H-1' x 2), 6.77 (d, 2H, J = 8.9 Hz, protons ortho to CH_3O of MMTr), 7.19–7.57 (m, 13H, aromatic protons of MMTr and H-6 of Ur), 8.32 (s, 1H, H-2 of Ad), 8.53 and 8.57 (two sets of s's, 1H, H-2 of Ad), 8.62 and 8.63 (two sets of s's, 1H, H-8 of Ad), 8.64 (s, 1H, H-8 of Ad); ^{31}P NMR ($CD_3OD/CDCl_3$) δ –2.66, –2.34, and –1.06.

Preparation of the Protected A(2'p5'G)3'p5'C ***27***.

The compound **27** was prepared in 55% overall yield from **17** and **23**: IR (KBr) 1759, 1701, 1615, 1508 cm^{-1}; UV (CH_3OH) λmax 249 nm (ε 46,200); ^{1}H NMR ($CD_3OD/CDCl_3$) δ 3.36–3.54 (m, 2H, H_2-5' of Ad at the branched point), 3.67 (s, 3H, CH_3O), 3.93–4.12 (br s, 3H, H-4' and H_2-5' of Cy), 4.28–4.81 (m, 20H, CH_2=CHC*H*$_2$ x 8, H-4' x 2, and H_2-5' of Gu), 5.16–5.55 (m, 19H, C*H*$_2$=CHCH_2 x 8, H-2', and H-3' x 2), 5.62–6.09 (m, 13H, CH_2=C*H*CH_2 x 8, H-1' x 2, H-2' x 2, and H-3' of Ad at the branched point), 6.24–6.30 (m, 1H, H-1'), 6.69–6.76 (m, 2H, protons ortho to CH_3O of MMTr), 7.02–7.35 (m, 13H, aromatic protons of MMTr and H-5 of Cy), 7.73 and 7.97 (two sets of d's, 1H, J = 6.8 Hz and J = 5.6 Hz, H-6 of Cy), 8.07 (s, 1H, H-8 of Gu), 8.19 and 8.21 (two sets of s's, 1H, H-2 of Ad), 8.58 (s, 1H, H-8 of Ad); ^{31}P NMR ($CD_3OD/CDCl_3$) δ –3.70 and –2.62.

Preparation of the Protected A(2'p5'G)3'p5'U ***28***.

The nucleotide **28** was prepared in 57% overall yield from **19** and **23**: IR (KBr) 1759, 1701, 1613, 1510 cm^{-1}; UV (CH_3OH) λmax 259 nm (ε 40,500); ^{1}H NMR ($CD_3OD/CDCl_3$) δ 3.34–3.53 (m, 2H, H_2-5' of Ad at the branched point), 3.74 and 3.75 (two sets of s's, 3H, CH_3O), 4.03–4.81 (m, 21H, CH_2=CHC*H*$_2$

x 7, H-4' x 3, and H-5' x 4), 5.17–6.08 (m, 30H, CH_2=CHCH$_2$ x 7, CH$_2$=C*H*CH$_2$ x 7, H-1' x 2, H-2' x 3, H-3' x 3, and H-5 of Ur), 6.27–6.31 (m, 1H, H-1'), 6.67–6.79 (m, 2H, protons ortho to CH$_3$O of MMTr), 7.56 and 8.01 (two sets of d's, 1H, J = 7.6 Hz and J = 1.9 Hz, H-6 of Ur), 8.11 (s, 1H, H-8 of Gu), 8.21 and 8.23 (two sets of s's, 1H, H-2 of Ad), 8.61 (s, 1H, H-8 of Ad); ^{31}P NMR (CD$_3$OD/CDCl$_3$) δ –3.90 and –2.69.

*Deprotection of the Protected Branch-Type Trimers **24–28**, Giving **1–5**. A General Procedure: Preparation of A(2'p5'A)3'p5'C (**1**).*

A mixture of **24** (2.09 g, 1.15 mmol) and dichloroacetic acid (2.0 mL, 3.13 g, 24 mmol) in dichloromethane (15 mL) was stirred for 5 min. The mixture was diluted with dichloromethane (200 mL) and washed with a sodium hydrogencarbonate solution (200 mL x 2) and brine (100 mL). The organic layer was concentrated and the resulting oil was subjected to chromatography on a silica gel column with a 5:1 mixture of ethyl acetate and hexane followed by a 7:1 mixture of dichloromethane and methanol as eluents to give a colorless foam (1.58 g). To a solution of this product in dichloromethane (30 mL) were added with vigorous stirring diethylammonium hydrogencarbonate (5.55 g, 41.1 mmol) followed by a solution of Pd[P(C$_6$H$_5$)$_3$]$_4$ (474.3 mg, 0.41 mmol) and P(C$_6$H$_5$)$_3$ (66.4 mg, 0.25 mmol) in dichloromethane (10 mL). Stirring was continued for 1 h. After dilution with dichloromethane (200 mL), the mixture was extracted with water (200 mL x 2). The aqueous extracts were concentrated to give a pale yellow solid, which was treated with 95% ethanol to afford **1** (993 mg, 32,300 OD$_{260}$, 74% overall yield) as a colorless powder: HPLC R_t 13.65 min (eluent: an 8:1.5:1 mixture of 0.1 M potassium dihydrogenphosphate–5 mM tetrabutylammonium bromide buffer/methanol/water). The ^{1}H and ^{31}P NMR signals are listed in Table 1.

*Preparation of A(2'p5'A)3'p5'G (**2**).*

The trimer **2** (23.6 mg, 780 OD$_{260}$, 73%) was obtained as a colorless powder from **25** (47.4 mg): HPLC R_t 13.08 min (eluent: an 8:2:1 mixture of 0.1 M potassium dihydrogenphosphate–5 mM tetrabutylammonium bromide buffer/methanol/water). The ^{1}H and ^{31}P NMR signals are listed in Table 1.

*Preparation of A(2'p5'A)3'p5'U (**3**).*

The trimer **3** (33.0 mg, 1,100 OD$_{260}$, 71%) was obtained as a colorless powder from **26** (66.0 mg): HPLC R_t 32.10 min (eluent: an 8:1.5:1 mixture of 0.1 M potassium dihydrogenphosphate–5 mM tetrabutylammonium bromide buffer/methanol/water). The ^{1}H and ^{31}P NMR signals are listed in Table 1.

*Preparation of A(2'p5'G)3'p5'C (**4**).*

The trimer **4** (40.7 mg, 1,185 OD$_{260}$, 78%) was obtained as a colorless powder from **27** (82.3 mg): HPLC R_t 7.54 min (eluent: an 8:2:1 mixture of 0.1 M potassium dihydrogenphosphate–5 mM tetrabutylammonium bromide buffer/methanol/water). Table 1 summarizes the ^{1}H and ^{31}P NMR signals.

*Preparation of A(2'p5'G)3'p5'U (**5**).*

The nucleotide **5** (39.0 mg, 1,077 OD$_{260}$, 66%) was obtained as a colorless powder from **28** (80.1 mg): HPLC R_t 10.35 min (eluent: an 8:2:1 mixture of 0.1 M potassium dihydrogenphosphate–5 mM tetrabutylammonium bromide buffer/methanol/water). The ^{1}H and ^{31}P NMR signals are shown in Table 1.

Digestion of the Branched Nucleotides A(2'p5'X)3'p5'Y 1–5 by Snake Venom Phosphodiestarase (SVP).

A solution of the branched nucleotide (1 OD_{260}) in water (0.4 mL) was incubated with a solution of SVP (1 unit) in water (2 mL) at 37 °C for 12 h. After heating at 100 °C for 3 min, the mixture was directly subjected to HPLC analysis. The HPLC indicated that, in all cases, the product is a mixture of adenosine and two kinds of nucleoside 5'-phosphates, pX and pY, in a reasonable ratio.

Treatment of the Branched Nucleotides 1–5 with RNase T_2.

A solution of the branched nucleotide (1 OD_{260}) in a 0.5 M ammonium acetate buffer (pH 4.5, 75 mL) was mixwd with a solution of RNase T_2 (1 unit) in water (1 mL) and the mixture was heated at 37 °C for 12 h and then at 100 °C for 3 min. HPLC analysis of the reaction mixture indicated that the branched nucleotide was not digested at all.

Reaction of the Branched Nucleotides 24–28 and Spleen Phosphodiesterase.

A solution of the branched nucleotide (1 OD_{260}) in a 0.5 M ammonium acetate buffer (pH 6.5, 75 mL) was mixed with a suspension of spleen phosphodiesterase (20 mg) in a 0.5 M ammonium sulfate buffer (10 mL) and left at 37 °C for 12 h and then at 100 °C for 3 min. HPLC analysis of the reaction mixture showed that no reaction took place in all cases.

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