lution of **52** (100 mg, 0.124 mmol) in 1 mL of CH_2Cl_2 at 0 °C was treated sequentially with EDCI (37 mg, 0.124 mmol) and pentafluorophenol (25 mg, 0.137 mmol, 1.1 equiv). The reaction mixture was warmed to 25 °C and was stirred for 24 h. The reaction mixture was diluted with CH_2Cl_2 (5 mL) and was washed with water (3 × 2 mL). The organic layer was dried ($MgSO_4$) and concentrated in vacuo. Short column chromatography (SiO_2, 2 × 15 cm, 7% MeOH–CH_2Cl_2 eluant) afforded **54** (91 mg, 120 mg theoretical yield, 76%) as a yellow oil: $[\alpha]^{22}_D$ −37.1° (*c* 1.1, MeOH); ^{1}H NMR ($CDCl_3$, 200 MHz, ppm) 7.4–6.7 (m, 12 H, Ar H), 3.80 (br s, 3 H, Tyr(OCH_3)), 1.45 (br s, 9 H, *t*-Boc CH_3), 1.34 (d, 3 H, *J* = 7 Hz, Ala $^\beta CH_3$), 1.26 (d, 3 H, *J* = 7 Hz, Ala $^\beta CH_3$); IR (neat) ν_{max} 3854, 3839, 3752, 3676, 3650, 3312, 2978, 2935, 2668, 1752, 1638, 1521, 1448, 1419, 1368, 1248, 1172, 1112, 1005, 855, 825, 788, 735 cm^{-1}.

A solution of **54** (91 mg, 0.094 mmol) in 1 mL of TFA/CH_2Cl_2 (1:1) at 25 °C was stirred for 2 h. The solvents were removed in vacuo to afford the crude trifluoroacetic acid salt of **55** as a hygroscopic, crystalline solid which was used directly in the following reaction.

A solution of the trifluoroacetic acid salt of **55** (92 mg, 0.094 mmol) in 1 mL of DMF was added dropwise over 2–3 h (using a motor driven syringe pump) to a warm (90 °C) solution of pyridine (313 mL). The resulting reaction mixture was stirred for an additional 5 h (90 °C). The solvent was removed in vacuo and the residue dissolved in 2 mL of EtOAc. The EtOAc solution was washed with water (3 × 1 mL), dried ($MgSO_4$), and concentrated in vacuo. Flash chromatography (SiO_2, 2 × 20 cm, 5–10% MeOH–CH_2Cl_2 eluant) afforded **11** (31 mg, 64 mg theoretical yield, 49%) as a clear yellow oil which solidified on standing.

***cyclo*-(D-Ala-Ala-*N*-Me-Tyr(OMe)-Ala) (12): Method A.** A solution of **13b** (35 mg, 0.067 mmol) in 1 mL of TFA/CH_2Cl_2 (1:1) at 25 °C was stirred 1.5 h. The solvents were removed in vacuo to afford the trifluoroacetic acid salt of **56** as an extremely hygroscopic, crystalline solid which was used directly in the following reaction. For **56**·CF_3CO_2H: $[\alpha]^{22}_D$ −21.6° (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$, 200 MHz, ppm) 7.05 (d, 2 H, *J* = 9 Hz, C2-H, and C6-H), 6.86 (m, 2 H, C3-H and C5-H), 3.80 (br s, 3 H, Tyr(OCH_3)), 1.6–1.2 (m, Ala $^\beta CH_3$); IR (neat) ν_{max} 3802, 3650, 3630, 2929, 1718, 1670, 1654, 1637, 1559, 1541, 1515, 1458, 1420, 1250, 1201, 1141, 1034, 799, 722 cm^{-1}.

A solution of the trifluoroacetic acid salt of **56** (56 mg, 0.067 mmol) in 0.4 mL of DMF was cooled to 0 °C and treated sequentially with $NaHCO_3$ (28 mg, 0.335 mmol, 5 equiv) and DPPA (19 μL, 0.087 mmol, 1.3 equiv). The reaction mixture was stirred for 72 h at 0 °C. The solvent was removed in vacuo and the residue was diluted with water (1 mL) and extracted with EtOAc (3 × 2 mL). The combined organic extracts were washed with water (2 × 2 mL) and saturated aqueous NaCl, dried ($MgSO_4$), and concentrated in vacuo. Flash chromatography (SiO_2, 2 × 15 cm, 7% MeOH–CH_2Cl_2 eluant) afforded **12** (18 mg, 27 mg theoretical yield, 68%) as a yellow oil which solidified on standing: mp 149–152 °C (MeOH–H_2O, light yellow needles); $[\alpha]^{22}_D$ −19.9° (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$, 200 MHz, ppm) 7.44 (d, 1 H, *J* = 8 Hz, NH), 7.18 and 7.12 (two s, 2 H, *J* = 9 Hz, C2-H and C6-H), 6.87 and 6.85 (two s, 2 H, *J* = 9 Hz, C3-H and C5-H), 6.40 (d, 1 H, *J* = 8 Hz, NH), 6.18 and 6.12 (two d, 1 H, *J* = 8 Hz, NH), 4.60 (m, 4 H, $^\alpha$CH), 3.80 (s, 3 H, Tyr(OCH_3)), 3.04 and 2.95 (two s, 3 H, NCH_3), 1.38 (d, 3 H, *J* = 7 Hz, Ala $^\beta CH_3$), 1.29 (d, 3 H, *J* = 7 Hz, Ala $^\beta CH_3$), 1.19 (d, *J* = 7 Hz, Ala $^\beta CH_3$); IR (KBr) ν_{max} 3754, 3290, 3062, 2984, 2936, 1655, 1514, 1492, 1452, 1406, 1378, 1301, 1249, 1208, 1179, 1108, 1033, 919, 824, 778, 735 cm^{-1}; CIMS (isobutane), *m/e* (relative intensity) 405 (M^+ + H, 1), 334 (base), 283 (42). Reverse-phase HPLC: 97.8%, t_R 12 min, 2.0 mL/min, 0–12% methanol–water gradient elution (0.5%/min).

***cyclo*-(D-Ala-Ala-*N*-Me-Tyr(OMe)-Ala) (12): Method B.** A solution of **13b** (54 mg, 0.104 mmol) in 1 mL of CH_2Cl_2 at 0 °C was treated sequentially with EDCI (31 mg, 0.104 mmol, 1.0 equiv) and pentafluorophenol (19 mg, 0.104 mmol, 1.0 equiv). The reaction mixture was allowed to warm to 25 °C and was stirred for 24 h. The reaction mixture was diluted with CH_2Cl_2 (3 mL), washed with water (3 × 2 mL), dried ($MgSO_4$), and concentrated in vacuo. Short column chromatography (SiO_2, 2 × 15 cm, 3% MeOH–CH_2Cl_2 eluant) afforded **57** (48 mg, 72 mg theoretical yield, 67%) as a yellow oil: $[\alpha]^{22}_D$ −22.9° (*c* 1.2, MeOH); ^{1}H NMR ($CDCl_3$, 200 MHz, ppm) 7.10 and 7.06 (two d, 2 H, *J* = 9 Hz, C2-H and C6-H), 6.86 and 6.80 (two d, 2 H, *J* = 9 Hz, C3-H and C5-H), 3.78 (s, 3 H, Tyr(OCH_3)), 3.00 and 2.86 (two s, 3 H, NCH_3), 1.68 and 1.63 (two d, 3 H, *J* = 7 Hz, Ala $^\beta CH_3$), 1.30 and 1.26 (two d, 3 H, *J* = 7 Hz, Ala $^\beta CH_3$), 0.42 (d, *J* = 7 Hz, Ala $^\beta CH_3$); IR (neat) ν_{max} 3286, 2937, 1793, 1685, 1654, 1636, 1519, 1457, 1368, 1256, 1167, 1100, 996 cm^{-1}.

A solution of **57** (48 mg, 0.069 mmol) in 2 mL of TFA/CH_2Cl_2 (1:1) at 25 °C was stirred for 1.2 h. The solvents were removed in vacuo to afford the trifluoroacetic acid salt of **58** as a hygroscopic, crystalline solid which was used directly in the following reaction.

A solution of the trifluoroacetic acid salt of **58** (49 mg, 0.069 mmol) in 5 mL of DMF was added dropwise over 8 h (using a motor driven syringe pump) to a warm (90 °C) solution of pyridine (230 mL). After the addition was complete the solvent was removed in vacuo and the residue was dissolved in 1 mL of EtOAc. The EtOAc solution was washed with water (3 × 1 mL), dried ($MgSO_4$), and concentrated in vacuo. Flash chromatography (SiO_2, 1 × 20 cm, 5% MeOH–CH_2Cl_2 eluant) afforded **12** (14 mg, 28 mg theoretical yield, 50%) as a yellow oil which solidified on standing.

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Synthesis of Various Branched Triribonucleoside Diphosphates by Site-Specific Modification of a Diphenylcarbamoyl-Protected Guanine Residue

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Three branched triribonucleotides, consisting of an adenosine linked at 3′ to a cytidine and at 2′ to a guanosine or to a 2-aminopurine ribonucleoside bearing on its 6-position a phenylthio or a dimethylamino group, have been synthesized from a common precursor. These compounds, which may prove to be useful for understanding RNA splicing, were unambiguously characterized by NMR and mass spectra analysis as well as by enzymatic hydrolysis.

It is now established that, during the splicing of eukaryotic messenger RNA precursors, the intervening sequences are excised in the form of lariat or tailed circular RNA molecules.[1] The branch point of these lariat

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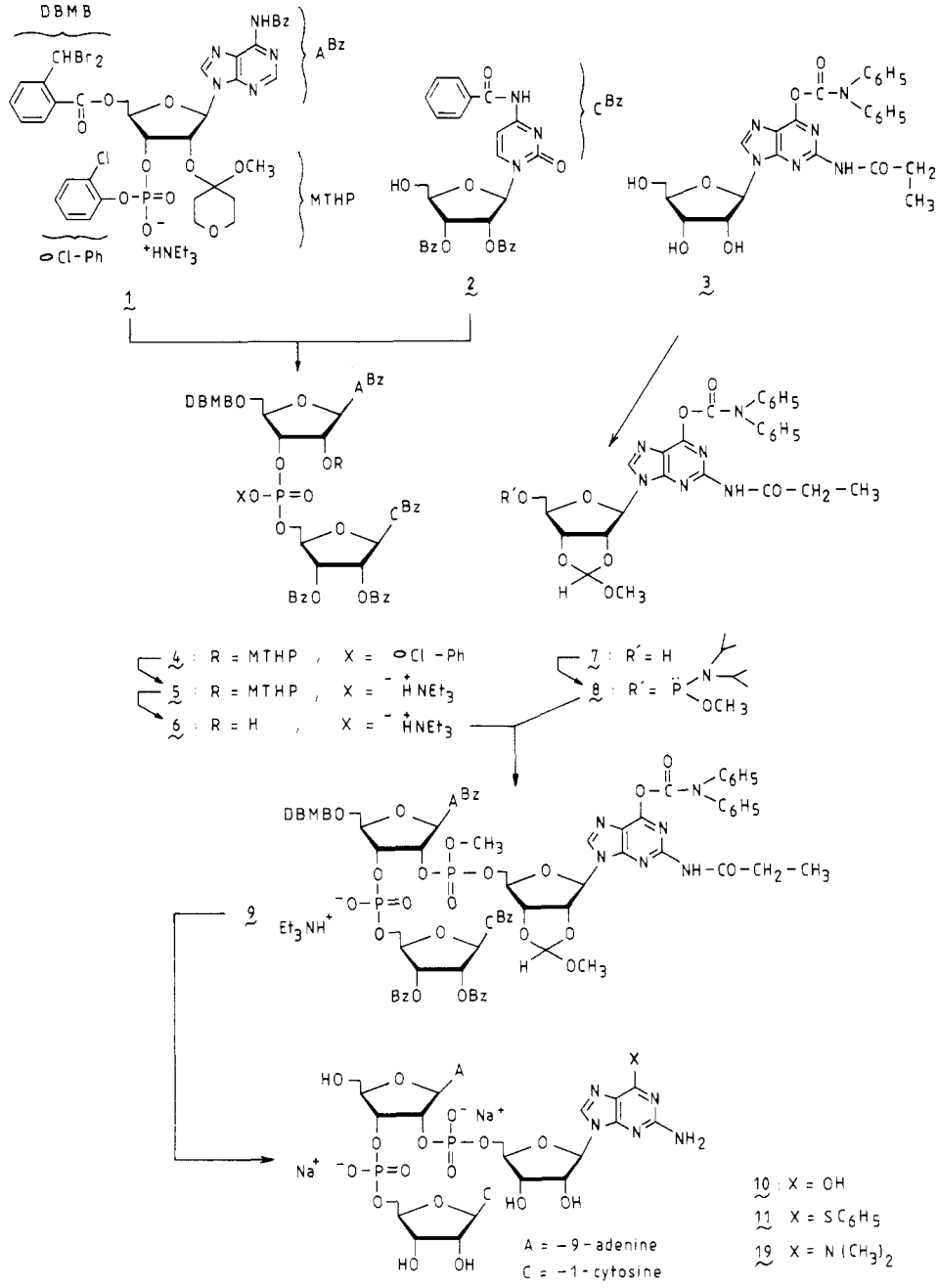
Scheme I



structures contains a modified triribonucleotide having vicinal 2′–5′ and 3′–5′ phosphodiester linkages, as depicted in Figure 1.

Despite their importance for elucidating the mechanism of RNA splicing, only a few reports have appeared describing the chemical synthesis of such unusual branched ribonucleotides.[2-5] In a preliminary paper,[6] we have briefly reported the isolation of two triribonucleoside diphosphates: one (**10**) is present at the branch point of the lariat structure of the human β-globin RNA;[7] the other (**11**) is an analogue of **10** in which the guanine residue is modified at the 6-position.

The present paper describes a detailed chemical synthesis from a common precursor of **10**, **11**, and a new modified trinucleotide, **19**. We also report our observation on the use of the diphenylcarbamoyl moiety as a leaving group for site-specific modifications at C-6 of guanine residues.

Results and Discussion

The strategy followed for the regiospecific chemical synthesis of the desired branched ribonucleotides **10**, **11**,

(1) For a review on mRNA splicing, see: Padgett, R. A.; Grabowski, P. J.; Konarska, M. M.; Seiler, S.; Sharp, P. A. *Annu. Rev. Biochem.* **1986**, *55*, 1119.
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Table I. Stability of Nucleosides 3 and 12 under Various Conditions and Products Obtained[a]

expt	conditions	products from starting compounds (%) 3	12
1	0.5 M tetrabutylammonium fluoride in tetrahydrofuran, 15 h at 25 °C	14 (100)	stable
2	0.5 M tetrabutylammonium fluoride in tetrahydrofuran–pyridine–water (8:1:1 v/v/v), 15 h at 25 °C	stable	stable
3	0.1 M sodium hydroxide in water–dioxane (1:1 v/v), 15 h at 25 °C	13 (100)	16 (90)
4	20% aqueous ammonia (*d* 0.92), 12 h at 50 °C	13 (100)	16 (85) 17 (10) 13 (5)
5	20% aqueous ammonia (*d* 0.92)–pyridine (4:1 v/v), 12 h at 50 °C	13 (100)	16 (100)
6	20% aqueous ammonia (*d* 0.92)–methanol (9:1 v/v), 12 h at 50 °C	13 (100)	16 (70) 15 (25) 13, 17 (traces)
7	ammonia in anhydrous methanol or dioxane,[b] 12 h at 50 °C	14 (>95)	stable
8	0.5 N methanolic sodium methoxide, 15 h at 25 °C	13 (50), 15 (50)	16 (75) 15 (25)
9	0.5 N methanolic sodium methoxide, 1.5 h at reflux	13 (60), 15 (40)	16 (30) 15 (70)
10	40% aqueous dimethylamine, 12 h at 50 °C	14 (76), 13 (13), 3 (11)	18 (100)

[a] For details, see the Experimental Section. [b] Methanol and dioxane were saturated by bubbling with ammonia at 0 and 20 °C, respectively.

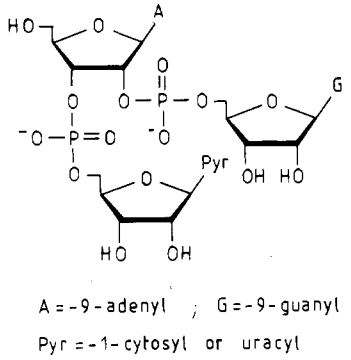

Figure 1.

and **19** was based in part on that first introduced by Caruthers et al.[4a] This strategy appeared appropriate with regard to both the specificity of sequence and the final yields.

A successful scheme for our purpose (Scheme I) required the triribonucleoside diphosphate **9** as a key intermediate. The synthesis of **9** started with preparation of the fully protected dinucleotide **4**. This compound was readily obtained in 88% yield by condensation of the phosphodiester synthon **1**, prepared as previously described,[8] with the partially benzoylated cytidine nucleoside **2**[9] with 1-(mesitylene-2-sulfonyl)-3-nitro-1,2,4-triazole[8] in pyridine as an activating agent. In accordance with literature results,[10,11] attempts to remove the acid labile 2′-methoxytetrahydropyranyl group from **4** failed due to the fast nucleophilic attack of the transiently free 2′-hydroxy function on the vicinal phosphotriester. However, partially protected dinucleoside **6** was easily obtained by first deprotecting the phosphotriester **4** with tetrabutylammonium fluoride in tetrahydrofuran–pyridine–water at room temperature to give **5**.[12-18] Removal of the 2′-acid-labile group from **5** with a 0.01 M solution of hydrochloric acid in dioxane–water at room temperature afforded **6**. ^{1}H and ^{31}P NMR spectra of purified **6** corroborated its structure.

Condensation of **6** with phosphoramidite **8** in dry acetonitrile in the presence of tetrazole and subsequent in situ oxidation of the phosphite triester intermediate with aqueous iodine afforded the key protected trimer **9**, which was purified by silica gel column chromatography.

Protection of the O^6- and N^2-positions of guanine in **8** seemed advisable due to the possible formation of O^6-phosphorylated side products, as has been reported by Ogilvie et al. in analogous condensations.[3,19] Preparation of the 5′-phosphoramidite derivative **8** required prior synthesis of nucleoside **7** from previously reported **3**.[20] Hitherto, **7** has been described as a noncrystalline compound.[21] We chose the diphenylcarbamoyl group to protect the O^6 oxygen of the guanine residue because its great lipophilicity facilitates chromatographic purification. Additionally, it can be introduced with good yield and selectively detected as dark blue spots on TLC upon heating.[20,22] With crystalline **7** as the starting material phosphoramidite **8** was easily prepared in satisfactory yield by using bis(diisopropylamino)methoxyphosphine[23] with diisopropylammonium tetrazolide[24] as catalyst in anhydrous acetonitrile.

In our preliminary paper[6] full deprotection of **9** was accomplished in a stepwise manner. Treatment of **9** with

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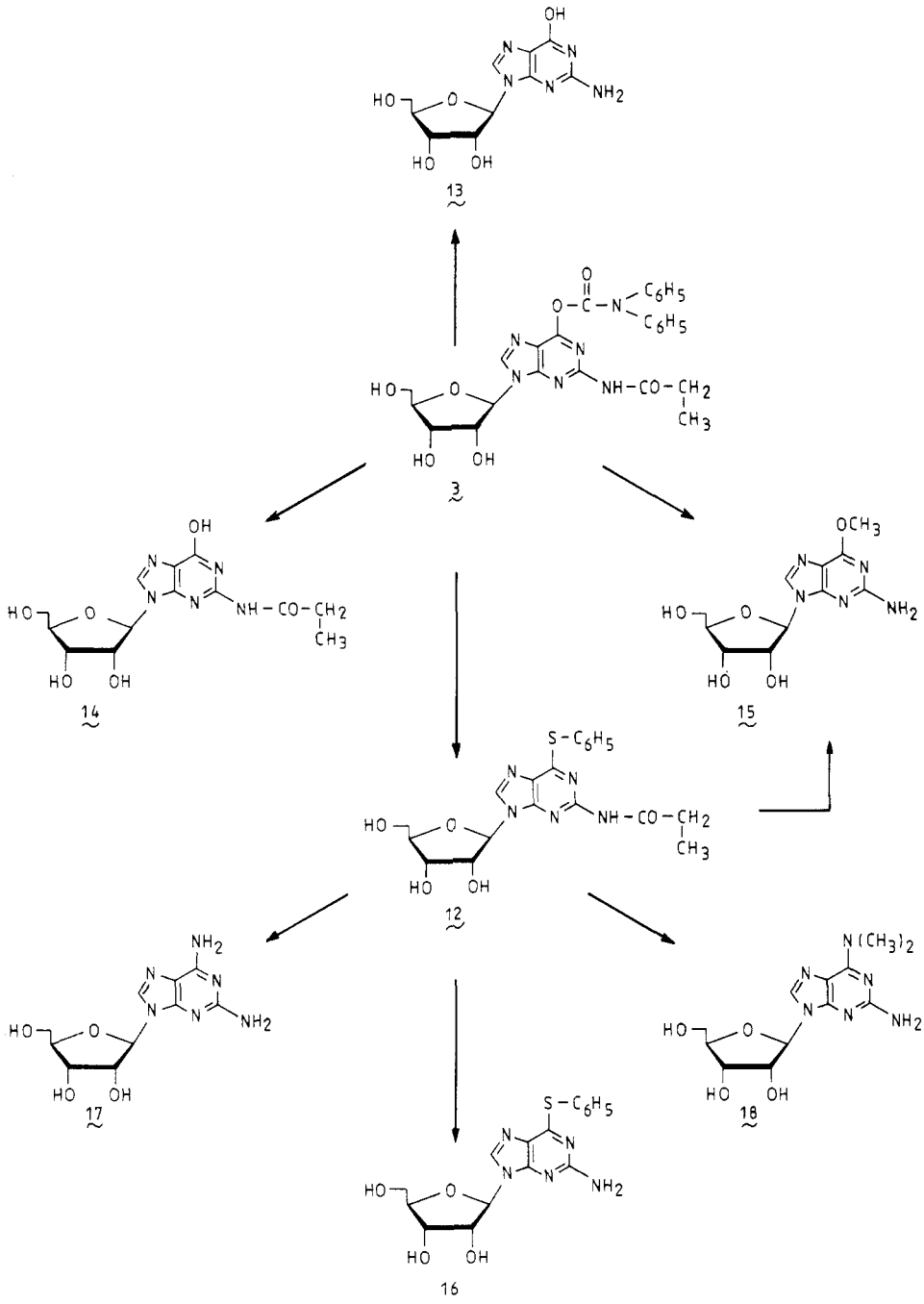
Scheme II



thiophenol–triethylamine to demethylate the phosphate ester[25,26] was followed by aqueous acetic acid to hydrolyze the methoxymethylene and then aqueous ammonia or a solution of aldoxime and tetramethylguanidine[27] to cleave the remaining protecting groups. This procedure resulted in **10** and **11**, unambiguously characterized by NMR and mass spectrometry.

The formation of both **10** and **11** appeared to result from a nucleophilic displacement of the diphenylcarbamoyl group by thiophenolate during the first step of deprotection of **9**. This possibility prompted us to examine the stabilities of the phenylthio and the diphenylcarbamate groups in order to find suitable conditions to obtain **10** selectively and to develop site-specific modifications of the guanine residue during the deprotection of **9**.

For this purpose, we prepared first **3** and **12** as model starting compounds and **14**–**18** as reference compounds. Except for **12**, **14**, and **16**, all of the other compounds were already known and were synthesized according published procedures. The behavior of **3** and **12** in the presence of various nucleophiles under a variety of conditions was then examined. The results are illustrated in Scheme II and summarized in Table I.

Compound **3** was quantitatively converted to guanosine (**13**) with sodium hydroxide (experiment 3) or aqueous ammonia (experiment 4–6). While under these conditions, **12** gave exclusively or preferentially 6-(phenylthio)-2-amino-9-β-D-ribofuranosylpurine (**16**). Also noteworthy was the quantitative conversion of **12** into the 6-(dimethylamino)-substituted derivative **18** (experiment 10).

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Taken together, these data suggested that the protected intermediate **9** could selectively give one of the three ribonucleotides **10**, **11**, or **19**, depending upon the deprotection conditions employed.

Thus, for the synthesis of **10**, the thiophenol treatment was avoided by reacting **9** with 20% aqueous ammonia, first for 5 h at room temperature to cleave the methyl phosphate ester, then for 15 h at 55 °C to remove all base-labile protecting groups.[28] Hydrolysis of the methoxymethylene was then accomplished with 0.01 N hydrochloric acid. The mixture obtained was submitted to alkaline phosphatase hydrolysis before being applied to a DEAE-Sephadex A-25 column. Elution with a linear gradient of triethylammonium bicarbonate gave pure **10** in 57% yield after transformation into its sodium salt form.

Synthesis of **11** and **19**, on the other hand, involved treatment of **9** first with thiophenol in dioxane–triethylamine to demethylate the phosphate triester and simultaneously to displace the diphenylcarbamate by the phenylthio function. The excess of thiophenol was removed by fast column chromatography, and the appropriate fractions were treated in two different ways. For the synthesis of **11**, subsequent reaction with aqueous ammonia in pyridine for 15 h at 55 °C removed base-labile groups but kept intact the phenylthio function. For the synthesis of **19**, treatment of the above appropriate fractions with 40% aqueous dimethylamine for 15 h at 55 °C removed base-labile groups and allowed substitution of phenylthio by dimethylamino function. The residues were then worked up as described for the synthesis of **10** (HCl, alkaline phosphatase, and DEAE chromatography). Further purification by HPLC afforded pure **11** and **19**, in 44% and 42% yields, respectively, after transformation into their sodium salts.

The three branched triribonucleotides **10**, **11**, and **19** were analyzed by TLC and HPLC and characterized by NMR (^{1}H, ^{31}P) spectrometry. Their assigned structures were corroborated by the results of their enzymatic digestions and by their UV absorption properties. Their physical properties are presented in Table II.

In summary, three branched ribonucleoside diphosphates were efficiently obtained by chemical syntheses from a common precursor **9**. Although chain extension in the 5′-direction was not intended in this study, this process appears feasible after selective removal[8,29] of the *o*-(dibromomethyl)benzoyl protecting group from **9**. The reported compounds **10**, **11**, and **19** may prove useful for understanding the mechanism of RNA splicing since they could be used both to isolate branch-specific proteins as debranching enzymes or antibodies and to serve as models for biophysical studies of branched RNAs.

Experimental Section

General Procedures. Evaporation of solvents was done with a rotary evaporator under reduced pressure. Melting points were determined on a Büchi 510 apparatus and are uncorrected. Ultraviolet spectra (UV) were recorded on a Cary 118 C spectrophotometer; numbers in parentheses are extinction coefficients ($\epsilon \times 10^{-3}$). Proton nuclear magnetic resonances were determined in DMSO-d_6 (or in D_2O) at ambient temperature on a Bruker WM 360 WB spectrometer. Chemical shifts are expressed in parts per million downfield from DMSO set at 2.49 ppm (or from HDO set at 4.79 ppm). ^{31}P NMR spectra were recorded with and without proton decoupling on a Bruker WP 200 SY instrument at 81.015 MHz; chemical shifts (parts per million) are reported relative to external H_3PO_4. Elemental analyses were determined by the Service de Microanalyse du CNRS, Division de Vernaison. Thin-layer chromatography (TLC) was performed on precoated aluminium sheets of silica gel 60 F_{254} (Merck, No. 5554), visualization of products being accomplished by UV absorbance followed by charring with 10% ethanolic sulfuric acid and heating; phosphorus-containing components were detected by spraying with molybdate Hanes reagent.[30] Short column chromatography was performed with silica gel 60 H (Merck, No. 7736) under weak nitrogen pressure (≃4 psi). High-pressure liquid chromatographic (HPLC) studies were carried out on two Waters Associate units: one (apparatus A) equipped with two Model 510 EF solvent delivery systems, a Model 720 solvent programmer, a Model U6K sample injector, a Model 481 UV-absorbance detector operating at 254 nm, a Model R401 differential refractometer, and a M-730 microprocessor-controlled data system; the other (apparatus B) equipped with two Model 6000 A solvent delivery systems, a Model 680 solvent programmer, a Model U6K sample injector, and (i) a Model 440 UV-absorbance detector operating at 254 nm, a Model R401 differential refractometer, a two-way Omniscribe recorder, or (ii) a Philips instrument composed of a PU 4021 multichannel detector, a PU 4850 videochromatography center and a PU 4900/20 printer/plotter. Snake venom phosphodiesterase (from Crotalus Durissus, E.C. 3.1.4.1) and calf spleen phosphodiesterase (E.C. 3.1.16.1) were from Boehringer Mannheim; ribonuclease T_2 (E.C. 3.1.27.1) and bacterial alkaline phosphatase (type III-R, E.C. 3.1.3.1) were from Sigma.

***P*-(*o*-Chlorophenyl)-*N*^{6}-benzoyl-2′-*O*-(4-methoxytetrahydropyran-4-yl)-5′-*O*-[*o*-(dibromomethyl)benzoyl]adenylyl-(3′→5′)-*N*^{4}-benzoyl-2′,3′-di-*O*-benzoylcytidine (4).** *N*^{6}-Benzoyl-2′-*O*-(4-methoxytetrahydropyran-4-yl)-5′-*O*-[*o*-(dibromomethyl)benzoyl]adenosine 3′-[(*o*-chlorophenyl)triethylammonium phosphate] (1;[8] 5.0 g, 4.75 mmol) and N^4-benzoyl-2′,3′-di-*O*-benzoylcytidine (2;[9] 2.4 g, 4.32 mmol) were first coevaporated three times with anhydrous pyridine and then dissolved in pyridine (24 mL), and 1-(mesitylene-2-sulfonyl)-3-nitro-1,2,4-triazole[8] (3.84 g, 12.96 mmol) was added to the stirred solution at room temperature. After 40 min, saturated aqueous sodium bicarbonate (6 mL) was added, and the stirring was continued for 15 min. The reaction mixture was poured into saturated aqueous sodium bicarbonate (300 mL) and extracted with chloroform (4 × 300 mL). The combined chloroform extracts were washed with water (2 × 200 mL), dried over sodium sulfate, filtered, and evaporated to dryness. The residue was reevaporated three times in toluene solvent and then chromatographed on a silica gel column with a stepwise gradient of ethyl acetate (50%–100%) in dichloromethane as eluent. The fractions containing the pure compound were pooled and evaporated to give **4** (5.7 g, 88%) as a foam: ^{31}P NMR ($CDCl_3$) δ −6.74 (18%) and −7.66 (82%), two diastereoisomers.

***P*-(Triethylammonio)-*N*^{6}-benzoyl-2′-*O*-(4-methoxytetrahydropyran-4-yl)-5′-*O*-[*o*-(dibromomethyl)benzoyl]adenylyl-(3′→5′)-*N*^{4}-benzoyl-2′,3′-di-*O*-benzoylcytidine (5).** The protected derivative **4** (5.0 g, 3.36 mmol) was dissolved in 0.05 M tetrabutylammonium fluoride in a mixture of tetrahydrofuran–pyridine–water (8:1:1 v/v/v; 201 mL).[12-18] After the mixture was stirred overnight at room temperature, Dowex 50 W cation exchange resin (pyridinium form, 150 g) was added, the resin was filtered off, and 25 mL of 1 M triethylammonium bicarbonate buffer was added to the filtrate. The solution was evaporated to dryness and reevaporated in ethanol solvent. Chromatography on a silica gel column with a stepwise gradient of methanol (2%–10%) in chloroform led to the isolation of pure **5** (4.5 g, 90%), ^{31}P NMR ($CDCl_3$) δ −0.80.

***P*-(Triethylammonio)-*N*^{6}-benzoyl-5′-*O*-[*o*-(dibromomethyl)benzoyl]adenylyl-(3′→5′)-*N*^{4}-benzoyl-2′,3′-di-*O*-benzoylcytidine (6).** The dinucleotide **5** (4.0 g, 2.7 mmol) was dissolved in a 0.01 M solution of HCl in dioxane–water (2:1 v/v, 80 mL), and the solution was stirred at room temperature for 3 h. After being cooled to 0 °C, the solution was neutralized to pH 5–6 by addition of aqueous 5% ammonium bicarbonate (ca. 12.4 mL). Water (200 mL) was added, and the solution was extracted with chloroform (4 × 200 mL). The organic layers were washed

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Table II. Relevant Data for the Properties of Unprotected Triribonucleotides 10, 11, and 19

	TLC[a] R_f values		HP-LC[b]	UV[c]					
	A	B	t_R, min	before hydrolysis	after hydrolysis	hyperchromicity, %	^{1}H NMR[d]	^{31}P NMR[d]	mass spectrum (FAB < 0)
10	0.22	0.23	13.3	λ_{max} 259 nm, λ_{min} 227 nm	λ_{max} 260 nm, λ_{min} 228 nm	10	8.31 [s, 1 H, H-8 (A)], 7.98 [s, 1 H, H-2 (A)], 7.94 (d, 1 H, H-6 (C), $J_{5,6}$ = 7.5 Hz], 7.81 [s, 1 H, H-8 (G)], 6.27 [d, 1 H, H-1′ (A), $J_{1',2'}$ = 5.8 Hz], 6.07 [d, 1 H, H-5 (C)], 6.02 [d, 1 H, H-1′ (C), $J_{1',2'}$ = 4.2 Hz], 5.74 [d, 1 H, H-1′ (G), $J_{1',2'}$ = 4.9 Hz]	−0.71, −1.39	916 $(M - H)^-$, 938 $(M + Na - H)^-$
11	0.41	0.37	19.4	λ_{max} 316 nm, 256 nm ($\epsilon_{316}/\epsilon_{256}$ = 0.48); λ_{min} 295 nm, 230 nm ($\epsilon_{295}/\epsilon_{230}$ = 0.35)	λ_{max} 316 nm, 259 nm ($\epsilon_{316}/\epsilon_{259}$ = 0.47); λ_{min} 294 nm, 237 nm ($\epsilon_{294}/\epsilon_{237}$ = 0.39)	17, 20	8.29 [s, 1 H, H-8 (A)], 8.05 [s, 1 H, H-8 (2,6-substituted purine)], 7.99 [s, 1 H, H-2 (A)], 7.92 [d, 1 H, H-6 (C), $J_{5,6}$ = 7.6 Hz], 7.78–7.75 and 7.64–7.62 [2 m, 2 and 3 H, SC_6H_5], 6.26 [d, 1 H, H-1′ (A), $J_{1',2'}$ = 5.2 Hz] 6.03 [d, 1 H, H-5 (C)], 5.99 [d, 1 H, H-1′ (C), $J_{1',2'}$ = 4.0 Hz], 5.85 [d, 1 H, H-1′ (2,6-substituted purine), $J_{1',2'}$ = 4.7 Hz]	−0.63, −1.28	1008 $(M - H)^-$, 1030 $(M + Na - H)^-$
19	0.36	0.29	15.8	λ_{max} 264 nm, 232 nm ($\epsilon_{264}/\epsilon_{232}$ = 1.07); λ_{min} 246 nm	λ_{max} 265 nm, 230 nm ($\epsilon_{265}/\epsilon_{230}$ = 1.09); λ_{min} 246 nm	18, 16	8.25 [s, 1 H, H-8 (A)], 7.98 [s, 1 H, H-2 (A)], 7.88 [d, 1 H, H-6 (C), $J_{5,6}$ = 7.6 Hz], 7.82 [s, 1 H, H-8 (2,6-substituted purine)], 6.22 [d, 1 H, H-1′ (A), $J_{1',2'}$ = 4.0 Hz], 5.98 [d, 1 H, H-5 (C)], 5.97 [d, 1 H, H-1′ (C), $J_{1',2'}$ = 3.5 Hz], 5.83 [d, 1 H, H-1′ (2,6-substituted purine), $J_{1',2'}$ = 4.3 Hz], 3.36 [s, 6 H, $N(CH_3)_2$]	−0.64, −1.44	943 $(M - H)^-$, 965 $(M + Na - H)^-$

[a] Eluents: A, 2-propanol–20% aqueous ammonia–water, 7:2:1 v/v/v; B, 1 M aqueous ammonium acetate–ethanol, 2:8 v/v. [b] Apparatus B: column C_{18} "Radial Pak" (100 × 8 mm i.d., 10-μm particle size) in a Waters Associates Radial Compression Module RCM 100, protected by a Precolumn C_{18} "Guard Pak". Solvent A: 0.1 M ammonium acetate buffer, pH 5.9. Solvent B: 50% acetonitrile in the same buffer. Convex gradient (curve 7) 0%–100% B in 30 min, flow rate 3 mL/min. [c] In the conditions of hydrolysis by snake venom phosphodiesterase; hyperchromicity, defined as $[(A_D - A_I)/A_I] \times 100$, was determined by measurement of the absorbance before (A_I) and after (A_D) digestion with snake venom phosphodiesterase, as described in the Experimental Section. [d] In D_2O.

with water (300 mL), dried over sodium sulfate, and filtered, and the filtrate was evaporated to dryness. The residue was chromatographed on a silica gel column with a stepwise gradient of methanol (2%–10%) in chloroform containing 1% triethylamine to afford pure **6** (2.7 g, 73%): ^{1}H NMR (Me_2SO-d_6) δ 11.3 and 11.1 (2 br s, 2 × 1 H, 2 NH), 8.67 and 8.58 (2 s, 2 × 1 H,H-2 and H-8 of A^{Bz}), 8.1–7.3 (m, 26 H, 4 × C_6H_5CO + $CHBr_2C_6H_4CO$ + H-6 of C^{Bz}), 6.37 (d, 1 H, H-1′ of A^{Bz} or C^{Bz}, $J_{1',2'}$ = 3.9 Hz), 6.12 (d, 1 H, H-5 of C^{Bz}, $J_{5,6}$ = 6.5 Hz), 5.84 (m, 2 H, of which H-1′ of A^{Bz} or C^{Bz}), 5.1–4.1 (m, 9 H, other H of sugars), 3.09 (q, 6 H, CH_2 of triethylammonium), 1.19 (t, 9 H, CH_3 of triethylammonium); ^{31}P NMR ($CDCl_3$ + CH_3OH) δ −0.63.

2′,3′-*O*-(Methoxymethylene)-*O*^{6}-(diphenylcarbamoyl)-*N*^{2}-propionylguanosine (7). A mixture of *O*^{6}-(diphenylcarbamoyl)-*N*^{2}-propionylguanosine (**3**;[20] 3.0 g, 5.61 mmol), trimethyl orthoformate (3.7 mL, 33.67 mmol), and toluene-*p*-sulfonic acid monohydrate (97 mg, 0.51 mmol) was stirred at room temperature for 30 min in anhydrous acetonitrile (5.6 mL). The resulting solution was neutralized by addition of a freshly prepared solution of 0.1 N sodium methoxide (6 mL) and then evaporated to dryness. To the residue was added chloroform (150 mL), and the suspension was filtered. The filtrate was washed with water (60 mL), dried over sodium sulfate, filtered, and evaporated to dryness. The residue was chromatographed on a silica gel column (eluent, chloroform) to afford pure **7** (2.7 g, 83%). The compound was crystallized from ethyl acetate: mp 184–186 °C (lit.[21] mp 168–169 °C, precipitated from dichloromethane with hexane–ether); UV (EtOH) λ_{max} 280 (12.4), 254 (sh, 19.1), 228 (29.8), λ_{min} 272 (11.8); ^{1}H NMR (Me_2SO-d_6) δ 10.72 and 10.70 (s and s, 1 H, N^2-H), 8.63 and 8.59 (s and s, 1 H, H-8), 7.5–7.3 (m, 10 H, 2 C_6H_5), 6.32 and 6.24 (d and d, 1 H, H-1′, $J_{1',2'}$ = 2.0 and 1.1 Hz, respectively), 6.16 and 6.06 (s and s, 1 H, C*H*(OCH_3)), 5.5 and 5.3 (m and m, 2 H, H-2′ and 3′), 4.87 (t, 1 H, OH-5′, J = 5.4 Hz), 4.31 and 4.16 (m and m, 1 H, H-4′), 3.5 (m, 2 H, H-5′, 5″), 3.36 and 3.26 (s and s, 3 H, OCH_3), 2.5 (m, 2 H, $COCH_2CH_3$), 1.1 (m, 3 H, $COCH_2CH_3$); two diastereoisomers (66% and 34%, respectively); the ^{1}H NMR spectrum was in little disagreement with literature data.[21] Anal. Calcd for $C_{28}H_{28}N_6O_8$: C, 58.33; H, 4.89; N, 14.58. Found: C, 57.98; H, 4.83; N, 14.35.

5′-*O*-[(Diisopropylamino)methoxyphosphino]-2′,3′-*O*-(methoxymethylene)-*O*^{6}-(diphenylcarbamoyl)-*N*^{2}-propionylguanosine (8). To a mixture of the protected nucleoside **7** (1.5 g, 2.6 mmol) and diisopropylammonium tetrazolide[23] (221.5 mg, 1.29 mmol) in anhydrous acetonitrile (13 mL) was added with stirring under argon bis(diisopropylamino)methoxyphosphine[24] (0.82 mL, 2.86 mmol). After 1.5 h, the resulting solution was poured into saturated aqueous sodium bicarbonate (60 mL), and the product was extracted into the organic phase with dichloromethane (3 × 60 mL). The combined organic layers were washed with saturated aqueous sodium chloride (2 × 50 mL), dried over sodium sulfate, and filtered, and the filtrate was evaporated to dryness. The residue was chromatographed on a silica gel column (eluent, ethyl acetate–hexane, 1:1 v/v, with 1% triethylamine) to afford pure **8** (1.56 g, 82%) as a white foam: ^{31}P NMR (CD_3CN) δ 150.20 (16%), 150.06 (35%), 149.75 (25%), 149.71 (24%), four diastereoisomers.

Chemical Synthesis of the Protected Branched Triribonucleotide 9. A mixture of diribonucleotide **6** (0.61 g, 0.44 mmol), phosphoramidite **8** (1.3 g, 1.77 mmol), and [^{1}H]tetrazole (0.18 g, 2.64 mmol) was first dried in vacuo for 10 h and then dissolved in anhydrous acetonitrile (3 mL). The solution was stirred at room temperature for 1 h. The reaction mixture was then oxidized by addition of 0.1 M iodine in tetrahydrofuran–lutidine–water (2:1:1 v/v/v, 2.2 mL). After 5 min, 1 M aqueous sodium bisulfite (55 mL) was added, and the solution was extracted with chloroform (4 × 100 mL). The combined organic layers were washed with saturated aqueous sodium bicarbonate (2 × 100 mL), dried over sodium sulfate, filtered, and evaporated to dryness. The residue was chromatographed on a silica gel column with a stepwise gradient of methanol (3%–15%) in chloroform as eluent to give **9** (0.44 g, 52%) as a powder after lyophilization from dioxane–water, ^{31}P NMR ($CDCl_3$) δ −1.9 (broad signal). This compound was sufficiently pure to be used without further purification for the preparation of **10**, **11**, and **19**.

6-(Phenylthio)-2-(propionylamino)-9-β-D-ribofuranosylpurine (12). To a stirred solution of nucleoside **3**[20] (1.8 g, 3.4 mmol) in dioxane–triethylamine (1:1 v/v, 68 mL) was added thiophenol (17 mL, 165 mmol). After 3 h at room temperature, the reaction mixture was partioned between water (500 mL) and chloroform (500 mL). The aqueous layer was washed with hot chloroform (8 × 300 mL), and then the combined organic layers were dried over sodium sulfate, filtered, and evaporated to dryness. The residue was chromatographed on a silica gel column with a stepwise gradient of methanol (0%–5%) in chloroform with 1% triethylamine as eluent to give pure **12** (1.1 g, 75%). The compound was crytallized from ethyl acetate: mp 134 °C; UV (EtOH) λ_{max} 303 (17.3), 249 (19.6), λ_{min} 280 (9.3); ^{1}H NMR (Me_2SO-d_6) δ 10.15 (s, 1 H, N^2-H), 8.55 (s, 1 H, H-8), 7.7 and 7.5 (m and m, 2 and 3 H, SC_6H_5), 5.88 (d, 1 H, H-1′, $J_{1',2'}$ = 5.8 Hz), 5.45 (d, 1 H, OH-2′, J = 5.8 Hz), 5.14 (d, 1 H, OH-3′, J = 4.7 Hz), 4.92 (t, 1 H, OH-5′, J = 5.5 Hz), 4.6 (m, 1 H, H-2′), 4.2 (m, 1 H, H-3′), 3.9 (m, 1 H, H-4′), 3.6 and 3.5 (m and m, 1 and 1 H,H-5′ and 5″), 2.18 (q, 2 H, $COCH_2CH_3$), 0.84 (t, 3 H, $COCH_2CH_3$). Anal. Calcd for $C_{19}H_{21}N_5O_5S$: C, 52.89; H, 4.91; N, 16.23; S, 7.43. Found C, 52.66; H, 5.01; N, 16.13; S, 7.23.

N^2-Propionylguanosine (14). Guanosine (1.0 g, 3.53 mmol) was first dried in vacuo for 24 h and then coevaporated three times with anhydrous pyridine and suspended in pyridine (18 mL). Propionic anhydride (6.4 mL, 50 mmol) and 4-(dimethylamino)pyridine (0.43 g, 3.52 mmol) were added, and the mixture, was heated with stirring at 70 °C for 3 h. After the mixture was cooled to 0 °C, methanol (4 mL) was added, and the stirring was continued for 10 min. The reaction mixture was concentrated to half its volume, diluted with methylene chloride (50 mL), and then successively washed with 0.2 M aqueous sodium dihydrogen phosphate (2 × 20 mL), water (25 mL), and saturated aqueous sodium bicarbonate (6 × 25 mL). The organic layer was dried over sodium sulfate, filtered, and evaporated, and the residue was coevaporated several times with toluene to remove traces of pyridine. The obtained 2′,3′,5′-tri-*O*-propionyl-*N*^{2}-propionylguanosine (1.8 g) was dissolved in pyridine (22 mL) and ethanol (15 mL) and then cooled to 0 °C, and 1 M aqueous potassium hydroxide (14.7 mL) was added with stirring. After 5 min, the solution was neutralized by the addition of a mixture of acetic acid–methanol (1:3 v/v, 3.9 mL). The reaction mixture was evaporated to dryness and coevaporated first with toluene and then with methanol–water (1:3 v/v). The residue was chromatographed on a silica gel column with a stepwise gradient of methanol (5%–40%) in chloroform to give pure **14** (0.9 g, 75%). The compound was crystallized from ethanol: mp 145 °C, start of decomposition; UV (EtOH) λ_{max} 282 (11.2), 259 (15.5), λ_{min} 276 (11.0), 229 (2.4); ^{1}H NMR (Me_2SO-d_6) δ 11.8 (br s, 2 H, 2 NH), 8.25 (s, 1 H, H-8), 5.80 (d, 1 H, H-1′, $J_{1',2'}$ = 5.6 Hz), 5.4, 5.1, and 5.0 (1 and 1 and 1 H, all br, OH-2′,3′, and 5′), 4.43 (m, 1 H, H-2′), 4.13 (m, 1 H, H-3′), 3.90 (m, 1 H, H-4′), 3.63 and 3.56 (m and m, 1 and 1 H, H-5′ and 5″), 2.49 (m, 2 H, $COCH_2CH_3$), 1.09 (t, 3 H, $COCH_2CH_3$, J = 7.5 Hz). Anal. Calcd for $C_{13}H_{17}N_5O_6 \cdot {}^2/_3H_2O$: C, 44.44; H, 5.26; N, 19.94. Found: C, 44.30; H, 5.31, N, 19.76.

6-(Phenylthio)-2-amino-9-β-D-ribofuranosylpurine (16). A solution of nucleoside **12** (0.5 g, 1.16 mmol) in 20% aqueous ammonia (*d* 0.92)–pyridine (4:1 v/v, 70 mL) was heated in a bomb at 50 °C for 15 h. After the reaction mixture was cooled to 0 °C, the bomb was opened, and the reaction mixture was evaporated under vacuum. After coevaporation with toluene, the residue was chromatographed on a silica gel column with a stepwise gradient of methanol (0%–8%) in chloroform to give pure **16** (0.36 g, 82%). The compound was crystallized from chloroform: mp 122 °C; UV (EtOH) λ_{max} 318 (13.5), 249 (12.1), λ_{min} 284 (4.1), 238 (10.7); ^{1}H NMR (Me_2SO-d_6) δ 8.21 (s, 1 H, H-8), 7.6 and 7.4 (m and m, 2 and 3 H, SC_6H_5), 6.34 (s, 2 H, NH_2), 5.78 (d, 1 H, H-1′, $J_{1',2'}$ = 5.9 Hz), 5.38 (d, 1 H, OH-2′, J = 5.8 Hz), 5.10 (d, 1 H, OH-3′, J = 4.6 Hz), 5.01 (t, 1 H, OH-5′, J = 5.5 Hz), 4.4 (m, 1 H, H-2′), 4.1 (m, 1 H, H-3′), 3.9 (m, 1 H, H-4′), 3.6 and 3.5 (m and m, 1 and 1 H, H-5′ and 5″). Anal. Calcd for $C_{16}H_{17}N_5O_4S \cdot {}^3/_8CHCl_3$: C, 46.80; H, 4.17, N, 16.67; s, 7.63. Found: C, 46.86; H, 4.29; N, 16.52; S, 7.63.

Stability Studies of Nucleosides 3 and 12 (Table I, Scheme II). Nucleoside samples (4.6 mg) were dissolved or suspended in various cold solutions (1 mL for experiments 3, 8, and 9 and 0.6 mL in other experiments). Except for experiment 9 (reflux 1.5 h), the samples were placed in screw-cap test tubes fitted with Pierce Teflon disks, and the sealed tubes were maintained at 25

°C or heated at 50 °C for 12–15 h as indicated in Table I. Then, the reaction mixtures were evaporated under vacuum after neutralization if necessary by addition of 0.1 N hydrochloric acid (experiments 3, 8, and 9). The residues were analyzed by analytical HPLC with the following conditions. Apparatus B: C_{18} "ultrasphere" XL 3 μ ODS ultrafast cartridge Beckman, 70 × 4.6 mm i.d., 3-μm particle size, protected by a precolumn XL ODS (5 × 4.6 mm i.d., 3 μm). Solvent A: 0.1 M ammonium acetate buffer, pH 5.9. Solvent B: 50% acetonitrile in the same buffer; linear gradient 0%–100% B in 20 min, flow rate 1.0 mL/min. Peaks were detected at 254 nm, and their UV spectra enregistred; they were identified, and their areas were quantified and calibrated by reference to the above synthesized (**12**, **14**, and **16**) or already reported (**15**,[31] **17**,[32–36] and **18**[31,32,37]) compounds.

Synthesis of Unprotected Triribonucleotide 10. Compound **9** (98 mg, 0.051 mmol) was dissolved in 20% aqueous ammonia (*d* 0.92, 30 mL) and stored in a sealed flask at room temperature for 5 h. After concentration under vacuum to a dry residue, another 30 mL of 20% aqueous ammonia was added, and the sealed flask was stored at 55 °C for 15 h. The volatile material was then removed, the residue, after three coevaporations with water, was dissolved in 0.01 N hydrochloric acid (30 mL), and the pH was adjusted to 2 with 1 N hydrochloric acid. After 6 h at room temperature, the reaction was neutralized by addition of diluted aqueous ammonia and evaporated to dryness. To the residue were added water (150 mL) and chloroform (70 mL). The aqueous layer was successively washed with chloroform (5 × 60 mL) and diethyl ether (2 × 60 mL) and then evaporated under vacuum. The residue was dissolved in 0.1 M triethylammonium bicarbonate buffer (TEAB, pH 7.5, 3 mL), and a suspension of alkaline phosphatase from Escherichia *coli* (0.1 mL) was added. After 15 h at 37 °C, the enzyme was inactivated by heating for 3 min at 90 °C. The reaction mixture was centrifuged, and the supernatant was evaporated in vacuo and reevaporated to dryness in water. The residue was then chromatographed on a DEAE-Sephadex A-25 column (25 × 2 cm) with TEAB buffer (pH 7.5, linear gradient from 0.002 to 0.25 M). The product **10** was eluted at 0.20 M TEAB. The appropriate fractions, which were found to be pure by TLC and HPLC, were combined, evaporated, and reevaporated in water, and then they were passed two times through a column of Dowex 50 W × 2 (sodium form) ion-exchange resin to yield the sodium salt of **10** (28 mg, 57% after lyophilization from water). The properties of **10** are presented in Table II.

Synthesis of Unprotected Triribonucleotide 11. To a stirred solution of compound **9** (80 mg, 0.041 mmol) in dioxane–triethylamine (1:1 v/v, 0.8 mL) was added thiophenol (0.2 mL, 0.95 mmol). After 3 h at room temperature, the reaction mixture was evaporated to dryness. To remove the excess of thiophenol, the residue was chromatographed on a silica gel column with a stepwise gradient of methanol (0%–8%) in chloroform with 1% triethylamine as eluent. Fractions containing ribonucleotide material were combined and evaporated to dryness. The residue was dissolved in 20% aqueous ammonia (*d* 0.92)–pyridine (4:1 v/v, 60 mL) and stored in a sealed flask at 55 °C for 15 h. The volatile material was then removed, and the residue was treated as described before for the synthesis of **10** (hydrochloric acid, alkaline phosphatase, and DEAE chromatography). The product **11** was eluted, with some impurities, with 0.22 M TEAB. Further purification of **11** was accomplished by HPLC. Apparatus A: column μ Bondapak C_{18} Waters, 150 × 19 mm i.d., 10-μm particle size, protected by a precolumn C_{18} "Guard Pak". Solvent A: 6% acetonitrile in 0.05 M ammonium hydrogen carbonate buffer, pH 7.5. Solvent B: 50% acetonitrile in the same buffer; linear gradient 0%–100% B in 15 min, flow rate 5 mL/min. Pure sodium salt of **11** (19 mg, 44%) was obtained after treatment with Dowex 50 W × 2 (sodium form) and lyophilization from water. The properties of **11** are presented in Table II.

Synthesis of Unprotected Triribonucleotide 19. Compound **9** (80 mg, 0.041 mmol) was treated with thiophenol as described before during the synthesis of **11**. The residue obtained by evaporating appropriate fractions from silica gel column chromatography was dissolved in 40% aqueous dimethylamine (30 mL) and stored in a sealed flask at 55 °C for 15 h. The volatile material was then removed, and the residue was treated as described before for the synthesis of **10** and **11** (hydrochloric acid, alkaline phosphatase, and DEAE chromatography). The product **19** was eluted, with some impurities, with 0.18 M TEAB. Further purification of **19** was accomplished by HPLC. Apparatus and column were the same as for the above purification of **11**: eluent 12.5% of acetonitrile in 0.05 M ammonium hydrogen carbonate buffer, pH 7.5, isocratic conditions, flow rate 5 mL/min. Pure sodium salt of **19** (17 mg, 42%) was obtained after treatment with Dowex 50 W × 2 (sodium form) and lyophilization from water. The properties of **19** are presented in Table II.

Enzymatic Hydrolysis Studies of 10, 11, and 19. Each unprotected triribonucleotide **10**, **11**, and **19** (≃1.3 mg) was dissolved in water (10 mL). *With ribonuclease T_2*: 1 mL of each stock solution was first lyophilized, dissolved in 0.1 M ammonium acetate buffer (pH adjusted to 4.5 with acetic acid, 0.3 mL), and incubated overnight at 37 °C with ribonuclease T_2 (2.0 μL of a solution containing 1000 Units/mL of H_2O). TLC and HPLC analysis showed no hydrolysis of any of the triribonucleotides **10**, **11**, and **19**. *With calf spleen phosphodiesterase*: To 0.8 mL of each stock solution were added 0.15 mL of a cocktail (6 mL of 1 M KH_2PO_4, pH adjusted to 6.1 with 1 M NaOH + 0.5 mL of 0.1 M EDTA + 6 mL of Tween 80 + 23 mL of H_2O) and 20 μL of calf spleen phosphodiesterase. The resulting solutions were maintained at 37 °C for 16 h. TLC and HPLC analysis showed no hydrolysis of the triribonucleotides. *With snake venom phosphodiesterase*: to 2 mL of each stock solution were added 0.56 mL of $MgCl_2$ (0.1 M) and 0.56 mL of Tris·HCl (1 M, pH adjusted to 8.9 with HCl), and the volume was adjusted to 10 mL with H_2O. Four milliliters of the resulting solutions were incubated with snake venom phosphodiesterase (20 μL) at 37 °C for 16 h. The enzyme was inactivated by heating for 3 min at 90 °C, and the enzymatic digests were analyzed qualitatively by TLC and quantitatively by HPLC. Complete conversion of triribonucleotides **10**, **11**, and **19** into the expected products was observed.

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