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Acid/Azole Complexes as Highly Effective Promoters in the Synthesis of DNA and RNA Oligomers via the Phosphoramidite Method

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Abstract: The utility of various kinds of acid salts of azole derivatives as promoters for the condensation of a nucleoside phosphoramidite and a nucleoside is investigated. Among the salts, *N*-(phenyl)imidazolium triflate, *N*-(*p*-acetylphenyl)imidazolium triflate, *N*-(methyl)benzimidazolium triflate, benzimidazolium triflate, and *N*-(phenyl)imidazolium perchlorate have shown extremely high reactivity in a liquid phase. These reagents serve as powerful activators of deoxyribonucleoside 3′-(allyl *N*,*N*-diisopropylphosphoramidite)s or 3′-(2-cyanoethyl *N*,*N*-diisopropylphosphoramidite)s employed in the preparation of deoxyribonucleotides, and 3′-*O*-(*tert*-butyldimethylsilyl)ribonucleoside 2′-(*N*,*N*-diisopropylphosphoramidite)s or 2′-*O*-(*tert*-butyldimethylsilyl)-ribonucleoside 3′-(*N*,*N*-diisopropylphosphoramidite)s used for the formation of 2′−5′ and 3′−5′ internucleotide linkages between ribonucleosides, respectively. The azolium salt has allowed smooth and high-yield condensation of the nucleoside phosphoramidite and a 5′-*O*-free nucleoside, in which equimolar amounts of the reactants and the promoter are employed in the presence of powdery molecular sieves 3A in acetonitrile. It has been shown that some azolium salts serve as excellent promoters in the solid-phase synthesis of oligodeoxyribonucleotides and oligoribonucleotides. For example, benzimidazolium triflate and *N*-(phenyl)imidazolium triflate can be used as effective promoters in the synthesis of an oligodeoxyribonucleotide, $^{5'}$CGACACCCAATTCT-GAAAAT$^{3'}$ (20mer), via a method using *O*-allyl/*N*-allyloxycarbonyl-protected deoxyribonucleoside 3′-phosphoramidites or *O*-(2-cyanoethyl)/*N*-phenoxyacetyl-protected deoxyribonucleotide 3′-phosphoramidite as building blocks, respectively, on high-cross-linked polystyrene resins. Further, *N*-(phenyl)imidazolium triflate is useful for the solid-phase synthesis of oligoribonucleotides, such as $^{5'}$AGCUACGUGACUACUACUUU$^{3'}$ (20mer), according to an allyl/allyloxycarbonyl-protected strategy. The utility of the azolium promoter has been also demonstrated in the liquid-phase synthesis of some biologically important substances, such as cytidine-5′-monophosphono-*N*-acetylneuraminic acid (CMP-Neu5Ac) and adenylyl(2′−5′)adenylyl(2′−5′)adenosine (2−5A core).

Introduction

Recent advances in and diversity of nucleic acid research have increased the importance of developing a chemical method capable of preparing oligonucleotides both with and without artificial modifications. Among the methods reported thus far, the phosphoramidite approach[1] is considered to be the most useful, as it allows for high-yield and high-purity preparations in both solution and solid phases. This approach is especially useful for the synthesis of medium-size oligonucleotides (longer

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than 20mers) with a modified backbone; these oligonucleotides include *H*-phosphonate,[2] arylphosphonate,[3] phosphotriester,[3,4] phosphoramidate,[5] phosphoramidimidate,[6] phosphorothioate,[7] phosphorodithioate,[8] phosphoroselenate,[9] and boranophosphate,[10] some of which are useful as antisense molecules.[11] In the phosphoramidite strategy, an important research objective is the invention of a promoter that is useful for the condensation of a nucleoside phosphoramidite and a nucleoside. A variety of promoters including 1*H*-tetrazole,[12] 5-(*p*-nitrophenyl)-1*H*-tetrazole (NPT),[13] 5-(ethylthio)-1*H*-tetrazole (ETT),[14] pyridinium chloride in the presence of imidazole,[15] pyridinium trifluoroacetate (PyTFA),[16] *N*-methylanilinium trifluoroacetate (*N*-MeANTFA),[17] pyridinium tetrafluoroborate (PyTFB),[8b] 2,4-dinitrophenol,[18] 4,5-(dicyano)imidazole (DCI),[19] 2-(bromo)-4,5-(dicyano)imidazole (BrDCI),[20] and 2-(mesityl)-4,5-(dicyano)-imidazole[21] have been reported to date. However, these reagents have some drawbacks. For example, the existing reagents do not sufficiently activate poorly reactive ribonucleoside phosphoramidites such as 3′-*O*-(*tert*-butyldimethylsilyl)ribonucleoside 2′-phosphoramidites and 2′-*O*-(*tert*-butyldimethylsilyl)-ribonucleoside 3′-phosphoramidites, which are most widely used as monomer units in the synthesis of 2′−5′- and 3′−5′-linked oligoribonucleotides, respectively.[22] Reactions using these phosphoramidites do not always take place smoothly in the presence of the existing promoters. In addition, the product yield is generally not high. Some reagents such as pyridinium chloride and PyTFA are hygroscopic. In the case of the tetrazole reagents, potential explosive and biohazadous characteristics[23] are problematic. With regard to NPT, its low solubility in acetonitrile (ca. 0.1 mol/L) is a drawback to the large-scale synthesis. To render the phosphoramidite method more useful, it is necessary to develop more efficient promoters that are capable of circumventing these disadvantages. This paper suggests certain acid salts of imidazole- and benzimidazole-related compounds as being such desirable promoters. Furthermore, the utility of these salts for the synthesis of various deoxyribonucleotides, ribonucleotides, and related compounds in solution and solid phases is described. A mechanism of the condensation of a deoxyribonucleoside 3′-phosphoramidite and a 5′-*O*-free deoxyribonucleoside using an acid/azole complex promoter is also briefly discussed on the basis of ^{31}P NMR study.

Results and Discussion

A variety of acid/azole complexes were prepared by combining acids and azoles that are commercially available at reasonable prices. The acids include methanesulfonic acid, trifluoromethanesulfonic acid, *p*-toluenesulfonic acid, trifluoroacetic acid, hydroperchloric acid, tetrafluoroboric acid, and hexafluorophosphoric acid. The azoles include imidazole, *N*-(methyl)imidazole, *N*-(phenyl)imidazole, *N*-(*p*-acetylphenyl)-imidazole, 2-(methyl)imidazole, 2-(phenyl)imidazole, 4-(methyl)-imidazole, 4-(phenyl)imidazole, benzimidazole, *N*-(methyl)benzimidazole, 2-(methyl)benzimidazole, and 2-(phenyl)benzimidazole.

Reactivity of Promoters and Preparation of Deoxyribonucleotides and Ribonucleotides in Solution. Noncrystalline and/or hygroscopic compounds are not suitable as promoters for the phosphoramidite method. Good solubility in acetonitrile is also a crucial requirement for achieving efficient synthesis. Accordingly, the following nonhygroscopic, crystalline promoters with high solubility (≥0.4 mol/L) were selected for the following examinations: imidazolium triflate (IMT), imidazolium perchlorate (IMP), imidazolium tetrafluoroborate, *N*-(methyl)imidazolium triflate (*N*-MeIMT),[24] *N*-(phenyl)imidazolium triflate (*N*-PhIMT), *N*-(phenyl)imidazolium perchlorate (*N*-PhIMP), *N*-(phenyl)imidazolium tetrafluoroborate (*N*-PhIMTFB), *N*-(*p*-acetylphenyl)imidazolium triflate (*N*-AcPhIMT), 2-(phenyl)imidazolium triflate (2-PhIMT), 4-(methyl)imidazolium triflate (4-MeIMT), 4-(methyl)imidazolium tosylate, 4-(methyl)imidazolium trifluoroacetate, 4-(phenyl)imidazolium triflate (4-PhIMT), 4-(phenyl)imidazolium trifluoroacetate, benzimidazolium triflate (BIT),[25] benzimidazolium tetrafluoroborate (BITFB), *N*-(methyl)benzimidazolium triflate (*N*-MeBIT), 2-

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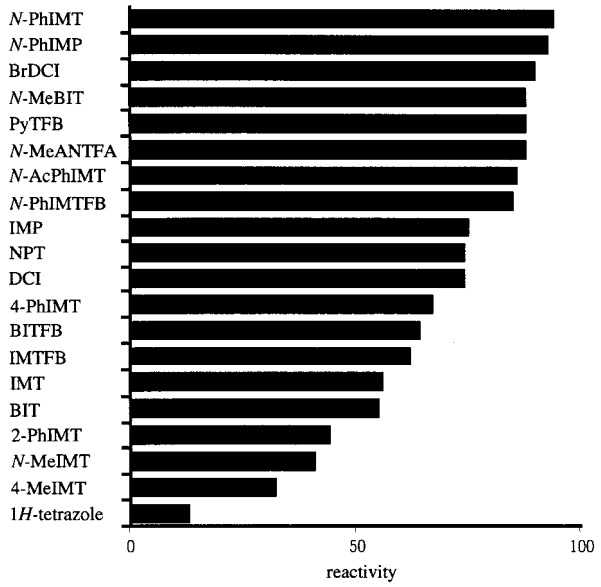

Figure 1. Reactivity of promoters estimated by yield of the product **15** (^{31}P NMR assay) obtained by the condensation of **4** and **11** with equimolar amounts (0.02 mmol) of these reactants and the promoter in a 0.02 M acetonitrile solution (25 °C, 1 min), followed by TBHP oxidation (25 °C, 5 min).

(phenyl)benzimidazolium triflate, and 2-(phenyl)benzimidazolium perchlorate. First, the reactivity of these azolium promoters was screened by the condensation of the thymidine 3′-(allyl *N*,*N*-diisopropylphosphoramidite) **4** and the 5′-*O*-free thymidine **11** in the presence of powdery molecular sieves (MS) 3A[26] at 25 °C, where the promoter, the phosphoramidite, and the nucleoside were used in 0.02 mmol amounts each in a 0.02 M acetonitrile solution. As the control experiment, condensation using 1*H*-tetrazole, NPT, PyTFA, PyTFB, *N*-MeANTFA, DCI, or BrDCI as the promoter was also carried out under similar conditions. The reaction was quenched after 1 min by the addition of a 1.0 M TBHP/toluene solution.[27] The mixture was then stirred at 25 °C for 5 min to oxidize the resulting dinucleoside phosphite to the phosphate **15**. The reactivity of promoters was evaluated on the basis of the yield of **15**, determined by the ^{31}P NMR assay. Figure 1 summarizes the reactivity of several promoters thus obtained. Among the azolium salts, *N*-PhIMT and *N*-PhIMP showed much better reactivity than existing promoters including BrDCI and PyTFB, which are among the most reactive promoters reported so far;[28] *N*-MeBIT, *N*-AcPhIMT, and *N*-PhIMTFB also demonstrated reactivity comparable to that of BrDCI or PyTFB. IMT[29] and BIT exhibited better reactivity than 1*H*-tetrazole, but the reactivities of these were lower than those of BrDCI, PyTFB, and NPT. *N*-MeIMT, which was previously reported as an efficient promoter,[30] has a low grade of reactivity among the azolium reagents.

Subsequently, we selected *N*-PhIMT, *N*-AcPhIMT, BIT, and *N*-MeBIT among the azolium promoters due to their high reactivity in the previous experiment and examined the utility of these azolium promoters for the liquid-phase preparation of dinucleoside phosphates on a larger scale and in a higher concentration of solution using the nucleoside 3′-(allyl phosphoramidite)s, **1**, **2**, **3**, and **4**, the nucleoside 3′-(2-cyanoethyl phosphoramidite)s, **5**, **6**, **7**, and **8**, and the 5′-*O*-free nucleosides, **9**, **10**, and **11**, as building blocks. The target product was prepared via the condensation of the phosphoramidite and the nucleoside by the use of these reactants and the promoter in 0.1 mmol amounts each in the presence of MS 3A in a 0.1 M acetonitrile solution at 25 °C, followed by 2-butanone peroxide oxidation.[31] In this process, the condensation of the phosphoramidite and the nucleoside was smoothly conducted by the use of every azolium promoter. Thus, the condensation using the allyl phosphoramidite was completed in 1−5 min, and the reaction using the cyanoethyl phosphoramidite required 3−10 min for completion. In both cases, the target compounds, **12**−**19**, were obtained in excellent yields after the oxidation. Further, none of the cases caused depurination in deoxyadenosine and deoxyguanosine derivatives nor cleavage of dimethoxytrityl (DMTr), allyl (All), allyloxycarbonyl (AOC), *tert*-butyldimethylsilyl (TBDMS), 2-cyanoethyl, and acyl protecting group. The results of the preparation using *N*-PhIMT as the promoter are representatively listed in Table 1.

Notable efficiency of the azolium promoters was also observed in the formation of 2′−5′ and 3′−5′ interribonucleotide linkage using 3′-*O*-(*tert*-butyldimethylsilyl)ribonucleoside 2′-(phosphoramidite)s and 2′-*O*-(*tert*-butyldimethylsilyl)ribonucleoside 3′-(phosphoramidite)s, respectively.[32] The reactivity of several azolium reagents, such as IMP, *N*-PhIMT, *N*-PhIMP, *N*-AcPhIMT, BIT, and *N*-MeBIT, was representatively examined by the condensation of **21** and **24**, conducted at 25 °C for 5 min by the use of 0.02 mmol each of **21**, **24**, and the promoter in a 0.1 M acetonitrile solution of the reactants and the promoter under conditions similar those described above for the reaction using the deoxyribonucleoside phosphoramidite. In addition, the reactivities of ETT, which is among the best invented so far

(26) We previously reported that it is necessary to use 1.2−1.5 equiv each of the nucleoside phosphoramidite and the promoter with respect to the nucleoside for obtaining satisfactory results.[25a] However, the current investigation revealed that the reaction in the presence of powdery MS 3A can be achieved by the use of only 1.0 equiv each of the nucleoside phosphoramidite, the promoter, and the nucleoside. This reaction was very cleanly performed; the molecular sieves cause no damage to the reactants, the promoter, and the nucleotide product. MS 3A may remove water contaminating the solvent and/or reactants and consequently prevent the decomposition of the moisture-sensitive phosphoramidite and/or intermediately formed reactive species. In fact, in the reaction without MS 3A, the nucleoside *H*-phosphonate resulting from hydrolysis of the phosphoramidite or the reactive intermediate was formed to some extent, thus decreasing the yield of the desired product. Details of the effect of MS 3A in the solution-phase phosphoramidite approach are now being investigated and will be reported in a separated paper in the future.

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(28) Caruthers et al. reported in ref 8b that PyTFB causes detritylation to some extent (ca. 5%) in cases using an excess amount of this promoter with respect to the 5′-*O*-dimethoxytrityl nucleoside phosphoramidite. Thus, this reagent seems to be unreliably useful for the phosphoramidite approach. However, according to our experiments, the detritylation does not take place in the reaction of 5′-*O*-dimethoxytrityl deoxyribonucleotide 3′-(allyl *N*,*N*-diisopropylphosphoramidite) or 3′-(2-cyanoethyl *N*,*N*-diisopropylphosphoramidite) and a 5′-O-free deoxyribonucleoside with a stoichiometric amount of PyTFB with respect to the phosphoramidite in the presence of MS 3A. In this manner, an excellent yield of the desired product is obtained. In addition, PyTFB can be prepared at a lower cost than the acid/azole complexes as a nonhygroscopic, stable powdery material. Therefore, PyTFB may be a very useful promoter for the solution-phase synthesis of deoxyribonucleotides via the procedure described in the present paper. However, this reagent is not useful for the solid-phase synthesis, which requires the use of an excess amount of the promoter; this case causes detritylation and related undesirable side reactions.

(29) Although IMT does not have extremely high reactivity, this reagent shows excellent chemoselectivity toward the *N*-unprotected derivatives, allowing for the facile oligodeoxyribonucleotide synthesis without nucleoside base protection (see ref 25f).

(30) According to ref 24b, *N*-MeIMT allowed for one-base elongation in an over 98% yield in the solid-phase synthesis of oligodeoxyribonucleotides via the 2-cyanoethyl-*N*,*N*-diisopropylphosphoramidite method. However, as shown Figure 1, this reagent is less reactive than related azolium reagents including *N*-PhIMT, *N*-PhIMP, *N*-MeBIT, *N*-AcPhIMT, *N*-PhIMTFB, IMT, and BIT in the solution-phase reaction carried out in our laboratory. The reactivity is also lower than some non-azolium salt-type reagents such as PyTFB, *N*-MeANTFA, and NPT.

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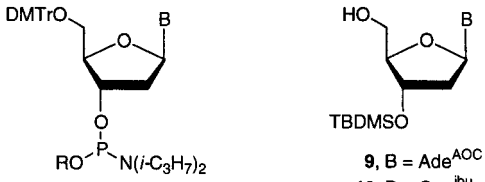
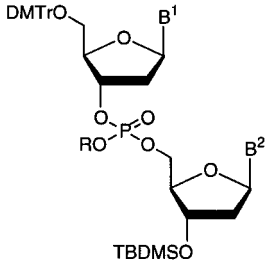
Table 1. Synthesis of Dideoxyribonucleoside Phosphates Using *N*-PhIMT as the Promoter[a]



9, B = Ade^{AOC}
10, B = Gua^{ibu}
11, B = Thy

1, B = Ade^{AOC}, R = All
2, B = Cyt^{AOC}, R = All
3, B = Gua^{All,AOC}, R = All
4, B = Thy, R = All
5, B = Ade^{Bz}, R = $NCCH_2CH_2$
6, B = Cyt^{Ac}, R = $NCCH_2CH_2$
7, B = Gua^{ibu}, R = $NCCH_2CH_2$
8, B = Thy, R = $NCCH_2CH_2$



12, B^1 = Ade^{AOC}, B^2 = Thy, R = All
13, B^1 = Cyt^{AOC}, B^2 = Ade^{AOC}, R = All
14, B^1 = Gua^{All,AOC}, B^2 = Ade^{AOC}, R = All
15, B^1 = Thy, B^2 = Thy, R = All
16, B^1 = Ade^{Bz}, B^2 = Gua^{ibu}, R = $NCCH_2CH_2$
17, B^1 = Cyt^{Ac}, B^2 = Thy, R = $NCCH_2CH_2$
18, B^1 = Gua^{ibu}, B^2 = Thy, R = $NCCH_2CH_2$
19, B^1 = Thy, B^2 = Thy, R = $NCCH_2CH_2$

amidite	nucleoside	reaction time, min	product	yield, %[b]
1	**11**	5	**12**	100, 99[c]
2	**9**	1	**13**	91
3	**9**	5	**14**	98, >95[c]
4	**11**	1	**15**	100, 99[c]
5	**10**	5	**16**	86
6	**11**	3	**17**	88
7	**11**	10	**18**	96, 89[c]
8	**11**	3	**19**	97

[a] The preparation was carried out on a 0.1-mmol scale in a 0.1 M solution of the promoter and reactants in acetonitrile. [b] Determined by ^{31}P NMR analysis unless otherwise noted. [c] Isolated yield.

for ribonucleotide synthesis, and BrDCI and PyTFB, which showed the highest reactivity among the existing promoters in the deoxyribonucleotide synthesis, were also checked in the same reaction under similar conditions, to serve as a control. The reactivity was estimated from the yield of the dinucleoside phosphate **28** obtained after TBHP oxidation of the resulting dinucleoside phosphite. The results are indicated in Figure 2. Among the promoters, *N*-PhIMT is the most reactive, completing the condensation in 5 min. *N*-MeBIT and BIT also serve as good promoters, showing similar reactivity. In comparison with these three reagents, IMP, *N*-PhIMP, and *N*-AcPhIMT are slightly less reactive. ETT, BrDCI, and PyTFB are much less reactive than all of the above, requiring longer periods for completion of the reaction. Similar results were obtained in reactions using other ribonucleoside 3′-phosphoramidites, such as **20**, **22**, and **23**, and nucleosides, such as **25** and **26**. Thus, the azolium reagent allowed for the general, efficient synthesis of diribonucleoside phosphates using stoichiometric amounts of starting materials. For example, **27**−**31** were prepared in excellent yields by the use of suitable building blocks among **20**−**26** and an azolium promoter such as *N*-PhIMT, BIT, and

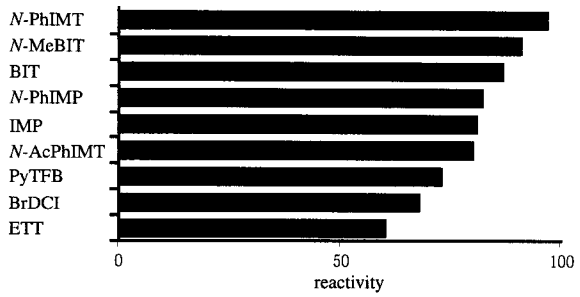
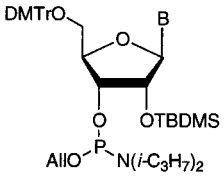
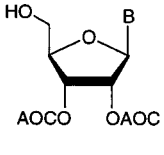


Figure 2. Reactivity of promoters estimated by yield of the product **28** (^{31}P NMR assay) obtained by the condensation of **21** and **24** by the stoichiometric (0.02 mmol each) use of these reactants and the promoter in a 0.1 M acetonitrile solution (25 °C, 5 min), followed by TBHP oxidation (25 °C, 5 min).

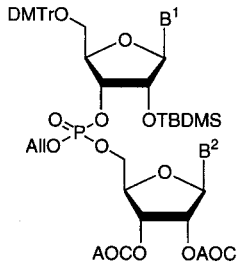
Table 2. Synthesis of Diribonucleoside Phosphates Using *N*-PhIMT as the Promoter[a]



20, B = Ade^{AOC}
21, B = Cyt^{AOC}
22, B = Gua^{All,AOC}
23, B = Ura



24, B = Ade^{AOC}
25, B = Cyt^{AOC}
26, B = Ura



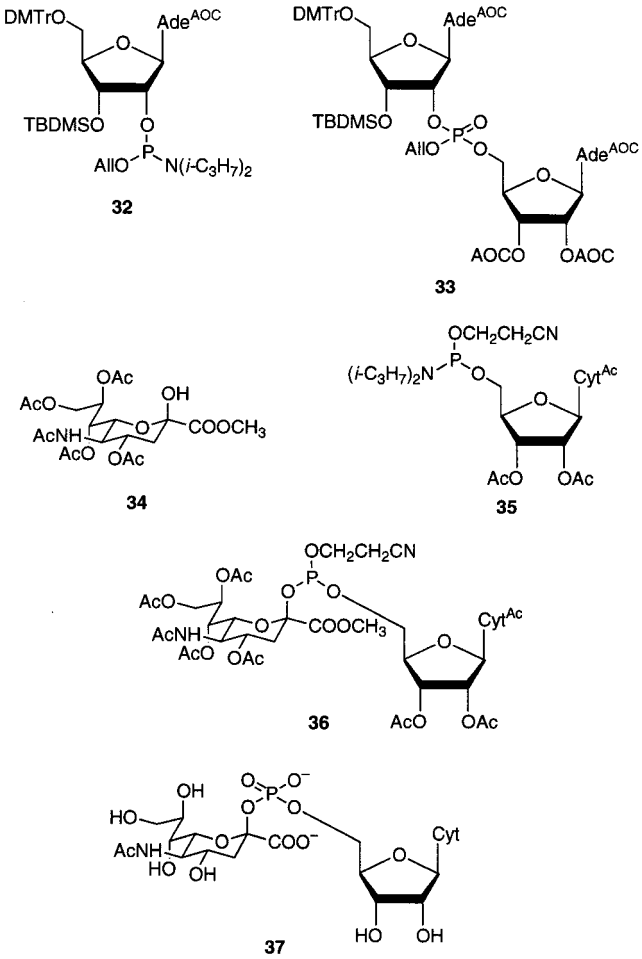
27, B^1 = Ade^{AOC}, B^2 = Ade^{AOC}
28, B^1 = Cyt^{AOC}, B^2 = Ade^{AOC}
29, B^1 = Cyt^{AOC}, B^2 = Ura
30, B^1 = Gua^{All,AOC}, B^2 = Cyt^{AOC}
31, B^1 = Ura, B^2 = Ura

amidite	nucleoside	reaction time, min	product	yield, %[b]
20	**24**	10	**27**	95
21	**24**	15	**28**	96
21	**26**	15	**29**	97
22	**25**	15	**30**	96
23	**26**	15	**31**	96

[a] The preparation was carried out on a 0.1-mmol scale. [b] Isolated yield.

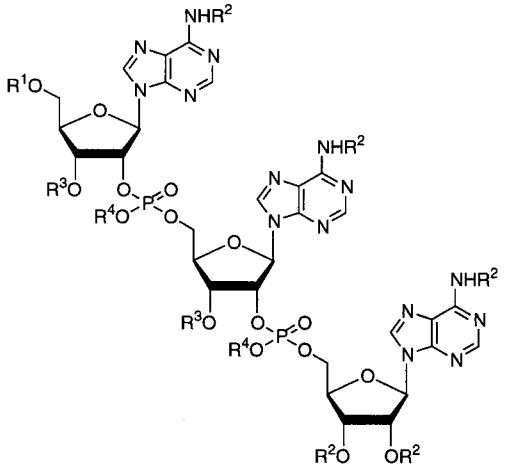
(32) Oligoribonucleotides joined by 2′−5′ linkages are an important class of substances, whose physicochemical and biochemical properties have recently attracted considerable attention. For representative reports on the synthesis and properties of 2′−5′-linked oligoribonucleotides, see: (a) Wu, T.; Ogilvie, K. K. *J. Org. Chem.* **1990**, *55*, 4717−4724. (b) Kierzek, R.; He, L.; Turner, D. H. *Nucleic Acids Res.* **1992**, *20*, 1685−1690. (c) Kandimalla, E. R.; Manning, A.; Zhao, Q.; Shaw, D. R.; Byrn, R. A.; Sasisekharan, V.; Agrawal, S. *Nucleic Acids Res.* **1997**, *25*, 370−378. (d) Wasner, M.; Arion, D.; Borkow, G.; Noronha, A.; Uddin, A. H.; Parniak, M. A.; Damha, M. J. *Biochemistry* **1998**, *37*, 7478−7486 and references therein. (e) Damha, M. J.; Noronha, A. *Nucleic Acids Res.* **1998**, *26*, 5152−5156. (f) Burlina, F.; Fourrey, J.-L.; Lefort, V.; Favre, A. *Tetrahedron Lett.* **1999**, *40*, 4559−4562.

N-MeBIT. Table 2 summarizes some examples of the preparation using *N*-PhIMT as the promoter. On the other hand, the condensation of the nucleoside **24** and the ribonucleoside 2′-phosphoramidite **32** for the formation of the 2′−5′ internucleotide linkage was achieved more slowly even with *N*-PhIMT, requiring 20 min for completion under similar conditions; the reaction, after oxidation, gave **33** in a 96% yield. In contrast, the ETT-promoted reaction of **24** and **32** was not completed for 20 min to give the desired coupling product in a 69% yield at this stage.



Application of the New Method to the Synthesis of Biologically Important Substances. The phosphoramidite approach with *N*-PhIMT was applied for the synthesis of some biologically attractive compounds, showing higher efficiency than the method using 1*H*-tetrazole. One notable example was the preparation of **36**, which is a precursor for the synthesis of cytidine-5′-monophosphono-*N*-acetylneuraminic acid (CMP-Neu5Ac) (**37**),[33,34] acting as a source of sialic acid in the sialyltransferase-catalyzed biosynthesis of sialyl oligosaccharides.[35] The compound **36** was provided via the condensation of **34** and **35**. This condensation has been previously achieved by the use of 1*H*-tetrazole as the promoter, where excess amounts of the phosphoramidite **35** and 1*H*-tetrazole with respect to the alcohol **34** are required for obtaining an acceptable yield of **36**.[34d,f] For example, it was reported that **36** was produced in an 83% yield by the reaction using 3.5 equiv each of **35** and 1*H*-tetrazole with respect to **34** (−40 to 25 °C, 30 min).[34f] In contrast, in the case using *N*-PhIMT as the promoter, **36** was obtained in a comparable yield by the use of stoichiometric amounts of **34** and **35** (25 °C, 5 min). The product **36** was converted to **37** by the reported procedure.[34f]

Another example of the utility of *N*-PhIMT for the synthesis of biologically important substances is the synthesis of adenylyl-(2′−5′)adenylyl(2′−5′)adenosine (2−5A core) (**39**).[36−38] Thus, the 5′-*O*-DMTr protecting group of the above-obtained product **33** was removed by exposure to dichloroacetic acid to provide the 5′-*O*-free derivative in a 91% yield. The 5′-*O*-free nucleoside was then reacted with **32** (1.0 equiv) with the assistance of *N*-PhIMT (1.0 equiv), and the resulting dinucleoside phosphite was oxidized by a TBHP/toluene solution (2.0 equiv) to give the fully protected 2−5A core **38**. Finally, **38** was deprotected by the standard method[39] to give the target compound **39** in about 60% overall yield from **33**. In this synthesis, the key 2′−5′ interribonucleotide linkage formation was performed to produce a higher yield by the method using *N*-PhIMT rather than by using ETT.



38, R^1 = DMTr, R^2 = AOC, R^3 = TBDMS, R^4 = All
39, $R^1 = R^2 = R^3 = R^4$ = H

Solid-Phase Synthesis of Oligodeoxyribonucleotides and Oligoribonucleotides. Some azolium salts also served as

(33) Schauer, R. *Adv. Carbohydr. Chem. Biochem.* **1982**, *40*, 131−234.

(34) For previous chemical syntheses of CMP-Neu5Ac and its analogues, see: (a) Simon, E. S.; Bednarski, M. D.; Whitesides, G. M. *J. Am. Chem. Soc.* **1988**, *110*, 7159−7163. (b) Kondo, H.; Ichikawa, Y.; Wong, C.-H. *J. Am. Chem. Soc.* **1992**, *114*, 8748−8750. (c) Martin, T. J.; Schmit, R. R. *Tetrahedron Lett.* **1993**, *34*, 1765−1768. (d) Makino, S.; Ueno, Y.; Ishikawa, M.; Hayakawa, Y.; Hata, T. *Tetrahedron Lett.* **1993**, *34*, 2775−2778. (e) Kajihara, Y.; Koseki, K.; Ebata, T.; Kodama, H.; Matsushita, H.; Hashimoto, H. *Carbohydr. Res.* **1994**, *264*, C1−C5. (f) Kajihara, Y.; Ebata, T.; Koseki, K.; Komada, H.; Matsushita, H.; Hashimoto, H. *J. Org. Chem.* **1995**, *60*, 5732−5735. (g) Chappell, M. D.; Halcomb, R. L. *Tetrahedron* **1997**, *53*, 11109−11120. (h) Kajihara, Y.; Ebata, T.; Kodama, H. *Angew. Chem., Int. Ed.* **1998**, *37*, 3166−3169. (i) Miyazaki, T.; Sakakibara, T.; Sato, H.; Kajihara, Y. *J. Am. Chem. Soc.* **1999**, *121*, 1411−1412. (j) Miyazaki, T.; Sato, H.; Sakakibara, T.; Kajihara, Y. *J. Am. Chem. Soc.* **2000**, *122*, 5678−5694. (k) Cohen, S. B.; Halcomb, R. *J. Org. Chem.* **2000**, *65*, 6145−6152.

(35) (a) Beyer, T. A.; Sadler, J. E.; Rearick, J. I.; Paulson, J. C.; Hill, R. L. *Adv. Enzymol. Relat. Areas Mol. Biol.* **1981**, *52*, 23−175. (b) Okamoto, K.; Goto, T. *Tetrahedron* **1990**, *46*, 5835−5857.

(36) (a) Roberts, W. K.; Hovanessian, A. G.; Brown, R. E.; Clemens, M. J.; Kerr, I. M. *Nature (London)* **1976**, *264*, 477−480. (b) Hovanessian, A. G.; Kerr, I. M. *Eur. J. Biochem.* **1978**, *84*, 149−159. (c) Kerr, I. M.; Brown, R. E. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 256−260. (d) Farrell, P. J.; Sen, G. C.; Dubois, M. F.; Ratner, L.; Slattery, E.; Lengyel, P. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 5893−5897. (e) Ball, L. A. *Virology* **1979**, *94*, 282−296.

(37) For the biological activities of the 2−5A core, see: (a) Williams, B. R. G.; Kerr, I. M. *Nature (London)* **1978**, *276*, 88−90. (b) Gresser, I.; Tovey, M. G. *Biochim. Biophys. Acta* **1978**, *516*, 231−247. (c) Devash, Y.; Gera, A.; Willis, D. H.; Reichman, M.; Pfleiderer, W.; Charubala, R.; Sela, I.; Suhadolnik, R. J. *J. Biol. Chem.* **1984**, *259*, 3482−3486. (d) Rhodes, C. J.; Taylor, K. W. *FEBS Lett.* **1985**, *180*, 69−73. (e) Tominaga, A.; Saito, S.; Kohno, S.; Sakurai, K.; Hayakawa, Y.; Noyori, R. *Microbiol. Immunol.* **1990**, *34*, 737−747.

efficient promoters for the solid-phase synthesis of DNA and RNA oligomers via the *O*-allyl/*N*-AOC-protected[40] and *O*-(2-cyanoethyl)/*N*-acetyl,phenoxyacetyl (PAC)-protected[41] phosphoramidite methods.

We first examined the synthesis of a DNA oligomer, $^{5'}$CGACACCCAATTCTGAAAAT$^{3'}$ (20mer) (**40**), via the allyl/AOC-protected approach using **1**, **2**, **3**, and **4** as monomer units, starting from thymidine **41** covalently attached at the 3′-hydroxyl to high-cross-linked polystyrene resins,[42] via a long-chain alkylamine spacer arm.[43] The synthesis was conducted on a 0.2-μmol scale. In this synthesis, selection of the promoter was very important for obtaining desirable results. For example, according to the trityl assay, chain elongation with BIT was performed to achieve an average yield of 99.5% (overall yield, 91.7%). In contrast, the average coupling yield in the elongation using the promoter *N*-PhIMT or *N*-MeBIT in place of BIT exceeded 100% and indicated the occurrence of some side reactions.[44] After the chain elongation, allylic protecting groups were removed on the solid supports by the $Pd_2[(C_6H_5CH{=}CH)_2CO]_3{\cdot}CHCl_3$-mediated reaction,[45] and then the target product **40** was detached from the supports by exposure to concentrated ammonia. The synthesis of **40**, according to the cyanoethyl/acetyl,PAC-protected strategy, was also investigated. The chain elongation was performed using **6**, **8**, **42**, and **43** as monomer units and *N*-PhIMT as the promoter. The average coupling yield was 99.9% (overall yield, 97.8%). The resulting product was treated with concentrated ammonia to give **40**. The structure of **40** was confirmed by MALDI time-of-flight (TOF) mass analysis (Figure 3). Capillary gel electrophoresis (CGE) (Figure 3) indicated that oligomers prepared by allyl/AOC-protected and cyanoethyl/acetyl,PAC-protected approaches have excellent purity, even in their crude forms.

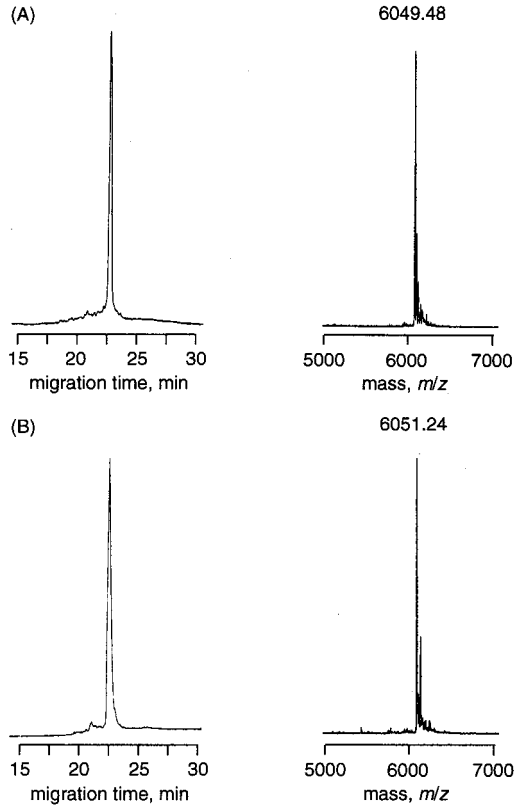
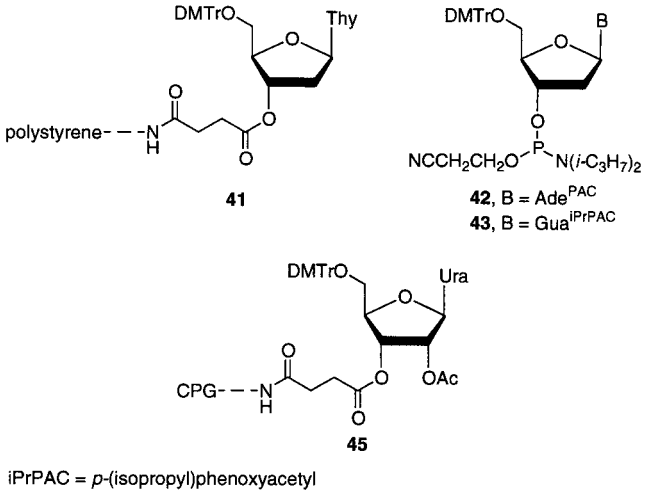


Figure 3. CGE and MALDI-TOF mass profiles of crude products of the DNA oligomer **40**. (A) Prepared via the *O*-allyl/*N*-AOC-protected method using BIT as the promoter. (B) Prepared via the *O*-cyanoethyl/*N*-acyl,PAC-protected method using *N*-PhIMT as the promoter. MALDI-TOF mass: calcd for $C_{194}H_{246}O_{114}N_{76}P_{19}$ (M + H) *m*/*z* 6052.08.



The synthesis of RNA oligomers on a solid support was efficiently achieved via the strategy using *N*-PhIMT as the promoter. First, we compared the effectiveness of *N*-PhIMT and

(38) For previously reported, representative synthesis of 2−5A-related compounds via the phosphoramidite approach, see: (a) Charubala, R.; Pfleiderer, W. *Helv. Chim. Acta* **1992**, *75*, 471−479. (b) Beigelman, L.; Matulic-Adamic, J.; Haeberli, P.; Usman, N.; Dong, B.; Silverman, R. H.; Khamnei, S.; Torrence, P. F. *Nucleic Acids Res.* **1995**, *23*, 3989−3994. (c) Hayakawa, Y.; Hirose, M.; Noyori, R. *Tetrahedron* **1995**, *51*, 9899−9916. (d) Schirmeister-Tichy, H.; Iacono, K. T.; Muto, N. F.; Homan, J. W.; Suhadolnik, R. J.; Pfleiderer, W. *Helv. Chim. Acta* **1999**, *82*, 597−613. (e) Cramer, H.; Player, M. R.; Torrence, P. F. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1049−1054.

(39) Deprotection was carried out by successive treatment with (1) dichloroacetic acid for removing the 5′-*O*-DMTr group, (2) a catalytic amount of $Pd[P(C_6H_5)_3]_4$ in the presence of $P(C_6H_5)_3$ and diethylammonium hydrogencarbonate in dichloromethane for eliminating the allyl and AOC protecting groups, and (3) tetrabutylammonium fluoride in THF for removal of the TBDMS protecting group.

(40) For the representative solid-phase synthesis of oligonucleotides and related compounds via the allyl-protected phosphoramidite method, see refs 3, 25c, and 25d. See also: (a) Matray, T. J.; Greenberg, M. M. *J. Am. Chem. Soc.* **1994**, *116*, 6931−6932. (b) Matray, T. J.; Haxton, K. J.; Greenberg, M. M. *Nucleic Acids Res.* **1995**, *23*, 4642−4648. (c) Bergmann, F.; Bannwarth, W. *Tetrahedron Lett.* **1995**, *36*, 1839−1842. (d) Greenberg, M. M.; Matray, T. J. *Biochemistry* **1997**, *46*, 14071−14079. (e) Greenberg, M. M.; Barvian, M. R.; Cook, G. P.; Goodman, B. K.; Matray, T. J.; Tronche, C.; Venkatesan, H. *J. Am. Chem. Soc.* **1997**, *119*, 1828−1839. (f) Bogdan, F. M.; Chow, C. S. *Tetrahedron Lett.* **1998**, *39*, 1897−1900. (g) Manoharan, M.; Lu, Y.; Casper, M. D.; Just, G. *Org. Lett.* **2000**, *2*, 243−246.

(41) For representative oligodeoxyribonucleotide synthesis via the PAC-protected phosphoramidite method in a solid phase, see: Schulhof, J. C.; Molko, D.; Teoule, R. *Nucleic Acids Res.* **1987**, *15*, 397−416. See also ref 25f.

(42) In the case of solid-phase synthesis, the choice of solid supports is rather important for obtaining high product yields. According to the trityl assay, the use of high-cross-linked polystyrene resins gave the best results in the synthesis of oligodeoxyribonucleotides, and the average yield of one-base elongation was generally >99.5%. By contrast, the use of controlled pore glass (pore size, 500 Å) decreased the average coupling yield to some extent (99.1% average).

(43) Efcavitch, J. W.; McBride, L. J.; Eadie, J. S. In *Biophosphates and Thier Analogues*: Bruzik, K. S., Stec, W. J., Eds.; Bioactive Molecules Series 3; Elsevier: Amsterdam, 1987; pp 65−70.

(44) According to some control experiments, treatment of a nucleoside 3′-(allyl *N*,*N*-diisopropylphosphoramidite) with *N*-PhIMT in acetonitrile generates some extremely reactive species which react with thymine base at the *N*(3) or O^4 position to give undesired products (structure of byproducts is not determined). Thus, it is conceived that coupling yields exceeding 100% arise from such byproducts formed by the use of excess amounts of the phosphoramidite and *N*-PhIMT.

(45) (a) Hayakawa, Y.; Kato, H.; Uchiyama, M.; Kajino, H.; Noyori, R. *J. Org. Chem.* **1986**, *51*, 2400−2402. (b) Hayakawa, Y.; Hirose, M.; Noyori, R. *Nucleosides Nucleotides* **1989**, *8*, 867−870. (c) Hayakawa, Y.; Wakabayashi, S.; Kato, H.; Noyori, R. *J. Am. Chem. Soc.* **1990**, *112*, 1691−1696.

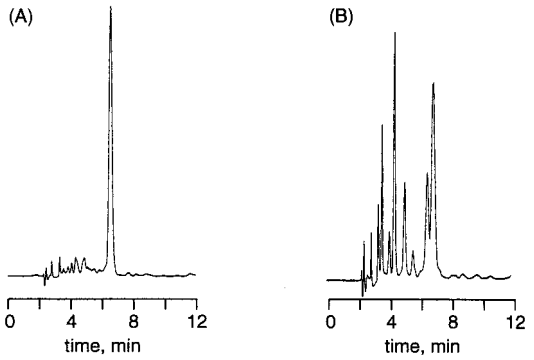


Figure 4. HPLC profiles of crude products of ACGACUACUU (**44**): (A) prepared using *N*-PhIMT as the promoter; (B) prepared using ETT as the promoter. Conditions: COSMOSIL 5C18-MS column (4.6 × 250 mm); buffer A, 5% acetonitrile−0.1 M ammonium acetate; buffer B, 25% acetonitrile−0.1 M ammonium acetate; gradient, linear 0 to 12.5% B in 30 min; detection: 260 nm; flow rate, 1.0 mL/min; temperature, 40 °C.

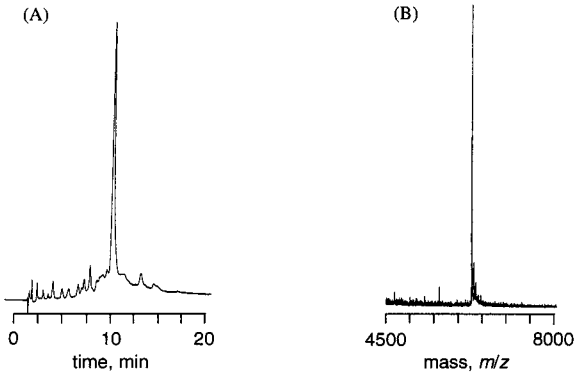
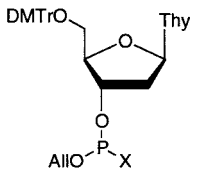


Figure 5. HPLC (A) and MALDI-TOF mass (B) profiles of crude products of AGCUACGUGACUACUACUUU (**46**). HPLC conditions: COSMOSIL 5C18-MS column (4.6 × 250 mm); buffer A, 5% acetonitrile−0.1 M ammonium acetate; buffer B, 25% acetonitrile−0.1 M ammonium acetate; gradient, linear 0 to 50% B in 30 min; detection, 260 nm; flow rate, 1.0 mL/min; temperature, 40 °C. MALDI-TOF mass: calcd for $C_{188}H_{235}O_{140}N_{69}P_{19}$ (M + H) *m*/*z* 6286.84, found *m*/*z* 6287.71.

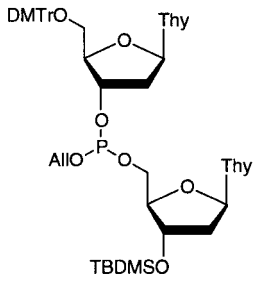
ETT in the preparation of a 10mer, $^{5'}$ACGACUACUU$^{3'}$ (**44**), on a 0.2-μmol scale with a 0.1 M acetonitrile solution of monomer units, **20**, **21**, **22**, and **23**, and a 0.1 M acetonitrile solution of the promoter.[46] Chain elongation was carried out, starting from the uridine derivative **45** anchored on controlled pore glass (CPG) supports (pore size, 500 Å).[47] The average coupling yield per elongation cycle was 99.4% in the synthesis using *N*-PhIMT; an overall coupling yield of 94.4% was achieved. On the other hand, the average and overall yields in the synthesis using ETT were 89.6% and 37.3%, respectively. These results indicate that *N*-PhIMT is much more effective than ETT. The target oligonucleotide **44** was isolated through (1) removal of allylic protectors using $Pd_2[(C_6H_5CH{=}CH)_2CO]_3{\cdot}CHCl_3$,[45] (2) detachment of the product from the solid supports using concentrated ammonia, and (3) deblocking of the silyl protecting groups of 2′-hydroxyls using triethylamine/3HF complex.[48] Figure 4 exhibits the HPLC profile of the crude products obtained via these two methods using *N*-PhIMT and ETT as the promoter. This result indicates that *N*-PhIMT-assisted synthesis affords a much purer product than does the ETT-assisted approach; these findings demonstrate the superiority of *N*-PhIMT over ETT. The method using *N*-PhIMT is also effective for the synthesis of longer oligomers. For example, $^{5'}$AGCUACGUGACUACUACUUU$^{3'}$ (**46**) (20mer) was similarly prepared in a high yield. The chain elongation was carried out with a 0.1 M acetonitrile solution of the phosphoramidite building blocks and a 0.25 M acetonitrile solution of the promoter in a 98.8% average coupling yield (overall yield, 78.8%). The purity of the fully deprotected product **46** was quite high, even in its crude form, as exhibited on the HPLC and MALDI-TOF mass profiles (Figure 5).

Explanation for the Higher Reactivity of the Azolium Promoter than 1*H*-Tetrazole. The reason that the acid/azole complex reagent shows higher reactivity than 1*H*-tetrazole may be explained as follows.

First, we carried out a typical condensation of **4** and **11** assisted by IMP in order to elucidate the mechanism of the azolium salt-promoted reaction, and the reaction profile was monitored by ^{31}P NMR measurement. Addition of an equimolar amount of IMP to a solution of **4** in CD_3CN in the absence of **11** eradicated the ^{31}P singlets due to **4** (two diastereomers) observed at δ 147.3 and 147.6 ppm (two diastereomers) within 3 min; a new broad ^{31}P singlet appeared at δ 125.8 ppm, suggesting the formation of the phosphorimidazolidite **47**. Subsequent addition of 1 equiv of **11** to this mixture instantaneously led to the disappearance of the signals due to **47**. Instead, two ^{31}P singlets appeared at δ 140.3 and 140.5 ppm, thus suggesting the formation of the dinucleoside phosphite **50** (two diastereomers). The ^{1}H NMR analysis also supported this



47, X = 1-imidazolyl
48, X = 1- or 2-tetrazolyl
49, X = 1-benzimidazolyl



50

pathway. Further, the IMP-promoted reaction of **4** and **11**, in which the reactants and the promoter were mixed together in equimolar amounts from the initial stage, was conducted in a 0.05 M solution in CD_3CN at 0 °C, and the reaction profile was monitored from the ^{31}P NMR spectrum. In this reaction, the signals due to **4** gradually decreased, and, according to the consumption of **4**, signals indicating the formation of **50** were increased; in contrast, no signals due to the phosphorimidazolidite **47** were observed at any time. These observations indicate that the consumption of **47** is faster than the formation of **47**. Thus, the rate-determining step in this reaction is the formation of **47**, i.e., the reaction of the protonated phosphoramidite and the azole. On the basis of these results, Scheme 1 is proposed as a mechanism for the condensation of a nucleoside phosphoramidite and a nucleoside using an azolium salt, AzH^+X^-

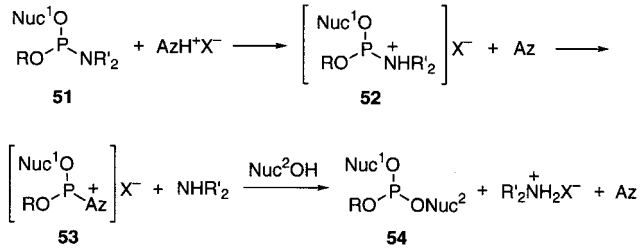
(46) To render the difference between the efficiency of *N*-PhIMT and ETT as plain as possible, the synthesis was conducted using a very low-concentration solution of the promoter. In this case, a clear result was obtained as expected, showing that *N*-PhIMT is more efficient than ETT. When a higher concentration (e.g., 0.25 M) solution of the promoter was employed, ETT also afforded a high yield of satisfactorily pure oligonucleotide.

(47) In the synthesis of oligoribonucleotides, CPG performs better than high-cross-linked polystyrene resins as the solid supports, thus providing a higher yield of the chain elongation.

(48) (a) Gasparutto, D.; Livache, T.; Bazin, H.; Duplaa, A.-M.; Guy, A.; Khorlin, A.; Molko, D.; Roget, A.; Téoule, R. *Nucleic Acids Res.* **1992**, *20*, 5159−5166. (b) Westman, E.; Strömberg, R. *Nucleic Acids Res.* **1994**, *22*, 2430−2341. (c) Pirrung, M. C.; Fallon, L.; Lever, D. C.; Shuey, S. W. *J. Org. Chem.* **1996**, *61*, 2129−2136.

(HX = a strong acid; Az = an azole). Thus, the promoter first acts as an acid, activating the phosphoramidite **51** by protonation and forming the activated species **52**. This activation cogenerates a free azole. Subsequently, the resulting azole, but not the less reactive conjugate base X^- of the acid, reacts with **52** to form the phosphorazolidite **53**. Finally, **53** condenses with a nucleoside (NucOH) to provide the dinucleoside phosphite **54** and to regenerate the free azole.[49] In this pathway, the second step is the slowest.

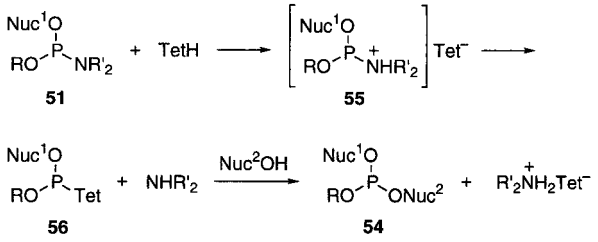
Scheme 1



In contrast, the 1*H*-tetrazole (TetH)-promoted condensation of a nucleoside phosphoramidite and a nucleoside proceeds through the mechanism illustrated in Scheme 2.[50] Thus, the 1*H*-tetrazole protonates the phosphoramidite **51** to produce **55**. Subsequently, **55** undergoes a nucleophilic attack by the tetrazolide anion that is generated in the first step; this process gives the reactive phosphorotetrazolidite **56**. Finally, **56** reacts with a nucleoside to afford **54**. In this pathway, the rate-determining step is the conversion of **55** to **56**, i.e., the reaction between the protonated phosphoramidite and the tetrazolide anion.[50a]

According to the mechanisms shown in Schemes 1 and 2, the reaction rate depends on the concentration of the protonated phosphoramidite, which is affected in both cases by the acidity of the promoter, and/or in the former and latter cases the reactivity of the azole and the tetrazolide anion, respectively, to the activated phosphoramidite. Therefore, we next attempted to elucidate the acidity of IMP, BIT, and *N*-PhIMT, as typical azolium salts, and 1*H*-tetrazole, and the nucleophilicity of benzimidazole, *N*-(phenyl)imidazole, and the tetrazolide anion. First, the acidities of these reagents were estimated by two methods. The pK_a values measured in aqueous solvents were as follows: IMP, 7.0 (H_2O); BIT, 4.5 (1:1 ethanol−H_2O); *N*-PhIMT, 6.2 (H_2O); and 1*H*-tetrazole, 4.8 (H_2O).[51] In addition, the energies of proton dissociation as calculated by ab initio methods[52] were found to be as follows: imidazole·H^+, 233.9 kcal mol^{-1}; benzimidazole·H^+, 236.2 kcal mol^{-1}; *N*-(phenyl)-

Scheme 2



imidazole·H^+, 239.7 kcal mol^{-1}; and 1*H*-tetrazole, 335.8 kcal mol^{-1}.[51] These results suggest that the acidity of azolium salts is higher than or comparable to that of 1*H*-tetrazole. Subsequently, the following examination indicated that the azole is more reactive than the tetrazolide anion toward the activated phosphoramidite. Thus, the reaction of **4** with a 1:1:1 mixture of 1*H*-tetrazole, benzimidazole,[53] and diisopropylammonium tetrazolide was carried out in acetonitrile (25 °C) and monitored from the ^{31}P NMR spectrum. In this reaction, 1*H*-tetrazole, which is more acidic than benzimidazole [pK_a 13 (H_2O)], acted as the proton-transferring agent to **4** to generate the corresponding protonated phosphoramidite and the tetrazolide anion. The activated phosphoramidite then underwent a nucleophilic attack from benzimidazole to give the phosphorobenzimidazolidite **49** (δ 129.3 and 130.4 ppm[25a]). In contrast, no formation of the phosphorotetrazolidite **48** (δ ca. 127 ppm[50a,b]), which results from the reaction between the phosphoramidite and the tetrazolide anion, was observed from the early stage to the last stage of the reaction. This result, particularly the lack of phosphorotetrazolidite production at the last stage despite the presence of a larger amount of the tetrazolide anion than benzimidazole, suggests that benzimidazole is more reactive than the tetrazolide anion toward the protonated phosphoramidite. Consequently, the azolium reagent may serve as a more reactive promoter than 1*H*-tetrazole in the phosphoramidite method.

Conclusion

We demonstrate the efficiency of certain acid salts of imidazole and benzimidazole derivatives such as *N*-PhIMT, *N*-AcPhIMT, BIT, and *N*-MeBIT as powerful promoters for synthesis of oligodeoxyribonucleotides and oligoribonucleotides via the phosphoramidite approach. The reactivity of these reagents is comparable to the reactivity of BrDCI and PyTFB and is higher than the reactivity of other existing promoters in the solution-phase deoxyribonucleotide synthesis using conventional phosphoramidites such as the allyl *N*,*N*-diisopropylphosphoramidite and the 2-cyanoethyl *N*,*N*-diisopropylphosphoramidite. Among the azolium promoters, *N*-PhIMT is highly efficient, overwhelming the capacity of both ETT and PyTFB in the construction of an inter-ribonucleotide bond with low reactive 2′-*O*-(*tert*-butyldimethylsilyl)ribonucleoside 3′-(*N*,*N*-diisopropylphosphoramidite)s and 3′-*O*-(*tert*-butyldimethylsilyl)-ribonucleoside 2′-(*N*,*N*-diisopropylphosphoramidite)s. One advantage of the present method using azolium promoters is that, even in the case using such a low reactive phosphoramidite, the smooth, high-yield reaction with a 5′-*O*-free nucleoside is achieved by the use of the phosphoramidite, the nucleoside, and the promoter in an equimolar ratio in a solution phase. In

(49) Since azoles such as imidazole and benzimidazole are generally less basic than amines such as diisopropylamine generated from the phosphoramidite as the reaction progresses, it is conceivable that the azole exists mainly in the unprotonated form, thus retaining its high nucleophilicity.

(50) For the mechanism of condensation of a nucleoside phosphoramidite and a nucleoside using 1*H*-tetrazole as a promoter, see: (a) Dahl, B. H.; Nielsen, J.; Dahl, O. *Nucleic Acids Res.* **1987**, *15*, 1729−1743. (b) Barone, A. D.; Tang, J.-Y.; Caruthers, M. H. *Nucleic Acids Res.* **1984**, *12*, 4051−4061. (c) Berner, S.; Mühlegger, K.; Seliger, H. *Nucleic Acids Res.* **1989**, *17*, 853−864. For related works, see: (d) Nurminen, E. J.; Mattinen, J. K.; Lönnberg, H. *J. Chem. Soc., Perkin Trans. 2* **1998**, 1621−1628. (e) Nurminen, E. J.; Mattinen, J. K.; Lönnberg, H. *J. Chem. Soc., Perkin Trans. 2* **1999**, 2551−2556. (f) Nurminen, E. J.; Mattinen, J. K.; Lönnberg, H. *J. Chem. Soc., Perkin Trans. 2* **2000**, 2238−2240.

(51) The pK_a strongly depends on the type of solvent. Accordingly, the numerical values obtained in aqueous solvents might not be same as those obtained in acetonitrile. However, the relative strength of acids in acetonitrile could be estimated from these values. Similarly, the proton dissociation energy values would tentatively indicate the relative strengths of the acids.

(52) Geometry optimization was carried out at the B3LYP/6-31+G** level.

(53) The ^{31}P NMR chemical shift of the phosphorimidazolidite is very similar to that of the phosphorotetrazolidite as described. Thus, in the reaction using imidazole, it might be very difficult to elucidate the structure of the product, i.e., the phosphorimidazolidite or the phosphorotetrazolidite, by ^{31}P NMR analysis. Therefore, benzimidazole was used as the azole in this experiment.

contrast, the methods using existing promoters such as 1*H*-tetrazole usually require excess amounts of the promoter and the phosphoramidite with respect to the nucleoside for gaining an acceptable reaction rate and for obtaining the target products in high yields.[54] Since the nucleoside phosphoramidites are generally expensive, the use of the phosphoramidite in excess is a serious economical disadvantage with regard to large-scale synthesis. In addition, even when the reaction is quantitatively performed, the excessively used phosphoramidites cause the formation of byproducts which must then be removed; this process is particularly troublesome in the liquid-phase synthesis. It is also of benefit that the azolium promoter causes no side reactions such as detritylation and depurination. The approach using *N*-PhIMT allows efficient synthesis of some biologically important substrates, such as CMP-Neu5Ac and 2−5A core. Furthermore, BIT is useful for the solid-phase synthesis of oligodeoxyribonucleotides on high-cross-linked polystyrene resins via the allyl/allyloxycarbonyl-protected approach, and *N*-PhIMT is effective for the synthesis of oligodeoxyribonucleotides on high-cross-linked polystyrene resins via the cyanoethyl/PAC-protected method and the synthesis of oligoribonucleotides on CPG via the allyl/allyloxycarbonyl-protected approach.

Experimental Section

General Methods. Melting points (mp) are uncorrected. IR spectra were measured in KBr on a JASCO FT/IR-5300 spectrometer. UV spectra were taken in MeOH on a JASCO Ubest-55/V-550 spectrometer. NMR spectra were obtained in $CDCl_3$ unless otherwise noted on a JEOL A-400 or JEOL EX-270 instrument. The ^{1}H NMR and ^{13}C NMR chemical shifts are described as δ values in ppm relative to $(CH_3)_4Si$. ^{31}P NMR chemical shifts are reported as δ values in ppm downfield from 85% H_3PO_4. High-performance liquid chromatography (HPLC) using a COSMOSIL 5C18-MS column (ODS-5 μm, 4.6 × 250 mm) was carried out on a JASCO PU-980 chromatograph with a JASCO UV-970-absorption detector. Solid-phase syntheses were conducted on a model 392 DNA/RNA synthesizer from Applied Biosystems. Capillary gel electrophoresis (CGE) was done with Ohtsuka Electronics CAPI-3200 capillary electrophoresis systems. Time-of-flight (TOF) mass analysis was achieved on a Voyager MDE from Applied Biosystems. Nacalai Tesque silica gel 60 (neutrality, 75 μm) was used for column chromatography. Unless otherwise stated, reactions were carried out at 25 °C. Acetonitrile was distilled from CaH_2. Powdery molecular sieves 3A (MS 3A) were used after drying the commercially supplied one (Nacalai Tesque) at 200 °C for 12 h. Other organic solvents were used after simple distillation of the commercially supplied ones.

Ab Initio Calculations. All ab initio calculations were carried out using the Gaussian 98 program.[52,55]

Materials. Imidazole (Nacalai Tesque), *N*-(methyl)imidazole (Nacalai Tesque), *N*-(phenyl)imidazole (Aldrich), *N*-(*p*-acetylphenyl)-imidazole (Aldrich), 2-(phenyl)imidazole (Nacalai Tesque), 4-(methyl)-imidazole (Kishida), 4-(phenyl)imidazole (Tokyo Kasei), benzimidazole (Nacalai Tesque), *N*-(methyl)benzimidazole (Wako), 2-(methyl)benzimidazole (Nacalai Tesque), 2-(phenyl)benzimidazole (Nacalai Tesque), trifluoromethanesulfonic acid (Central Glass), *p*-toluenesulfonic acid (Aldrich), trifluoroacetic acid (Kishida), hydroperchloric acid (Wako), tetrafluoroboric acid (Aldrich), hexafluorophosphoric acid (Aldrich), N^6-(benzoyl)-5′-*O*-(*p*,*p*′-dimethoxytrityl)-2′-deoxyadenosine 3′-(2-cyanoethyl *N*,*N*-diisopropylphosphoramidite) (**5**) (Glen Research), N^4-(acetyl)-5′-*O*-(*p*,*p*′-dimethoxytrityl)-2′-deoxycytidine 3′-(2-cyanoethyl *N*,*N*-diisopropylphosphoramidite) (**6**) (Glen Research), N^2-(isobutyryl)-5′-*O*-(*p*,*p*′-dimethoxytrityl)-2′-deoxyguanosine 3′-(2-cyanoethyl *N*,*N*-diisopropylphosphoramidite) (**7**) (Glen Research), and 5′-*O*-(*p*,*p*′-dimethoxytrityl)thymidine 3′-(2-cyanoethyl *N*,*N*-diisopropylphosphoramidite) (**8**) (Glen Research), Primer Support T (**41**) (Amersham Pharmacia Biotech), N^6-(phenoxyacetyl)-5′-*O*-(*p*,*p*′-dimethoxytrityl)-2′-deoxyadenosine 3′-(2-cyanoethyl *N*,*N*-diisopropylphosphoramidite) (**42**) (Amersham Pharmacia Biotech), N^2-(*p*-isopropylphenoxyacetyl)-5′-*O*-(*p*,*p*′-dimethoxytrityl)-2′-deoxyguanosine 3′-(2-cyanoethyl *N*,*N*-diisopropylphosphoramidite) (**43**) (Amersham Pharmacia Biotech), the uridine derivative anchored on CPG (**45**) (pore size, 500 Å, Milligen or Glen Research), a 2-butanone peroxide/dimethyl phthalate solution (Kishida), and $Pd[P(C_6H_5)_3]_4$ (Aldrich) were commercially supplied. The following compounds and a solution are reported in the literature or prepared by reported methods: azolium salts imidazolium triflate,[25f] imidazolium perchlorate[56] *N*-(methyl)imidazolium triflate,[24] *N*-(phenyl)imidazolium perchlorate,[56] and benzimidazolium triflate;[25a] protected 2′-deoxyribonucleoside 3′-phosphoramidites **1**,[45c,57] **2**,[45c,57] **3**,[45c,57] and **4**;[45c] 3′-*O*-protected 2′-deoxyribonucleosides **9**,[45a] **10**,[58] and **11**;[59] dideoxyribnonucleoside phosphates **17**,[31] **18**,[31] and **19**;[25a] protected ribonucleoside 3′-phosphoramidites **20**,[57] **21**,[40f] and **22**;[57] 2′,3′-*O*-protected ribonucleosides **24**,[38c] **25**,[38c] and **26**;[38c] 2′-*O*-(*tert*-butyldimethylsilyl)-5′-*O*-(*p*,*p*′-dimethoxytrityl)uridine;[60] 3′-*O*-(*tert*-butyldimethylsilyl)-5′-*O*-(*p*,*p*′-dimethoxytrityl)-adenosine;[57] a 1.0 M *tert*-butyl hydroperoxide/toluene solution;[27,61] (allyloxy)bis(diisopropylamino)-phosphine;[45c] $Pd_2[(C_6H_5CH{=}CH)_2CO]_3{\cdot}CHCl_3$;[45c] and diethylammonium hydrogencarbonate.[45a]

A General Procedure for Preparation of Acid/Azole Complexes. An acid (34 mmol) was added over 30 min to a solution of an azole (34 mmol) in dichloromethane (25 mL), and the mixture was stirred for 30 min. The reaction mixture was diluted with diethyl ether (20 mL). The resultant precipitate was collected by filtration. Melting points and spectral data of representative new compounds are shown below.

Imidazolium tetrafluoroborate: mp 175−177 °C; IR 3133, 2976, 2843, 2623, 1580, 1418, 1304 cm^{-1}; ^{1}H NMR (CD_3OD) 7.55 (s, 2H), 8.82 (s, 1H); ^{13}C NMR (CD_3OD) 120, 135.

***N*-(Phenyl)imidazolium triflate:** mp 126−127 °C; IR 3252, 3150, 3127, 1549, 1285, 1252 cm^{-1}; ^{1}H NMR (CD_3OD) 7.58−7.67 (m, 3H), 7.71−7.74 (m, 2H), 7.76 (t, J = 1.5 Hz, 1H), 8.06 (t, J = 1.5 Hz, 1H), 9.43 (t, J = 1.5 Hz, 1H); ^{13}C NMR (CD_3OD) 120, 122, 123, 124, 131.3, 131.5, 135.6, 136.5.

***N*-(Phenyl)imidazolium tetrafluoroborate:** mp 96−98 °C; IR 3407, 3094, 2845, 2623, 1599, 1543, 1495, 1337 cm^{-1}; ^{1}H NMR (CD_3OD) 7.55−7.79 (m, 6H), 8.03 (s, 1H), 9.36 (s, 1H); ^{13}C NMR (CD_3OD) 122, 122.6, 123.5, 131.2, 131.4, 135.5, 136.4.

***N*-(*p*-Acetylphenyl)imidazolium triflate:** mp 129−131 °C; IR 3137, 2980, 2886, 2662, 1686, 1605, 1547, 1433, 1368, 1345, 1246 cm^{-1}; ^{1}H NMR (CD_3OD) 2.66 (m, 3H), 7.70−8.27 (m, 7H), 9.54 (s, 1H); ^{13}C NMR (CD_3OD) 26.9, 122, 124, 130, 132, 135.6, 139, 199.

2-(Phenyl)imidazolium triflate: mp 106−107 °C; IR 3455, 3208, 3146, 3034, 2774, 1634, 1285, 1252 cm^{-1}; ^{1}H NMR (CD_3OD) 7.64 (s, 2H), 7.61−7.71 (m, 3H), 7.92 (dd, J = 1.5, 7.8 Hz, 2H); ^{13}C NMR (CD_3OD) 120, 121, 123, 124, 128, 134, 146.

4-(Methyl)imidazolium triflate: mp 92−93 °C; IR 3152, 1632, 1462, 1258 cm^{-1}; ^{1}H NMR (CD_3OD) 2.35 (d, J = 1.0 Hz, 3H), 7.25

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(s, 1H), 8.72 (d, $J = 1.0$ Hz, 1H); ^{13}C NMR (CD_3OD) 9.6, 117, 120, 123, 131, 134.

4-(Methyl)imidazolium tosylate: mp 112−113 °C; IR 3430, 1632, 1458, 1209 cm^{-1}; ^{1}H NMR (CD_3OD) 2.33 (d, $J = 1.0$ Hz, 3H), 2.35 (s, 3H), 7.22 (d, $J = 8.3$ Hz, 2H), 7.23 (s, 1H), 7.70 (d, $J = 8.3$ Hz, 2H), 8.71 (d, $J = 1.0$ Hz, 1H); ^{13}C NMR (CD_3OD) 9.6, 21.3, 117, 127, 130, 131, 134, 143, 144.

4-(Methyl)imidazolium trifluoroacetate: mp 92−94 °C; IR 3171, 3131, 3017, 2768, 1915, 1667, 1480, 1431, 1208 cm^{-1}; ^{1}H NMR (CD_3OD) 2.35 (s, 3H), 7.24 (s, 1H), 8.72 (s, 1H); ^{13}C NMR (CD_3OD) 9.5, 117, 120, 131, 134, 163.

4-(Phenyl)imidazolium triflate: mp 168−169 °C; IR 3185, 3146, 3086, 3044, 2928, 2903, 1630, 1601, 1508, 1474, 1287, 1240 cm^{-1}; ^{1}H NMR (CD_3OD) 7.44−7.54 (m, 3H), 7.72 (d, $J = 6.8$ Hz, 2H), 7.89 (d, $J = 1.0$ Hz, 1H), 8.97 (d, $J = 1.0$ Hz, 1H); ^{13}C NMR (CD_3OD) 116, 120, 123, 127, 128, 130.5, 130.9, 135, 136.

4-(Phenyl)imidazolium trifluoroacetate: mp 111−113 °C; IR 3426, 3167, 3054, 3007, 2772, 2627, 1921, 1653, 1483, 1433, 1271 cm^{-1}; ^{1}H NMR (CD_3OD) 7.43−7.52 (m, 3H), 7.72 (d, $J = 7.2$ Hz, 2H), 7.87 (s, 1H), 8.94 (s, 1H); ^{13}C NMR (CD_3OD) 116, 120, 127, 128, 130, 131, 135, 136, 163.

Benzimidazolium tetrafluoroborate: mp 168−170 °C; IR 3418, 3003, 2967, 2363, 1618, 1528, 1449, 1377, 1233 cm^{-1}; ^{1}H NMR (CD_3OD) 7.61 (dd, $J = 3.6$, 6.4 Hz, 2H), 7.82 (dd, $J = 3.6$, 6.4 Hz, 2H), 9.29 (d, $J = 12.4$ Hz, 1H); ^{13}C NMR (CD_3OD) 115, 128, 132, 141.

***N*-(Methyl)benzimidazolium triflate:** mp 129−130 °C; IR 3079, 1562, 1468, 1449, 1294, 1237 cm^{-1}; ^{1}H NMR (CD_3OD) 4.15 (s, 3H), 7.79−7.86 (m, 1H), 7.89−7.95 (m, 1H), 9.34 (s, 1H); ^{13}C NMR (CD_3OD) 33.6, 114, 116, 120, 123, 127.8, 128.2, 132, 133, 142.

2-(Phenyl)benzimidazolium triflate: mp 224−225 °C; IR 3067, 2992, 1636, 1462, 1289, 1236 cm^{-1}; ^{1}H NMR (CD_3OD) 7.63 (dd, $J = 2.9$, 6.3 Hz, 2H), 7.72−7.81 (m, 3H), 7.83 (dd, $J = 2.9$, 6.3 Hz, 2H), 8.13 (m, 2H); ^{13}C NMR (CD_3OD) 115, 120, 124, 128, 129, 131, 133, 135, 151.

2-(Phenyl)benzimidazolium perchlorate: mp 205−206 °C; IR 3424, 2961, 2724, 1630, 1460, 1375, 1265 cm^{-1}; ^{1}H NMR (CD_3OD) 7.59−7.62 (m, 2H), 7.70−7.83 (m, 5H), 8.11 (dd, $J = 6.4$, 6.8 Hz, 2H); ^{13}C NMR (CD_3OD) 115, 124, 128, 129, 131, 133, 135, 151.

Screening of the Reactivity of Promoters. A mixture of the phosphoramidite **4** (14.6 mg, 0.02 mmol), the nucleoside **11** (7.1 mg, 0.02 mmol), and MS 3A (20 mg) in dry acetonitrile (1.0 mL) was stirred for 30 min. To this mixture was added a promoter (0.02 mmol). After 1 min, the reaction was quenched by the addition of a 1.0 M toluene solution of TBHP (0.04 mL, 0.04 mmol), and stirring was continued for 5 min. Insoluble material was removed by filtration. To the solution was added a 0.1 M acetonitrile solution of triphenylphosphine oxide (200 μL) as a standard for determining the yield of the target dinucleoside phosphate. After concentration of the mixture, the residue was dissolved in $CDCl_3$ (1 mL). An aliquot of the solution was subjected to the ^{31}P NMR analysis to obtain the yield.

Preparation of Allyl [*N*^{6}-(Allyloxycarbonyl)-5′-*O*-(*p*,*p*′-dimethoxytrityl)-2′-deoxyadenylyl](3′−5′)[3′-*O*-(*tert*-butyldimethylsilyl)-thymidine] (12). A Typical Procedure for the Preparation of Dideoxyribonucleoside Phosphates by the Use of Stoichiometric Amounts of a Nucleoside 3′-Phosphoramidite, a Nucleoside, and a Promoter in a Solution Phase. A mixture of the phosphoramidite **1** (82.4 mg, 0.10 mmol), the nucleoside **11** (35.9 mg, 0.10 mmol), and MS 3A (20 mg) in dry acetonitrile (1.0 mL) was stirred for 30 min. *N*-(Phenyl)-imidazolium triflate (29.6 mg, 0.10 mmol) was then added to the mixture, which was stirred for an additional 1 min. To this mixture was added a 6.7% toluene solution of 2-butanone peroxide (0.20 mL), and stirring was continued for 5 min. Insoluble material was filtered off. Concentration of the filtrate afforded crude product, which was dissolved in dichloromethane (5 mL). The resulting solution was poured into a vigorously stirred petroleum ether (50 mL) to precipitate **12** as a colorless powder, which was collected by filtration (109 mg, 99% yield): IR 1752, 1701, 1613, 1586, 1510, 1466 cm^{-1}; UV λ_{max} 237 (ϵ 24 500), 267 nm (26 500); ^{1}H NMR 0.12 (s, 6H), 0.96 (s, 9H), 1.83 (m, 1H), 1.96 (d, $J = 14.8$ Hz, 3H), 2.24 (m, 1H), 2.33 (m, 1H), 2.87 (m, 1H), 3.17 (m, 1H), 3.49 (m, 2H), 3.83 (s, 6H), 4.08 (m, 1H), 4.30 (m, 1H), 4.36 (m, 1H), 4.50 (m, 1H), 4.66 (m, 2H), 4.82 (m, 2H), 5.32 (m, 2H), 5.40 (m, 1H), 5.45 (m, 1H), 5.51 (m, 1H), 6.03 (m, 2H), 6.33 (m, 1H), 6.53 (m, 1H), 6.85 (d, $J = 8.0$ Hz, 6H), 7.31 (m, 5H), 7.42 (d, $J = 7.6$ Hz, 4H), 7.87 (br s, 1H), 8.23 (d, $J = 6.4$ Hz, 1H), 8.71 (d, $J = 2.0$ Hz, 1H), 8.86 (br s, 2H); ^{31}P NMR −1.2, −1.1; TOF-MS of the Na salt, calcd for $C_{52}H_{60}N_7NaO_{14}PSi$ *m*/*z* 1118.41, found *m*/*z* 1119.44.

Allyl [*N*^{4}-(allyloxycarbonyl)-5′-*O*-(*p*,*p*′-dimethoxytrityl)-2′-deoxycytosylyl](3′−5′)[*N*^{6}-(allyloxycarbonyl)-3′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyadenosine] (13): IR 1750, 1665, 1615, 1584, 1508, 1466, 1400 cm^{-1}; UV λ_{max} 238 (ϵ 38 200), 267 nm (22 800); ^{1}H NMR 0.11, 0.12 (2 s, 6H), 0.91, 0.92 (2 s, 9H), 1.26 (s, 2H), 2.30 (m, 1H), 2.48 (m, 1H), 2.85 (m, 2H), 3.42 (m, 2H), 3.79, 3.80 (2 s, 6H), 4.12−4.51 (m, 6H), 4.65 (m, 1H), 4.68 (d, $J = 6.0$ Hz, 2H), 4.75 (m, 2H), 5.18−5.43 (m, 7H), 5.78−6.02 (m, 3H), 6.28 (m, 1H), 6.45 (m, 1H), 6.82−7.36 (m, 13H), 8.06 (m, 1H), 8.16 (s, 1H), 8.24 (s, 1H), 8.42, 8.54 (2 s, 1H), 8.74, 8.75 (2 s, 1H); ^{31}P NMR −1.2, −1.1; TOF-MS of the Na salt, calcd for $C_{57}H_{69}N_8NaO_{15}PSi$ *m*/*z* 1187.43, found *m*/*z* 1188.61.

Allyl [*N*^{2}-(allyloxycarbonyl)-*O*^{6}-(allyl)-5′-*O*-(*p*,*p*′-dimethoxytrityl)-2′-deoxyguanylyl](3′−5′)[*N*^{6}-(allyloxycarbonyl)-3′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyadenosine] (14): IR 1753, 1611, 1510, 1464, 1416 cm^{-1}; UV λ_{max} 238 (ϵ 28 300), 268 nm (31 000); ^{1}H NMR 0.15 (s, 6H), 0.94 (s, 9H), 2.48 (m, 1H), 2.76 (m, 1H), 2.98 (m, 2H), 3.34 (m, 1H), 3.41 (m, 1H), 3.78 (s, 6H), 4.21 (m, 2H), 4.33 (m, 1H), 4.40 (m, 1H), 4.49 (m, 1H), 4.55 (m, 1H), 4.73 (m, 6H), 5.11 (m, 2H), 5.23−5.51 (m, 8H), 5.87 (m, 1H), 5.97 (m, 2H), 6.16 (m, 1H), 6.46 (m, 2H), 6.78 (t, $J = 8.4$, 8.8 Hz, 4H), 7.26 (m, 7H), 7.36 (t, $J = 7.6$, 8.4 Hz, 2H), 7.94 (s, 1H), 8.25 (d, $J = 7.6$ Hz, 1H), 8.82 (d, $J = 7.6$ Hz, 1H); ^{31}P NMR −1.3, −1.1; TOF-MS of the Na salt, calcd for $C_{61}H_{73}N_{10}NaO_{15}PSi$ *m*/*z* 1267.47, found *m*/*z* 1268.63.

Allyl [5′-*O*-(*p*,*p*′-dimethoxytrityl)thymidylyl](3′−5′)[3′-*O*-(*tert*-butyldimethylsilyl)thymidine] (15): IR 1698, 1609, 1510, 1468 cm^{-1}; UV λ_{max} 235 (ϵ 25 900), 266 nm (20 700); ^{1}H NMR 0.07−0.09 (m, 6H), 0.88, 0.89 (2 s, 9H), 1.38 (s, 3H), 1.90 (s, 3H), 2.05−2.29 (m, 2H), 2.41 (m, 1H), 2.62 (m, 1H), 3.37 (m, 1H), 3.51 (m, 1H), 3.79 (s, 6H), 3.96−4.01 (m, 1H), 4.15−4.22 (m, 3H), 4.35−4.57 (m, 3H), 5.16−5.38 (m, 3H), 5.80−5.94 (m, 1H), 6.22 (q, $J = 6.8$, 13.6 Hz, 1H), 6.43 (m, 1H), 6.84 (d, $J = 8.8$ Hz, 4H), 7.23−7.36 (m, 8H), 7.56 (dd, $J = 9.2$, 10.4 Hz, 1H), 8.62 (br s, 2H); ^{31}P NMR −1.1; TOF-MS of the Na salt, calcd for $C_{50}H_{63}N_4NaO_{14}PSi$ *m*/*z* 1025.37, found *m*/*z* 1026.27.

2-Cyanoethyl [*N*^{6}-(benzoyl)-5′-*O*-(*p*,*p*′-dimethoxytrityl)-2′-deoxyadenylyl](3′−5′)[*N*^{2}-(isobutyryl)-3′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine] (16): IR 1686, 1611, 1510, 1462, 1402 cm^{-1}; UV λ_{max} 235 (ϵ 35 000), 279 nm (28 800); ^{1}H NMR 0.10, 0.11, 1.12 (3 s, 6H), 0.91 (s, 9H), 1.12−1.28 (m, 8H), 1.73−1.80 (m, 1H), 2.20−2.23 (m, 1H), 2.62−2.80 (m, 3H), 2.87−3.07 (m, 1H), 3.33−3.43 (m, 2H), 3.75 (s, 6H), 4.09−4.30 (m, 4H), 4.42−4.53 (m, 3H), 5.23−5.33 (m, 1H), 6.11−6.19 (m, 1H), 6.33−6.51 (m, 1H), 7.10−7.81 (m, 17H), 7.99 (t, $J = 6.8$, 7.2 Hz, 2H), 8.15, 8.21 (2 s, 1H), 8.59, 8.63 (2 s, 1H); ^{31}P NMR −2.6, −2.5; TOF-MS of the Na salt, calcd for $C_{61}H_{70}N_{11}NaO_{13}PSi$ *m*/*z* 1246.46, found *m*/*z* 1247.54.

2′-*O*-(*tert*-Butyldimethylsilyl)-5′-*O*-(*p*,*p*′-dimethoxytrityl)uridine 3′-*O*-(Allyl *N*,*N*-diisopropylphosphoramidite) (23). To a mixture of 2′-*O*-(*tert*-butyldimethylsilyl)-5′-*O*-(*p*,*p*′-dimethoxytrityl)uridine (2.54 g, 3.84 mmol), (allyloxy)bis(diisopropylamino)phosphine (1.26 g, 1.40 mL, 4.38 mmol), and MS 3A (50 mg) in dichloromethane (10 mL) was added *N*-(methyl)imidazolium triflate (888 mg, 3.82 mmol), and the resulting mixture was stirred for 90 min. Insoluble material was removed by filtration. The filtrate was diluted with dichloromethane (100 mL) and washed with an aqueous solution saturated with sodium hydrogencarbonate (10 mL) followed by brine (10 mL). The organic solution was dried and concentrated to give a viscous oil. The crude product was subjected to column chromatography on silica gel (80 g) eluted with a 1:3 mixture of ethyl acetate and hexane containing a trace amount of triethylamine to give **23** as a mixture of two diastereomers (2.92 g, 90% yield): IR 1696, 1609, 1510, 1462, 1366, 1302 cm^{-1}; UV λ_{max} 235 (ϵ 21 300), 267 nm (10 600); ^{1}H NMR 0.12 (s, 3H), 0.14 (s, 3H), 0.89, 0.91 (2 s, 9H), 1.02−1.20 (m, 12H), 3.40−3.46 (m, 1H), 3.51−3.65 (m, 3H), 3.79 (s, 6H), 3.94−4.40 (m, 5H), 4.98−5.30 (m, 3H), 5.73−6.00 (m, 2H), 6.80−6.90 (m, 4H), 7.20−7.34 (m, 9H), 7.90−8.08 (m, 2H); ^{31}P NMR 149.5, 150.1.

Preparation of Allyl [N^6-(Allyloxycarbonyl)-2′-*O*-(*tert*-butyldimethylsilyl)-5′-*O*-(*p*,*p*′-dimethoxytrityl)adenylyl](3′−5′)[N^6,2′,3′-*O*-tri(allyloxycarbonyl)adenosine] (27). A Typical Procedure for the Preparation of 2′−5′- or 3′−5′-Linked Diribonucleoside Phosphates by the Use of Equimolar Amounts of a Nucleoside 2′- or 3′-Phosphoramidite, a Nucleoside, and a Promoter in a Solution Phase. To a stirred mixture of the adenosine 3′-phosphoramidite **20** (96 mg, 0.10 mmol), the 5′-*O*-free adenosine **24** (52.0 mg, 0.10 mmol), and molecular sieves 3A (20 mg) in acetonitrile (1.0 mL) was added *N*-(phenyl)imidazolium triflate (29.4 mg, 0.10 mmol). After 10 min, to the reaction mixture was added a 1.0 M solution of TBHP in toluene (0.20 mL, 0.20 mmol), and the mixture was stirred for 5 min. The insoluble material was removed by filtration, and the filtrate was washed with ethyl acetate. The filtrate was diluted with ethyl acetate (10 mL). The resulting organic solution was washed with an aqueous sodium hydrogencarbonate-saturated solution (2 mL) followed by brine (2 mL), dried, and concentrated to give a glassy oil. This crude product was subjected to column chromatography on silica gel (20 g) using a 1:2 to 2:3 mixture of ethyl acetate and hexane as the eluent to provide the desired nucleotide **27** (132 mg, 95% yield) as an amorphous solid: IR 1759, 1613, 1510, 1466 cm^{-1}; UV λ_{max} 235 (ϵ 27 000), 267 nm (33 900); ^{1}H NMR −0.31, −0.30 (2 s, 6H), 0.08, 0.09 (2 s, 9H), 3.20−3.55 (m, 1H), 3.58−3.70 (m, 1H), 3.68, 3.73 (2 s, 6H), 4.07−4.80 (m, 15H), 4.85−5.02 (m, 1H), 5.10−5.45 (m, 11H), 5.60−6.31 (m, 8H), 6.69−6.85 (m, 4H), 7.28−7.70 (m, 9H), 8.10−8.20 (m, 4H), 8.57, 8.60 (2 s, 1H), 8.76, 8.77 (2 s, 1H); ^{31}P NMR −0.95, −0.29; TOF-MS calcd for $C_{66}H_{77}N_{10}O_{20}PSi$ *m*/*z* 1388.48, found *m*/*z* 1390.35.

Allyl [N^4-(allyloxycarbonyl)-2′-*O*-(*tert*-butyldimethylsilyl)-5′-*O*-(*p*,*p*′-dimethoxytrityl)cytosylyl](3′−5′)[N^6,2′,3′-*O*-tri(allyloxycarbonyl)adenosine] (28): IR 1759, 1676, 1613, 1508, 1466 cm^{-1}; UV λ_{max} 238 (ϵ 31 300), 266 nm (20 400); ^{1}H NMR −0.12, −0.18 (2 m, 6H), 0.84, 0.86 (2 s, 9H), 3.38−3.52 (m, 1H), 3.60−3.72 (m, 1H), 3.76, 3.78 (2 s, 6H), 4.07−4.80 (m, 14H), 4.75 (d, J = 6.0 Hz, 2H), 4.83−4.92 (m, 1H), 5.10−5.42 (m, 10H), 5.51−6.03 (m, 8H), 6.17 (d, J = 5.4 Hz, 1H), 6.70−6.88 (m, 5H), 7.17−7.48 (m, 9H), 7.99, 8.14 (2 s, 1H), 8.40 (d, J = 7.2 Hz, 1H), 8.65−8.77 (m, 1H), 8.97−9.07 (br s, 1H); ^{31}P NMR −0.81; TOF-MS of the Na salt, calcd for $C_{65}H_{77}N_8NaO_{21}PSi$ *m*/*z* 1387.46, found *m*/*z* 1388.34.

Allyl [N^4-(allyloxycarbonyl)-2′-*O*-(*tert*-butyldimethylsilyl)-5′-*O*-(*p*,*p*′-dimethoxytrityl)cytosylyl](3′−5′)[2′,3′-*O*-di(allyloxycarbonyl)-uridine] (29): IR 1757, 1698, 1628, 1559, 1508, 1460 cm^{-1}; UV λ_{max} 238 nm (ϵ 31 600); ^{1}H NMR 0.14, 0.22 (2 s, 6H), 0.88, 0.89 (2 s, 9H), 3.46−3.52 (m, 1H), 3.72−3.78 (m, 1H), 3.79, 3.80 (2 s, 6H), 3.96−4.13 (m, 1H), 4.14−4.33 (m, 2H), 4.35−4.45 (m, 2H), 4.50−4.66 (m, 9H), 4.86−4.94 (m, 1H), 5.17−5.39 (m, 10H), 5.57−5.97 (m, 7H), 6.72−6.90 (m, 5H), 7.18−7.48 (m, 9H), 8.15−8.36 (br s, 1H), 8.37−8.44 (m, 1H), 9.46−9.55 (br s, 1H); ^{31}P NMR −0.66, −0.51; TOF-MS of the Na salt, calcd for $C_{60}H_{72}N_5NaO_{21}PSi$ *m*/*z* 1280.41, found *m*/*z* 1281.14.

Allyl [N^2-(allyloxycarbonyl)-*O*^{6}-(allyl)-2′-*O*-(*tert*-butyldimethylsilyl)-5′-*O*-(*p*,*p*′-dimethoxytrityl)guanylyl](3′−5′)[N^4,2′,3′-*O*-tri(allyloxycarbonyl)cytidine] (30): IR 1759, 1672, 1609, 1557, 1510, 1462, 1416 cm^{-1}; UV λ_{max} 206 (ϵ 82 600), 238 nm (41 100); ^{1}H NMR −0.26 (s, 3H), −0.03, −0.02 (2 s, 3H), 0.71, 0.74 (2 s, 9H), 3.35−3.60 (m, 2H), 3.74, 3.76 (2 s, 6H), 4.20−4.75 (m, 15H), 5.04−5.52 (m, 18H), 5.73−6.25 (m, 8H), 6.70−6.83 (m, 4H), 7.15−7.49 (m, 9H), 7.63 (br s, 1H), 7.83 (d, J = 7.3 Hz, 1H), 8.00, 8.03 (2 s, 1H); ^{31}P NMR −0.84, −0.51; TOF-MS of the Na salt, calcd for $C_{68}H_{81}N_8NaO_{22}PSi$ *m*/*z* 1443.49, found *m*/*z* 1448.49.

Allyl [2′-*O*-*tert*-butyldimethylsilyl)-5′-*O*-(*p*,*p*′-dimethoxytrityl)uridylyl](3′−5′)[2′,3′-*O*-di(allyloxycarbonyl)uridine] (31): IR 1759, 1698, 1607, 1510, 1460 cm^{-1}; UV λ_{max} 236 nm (ϵ 30 500); ^{1}H NMR 0.17, 0.18 (2 s, 6H), 0.86, 0.87 (2 s, 9H), 3.38−3.66 (m, 2H), 3.76 (s, 6H), 4.16−4.70 (m, 11H), 4.87−4.96 (m, 1H), 5.18−5.37 (m, 10H), 5.62−5.98 (m, 6H), 6.70−6.87 (m, 4H), 7.21−7.40 (m, 9H), 7.82 (d, J = 7.2 Hz, 1H), 9.32−9.48 (m, 2H); ^{31}P NMR −0.99, −0.77; TOF-MS of the Na salt, calcd for $C_{56}H_{67}N_4NaO_{20}PSi$ *m*/*z* 1197.38, found *m*/*z* 1197.89.

N^6-(Allyloxycarbonyl)-3′-*O*-(*tert*-butyldimethylsilyl)-5′-*O*-(*p*,*p*′-dimethoxytrityl)adenosine 2′-*O*-(Allyl *N*,*N*-diisopropylphosphoramidite) (32). A mixture of 3′-*O*-(*tert*-butyldimethylsilyl)-5′-*O*-(*p*,*p*′-dimethoxytrityl)adenosine (1.00 g, 1.30 mmol), (allyloxy)bis(diisopropylamino)phosphine (0.52 mL, 0.47 g, 1.63 mmol), *N*-(methyl)-imidazolium triflate (341 mg, 1.47 mmol), and MS 3A (50 mg) in dichloromethane (3.5 mL) was stirred for 75 min. The reaction mixture was filtered, and the filtrate was diluted with dichloromethane (100 mL). The organic solution was washed with a sodium hydrogencarbonate-saturated solution (10 mL) followed by brine (10 mL), dried, and concentrated to give a viscous oil. This oil was subjected to chromatography on a silica gel (35 g) column eluted with a 1:3 mixture of ethyl acetate and hexane containing a trace amount of triethylamine to give **32** (1.06 g, 85% yield): IR 1611, 1586, 1510, 1464 cm^{-1}; UV λ_{max} 236 (ϵ 23 000), 267 nm (19 100); ^{1}H NMR 0.03, 0.04, 0.09, 0.11 (4 s, 6H), 0.85, 0.87 (2 s, 9H), 1.01−1.22 (m, 14H), 3.25−3.32 (m, 1H), 3.45−3.56 (m, 4H), 3.77, 3.78 (2 s, 6H), 3.92−4.25 (m, 2H), 4.50−4.59 (m, 1H), 4.76 (d, J = 5.6 Hz, 2H), 4.88−5.13 (m, 3H), 5.29 (dd, J = 1.0, 10.2 Hz, 1H), 5.41 (d, J = 17.1 Hz, 1H), 5.51−5.63 (m, 1H), 5.80−6.05 (m, 1H), 6.16−6.22 (m, 1H), 6.76−6.81 (m, 4H), 7.18−7.42 (m, 9H), 8.02 (s, 1H), 8.13, 8.17 (2 s, 1H), 8.65, 8.66 (2 s, 1H); ^{31}P NMR 150.2, 150.4.

Allyl [N^6-(allyloxycarbonyl)-3′-*O*-(*tert*-butyldimethylsilyl)-5′-*O*-(*p*,*p*′-dimethoxytrityl)adenylyl](2′−5′)[N^6,2′,3′-*O*-tri(allyloxycarbonyl)adenosine] (33): IR 1759, 1613, 1589, 1510, 1466 cm^{-1}; UV λ_{max} 239 (ϵ 24 000), 267 nm (30 600); ^{1}H NMR 0.79, 0.80 (2 s, 9H), 3.24−3.26 (m, 1H), 3.50−3.53 (m, 1H), 3.72 (s, 6H), 4.17−4.73 (m, 16H), 4.99−5.38 (m, 10H), 5.50−5.61 (m, 2H), 5.78−5.98 (m, 5H), 6.17−6.28 (m, 2H), 6.63−6.76 (m, 4H), 7.13−7.46 (m, 9H), 8.14−8.23 (m, 2H), 8.60−8.73 (m, 3H), 8.91 (br s, 1H); ^{31}P NMR −0.51, −0.73; TOF-MS of the Na salt, calcd for $C_{66}H_{77}N_{10}NaO_{20}PSi$ *m*/*z* 1411.47, found *m*/*z* 1412.64.

Condensation of the Pentaacetyl Neuraminic Acid Methyl Ester 34 and the Cytidine 5′-Phosphoramidite 35, Producing the Phosphite 36. To a mixture of **34** (48 mg, 0.1 mmol) and **35** (56 mg, 0.1 mmol) in the presence of molecular sieves 3A (20 mg) in acetonitrile (1.0 mL) was added *N*-(phenyl)imidazolium triflate (29 mg, 0.1 mmol). The resulting mixture was stirred for 5 min. The reaction was quenched by the addition of triethylamine (0.2 mL), and the mixture was diluted with ethyl acetate (20 mL). The insoluble powder was removed by filtration and washed with ethyl acetate (5 mL). The organic filtrate was diluted with ethyl acetate (20 mL) and washed with an aqueous sodium hydrogencarbonate-saturated solution (10 mL) followed by brine (10 mL), dried, and concentrated to give a residual oil. This material was dissolved in dichloromethane (5 mL), and the resulting solution was poured into petroleum ether (50 mL) to give a colorless precipitate. The precipitate was collected and dried in vacuo. The ^{1}H NMR spectrum indicated that the solid product (101 mg) was **36**,[34f] containing a small amount (ca. 5%) of impurities such as *N*-(phenyl)imidazole and triethylamine. Thus, the yield of **36** was ca. 95%. This compound without further purification could be converted to CMP-Neu5Ac (**37**) via the reported procedure.[34f]

Synthesis of Adenylyl(2′-5′)adenylyl(2′−5′)adenosine (2−5A Core) (39) from the 2′−5′-Linked Adenylyl Dimer 33. To a solution of **33** (1.32 g, 0.96 mmol) in dichloromethane (14 mL) was added dichloroacetic acid (1.60 mL, 2.50 g, 19 mmol), and the mixture was stirred at 0 °C for 10 min. The reaction mixture was diluted with dichloromethane (50 mL) and washed with an aqueous solution saturated with sodium hydrogencarbonate (50 mL) and brine (50 mL). After drying, the organic layer was concentrated to give an amorphous solid. This material was chromatographed on silica gel (50 g) eluted with a 1:2 acetone−hexane mixture to afford the 5′-*O*-free adenylyl dimer (947 mg, 91% yield): IR 1759, 1614, 1589, 1530, 1468, 1424 cm^{-1}; UV λ_{max} 267 nm (ϵ 32 000); ^{1}H NMR 0.90, 0.92 (2 s, 9H), 3.65−3.78 (m, 1H), 3.90−3.95 (m, 1H), 4.07−4.28 (m, 6H), 4.56−4.68 (m, 5H), 4.77−4.81 (m, 4H), 5.03−5.14 (m, 2H), 5.25−5.61 (m, 11H), 5.71−6.09 (m, 6H), 6.17−6.28 (m, 1H), 8.05−8.24 (m, 2H), 8.42−8.58 (m, 2H), 8.67−8.76 (m, 2H); ^{31}P NMR −0.77, −1.21. This product (793 mg, 0.73 mmol) was dissolved in acetonitrile (7.3 mL). To the resulting solution were successively added MS 3A (50 mg), the phosphoramidite **32** (697 mg, 0.73 mmol), and *N*-(phenyl)imidazolium triflate (215 mg, 0.73 mmol). The mixture was stirred for 40 min. To this mixture was added a 1.0 M toluene solution of TBHP (1.5 mL, 1.5 mmol), and stirring was continued for an additional 5 min. The insoluble material

was filtered off and washed with ethyl acetate. The filtrate was diluted with ethyl acetate (50 mL) and washed with an aqueous sodium hydrogencarbonate-saturated solution (10 mL) and brine (10 mL). After drying, concentration of the organic layer furnished an amorphous solid. This crude material was subjected to column chromatography on silica gel (40 g) eluted with a 1:2 to 2:3 mixture of ethyl acetate and hexane to provide an amorphous solid (1.36 g) including the fully protected adenylyl trimer **38** (a mixture of four diastereomers) as the main component and some undesired products as the minor component. The ^{1}H NMR spectral data of the main product **38** are as follows: 0.02−0.15 (m, 12H), 0.78−0.94 (m, 18H), 3.21−3.59 (m, 2H), 3.75, 3.76 (2 s, 6H), 3.92−4.81 (m, 27H), 4.92−5.59 (m, 14H), 5.60−6.10 (m, 8H), 6.13−6.31 (m, 2H), 6.72−6.81 (m, 4H), 7.10−7.41 (m, 9H), 8.16−8.35 (m, 4H), 8.42−8.56 (m, 1H), 8.62−8.76 (m, 2H), 8.89−8.93 (m, 1H), 9.10−9.16 (m, 1H). This material was used without further purification in the following reaction. To a solution of the amorphous product (1.30 g, 0.65 mmol) was added dichloroacetic acid (1.1 mL, 1.69 g, 13 mmol), and the solution was stirred at 0 °C for 10 min. The reaction mixture was diluted with dichloromethane (50 mL), washed with an aqueous sodium hydrogencarbonate-saturated solution (50 mL) and brine (50 mL), dried, and concentrated to give an amorphous solid. Silica gel (40 g) column chromatography of the product with a 1:2 acetone−hexane mixture as the eluent gave an amorphous solid (1.01 g). The product was dissolved in dichloromethane (18 mL), and to the resulting solution was added with stirring diethylammonium hydrogencarbonate (2.36 g, 17 mmol). After 5 min, to the mixture was added a solution of $Pd[P(C_6H_5)_3]_4$ (202 mg, 0.17 mmol) and triphenylphosphine (41 mg, 0.16 mmol) in dichloromethane (6 mL), and stirring was continued for an additional 1 h. The reaction mixture was diluted with dichloromethane (100 mL) and extracted with water (100 mL × 2). Concentration of the combined aqueous extracts provided a colorless powder (672 mg). This material (658 mg) was dissolved in THF (10 mL). The solution was mixed with a 1.0 M tetrabutylammonium fluoride−THF solution (11.3 mL, 11.3 mmol) and stirred for 18 h. The reaction mixture was concentrated to give a viscous oil. The oily product was diluted with a 0.01 M triethylammonium hydrogencarbonate buffer solution (100 mL) and washed with ether (30 mL × 2). The aqueous layer was concentrated to give a viscous oil, which was dissolved in ethanol and evaporated. The resulting oil was subjected to DEAE cellulose ion-exchange column chromatography (40 g, 2.4 × 22 cm, HCO_3^- form). Elution with a triethylammonium hydrogencarbonate buffer solution (pH 7.6, 0.001−0.3 M linear gradient, 1000 mL/1000 mL) afforded **39** (60% overall yield from **33** according to the UV analysis), which was identical with an authentic sample by HPLC.[62]

Solid-Phase Synthesis of Oligonucleotides. The syntheses of oligodeoxyribonucleotides and oligoribonucleotides were carried out according to the reaction cycle shown in Tables 3 and 4, respectively, using suitable phosphoramidites as monomer units. In the synthesis via the allyl/AOC-protected phosphoramidite approach, the solid-anchored product after chain elongation was treated with a mixture of $Pd_2[(C_6H_5CH{=}CH)_2CO]_3{\cdot}CHCl_3$ and $(C_6H_5)_3P$ in the presence of diethylammonium carbonate in THF at 50 °C for 60 min and then with concentrated ammonia at 25 °C for 60 min to give the target oligomer. In the synthesis according to the cyanoethyl/acetyl,PAC-protected method, the chain-elongation product was exposed to concentrated ammonia at 25 °C for 60 min and then at 65 °C for 30 min to afford the target oligomer.

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Table 3. Reaction Sequence of the Solid-Phase Synthesis of DNA Oligomers

step	operation	reagent(s)	time, min
1	washing	CH_3CN	0.4
2	detritylation	3% Cl_3CCOOH/CH_2Cl_2	1.3
3	washing	CH_3CN	0.8
4	coupling	0.1 M amidite/CH_3CN + 0.1 M promoter/CH_3CN	1.0
5	washing	CH_3CN	0.2
6	capping	Ac_2O/2,6-lutidine/THF (1:1:8) + *N*-methylimidazole/THF	0.3
7	washing	CH_3CN	0.2
8	oxidation	1.0 M *t*-C_4H_9OOH/toluene	1.0
9	washing	CH_3CN	0.6

Table 4. Reaction Sequence of the Solid-Phase Synthesis of RNA Oligomers

step	operation	reagent(s)	time, min	
1	washing	CH_3CN	0.4	
2	detritylation	3% Cl_3CCOOH/CH_2Cl_2	1.3	
3	washing	CH_3CN	0.5	
4	coupling	0.1 M amidite/CH_3CN + 0.1 or 0.25 M promoter/CH_3CN	0.2	× 2
5	wait	CH_3CN	2.0	
6	washing	CH_3CN	0.2	
7	capping	Ac_2O/2,6-lutidine/THF (1:1:8) + *N*-methylimidazole/THF	0.3	
8	oxidation	1.0 M *t*-C_4H_9OOH/toluene	1.0	
9	washing	CH_3CN	0.6	

Supporting Information Available: Characterization data including IR, ^{1}H NMR, ^{13}C NMR, and/or ^{31}P NMR spectral charts for new compounds including **12**, **13**, **14**, **15**, **16**, **23**, **27**, **28**, **29**, **30**, **31**, **32**, and **33**; acid/azole complexes examined in this work and their melting points and the solubility in acetonitrile; examples of solution-phase synthesis of deoxyribonucleoside and ribonucleoside phosphates using promoters other than *N*-PhIMT for the condensation of a nucleoside phosphoramidite and a nucleoside; the MALDI-TOF mass spectrum of the oligoribonucleotide **44** prepared by the method using *N*-PhIMT as the promoter; the ^{31}P NMR spectrum of the reaction of **4**, **11**, and IMP (1 equiv each) in a 0.05 M acetonitrile solution after 40-min treatment at 0 °C (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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