Benzimidazolium Triflate as an Efficient Promoter for Nucleotide Synthesis via the Phosphoramidite Method

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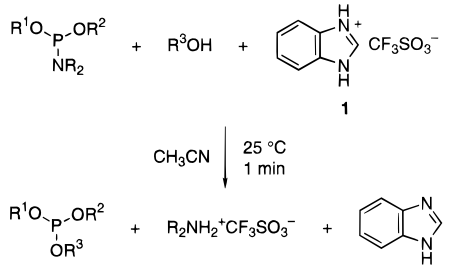
The condensation of a nucleoside phosphoramidite and a nucleoside is an essential step in oligonucleotide synthesis.[1] This reaction usually requires an azole promoter such as 1*H*-tetrazole,[2] 5-(*p*-nitrophenyl)-1*H*-tetrazole (NPT),[3] and 5-(ethylthio)-1*H*-tetrazole.[4] None of these reagents, however, is sufficiently effective for nucleoside phosphoramidites such as arylated deoxyribonucleoside *N*,*N*-diisopropylphosphoramidites or phosphoromorpholidites and sterically crowded ribonucleoside *N*,*N*-diisopropylphosphoramidites. The lack of reactivity of these amidites prevents the preparation of some oligonucleotides with modified backbones that are attractive as antisense molecules.[5] Thus, the invention of new reagents with a potent promotion ability is highly desirable. We disclose here that benzimidazolium triflate (**1**)[6] perfectly meets such a requirement (Scheme 1).

The reagent **1**, mp 188−190 °C, was quantitatively obtained by mixing benzimidazole and trifluoromethanesulfonic acid in equimolar amounts in dichloromethane at room temperature. This reagent can be stored at ambient temperature under atmosphere without decomposition. This salt has good solubility in acetonitrile, ca. 0.4 mol/L, which is comparable to that of 1*H*-tetrazole (ca. 0.5 mol/L) and higher than that of NPT (ca. 0.1 mol/L).[3]

The triflate **1** is an extremely reactive promoter, generally accomplishing the condensation of a nucleoside phosphoramidite and a nucleoside within 1 min at 25 °C, even with normally weakly reactive amidites such as arylated deoxyribonucleoside 3′-phosphoromorpholidites or sterically crowded ribonucleoside *N*,*N*-diisopropylphosphoramidites. Table 1 lists several examples of the preparation of phosphates via the condensation of the amidites, **2**−**6** and **10**−**12**, and the nucleosides, **13**−**15**, followed by *tert*-butyl hydroperoxide (TBHP) oxidation.[7] The high reactivity of **1** allowed rapid condensation of phosphoramidites with an electron-withdrawing substituent such as *o*-chlorophenyl.[8,9] The sterically crowded ribonucleoside phosphoramidites could also be used. A

Scheme 1



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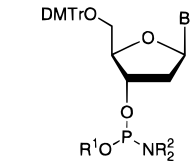
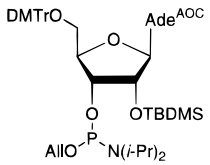
slight excess of the phosphoramidite and the promoter (1.2 equiv each) to the nucleoside (1 equiv) was employed to produce a reasonable rate of reaction.[10] The benzimidazolium reagent activated the phosphoramidite in a chemoselective manner without noticeable deterioration to effect the reaction of the substrates having an (allyloxy)carbonyl,[11] *tert*-butyldimethylsilyl, or even mono-



2, B = Ade^{Bz}; R^1 = *o*-ClC$_6$H$_4$
R^2–R^2 = O(CH$_2$CH$_2$)$_2$
3, B = Cyt^{Bz}; R^1 = *o*-ClC$_6$H$_4$
R^2–R^2 = O(CH$_2$CH$_2$)$_2$
4, B = Gua^{Ibu}; R^1 = *o*-ClC$_6$H$_4$
R^2–R^2 = O(CH$_2$CH$_2$)$_2$
5, B = Thy; R^1 = *o*-ClC$_6$H$_4$
R^2–R^2 = O(CH$_2$CH$_2$)$_2$
6, B = Thy; R^1 = CNCH$_2$CH$_2$
R^2 = *i*-Pr
7, B = Ade^{AOC}; R^1 = All
R^2 = *i*-Pr
8, B = Cyt^{AOC}; R^1 = All
R^2 = *i*-Pr
9, B = Gua^{AOC}; R^1 = All
R^2 = *i*-Pr
10, B = Thy; R^1 = All
R^2 = *i*-Pr



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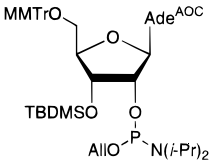

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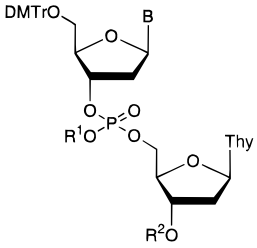
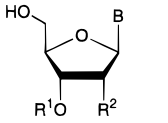


16, B = Ade^{Bz}; R^1 = *o*-ClC$_6$H$_4$
R^2 = TBDMS
17, B = Cyt^{Bz}; R^1 = *o*-ClC$_6$H$_4$
R^2 = TBDMS
18, B = Gua^{Ibu}; R^1 = *o*-ClC$_6$H$_4$
R^2 = TBDMS
19, B = Thy; R^1 = *o*-ClC$_6$H$_4$
R^2 = TBDMS
20, B = Thy; R^1 = CNCH$_2$CH$_2$
R^2 = TBDMS
21, B = Thy; R^1 = All
R^2 = AOC



13, B = Thy; R^1 = TBDMS
R^2 = H
14, B = Thy; R^1 = AOC
R^2 = H
15, B = Ade^{AOC}; R^1 = AOC
R^2 = AOCO

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(6) Pyridinium tetrafluoroborates and *N*-methylimidazolium trifluoroacetate are used as the activator for the weakly reactive amidites including the phosphorothioamidite, but these reagents cause considerable cleavage of the dimethoxytrityl protector. See: Brill, W. K.-D.; Nielsen, J.; Caruthers, M. H. *J. Am. Chem. Soc.* **1991**, *113*, 3972−3980.

(7) Hayakawa, Y.; Uchiyama, M.; Noyori, R. *Tetrahedron Lett.* **1986**, *27*, 4191−4194.

(8) Use of benzimidazolium mesylate, mp 214−215 °C, less soluble in acetonitrile (<0.1 mol/L), usually completes the reaction of *o*-chlorophenyl phosphoramidites within 45 min. Benzimidazolium tosylate, mp 222−223 °C, is scarcely soluble in acetonitrile.

(9) (a) Dahl, B. H.; Nielsen, J.; Dahl, O. *Nucleic Acids Res.* **1987**, *15*, 1729−1743. (b) Schwarz, M. W.; Pfleiderer, W. *Tetrahedron Lett.* **1984**, *25*, 5513−5516.

(10) In usual cases, the reaction requires 2−4 equiv of a promoter for gaining an acceptable reaction rate. See: Stec, W. J.; Zon, G. *Tetrahedron Lett.* **1984**, *25*, 5279−5282.

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Table 1. Synthesis of Dinucleoside Phosphates[a]

			product yield,[b,c] %		
amidite	nucleoside	product	reagent **1**	NPT	1*H*-tetrazole
2	**13**	**16**	97	71 (45)	29 (13)
3	**13**	**17**	98	72 (45)	32 (16)
4	**13**	**18**	97	68 (43)	26 (10)
5	**13**	**19**	98	72 (47)	30 (12)
6	**13**	**20**	99	98 (85)	69 (44)
10	**14**	**21**	99	99 (88)	75 (49)
11	**15**	**22**	99	88 (74)	49 (32)
12	**15**	**23**	98	87 (71)	51 (29)

[a] Condensation was carried out using amidite, nucleoside, and promoter in a 1.2:1:1.2 molar ratio in a promoter-saturated (**1**, ca. 0.4 M; NPT, ca. 0.1 M; 1*H*-tetrazole, ca. 0.5 M) acetonitrile solution at 25 °C. The resulting phosphite was directly oxidized by *tert*-butyl hydroperoxide (in acetonitrile−toluene, 25 °C, 5 min) to the phosphate. [b] Isolated yield obtained via the phosphitylation with **1**, NPT, and 1*H*-tetrazole for 1, 30, and 60 min, respectively, unless otherwise stated. [c] Yields in parentheses were obtained via 1-min condensation.

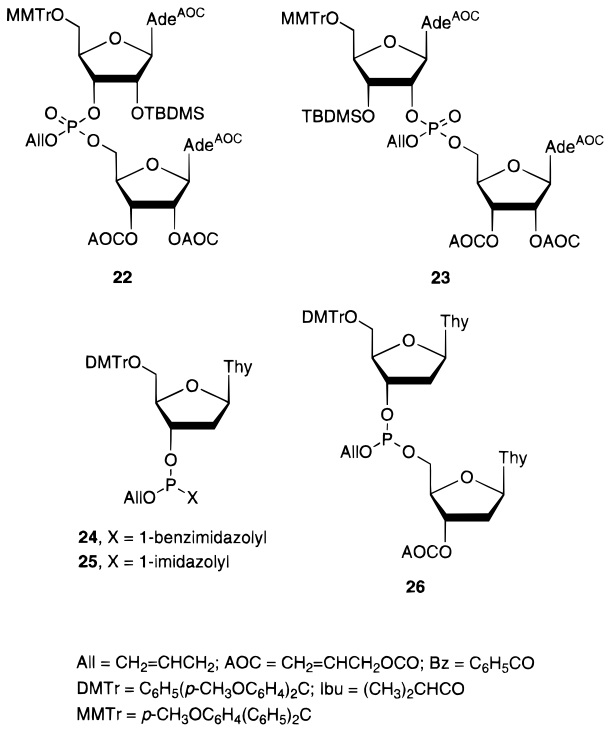
methoxy- or dimethoxytrityl protector. No depurination occurred in the reaction of the deoxyadenosine and deoxyguanosine derivatives.

The following is a typical procedure for the preparation of dinucleoside phosphates. The salt **1** (0.644 g, 2.40 mmol), the phosphoramidite **5** (1.89 g, 2.40 mmol), and the nucleoside **13** (0.713 g, 2.00 mmol) were dissolved in dry acetonitrile (6.0 mL) and the resulting solution was stirred at room temperature for 1 min. To this was added a 1.0 M toluene solution of TBHP (4.00 mL, 4.00 mmol), and stirring was continued for 5 min. The reaction mixture was diluted with ethyl acetate and washed with brine. The organic layer was concentrated to give an oil, which was chromatographed on silica gel (40 g) with a 1:5 mixture of hexane and ethyl acetate to afford **19** (2.10 g, 98% yield) as an amorphous solid.

When **5** and **13** were combined with a conventional tetrazole reagent, NPT or 1*H*-tetrazole, with similar stoichiometry and maximum promoter concentration, the reaction proceeded much slower. In the reaction using NPT, for example, the complete consumption of the amidite **5** needed ca. 60 min. The reaction with 1*H*-tetrazole did not finish even after 180 min. In these cases, the highest yields of **19** after TBHP oxidation were 72% after 30 min (NPT) and 30% after 60 min (1*H*-tetrazole), despite the remaining unreacted amidite; the longer reaction caused decomposition of the product, which decreased the yield.

Notably, the new promoter **1** can be used for the solid-phase synthesis of oligodeoxyribonucleotides. For example, d(ACGTACGTAT) was prepared in 92% overall yield (99.1% average coupling yield) according to the reported procedures[11b] using **7**−**10** as building blocks.

NMR studies revealed that the condensation of Scheme 1 proceeds via phosphorobenzimidazolidite intermediates. The reaction of equimolar amounts of the phosphoramidite **10** (diastereomers; ^{31}P singlets: δ 147.3 and 147.6 ppm) and **1** in the absence of any nucleoside in CD_3CN formed **24** within 1 min, with an ^{1}H singlet due to theH-2 of the imidazole ring at δ 8.31 ppm and two ^{31}P singlets at δ 128.9 and 130.2 ppm due to the two diastereomers (dinucleoside phosphorotetrazolidites: δ ~127 ppm[12]). Quantitative formation of diisopropylammonium triflate was proved by the ^{1}H signals at δ 3.54 (heptet) and at δ 1.36 ppm (doublet) due to $(CH_3)_2CH$ and $(CH_3)_2CH$, respectively. Addition of 1 equiv of the nucleoside **14** to this mixture instantaneously consumed **24** to give the dinucleoside phosphite **26**, displaying two ^{31}P singlets at δ 139.9 and 140.5 ppm (diastereomers) and benzimidazole showing an ^{1}H singlet at δ 8.28 ppm due to H-2. The reagent **1** satisfies the crucial requirements for an efficient promoter. It has sufficient acidity[13] to activate the phosphoramidite, whereas benzimidazole, the conjugate base, possesses a high nucleophilicity displacing the dialkylamine from the phosphorus atom, giving the benzimidazolidite intermediate. Neutral benzimidazole is too weak an acid[13] to be an activator of the amidite. Imidazolium triflate is less acidic[13] than **1**, resulting in slower condensation. For example, the complete formation of **25** (a ^{31}P singlet at δ 127.1 ppm) from **10** needed 5 min. The reaction using NPT or NPT together with benzimidazole was much slower, while a mixture of triflic acid and NPT caused various side reactions that gave none of the desired product. No reaction took place by the use of a 1*H*-tetrazole−benzimidazole mixture.



All = CH_2=$CHCH_2$; AOC = CH_2=$CHCH_2OCO$; Bz = C_6H_5CO
DMTr = $C_6H_5(p\text{-}CH_3OC_6H_4)_2C$; Ibu = $(CH_3)_2CHCO$
MMTr = $p\text{-}CH_3OC_6H_4(C_6H_5)_2C$

The benzimidazolium reagent **1** overcomes several drawbacks of the currently employed tetrazole promoters including explosiveness and harmfulness to health.[14] We highly recommend the use of **1** based on its favorable properties.

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Supporting Information Available: Characterization data for all new compounds (33 pages).

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(13) The pK_a values in aqueous solvents are as follows: NPT, 3.7 (1:1 ethanol−H_2O); benzimidazolium triflate, 4.5 (1:1 ethanol−H_2O); 1*H*-tetrazole, 4.8 (H_2O); imidazolium triflate, 6.9 (H_2O); benzimidazole, 13 (H_2O).

(14) *Sigma−Aldrich Library of Chemical Safety 2*; Sigma−Aldrich: Milwaukee, 1990; p 3313D (Sigma−Aldrich Material Safety Data Sheets; Product No. 33644-0).