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O^6-(Benzotriazol-1-yl)inosine Derivatives: Easily Synthesized, Reactive Nucleosides

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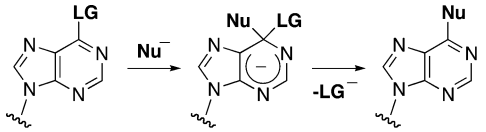
Abstract: A novel class of O^6-(benzotriazol-1-yl)inosine as well as the corresponding 2′-deoxy derivatives can be conveniently prepared by a reaction between sugar-protected or -unprotected inosine or 2′-deoxyinosine nucleosides and 1*H*-benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP). The reaction appears to proceed via a nucleoside phosphonium salt, and in the absence of any additional nucleophile, the released 1-hydroxybenzotriazole undergoes reaction with the formed phosphonium salt leading to the requisite O^6-(benzotriazol-1-yl)inosine or 2′-deoxyinosine derivatives. Isolation and characterization of the phosphonium salt as well as analysis by $^{31}P\{^1H\}$ NMR appear to be consistent with this reaction pathway. The resulting O^6-(benzotriazol-1-yl)inosine derivatives are effective as electrophilic nucleosides, undergoing facile reactions with a variety of nucleophiles such as alcohols, phenols, amines, and a thiol. Unusual and challenging nucleoside derivatives such as an aryl-bridged dimer, a nucleoside−amino acid conjugate, and a nucleoside−nucleoside dimer have also been synthesized from the O^6-(benzotriazol-1-yl)-2′-deoxyinosine derivative. Finally, a fully protected DNA building block, the O^6-(benzotriazol-1-yl)-2′-deoxyinosine 5′-*O*-DMT 3′-*O*-phosphoramidite, has been prepared and a preliminary evaluation of its use for DNA modification has been performed. Results from these studies indicate several important facts: A single, simple methodological approach provides a class of stable, isolable ribo and 2′-deoxyribonucleoside derivatives that possess excellent reactivity for S_NAr chemistry with a wide range of nucleophiles. Also, a benzotriazolyl nucleoside phosphoramidite appears to be a suitable reagent for incorporation into DNA for purposes of site-specific DNA modification.

Introduction

Convenient access to modified nucleosides continues to be of high importance due to the wide-ranging pharmacological, biochemical, and biological importance of these compounds. For nucleoside modification, electrophilic nucleoside derivatives are an important class of compounds because they serve as precursors to other nucleoside analogues via nucleophilic displacement (S_NAr) reactions (Scheme 1).[1]

For this reason, substantial research effort has been directed toward the synthesis of stable but relatively reactive nucleoside derivatives bearing leaving groups. For modification at the C-6 position of purine nucleosides, the most common electrophilic precursors are the halo derivatives,[2−5] but phenoxy,[6] aryl, and alkyl sulfonyl,[6c,7] pyridyl,[8] sulfone,[9] and imidazolyl[9,10] derivatives have all found applications. Syntheses of many of these compounds are nontrivial and can pose significant preparative challenges. Because of these reasons, recently there were two separate reports on the utility of nucleoside phosphonium salts as reactive, electrophilic derivatives. In one methodology, the C-6 amide carbonyl of inosine nucleosides was converted to the putative triphenylphosphonium salt **2** via reaction with PPh_3/

Scheme 1. Nucleoside Modification by Nucleophilic Displacement



(1) For some representative examples on the use of S_NAr chemistry over the years for nucleoside modification, please see: (a) *Nucleosides and Nucleotides*; Townsend, L. B., Ed.; Plenum Press: New York, 1988; Vol. 1, Chapter 2, pp. 113−281. (b) Gerster, J. F.; Robins, R. K. *J. Am. Chem. Soc.* **1965**, *87*, 3752−3759. (c) Lakshman, M.; Lehr, R. E. *Tetrahedron Lett.* **1990**, *31*, 1547−1550. (d) Samano, V.; Miles, R. W.; Robins, M. J. *J. Am. Chem. Soc.* **1994**, *116*, 9331−9332. (e) Kim, S. J.; Jajoo, H. K.; Kim, H.-Y.; Zhou, L.; Horton, P.; Harris, C. M.; Harris, T. M. *Bioorg. Med. Chem.* **1995**, *3*, 811−822. (f) Chaturvedi, S.; Lakshman, M. K. *Carcinogenesis* **1996**, *17*, 2747−2752. (g) Choi, Y.; George, C.; Strazewski, P.; Marquez, V. E. *Org. Lett.* **2002**, *4*, 589−592. (h) Jeong, L. S.; Jin, D. Z.; Kim, H. O.; Shin, D. H.; Moon, H. R.; Gunaga, P.; Chun, M. W.; Kim, Y.-C.; Melman, N.; Gao, Z.-G.; Jacobson, K. A. *J. Med. Chem.* **2003**, *46*, 3775−3777.

(2) Examples of chloro nucleoside synthesis: (a) Robins, M. J.; Basom, G. L. *Can. J. Chem.* **1973**, *51*, 3161−3169. (b) Robins, M. J.; Uznański, B. *Can. J. Chem.* **1981**, *59*, 2601−2607. (c) Véliz, E. A.; Beal, P. A. *Tetrahedron Lett.* **2000**, *41*, 1695−1697. (d) Francom, P.; Janeba, Z.; Shibuya, S.; Robins, M. J. *J. Org. Chem.* **2002**, *67*, 6788−6796. (e) Francom, P.; Robins, M. J. *J. Org. Chem.* **2003**, *68*, 666−669.

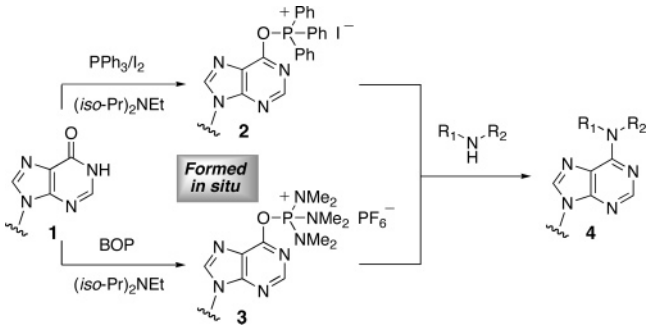
(3) Examples of bromo nucleoside synthesis: (a) Nair, V.; Richardson, S. G. *J. Org. Chem.* **1980**, *45*, 3969−3974. (b) Lakshman, M. K.; Keeler, J. C.; Hilmer, J. H.; Martin, J. Q. *J. Am. Chem. Soc.* **1999**, *121*, 6090−6091. (c) Véliz, E. A.; Beal, P. A. *J. Org. Chem.* **2001**, *66*, 8592−8598. (d) Lagisetty, P.; Russon, L. M.; Lakshman, M. K. *Angew. Chem., Int. Ed.* **2006**, *45*, 3660−3663.

(4) Examples of iodo nucleoside synthesis: (a) Cosstick, R.; Douglas, M. E. *J. Chem. Soc., Perkin Trans. 1* **1991**, 1035−1040. (b) van der Wenden, E. M.; von Frijtag Drabbe Künzel, J. K.; Mathôt, R. A. A.; Danhof, M.; IJzerman, A. P.; Soudijn, W. *J. Med. Chem.* **1995**, *38*, 4000−4006. (c) Liu, J.; Janeba, Z.; Robins, M. J. *Org. Lett.* **2004**, *6*, 2917−2919.

(5) Examples of fluoro nucleoside synthesis: ref 2a. Robins, M. J.; Uznański, B. *Can. J. Chem.* **1981**, *59*, 2608−2611.

10.1021/ja064682n CCC: $37.00

Scheme 2. In situ Formation of Nucleoside Phosphonium Salts as Electrophilic Nucleosides



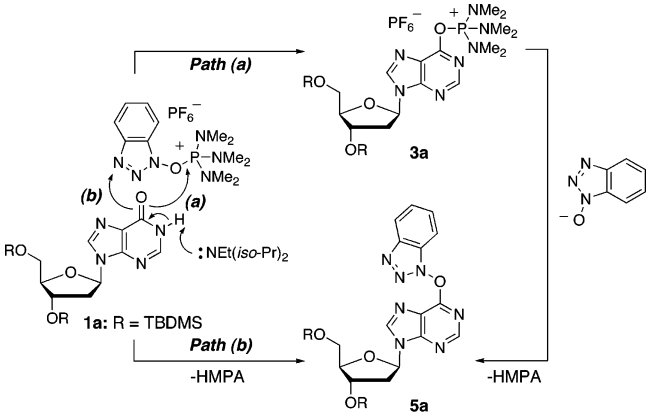
I_2/(*iso*-Pr)$_2$NEt that underwent displacement chemistry in the presence of an amine, to yield adenine derivatives.[9] In the second method, reaction of the C-6 amide carbonyl with 1*H*-benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP) and (*iso*-Pr)$_2$NEt led to the postulated formation of a tris(dimethylamino)phosphonium salt **3** that could be converted to adenine derivatives **4** with an amine.[11] Both methods were one-pot procedures that do not allow for the isolation of the phosphonium salt. Scheme 2 shows these two parallel approaches. In this context, a serendipitous discovery was reported wherein guanine residues undergo reaction with PyBOP at the C-6 position during PNA assembly, resulting in a similar phosphonium salt that then undergoes substitution by piperidine during Fmoc cleavage.[12]

It was at this point that we became interested in the reactions of inosine nucleosides with BOP, and during the course of these investigations, we have discovered the novel class of *O*6-(benzotriazol-1-yl)inosine derivatives that are effective, electrophilic nucleosides. For many applications, these new stable, isolable derivatives could replace other electrophilic nucleosides that are more difficult to access. Furthermore, these compounds can potentially be used in reactions with a wide range of nucleophiles that are perhaps not possible with methodology involving the in situ generation of phosphonium salts. This report describes the synthesis of these novel benzotriazolyl inosine and 2′-deoxyinosine derivatives, a plausible mechanism of formation, their reactions with a range of nucleophiles, and the synthesis of a suitable building block for DNA assembly and its potential use for site-specific DNA modification.

Results and Discussion

Because it had been documented in the literature that PPh_3/I_2 and BOP underwent reaction with inosine nucleosides leading to adenine derivatives in the presence of an amine, we questioned whether other nucleophiles could be utilized in this reaction. Oxygen nucleophiles were interesting, but competing reactions at the phosphorus atom in the formed phosphonium salt were something that elicited our concern. In conjunction with the possibility of introducing an oxygen nucleophile, we also wondered if a moiety could be introduced that would serve as a leaving group in subsequent S_NAr displacement reactions. It has been reported that 1-hydroxybenzotriazole (HOBT) derived adducts were observed by LC/MS in the reactions of inosine with BOP and aryl amines.[11,13] This led us initially to question the mechanism of this reaction.

Scheme 3. Two Possible Reaction Modes of Inosine Nucleosides with BOP



As shown in the example in Scheme 3, there are two possible modes by which the hypoxanthine core of inosine nucleosides could react with BOP. In pathway (a), the reaction could be initiated via attack of the amide oxygen of the nucleoside at the phosphorus atom of BOP, and alternatively in pathway (b), the reaction could occur between the amide oxygen and the ring nitrogen of BOP (S_N2'-like). In pathway (a), the intermediate would indeed be the hexamethylphosphonium salt **3a**, whereas in pathway (b), the *O*6-(benzotriazol-1-yl) product **5a** would be expected to result from expulsion of HMPA in a single step. In pathway (a), the alkoxide formed from HOBT could then displace HMPA from the hexamethylphosphonium salt **3a**, also leading to the *O*6-benzotriazol-1-yl product **5a**.

To make an assessment, two courses of action were considered: (i) ascertain whether the hexamethylphosphonium salt could be observed under short reaction periods and (ii) evaluate the course of the reaction by $^{31}P\{^1H\}$ NMR to look for intermediates and products. First, 3′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyinosine (**1a**) was allowed to react with 2 molar equiv of BOP and 1.5 molar equiv of (*iso*-Pr)$_2$NEt in dry THF at room temperature for 4 h. Workup and purification of the products by preparative TLC led to the isolation of phosphonium salt **3a**, albeit in low yield. Compound **3a** displayed a doublet for the $N(CH_3)_2$ resonances centered at δ 2.92 ppm (J_{P-H} = 11.2 Hz). In comparison, the $N(CH_3)_2$ resonance of BOP appears at δ 2.86 ppm (J_{P-H} = 10.3 Hz).[14] This confirmed the presence

(6) (a) *O*6-(Pentafluorophenyl)-2′-deoxyguanosine: Gao, H.; Fathi, R.; Gaffney, B. L.; Goswami, B.; Kung, P.-P.; Rhee, Y.; Jin, R.; Jones, R. A. *J. Org. Chem.* **1992**, *57*, 6954−6959. (b) *O*6-(4-Bromophenyl)-2′-deoxyinosine and *O*6-(phenyl)-2′-deoxyinosine: Lakshman, M. K.; Zajc, B. *Nucleosides Nucleotides* **1996**, *15*, 1029−1039. (c) *O*6-(4-Chlorophenyl)inosine: Allerson, C. R.; Chen, S. L.; Verdine, G. L. *J. Am. Chem. Soc.* **1997**, *119*, 7423−7433.

(7) (a) Hayakawa, Y.; Hirose, M.; Noyori, R. *J. Org. Chem.* **1993**, *58*, 5551−5555. (b) Nagatsugi, F.; Uemura, K.; Nakashima, S.; Maeda, M.; Sasaki, S. *Tetrahedron* **1997**, *53*, 3035−3044.

(8) (a) Adamiak, R. W.; Biała, E.; Skalski, B. *Nucleic Acids Res.* **1985**, *13*, 2989−3003. (b) Fathi, R.; Goswami, B.; Kung, P.-P.; Gaffney, B. L.; Jones, R. A. *Tetrahedron Lett.* **1990**, *31*, 319−322.

(9) Lin, X.; Robins, M. J. *Org. Lett.* **2000**, *2*, 3497−3499.

(10) Janeba, Z.; Lin, X.; Robins, M. J. *Nucleosides Nucleotides Nucleic Acids* **2004**, *23*, 137−147.

(11) Wan, Z.-K.; Binnun, E.; Wilson, D. P.; Lee, J. *Org. Lett.* **2005**, *7*, 5877−5880.

(12) Pritz, S.; Wolf, Y.; Klemm, C.; Bienert, M. *Tetrahedron Lett.* **2006**, *47*, 5893−5896.

(13) While this work was in progress, it was reported that reaction of 4-hydroxyquinazoline with BOP and DBU in CH_3CN led to the formation of 4-(benzotriazol-1-yloxy)quinazoline: Wan, Z.-K.; Wacharasindhu, S.; Binnun, E.; Mansour, T. *Org. Lett.* **2006**, *8*, 2425−2428. In reactions with the less nucleophilic aryl amine, HOBT adducts were also observed in this report.

(14) The $N(CH_3)_2$ resonance of HMPA appears at δ 2.63 ppm (J_{P-H} = 9.5 Hz), and that of HMPT appears at δ 2.47 ppm (J_{P-H} = 9.2 Hz).

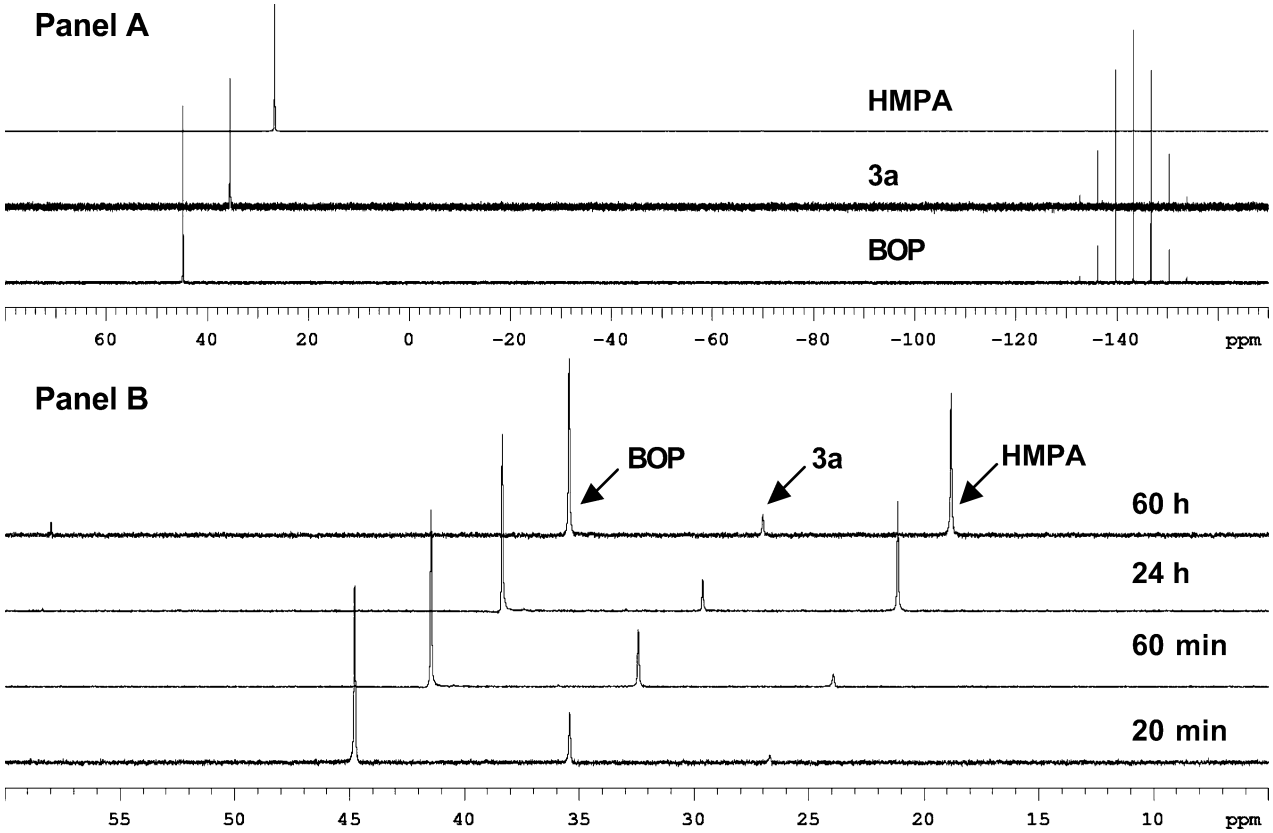
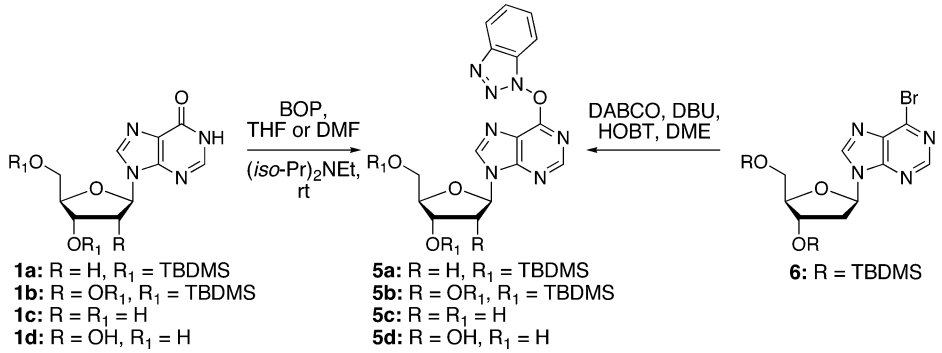


Figure 1. $^{31}P\{^1H\}$ NMR studies. Panel A: NMR spectra of BOP, phosphonium salt **3a**, and HMPA. Panel B (spectra offset to the right): time course of the reaction between **1a**, BOP, and (*iso*-Pr)$_2$NEt.

Scheme 4. Synthesis of O^6-(Benzotriazol-1-yl)inosine Derivatives



of the phosphonium salt (**3a** in Scheme 3) in the reaction mixture and indicated that pathway (a) was likely in operation.

To conduct an analysis of the reaction by $^{31}P\{^1H\}$ NMR, the ^{31}P chemical shifts of BOP, nucleoside phosphonium salt **3a**, and HMPA (Figure 1, panel A) were unambiguously ascertained. Next, the $^{31}P\{^1H\}$ NMR spectra of the reaction mixture were recorded as a function of time (Figure 1, panel B). For this, a reaction among **1a**, 2 molar equiv of BOP, and 1.5 molar equiv of (*iso*-Pr)$_2$NEt was conducted in $CDCl_3$ at room temperature in an NMR tube. As can be seen from the stacked NMR plots, at 20 min there is a clear discernible resonance corresponding to salt **3a** at δ 35.44 ppm, but the extent of formation of HMPA remains very minor. At 1 h, as the resonance from **3a** becomes more intense, the HMPA resonance continues to remain minor. Finally, at 24 h and then at 60 h, the resonance from **3a** decreases and the HMPA resonance is prominent.

These results clearly indicated that HMPA formation is not a rapid occurrence and that there is intermediacy of the phosphonium salt **3a**. Notably, even at 60 h in $CDCl_3$, a small trace of **3a** persists. With these data in hand, the next step was the determination of structure of the product in the reaction of BOP with **1a** (Scheme 4). Prolonged reaction (23 h) of **1a** with BOP and (*iso*-Pr)$_2$NEt in THF yielded a single compound in 83% isolated yield. The ^1H and ^{13}C NMR data of this product were consistent with the O^6-(benzotriazol-1-yl) derivative **5a**.

Unambiguous structural characterization of **5a** came via the reaction of bromo nucleoside **6** with HOBT under conditions we have previously utilized for the synthesis of O^6-alkyl and aryl 2′-deoxyguanosine derivatives.[15] The product derived from the reaction of **6** with HOBT was identical to that derived from

(15) Lakshman, M. K.; Ngassa, F. N.; Keeler, J. C.; Dinh, Y. Q. V.; Hilmer, J. H.; Russon, L. M. *Org. Lett.* **2000**, *2*, 927−930.

the reaction of **1a** with BOP. With a mechanistic understanding and structural proof completed, the next stage was the synthesis of other related benzotriazolyl nucleoside derivatives. Using the optimized conditions, we converted fully protected inosine to the O^6-(benzotriazol-1-yl) derivative **5b** in 80% yield. Because direct BOP-mediated aminations of unprotected nucleosides have been reported (seven examples with inosine and one with 2′-deoxyinosine),[11] it was reasoned that the hydroxyl unprotected O^6-benzotriazolyl derivatives should also be available via this procedure. Correspondingly, both **5c** and **5d** could be obtained in 57% and 53% yields, respectively (it is possible that some losses could have occurred due to water solubility). However, for solubility considerations, these two reactions were performed in DMF rather than in THF. Generally, these transformations appear to be quite scalable and have been performed on the 0.5−3 g scales.

With the syntheses of O^6-(benzotriazol-1-yl)inosine derivatives completed, the next stage was an assessment of their use as substrates for S_NAr displacement reactions. Displacement of HOBT from the C-6 position of a purine with amines has been reported in the preparation of a HOBT linker for heterocycle synthesis.[16] For the present study a range of alcohols, phenols, amines, and a thiol were selected for displacement reactions. For the synthesis of simple alkyl ethers, alcohols were chosen as the reaction medium, whereas with other nucleophiles, toluene or DME was used. The reaction temperature ranged from room temperature to 105 °C depending upon the nucleophile; Cs_2CO_3 was used as the base for all reactions except in the case of *p*-toludine as well as some reactions with morpholine and benzylamine. The results from these experiments are compiled in Table 1.

Simple nucleophiles underwent smooth coupling with nucleoside derivatives **5a**−**d**, and some observations are noteworthy. Reaction of **5a** with MeOH does not proceed at room temperature (18 h) or at 85 °C (24 h) in the absence of a base, clearly indicating that these substrates are stable to solvolysis. On the other hand, as can be anticipated, **5a** and **5b** undergo reaction with stronger nucleophiles such as morpholine and benzyl amine in the absence of base. With *p*-toluidine (entries 23−26), after testing several conditions, the use of EtOH/(*iso*-Pr)$_2$NEt proved optimal. Solvents such as DMF and DME led to little desirable product, and the combination of EtOH/Cs_2CO_3 could not be used. In the previously reported one-pot reaction of inosine with BOP and aryl amines, it has been noted, albeit in the Supporting Information, that the reactions were conducted in DMF followed by dilution with EtOH and heating.[11] Although this procedural aspect was initially not clear to us, it is now obvious that EtOH is indeed a suitable solvent for the displacement step of this reaction, and as we have demonstrated with MeOH, simple alcoholysis of the benzotriazolyl nucleoside does not occur. Also, notably, it has been reported that a trissilyl-protected 6-bromopurine nucleoside was unreactive toward *p*-toluidine and that the 6-chloro analogue was poorer reacting in S_NAr displacements compared to the bromo nucleoside.[3c] Even with morpholine, the trissilyl 6-bromopurine nucleoside was slow to react and underwent reaction only in 9 h in DME.[3c] In contrast to these results, facile reactions were attained with both **5a** and **5b** in this study, perhaps indicating the superiority of these benzotriazolyl nucleosides over the corresponding bromo and chloro analogues as substrates for displacement reactions. The unprotected nucleosides were also tested for their reactivity (entries 16, 17, 25, and 26), and although the yields are lower, possibly due to high polarity of the products, successful reactions were attained.

The reactivity of **5a** was then challenged via its use in unusual reactions. As shown in Scheme 5, we queried the reactions of **5a** with a bisphenol (hydroquinone **11**), an amino acid [(*S*)-*N*-Boc-tyrosine methyl ester **12**], and a nucleoside (3′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyinosine **1a**).

With 2.5 molar equiv each of **5a** and Cs_2CO_3, the reaction of hydroquinone (**11**) was complete within 3 h in DME at 85 °C, affording the aryl-bridged dimer **13** in 68% yield. In the reaction with (*S*)-*N*-Boc-tyrosine methyl ester (**12**), epimerization is possible under the basic reaction conditions. Therefore, the optical rotation of **12** was initially determined under various conditions (Table 2). These experiments showed that partial epimerization did occur under the reaction conditions with a greater loss of chirality occurring with Cs_2CO_3. However, no loss of chirality was experienced with $NaHCO_3$.

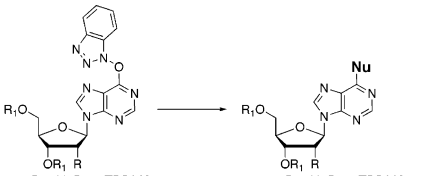
Having ascertained this, reactions of **5a** with **12** were conducted. In DME with Cs_2CO_3 at 85 °C, a 64% yield of **14** was obtained over 1 h whereas use of $NaHCO_3$ in place of Cs_2CO_3 led to a longer (24 h) reaction and a lowered 41% yield. Although the ^{1}H NMR spectrum of **14** resulting from the Cs_2CO_3-mediated reaction did not show distinct signals corresponding to two diastereomers, results in Table 2 indicate possible epimerization. On the other hand, **14** arising from the $NaHCO_3$-mediated reaction is most likely a single diastereomer. The point of this experiment was to demonstrate that unusual peptide−nucleoside conjugates can also be prepared via the method. Another observation that emerged from the reactions of **5a** with **12** was that base was needed for this reaction with a phenolic hydroxyl group as well, and no reaction was observed in the absence of a base over 17 h at 85 °C. Finally, the reaction of **5a** with **1a** was conducted in the presence of Cs_2CO_3 in DME at 85 °C, resulting in a 60% yield of the unsymmetrical dimer **15**. Use of K_3PO_4 in this reaction proved to be better, providing **15** in 92% yield. The structure of the unsymmetrical dimer was obvious from its ^{1}H NMR spectrum, where dual resonances were observed. A symmetrical dimeric structure at the O atom would have produced only a single set of resonances. Interestingly, **5a** and **5b** failed to produce any product in reactions with 3′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyadenosine.

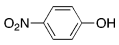
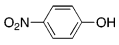
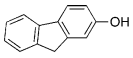
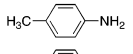
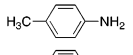
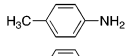
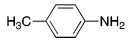
Once the displacement reactions were completed, synthesis of a suitable DNA building block bearing the O^6-(benzotriazol-1-yl) unit was assessed. For this, two options were available: (a) conversion of 5′-*O*-DMT-2′-deoxyinosine to the BOP derivative or (b) conversion of O^6-(benzotriazol-1-yl)-2′-deoxyinosine (**5c**) to the 5′-*O*-DMT derivative, followed in each case by conversion to the 3′-*O*-phosphoramidite. Alternative (a) was selected because this would also allow a test of the compatibility of the DMT protecting group with the reaction conditions leading to the benzotriazolyl nucleoside derivative. As shown in Scheme 6, reaction of the known 5′-*O*-DMT-2′-deoxyinosine **16**[17] with 2 mol equiv each of BOP and (*iso*-Pr)$_2$NEt at room temperature over 40 h led to the O^6-(benzotriazol-1-yl)-5′-*O*-DMT-2′-deoxyinosine **17** in 85% isolated yield. Finally, conver-

(16) Scicinski, J. J.; Congreve, M. S.; Jamieson, C.; Ley, S. V.; Newman, E. S.; Vinader, V. M.; Carr, R. A. E. *J. Comb. Chem.* **2001**, *3*, 387−396.

(17) Seela, F.; Kaiser, K. *Nucleic Acids Res.* **1986**, *14*, 1825−1844.

Table 1. Reactions of Nucleophiles with O^6-(Benzotriazol-1-yl)inosine Nucleosides **5a**−**d**[a]



entry	compound	nucleophile	conditions	product, yield
1	**5a**	CH_3OH	CH_3OH, Cs_2CO_3, rt, 1 h	**7a:** 77%
2	**5a**	CH_3CH_2OH	CH_3CH_2OH, Cs_2CO_3, rt, 1 h	**7b:** 95%
3	**5a**	$(CH_3)_2CHOH$	$(CH_3)_2CHOH$, Cs_2CO_3, rt, 10 h	**7c:** 84%
4	**5b**	$(CH_3)_2CHOH$	$(CH_3)_2CHOH$, Cs_2CO_3, rt, 24 h, then 82 °C, 3 h	**8a:** 70%
5	**5a**	CH_2=$CHCH_2OH$	CH_2=$CHCH_2OH$, Cs_2CO_3, rt, 2 h	**7d:** 89%
6	**5a**		PhMe, Cs_2CO_3, 105 °C, 2 h	**7e:** 86%
7	**5a**		PhMe, Cs_2CO_3, 105 °C, 20 h	**7f:** 75%
			DME, Cs_2CO_3, 85 °C, 2 h	**7f:** 78%
8	**5b**		DME, Cs_2CO_3, 85 °C, 1 h	**8b:** 81%
9	**5a**		DME, Cs_2CO_3, 85 °C, 2 h	**7g:** 82%
10	**5a**		DME, Cs_2CO_3, 85 °C, 2 h	**7h:** 81%
11	**5a**		DME, Cs_2CO_3, 85 °C, 1 h	**7i:** 87%
12	**5a**		DME, Cs_2CO_3, 85 °C, 1 h	**7j:** 79%
13	**5b**		DME, Cs_2CO_3, 85 °C, 1 h	**8c:** 72%
14	**5a**		DME, Cs_2CO_3, rt, 1 h	**7k:** 78%
			DME, rt, 1 h	**7k:** 82%
15	**5b**		DME, Cs_2CO_3, rt, 1 h	**8d:** 85%
			DME, rt, 1 h	**8d:** 90%
16	**5c**		DME, Cs_2CO_3, rt, 1 h	**9a:** 52%
17	**5d**		DME, Cs_2CO_3, rt, 1 h	**10a:** 58%
18	**5a**		DME, Cs_2CO_3, rt, 6 h	**7l:** 84%
			DME, rt, 8 h	**7l:** 75%
19	**5b**		DME, Cs_2CO_3, rt, 7 h	**8e:** 87%
			DME, rt, 9 h	**8e:** 83%
20	**5a**		DME, Cs_2CO_3, 85 °C, 4 h	**7m:** 60%
21	**5a**		DME, Cs_2CO_3, rt, 1 h	**7n:** 85%
22	**5b**		DME, Cs_2CO_3, rt, 1 h	**8f:** 93%
23	**5a**		EtOH, (*iso*-Pr)$_2$NEt, 60 °C, 24 h	**7o:** 74%
24	**5b**		EtOH, (*iso*-Pr)$_2$NEt, 60 °C, 24 h	**8g:** 96%
25	**5c**		EtOH, (*iso*-Pr)$_2$NEt, 60 °C, 24 h	**9b:** 70%
26	**5d**		EtOH, (*iso*-Pr)$_2$NEt, 60 °C, 24 h	**10b:** 56%

[a] See the Supporting Information for details on specific reactions.

Scheme 5. Reactions of **5a** with Hydroquinone, (*S*)-*N*-Boc-tyrosine Methyl Ester, and 2′-Deoxyinosine

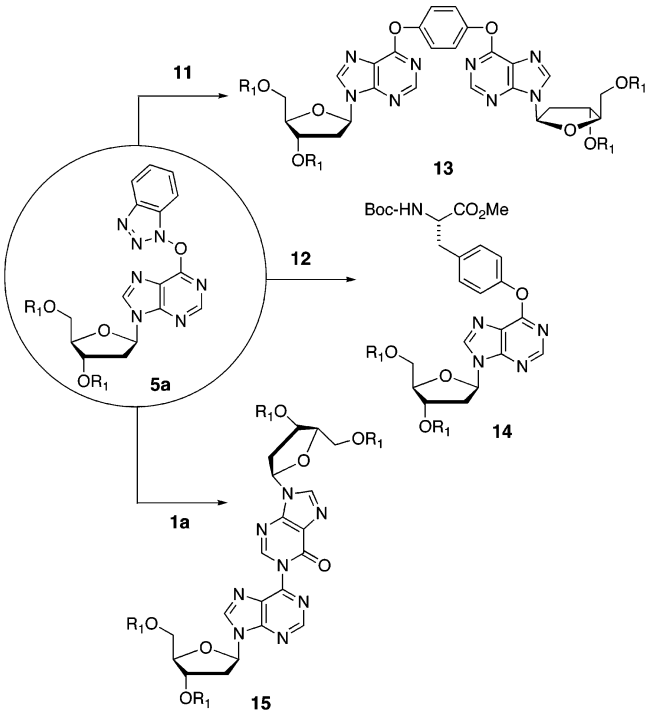


Table 2. Exposure of (*S*)-*N*-Boc-tyrosine Methyl Ester **12** to Various Conditions[a]

entry	solvent	base	temp	time	$[\alpha]_D$
1	–	–	rt	–	+5.24 (c = 0.00248, MeOH)
2	DME	–	85 °C	1 h	+5.64 (c = 0.00248, MeOH)
3	DME	Cs_2CO_3	85 °C	1 h	+1.61 (c = 0.00248, MeOH)
4	DME	K_3PO_4	85 °C	1 h	+3.63 (c = 0.00248, MeOH)
5	DME	$NaHCO_3$	85 °C	24 h	+5.24 (c = 0.00248, MeOH)

[a] Supplier-provided Certificate of Analysis reports that $[\alpha]_D = +5.20$ (c = 1, MeOH).

sion of **17** to the phosphoramidite **18** was accomplished by conventional methods (47% yield).

To demonstrate the utility of **18** for DNA modification, we have undertaken preliminary experiments to incorporate **18** into the 11-mer oligonucleotide **19** using standard phosphoramidite chemistry. The support-bound reactive DNA **19** was then exposed to a DMF solution of morpholine for 24 h at room temperature. The DNA was subjected to standard cleavage from the support and deprotection followed by LC/MS analysis. This analysis showed the presence of the desired morpholine modified DNA oligomer **20** (mass 3665.7) and the T_{10} oligomer (mass 2980.3) in a nearly 1:1 ratio indicating the coupling efficiency of **18** to be ~50%. However, it is important to note that DNA assembly was performed with only a very small amount of **18** (6 mg). Coupling efficiency can be substantially improved by altering factors such as increased concentrations of **18**, longer coupling times, etc. Most important is the fact that only the desired morpholine modified oligomer **20** was obtained in the reaction, and none of the ammonia displacement product was observed. The latter would have been formed in the ammonia cleavage step had incomplete displacement by morpholine occurred. This showed that the DNA containing the reactive nucleoside underwent conversion in essentially quantitative yield to **20**. The results from these experiments clearly indicate the suitability of phosphoramidite **18** for DNA incorporation and for displacement reactions post-assembly. Additional detailed studies on the use of **18** for DNA synthesis and post-assembly modification of DNA, as well as other applications of these modified nucleosides, are currently being considered.

In summary, we have demonstrated that a family of novel O^6-(benzotriazol-1-yl)inosine nucleosides can be synthesized in a facile and simple manner, where a unified method provides both ribo and deoxyribo derivatives. These stable, storable compounds possess excellent reactivity toward a range of nucleophiles, perhaps superior to the corresponding bromo and chloro derivatives, making them well suited for nucleoside and DNA modification. The ease of preparation of these compounds is also noteworthy from several other angles. Protection–deprotection strategies for the saccharide can be circumvented. When compared to the synthesis of reactive O^6-sulfonyl derivatives of inosine nucleosides, where competing sulfonylation at N^1 and O^6 is observed,[6c,18] reactions leading to the benzotriazolyl nucleoside derivatives occur exclusively at the O^6. This particular feature avoids loss of a valuable precursor. Finally, the method is compatible with the DMT protecting group and is well suited for the synthesis of a DNA building block that can in turn be used for DNA assembly and post-assembly DNA modification. It is anticipated that the chemistry described will lead to the commercial availability of these modified nucleosides.[19]

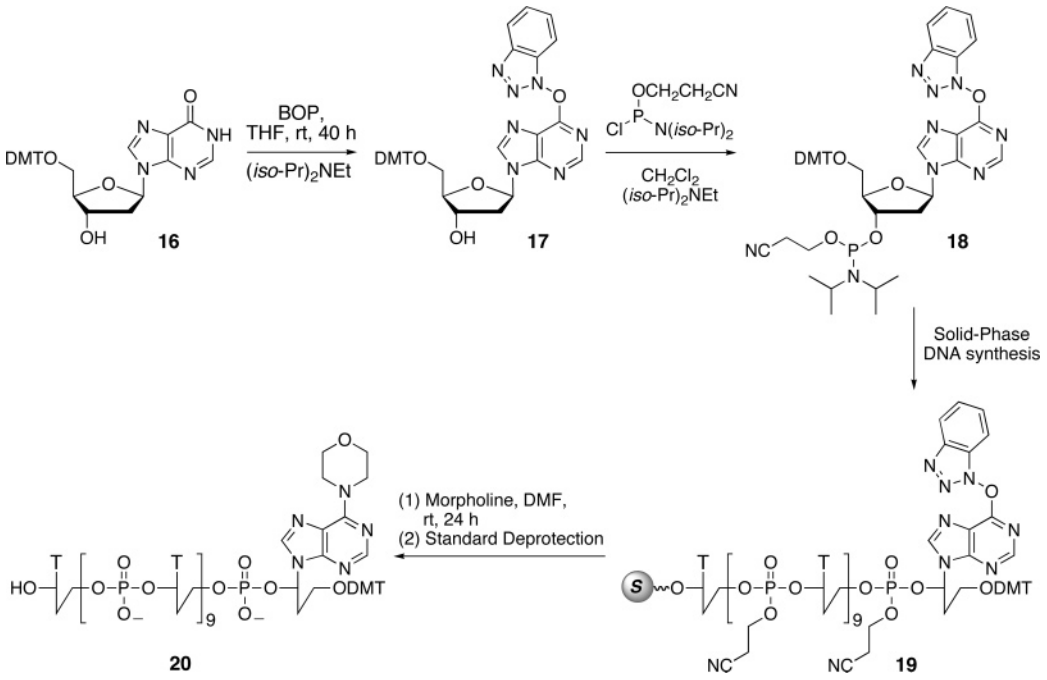
Experimental Section

Reactions were monitored by TLC (silica gel, 250 μm), and column chromatographic purifications were performed on 200–300 mesh silica gel. Solvents used for eluting the compounds, as well as TLC conditions and R_f values, are provided under individual compound headings. THF was distilled from $LiAlH_4$ and redistilled over Na when needed. CH_2Cl_2 was distilled over $CaCl_2$, and (*iso*-Pr)$_2$NEt was distilled over CaH_2. All other reagents were obtained from commercial sources and used without further purification. ^{1}H NMR spectra were recorded at 500 MHz, and ^{13}C NMR spectra were recorded at 126 MHz. All were referenced to the residual protonated solvent. $^{31}P\{^1H\}$ NMR spectra were obtained at 202 MHz and were referenced to 85% H_3PO_4 as an external standard. Chemical shifts are reported in parts per million (δ), and coupling constants are in hertz. The sugar protons are numbered 1′–5′ beginning at the anomeric carbon and proceeding via the carbon chain to the primary carbinol carbon.

9-[2-Deoxy-3,5-bis-*O*-(*tert*-butyldimethylsilyl)-β-D-*erythro*-pentofuranosyl]purin-6-yloxy-tris(dimethylamino)phosphonium Hexafluorophosphate (3a). In a clean, dry reaction vial equipped with a stirring bar were placed 3′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyinosine (**1a**) (0.100 g, 0.208 mmol) and BOP (0.184 g, 0.416 mmol). Dry THF (2.0 mL) and (*iso*-Pr)$_2$NEt (54.3 μL, 0.312 mmol) were added, and the mixture was allowed to stir at room temperature for 4 h and then evaporated. The crude material was taken up in CH_2Cl_2 and washed with water. The organic layer was separated, dried over Na_2SO_4, and concentrated. Purification by preparative thin layer chromatography (SiO_2, 5% MeOH in CH_2Cl_2) afforded ~10 mg (6% yield) of the product as a clear gum. R_f (5% MeOH in CH_2Cl_2) = 0.26. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.68 (s, 1H, Ar–H), 8.50 (s, 1H, Ar–H), 6.54 (t, 1H, H–1′, J = 6.4), 4.63 (m, 1H, H–3′), 4.06 (q, 1H, H–4′, J = 3.3), 3.89 (dd, 1H, H–5′, J = 11.2, 3.7), 3.79 (dd, 1H, H–5′, J = 11.2, 2.9), 2.66 (app quint, 1H, H–2′, J_{app} ~ 6.4), 2.52 (ddd, 1H, H–2′, J = 13.2, 5.9, 3.9), 2.92 (d, 18H, NCH_3, J_{H-P} = 11.2), 0.92, 0.90 (2s,

(18) Ferentz, A. E.; Verdine, G. L. *Nucleosides Nucleotides* **1992**, *11*, 1749–1763.
(19) Bae, S.; Lakshman, M. K. Provisional Patent Application 60/829,168, 2006.

Scheme 6. Synthesis of a DNA Building Block and Site-Specific DNA Modification



18H, *tert*-Bu), 0.11, 0.09, 0.08 (3s, 12H, $SiCH_3$). ^{13}C NMR (126 MHz, $CDCl_3$): δ 154.4, 152.7, 151.5, 144.5, 123.0, 88.4, 85.2, 71.9, 62.7, 41.4, 37.3 (d, $J_{C-P} = 4.5$), 25.9, 25.7, 18.4, 18.0, −4.7, −4.8, −5.4, −5.5. $^{31}P\{^1H\}$ NMR (202 MHz, $CDCl_3$): δ 35.44 (s, $P[N(CH_3)_2]_3$), −143.31 (septet, PF_6, $J_{P-F} = 712$).

***O*^{6}-(Benzotriazol-1-yl)-3′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyinosine (5a).** In a 50 mL round-bottom flask equipped with a stirring bar were placed 3′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyinosine (**1a**) (1.005 g, 2.090 mmol) and BOP (1.850 g, 4.181 mmol). THF (20 mL) and (*iso*-Pr)$_2$NEt (0.55 mL, 3.135 mmol) were added, and the mixture was allowed to stir at room temperature for 23 h. The mixture was evaporated, and CH_2Cl_2 (50 mL) was added. The mixture was washed with water, dried over Na_2SO_4, and concentrated. Chromatographic purification (SiO_2, elution with 20% EtOAc in hexanes) afforded 1.038 g (83% yield) of compound **5a** as a white foam. R_f (20% EtOAc in hexanes) = 0.14. ^1H NMR (500 MHz, $CDCl_3$): δ 8.54 (s, 1H, Ar−H), 8.41 (s, 1H, Ar−H), 8.14 (d, 1H, Ar−H, $J = 8.3$), 7.55−7.41 (m, 3H, Ar−H), 6.55 (t, 1H, H-1′, $J = 6.1$), 4.65 (m, 1H, H-3′), 4.06 (q, 1H, H−4′, $J = 3.1$), 3.92 (dd, 1H, H−5′, $J = 11.2$, 3.9), 3.80 (dd, 1H, H−5′, $J = 11.2$, 2.7), 2.65 (app quint, 1H, H−2′, $J_{app} \sim 6.3$), 2.52 (m, 1H, H−2′), 0.93, 0.92 (2s, 18H, *tert*-Bu), 0.11 (s, 12H, $SiCH_3$). ^{13}C NMR (126 MHz, $CDCl_3$): δ 159.0, 153.6, 151.3, 143.5, 128.9, 128.7, 124.8, 120.6, 120.0, 108.6, 88.2, 85.0, 71.6, 62.6, 41.7, 25.9, 25.7, 18.4, 18.0, −4.7, −4.8, −5.4, −5.5. FAB HRMS calcd for $C_{28}H_{44}N_7O_4Si_2$ ($M^+ + H$) 598.2993, found 598.2986.

***O*^{6}-(Benzotriazol-1-yl)-2′,3′,5′-tris-*O*-(*tert*-butyldimethylsilyl)-inosine (5b).** As described for the synthesis of **5a**, **5b** was prepared by a reaction of 2′,3′,5′-tris-*O*-(*tert*-butyldimethylsilyl)inosine **1b** (3.055 g, 5.00 mmol), BOP (4.425 g, 10.0 mmol), and (*iso*-Pr)$_2$NEt (1.31 mL, 7.5 mmol) in dry THF (50.0 mL) at room temperature over 43 h. Chromatographic purification (SiO_2, elution with 20% EtOAc in hexanes) afforded 2.898 g (80% yield) of compound **5b** as a white, foamy solid. R_f (20% EtOAc in hexanes) = 0.32. ^1H NMR (500 MHz, $CDCl_3$): δ 8.63 (s, 1H, Ar−H), 8.40 (s, 1H, Ar−H), 8.15 (d, 1H, Ar−H, $J = 8.3$), 7.56−7.45 (m, 3H, Ar−H), 6.16 (d, 1H, H−1′, $J = 4.4$), 4.58 (t, 1H, H−2′, $J = 4.4$), 4.34 (t, 1H, H−3′, $J = 4.2$), 4.18 (app q, 1H, H−4′, $J_{app} \sim 3.1$), 4.06 (dd, 1H, H−5′, $J = 11.7$, 3.4), 3.82 (dd, 1H, H−5′, $J = 11.7$, 2.4), 0.98, 0.94, 0.82 (3s, 27H, *tert*-Bu), 0.17, 0.16, 0.11, 0.10, 0.0, −0.17 (6s, 18H, $SiCH_3$). ^{13}C NMR (126 MHz, $CDCl_3$): δ 159.0, 153.9, 151.4, 143.9, 143.5, 129.0, 128.7, 124.8, 120.6, 120.0, 108.6, 88.9, 85.5, 76.4, 71.6, 62.2, 26.1, 25.8, 25.6, 18.5, 18.0, 17.8, −4.4, −4.7, −4.73, −5.0, −5.3, −5.4. FAB HRMS calcd for $C_{34}H_{58}N_7O_5Si_3$ ($M^+ + H$) 728.3807, found 728.3818.

***O*^{6}-(Benzotriazol-1-yl)-2′-deoxyinosine (5c).** In a 50 mL round-bottom flask equipped with a stirring bar were placed 2′-deoxyinosine **1c** (0.504 g, 2.00 mmol) and BOP (1.770 g, 4.00 mmol). DMF (20 mL) and (*iso*-Pr)$_2$NEt (0.70 mL, 4.00 mmol) were added, and the mixture was allowed to stir at room temperature for 26 h. The reaction mixture was evaporated with toluene several times. The crude product was dissolved in EtOAc and washed with water. The organic layer was separated, dried over Na_2SO_4, and concentrated. Chromatographic purification (SiO_2, elution with 10% MeOH in CH_2Cl_2) afforded 0.421 g (57% yield) of compound **5c** as a pale brownish-white foam. R_f (10% MeOH in CH_2Cl_2) = 0.38. ^1H NMR (500 MHz, $CDCl_3$): δ 8.43 (s, 1H, Ar−H), 8.24 (s, 1H, Ar−H), 8.16−8.14 (m, 1H, Ar−H), 7.57−7.54 (m, 1H, Ar−H), 7.49−7.46 (m, 1H, Ar−H), 6.46 (dd, 1H, H−1′, $J = 9.2$, 5.5), 5.10 (dd, 1H, OH, $J = 11.3$, 2.3, CD_3OD exchangeable), 4.83 (br m, 1H, H−3′), 4.25 (br s, 1H, H−4′), 3.97 (dt, 1H, H−5′, $J = 12.8$, 2.0), 3.82 (app td, 1H, H−5′, $J_{app} \sim 11.9$, 1.8), 3.07 (ddd, 1H, H−2′, $J = 13.4$, 9.5, 4.9), 2.42 (dd, 1H, H−2′, $J = 13.6$, 5.7), 2.05 (d, 1H, OH, CD_3OD exchangeable). ^{13}C NMR (126 MHz, $CDCl_3$): δ 159.1, 153.1, 151.1, 144.8, 143.2, 129.1, 128.8, 125.2, 120.5, 120.3, 108.7, 88.9, 86.9, 72.2, 62.7, 40.9. FAB HRMS calcd for $C_{16}H_{16}N_7O_4$ ($M^+ + H$) 370.1264, found 370.1258.

***O*^{6}-(Benzotriazol-1-yl)inosine (5d).** In a reaction vial equipped with a stirring bar were placed inosine **1d** (0.100 g, 0.373 mmol) and BOP (0.330 g, 0.746 mmol). DMF (3.7 mL) and (*iso*-Pr)$_2$NEt (97.3 μL, 0.559 mmol) were added. The reaction vial was flushed with N_2, and the mixture was allowed to stir at room temperature for 68 h. Water was added to the reaction mixture, and the mixture was extracted with CH_2Cl_2. The organic layer was separated, washed with brine, dried over Na_2SO_4, and concentrated. Because the crude product contained residual DMF, toluene was added and evaporated several times. Chromatographic purification (SiO_2, elution with 5% MeOH in CH_2Cl_2) afforded 76 mg (53% yield) of **5d** as a white powder. R_f (5% MeOH in CH_2Cl_2) = 0.09. ^1H NMR (500 MHz, DMSO-d_6): δ 8.95 (s, 1H, Ar−H), 8.53 (s, 1H, Ar−H), 8.21 (d, 1H, Ar−H, $J = 8.8$), 7.80 (d, 1H, Ar−H, $J = 8.3$), 7.66 (t, 1H, Ar−H, $J = 7.6$), 7.56 (t, 1H, Ar−H, $J = 7.6$), 6.09 (d, 1H, H−1′, $J = 5.4$), 5.55 (d, 1H, OH, $J = 5.9$, D_2O exchangeable), 5.24 (d, 1H, OH, $J = 5.4$, D_2O exchangeable), 5.07 (t, 1H, OH, $J =$

5.6, D_2O exchangeable), 4.62 (q, 1H, H−2′, J = 5.4), 4.21 (q, 1H, H−3′, J = 4.7), 4.01 (m, 1H, H−4′), 3.71 (m, 1H, H−5′), 3.60 (m, 1H, H−5′). ^{13}C NMR (126 MHz, DMSO-d_6): δ 159.0, 154.8, 151.9, 146.1, 143.5, 130.1, 129.3, 126.1, 120.7, 119.8, 110.2, 88.9, 86.5, 74.7, 70.9, 61.8. FAB HRMS calcd for $C_{16}H_{16}N_7O_5$ (M^+ + H) 386.1213, found 386.1191.

***O*^{6}-(Benzotriazol-1-yl)-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyinosine (17).** In a 100 mL round-bottom flask equipped with a stirring bar were placed 5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyinosine[17] (**16**) (0.7 g, 1.262 mmol), BOP (1.117 g, 2.524 mmol), (*iso*-Pr)$_2$NEt (0.44 mL, 2.524 mmol), and dry THF (50.0 mL). The mixture was allowed to stir at room temperature, under a N_2 balloon, for 40 h. The reaction mixture was evaporated, diluted with EtOAc (200 mL), and washed with brine. The organic layer was dried over Na_2SO_4 and concentrated. Chromatographic purification (SiO_2, packed with 1% (*iso*-Pr)$_2$NEt in EtOAc and elution with EtOAc) afforded 0.718 g (85% yield) of compound **17** as a white foam. R_f (EtOAc) = 0.31. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.31 (s, 1H, Ar−H), 8.28 (s, 1H, Ar−H), 8.13 (d, 1H, Ar−H, J = 8.2), 7.55−7.45 (m, 3H, Ar−H), 7.38 (d, 2H, Ar−H, J = 7.3), 7.29−7.19 (m, 7H, Ar−H), 6.80 (d, 4H, Ar−H, J = 8.9), 6.52 (t, 1H, H−1′, J = 6.4), 4.73 (br m, 1H, H−3′), 4.20 (q, 1H, H−4′, J = 4.2), 3.77 (s, 6H, OCH_3), 3.45 (dd of $AB_{quartet}$, 1H, H−5′, J = 10.4, 4.6), 3.40 (dd of $AB_{quartet}$, 1H, H−5′, J = 10.4, 5.0), 2.87 (app quint, 1H, H−2′, $J_{app} \sim$ 6.6), 2.62 (ddd, 1H, H−2′, J = 13.4, 6.2, 4.3), 2.39 (d, 1H, 3′−OH, J = 3.1). ^{13}C NMR (126 MHz, $CDCl_3$): δ 159.1, 158.6, 153.5, 151.4, 144.4, 143.5, 135.5, 135.4, 130.0, 128.9, 128.8, 128.0, 127.9, 127.1, 124.8, 120.6, 120.1, 113.2, 108.7, 86.8, 86.3, 85.0, 72.6, 63.5, 55.2, 40.3. FAB HRMS calcd for $C_{37}H_{34}N_7O_6$ (M^+ + H) 672.2571, found 672.2583.

***O*^{6}-(Benzotriazol-1-yl)-3′-*O*-[(*N*,*N*-diisopropylamino)(β-cyanoethoxy)phosphinyl]-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyinosine (18).** In a clean, dry vial equipped with a stirring bar was placed O^6-(benzotriazol-1-yl)-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyinosine (**17**) (0.030 g, 0.0447 mmol), and the vial was transferred to a glove bag maintained under N_2. Dry CH_2Cl_2 (0.5 mL), (*iso*-Pr)$_2$NEt (19.5 μL, 0.112 mmol), and 2-cyanoethyl diisopropylchlorophosphoramidite (19.9 μL, 0.0894 mmol) were added. The mixture was removed from the glove bag and allowed to stir at room temperature for 1 h under a N_2 atmosphere. The reaction mixture was diluted with CH_2Cl_2 and washed with saturated $NaHCO_3$ followed by brine. The organic layer was dried over Na_2SO_4 and concentrated. Chromatographic purification (SiO_2 packed with 50:50:1 EtOAc/hexanes/(*iso*-Pr)$_2$NEt and eluted with 50% EtOAc in hexanes) afforded 18.4 mg (47% yield) of compound **18** as a white, foamy solid. R_f (50:50:1 EtOAc/hexanes/(*iso*-Pr)$_2$NEt) = 0.51 and 0.45 for the two phosphoramidite diastereomers. ^{31}P{^{1}H} NMR (202 MHz, $CDCl_3$): δ 150.08 and 150.00.

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Supporting Information Available: Synthetic procedures and spectral data not described in the manuscript as well as ^{1}H NMR spectra of **3a**, **5a**−**d**, **7a**−**o**, **8a**−**g**, **9a**,**b**, **10a**,**b**, **13**−**15**, **17**, and **18** and the ^{31}P{^{1}H} NMR spectrum of **18**. This material is available free of charge via the Internet at http://pubs.acs.org.

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