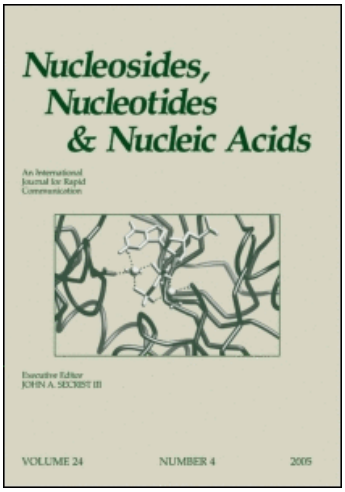


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

INCORPORATION OF 8-HISTAMINYL-DEOXYADENOSINE [8-(2-(4-IMIDAZOLYL)ETHYLAMINO)-2'-DEOXYRIBOADENOSINE] INTO OLIGODEOXYRIBONUCLEOTIDES BY SOLID PHASE PHOSPHORAMIDITE COUPLING

Leonard Lermer[a]; John Hobbs[b]; David M. Perrin[a]
[a] Department of Chemistry, University of British Columbia, Vancouver, Canada [b] NAPS (Nucleic Acid and Peptide Sequencing) Unit, University of British Columbia, Vancouver, Canada

Online publication date: 11 December 2002

To cite this Article Lermer, Leonard , Hobbs, John and Perrin, David M.(2002) 'INCORPORATION OF 8-HISTAMINYL-DEOXYADENOSINE [8-(2-(4-IMIDAZOLYL)ETHYLAMINO)-2'-DEOXYRIBOADENOSINE] INTO OLIGODEOXYRIBONUCLEOTIDES BY SOLID PHASE PHOSPHORAMIDITE COUPLING', Nucleosides, Nucleotides and Nucleic Acids, 21: 10, 651 — 664
To link to this Article: DOI: 10.1081/NCN-120015723
URL: http://dx.doi.org/10.1081/NCN-120015723

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS
Vol. 21, No. 10, pp. 651–664, 2002

INCORPORATION OF 8-HISTAMINYL-DEOXYADENOSINE [8-(2-(4-IMIDAZOLYL)ETHYLAMINO)-2′-DEOXYRIBOADENOSINE] INTO OLIGODEOXYRIBONUCLEOTIDES BY SOLID PHASE PHOSPHORAMIDITE COUPLING

Leonard Lermer,[1] John Hobbs,[2] and David M. Perrin[1,*]

[1]Department of Chemistry, 2036 Main Mall, and
[2]NAPS (Nucleic Acid and Peptide Sequencing) Unit,
6174 University Blvd., University of British Columbia,
Vancouver, B. C., V6T-1Z1 Canada

ABSTRACT

The 3′phosphoramidite of 8-histaminyl deoxyadenosine has been prepared and successfully incorporated into a short oligodeoxyribonucleotide. The synthetic methodology leading to this preparation is given and the implications for developing new DNAzymes as well as probing unusual nucleic acid structures are discussed.

*Corresponding author. E-mail: dperrin@chem.ubc.ca

DOI: 10.1081/NCN-120015723 1525-7770 (Print); 1532-2335 (Online)

INTRODUCTION

The imidazole functionality is of special interest to structural biologists, enzymologists, and medicinal chemists alike as this functionality plays a unique and important role in terms of catalysis, recognition, and drug delivery in part because of its pK_a that lies in the range of 6–7. The imidazole is found almost ubiquitously at the active sites of enzymes, especially those that operate on phosphate-derived substrates where it chelates metals and/or plays the role of a general acid and/or a general base.[1] In other enzymes, it strongly chelates redox active metals so as to position them in proximity to substrate and/or alter their redox potentials.[2,3] In the case of Zn-finger proteins, imidazole side-chains of histidine chelate divalent zinc to properly structure the DNA binding domain so as to permit sequence specific high affinity recognition of DNA sequences.[4] When DNA is condensed with cationic co-block polymers prepared from imidazole-modified monomers, cellular penetration and uptake of DNA is enhanced.[5–7] The imidazole has been appended directly to oligonucleotides in the study of pH dependent uptake and cellular fate[8] and to develop oligonucleotides that can recognize mRNA complements and effect site-specific cleavage via an RNaseA-like activity.[9–15] Finally imidazoles have been appended to monomer nucleotide triphosphate that can be enzymatically polymerized to give modified DNA, which in turn can be PCR amplified.[16–20] Such methodology would indicate the possibility of introducing this very important functionality in a combinatorial manner for the discovery of modified oligonucleotides that may use imidazoles for activities ranging from recognition to catalysis to cellular penetration. In particular, one of the first of such methodological reports (and the first to report on the simultaneous incorporation of two modified nucleotides) involved an examination of the enzymatic uptake of 8-histaminyl-dATP i.e., 8-(2-(4-Imidazolyl)ethylamino)-2′-deoxyriboadenosine triphosphate.[21] As the incorporation of imidazoles into biopolymers is of great interest and relates to many diverse subjects, we wish to report the synthesis of the phosphoramidite corresponding to 8-histaminyl-dATP: (6N-benzoyl-8-(2-(4-Imidazolyl)ethylamino)adenyl))-3′-*O*-(2-cyanoethyl-*N,N*-diisopropylphosphoramidyl)-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxy-β-D-ribofuranose (Fig. 1) and its incorporation into oligonucleotides by standard solid phase oligonucleotide chemistries.

Although a great number of modified pyrimidine and purine derivatives have been investigated with linkages to both the base and sugar moieties,[22–28] very few reports exist of an 8-amino-linked purine that presents an imidazole for use in solid phase synthesis.[29] Besides the immediate interest in a solid phase synthesis of a recently reported metal-independent DNAzyme that mimics RNaseA,[30] this report expands the general methodologies for positioning imidazoles at different positions on the purine and sugar moieties. Of further interest are the recent reports of novel triplex and parallel

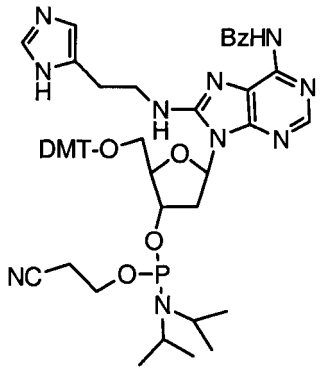


Figure 1.

duplex structures that are induced by 8-amino-substituted purines via Hoogsteen base pairing.[31,32]

RESULTS

After investigating several alternate approaches to protection, the phosphoramidite shown in Fig. 1 was ultimately synthesized in the order shown in Sch. 1. Compounds 6 & 7 are addressed in the discussion pertaining to the formulation of the synthetic route. This scheme differs from our initial approach which had been to *i*) condense 8-bromo-deoxyadenosine[33] with histamine, *ii*) perbenzoylate, *iii*) selectively saponify the 3′,5′ benzoyl esters,[34] *iv*) dimethoxytritylate and *v*) install the phosphoramidite. Initially, this seemed to be the most straightforward approach, particularly since displacement of the unprotected bromide with 5 eq. histamine normally proceeded over a period of 3–7 days in refluxing ethanol, and such conditions we suspected might otherwise have cleaved the N6-benzoyl group.[35] In the initial synthetic route, the DMT and benzoyl groups were installed *after* histamine displacement. This initial strategy was guided in part by *i*) a desire to monitor reaction completion by a characteristic λ-max shift from 263 nm to 280 nm, and *ii*) to take advantage of the very large R_f difference that was observed between the unprotected Br-dA and the corresponding histaminyl adduct, which we thought would aid in purification. Following displacement by histamine, treatment with benzoyl chloride gave a monobenzoylated histaminyl adduct. ^{1}H-NMR revealed a broad singlet at 9.05 ppm characteristic of an amide proton thus indicating benzoylation had transpired at the intended N6 position and not at the N8 position, as has been observed by others in the context of C8-NH guanine derivatives.[36]

The N6-benzoyl-8-histaminyl-dA rapidly reacted with dimethoxytrityl chloride in pyridine to yield a chromatographically pure compound that was

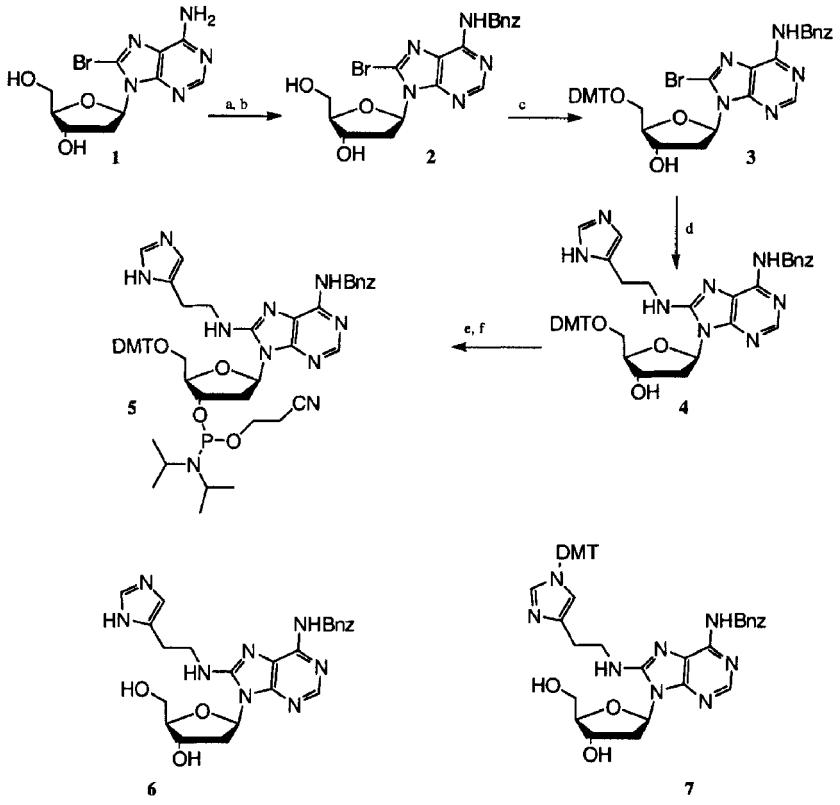
initially taken to be **4** as ESI indicated the presence of one dimethoxytrityl, one benzoyl, and one histaminyl group, each on the parent deoxyriboadenosine. As DMT can be used to protect imidazoles,[37,38] we entertained the possibility that the DMT had reacted exclusively on the imidazole to give an unwanted structural isomer **7**. Our suspicions in this regard were further aroused when the product was treated with 2-cyanoethyldiisopropylchlorophosphoramidite as two phosphorus adducts were detected by ESI and 4 singlets detected by ^{31}P NMR (data not shown).

Initially, two hypotheses were developed to explain this observation: 1) The dimethoxytrityl chloride reacted with the imidazole leaving two free hydroxyls to react with the oxophilic phosphonamidyl chloride or 2) the dimethoxytrityl was indeed properly installed on the 5′OH but both the 3′OH as well as the imidazole were phosphatidylated. Previous reports by Bashkin et al. used DMT-protected nucleosides that were subsequently appended with peptides containing NBoc-, FMOC- and DMT-protected histidines, presumably to avoid reaction of the imidazole with the phosphatidylating reagent used to prepare peptide-modified phosphoramidite monomers.[39] These early studies guided our thinking in part. More recent work by Joyce et al. however described the preparation of a urocanamido-allyl-dU phosphoramidite that did not require imidazole protection.[40] A doubly DMT-protected compound, similar to those investigated by Bashkin and coworkers also would have been amenable to phosphoramidite synthesis and subsequent oligonucleotide coupling, and moreover would have obviated concerns regarding the placement of the first DMT. We attempted to prepare such a derivative by retreating the product in question with excess DMT-chloride, however this only resulted in decomposition and further attempts to protect both the 5′OH *and* the imidazole were abandoned. Given the large number of aromatic protons on the target, we were unable to readily confirm the position of the DMT group by ^{1}H-NMR. For example, in d_6-DMSO, the 5′-methylene protons were found to overlap with the methylene protons of the histamine such that coupling to the nonexchangeable 5′OH proton (and subsequent decoupling by D_2O addition) was not easily detected. Nevertheless the two D_2O-exchangeable protons were detected in the alcohol region (t, 5.74 ppm J = 3.8 Hz, 1H; d 5.34 ppm J = 4.1 Hz).

The questions regarding the chemoselectivity of dimethoxytritylation caused us to reformulate the final synthetic strategy shown in Sch. 1. In this case, **3** was reacted with histamine to give **4**. Remarkably, this reaction went to completion within minutes rather than days as had been observed with the free bromide. Continued refluxing in ethanol with excess histamine did in fact result in a debenzoylation (data not shown). Given that histamine displacement on the unprotected bromide proceeded over a period of days, it was unexpected that the displacement on **3** would be *i*) so dramatically accelerated by the introduction of protecting groups, and *ii*) so selective for attack at the 8 position without any observable debenzoylation (little if any 5′-DMT-8-

bromo-deoxyadenosine was detected prior to complete displacement by histamine at the 8 position). With **4** in hand, detailed HMQC/HMBC NMR analysis finally indicated that reaction of **6** with DMT-Cl gave exclusive protection of the imidazole resulting in an unwanted structural isomer **7**.

Briefly stated, the final synthetic approach (Sch. 1) involved bromination, benzoylation, limited saponification, dimethoxytritylation, bromide displacement, and finally phosphatidylation. Product **4** was found to have a very different R_f from the previously obtained DMT-protected material **7** that was initially thought to be **4**. Chemical correlation distinguished **4** from **7** and phosphatidylation proceeded cleanly to give one product with two closely spaced singlets in the ^{31}P-NMR spectrum indicative of one diastereomeric trivalent phosphorus atom. It was fortuitous that the bromide displacement occurred rapidly and without debenzoylation on the fully protected bromide. At the outset of this undertaking, there appeared to be several ways to assemble the desired target, and no way of a-priori deciding



Scheme 1. a) Excess benzoylchloride/pyridine, b) aqueous NH_4OH, c) 1.1 eq. DMT-Cl/pyridine, d) 5 eq. histamine/ethanol, e) 1.1–1.3 eq. cyanoethyldiisopropylchlorophosphoramidite/ 3 eq. ethyldiisopropylamine, f) aq. K_2CO_3:CH_2Cl_2 extraction.

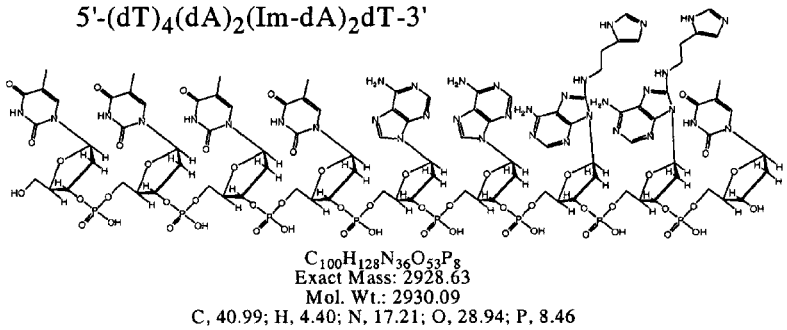
on the most efficacious strategy. We hope other investigators seeking to prepare imidazole-modified oligonucleotides will find the preceding discussion germane to their efforts. As such, we have characterized both the ultimate target of this work as well as the unanticipated structural isomer.

Once in hand, the phosphoramidite was successfully introduced into a small oligonucleotide of sequence d(TTTTAAA^{im}A^{im}T) on a 40 nmol scale using standard CPG oligonucleotide synthesis procedures on an ABI automated synthesizer with 10 min coupling times: tetrazole activation, coupling, iodine-collidine-water oxidation, acetic anhydride capping, and DCA detritylation. The dimethoxytrityl cation in the efflux of each coupling step was quantified by reactivation with addition of toluenesulfonic acid and absorbance noted at 450 nm, Yields were found to be greater than 90% per coupling step based on UV-vis absorbance analysis (498 nm) of the detritylation fractions and no appreciable difference was observed when the modified phosphoramidite was introduced. The oligonucleotide was cleaved from the CPG by overnight treatment with concentrated ammonium hydroxide at 55°C. Following lyophilization, the crude oligonucleotide was subjected to MALDI analysis (negative ion mode) shown in Fig. 2.

Although a small amount of an internally deleted product was detected by MALDI (2506.510), it was not observed by 20% D-PAGE (vide infra). The signal at 1859.269 represents a fragmentation product of either the three bases at the 3′ end of the full-length product or of the last two bases at the 3′ end of an n-1 internally deleted product. The 20% D-PAGE analysis (29:1 monomer:bis, 1X TBE pH 8.3) on the crude (Fig. 3) indicated no impurity by UV shadowing. The R_f values in the gel are given in centimeters measured from the top of the well, with lanes 1 and 3 containing only marker dyes as shown and lane 2 containing only product.

CONCLUSIONS AND DISCUSSION

Phosphoramidite **5** will allow for the solid phase synthetic scale-up of imidazole-modified oligonucleotides with altered recognition and uptake properties as well as catalytic activities. More specifically **5** will allow the synthesis of a candidate DNAzyme that had been initially polymerized in picomol amounts by DNA polymerase in the presence of 8-histaminyl-dATP in lieu of dATP.[41] This polymerization in conjunction with a combinatorial selection has resulted in the report of a putative RNaseA activity.[42] The phosphoramidite reported herein will likely contribute to defining interesting structure-function relationships on which this hydrolytic activity appears to depend. While this activity is possibly the first such RNaseA mimic, its discovery also indicated some of the methodological limitations that are encountered when using modified nucleotides to expand the catalytic repertoire of nucleic acids. One of these constraints is polymerase uptake. The polymerization yields of Im-dATP using Sequenase 2.0^{TM} diminished



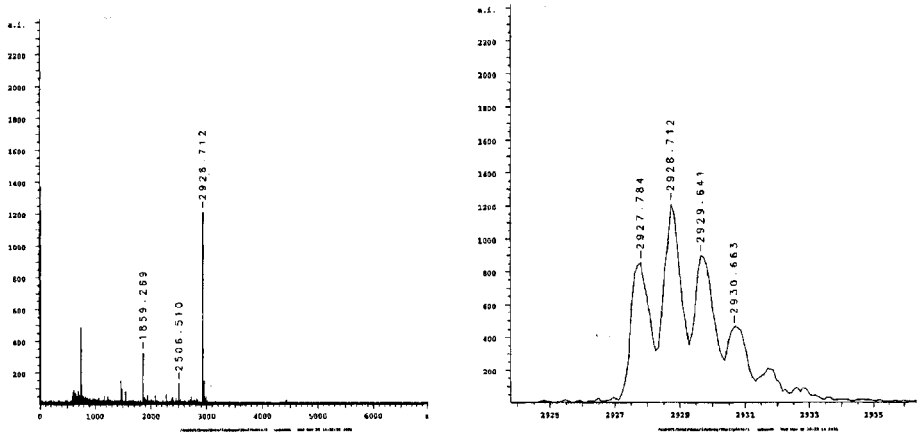


Figure 2.

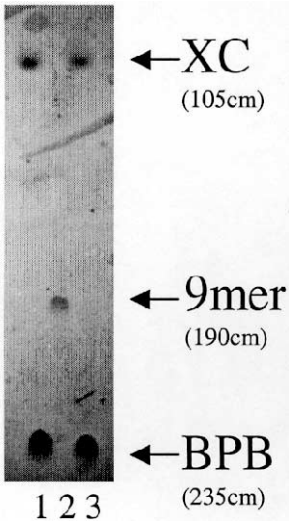


Figure 3.

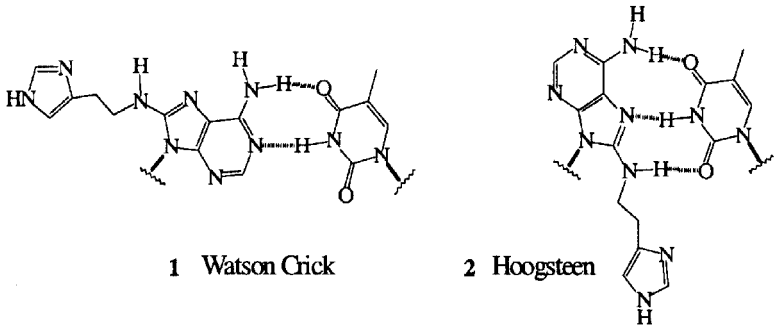


Figure 4.

precipitously after 3–5 isosequential incorporations. Polymerizations using Klenow enzymes exo$^{+/-}$ did not exhibit incorporation beyond two consecutive bases (unpublished observations). This represents a serious limitation in terms of the number of potential sequences that may be interrogated during the combinatorial selection of DNAzymes. One hypothesis for explaining this observation is that the N8 delivers 3 hydrogen bonds between the template thymidine and the Hoogsteen face of the Im-dA as shown schematically in Fig. 4.

Such Hoogsteen bonding, which would be favored by the C8-NH proton, has been observed in the context of triple helices.[43] More recently, strands rich in 8-amino purines were found to recognize their complements in a preferred parallel orientation.[44] If this Hoogsteen structure were to occur either within the active site of the polymerase during polymerization, or when polymerase transiently dissociates from the template, non-extendable dead-end complexes will result, thus explaining the limited incorporation of the corresponding triphosphate. Biophysical studies including gel-retardation and hyperchromic melting studies using synthetically prepared oligonucleotides generated from the aforementioned Im-dA phosphoramidite will also provide evidence for or against this hypothesis. In addition, primers as well as templates containing varying proportions of this modification vs. unmodified adenosine can now be prepared to address fine-structure kinetic aspects of polymerase uptake. In addition, the ability of this modified nucleotide to confer novel properties to oligonucleotides in the context of double and triple helices, and to cell up-take studies will be investigated.

MATERIALS AND METHODS

Materials

Deoxyadenosine, bromine, dimethoxytrityl chloride, benzoyl chloride, histamine, diisopropylethylamine, pyridine, and acetonitrile were purchased

from Sigma-Aldrich. 2-cyanoethyldiisopropylchlorophosphoramidite was obtained from Lancaster. Flash chromatography was carried out using Silica gel 60, 230–400 mesh, supplied by E. Merck Co. Silica gel 60 F_{254} on aluminum sheets (E. Merck, type 5554) was used for TLC analysis. Nuclear Magnetic Resonance spectra (^1H, ^{13}C and ^{31}P) were obtained on a Bruker AV-300 or Bruker AV-400 instrument. Solvent, signal multiplicity, coupling constants, and integration ratios are indicated in parentheses. An external standard of 85% H_3PO_4 was used for ^{31}P NMR. (ESI) MS were obtained on Bruker Esquire-LC instrument. Maldi-MS were obtained on Bruker Biflex instrument in negative ion mode. All reactions were carried out under positive argon pressure and dark conditions. Solvents used in modified phosphoramidite synthesis were distilled to dryness. Unmodified phosphoramidites were purchased in conjunction with the NAPS (Nucleic Acid Peptide Sequencing) Unit of UBC. Compounds **1**, **2** and **3** were prepared following a literature procedure.[45,46] The syntheses of compounds **4**, **5**, and **7** are described.

6N-Benzoyl-5′-O-(4,4′-dimethoxytrityl)-8-(2-(4-Imidazolyl)ethylamino)-2′deoxyadenosine (4): A solution of Compound **3** 0.9081 g (1.234 mmol) and 0.6886 g (6.168, 5 eq.) of histamine in 40 mL of absolute ethanol was degassed prior to warming to 80°C. The reaction was monitored by TLC developed with 10% MeOH in $CHCl_3$ ($R_f = 0.12$). After 2 h the reaction mixture was concentrated and purification by silica gel flash chromatography, eluted with 10% MeOH in $CHCl_3$ to afford 0.6805 g (72%) of compound **4** as a solid white foam. ^1H NMR (CD_3OD, 400 ppm): 8.28 (s, 1H, H2), 8.02 (d, J = 7.5 Hz, 2H, o-Bz-H), 7.54 (t, J = 7.5 Hz, 1H, p-Bz-H), 7.51 (s, 1H, Im-H2), 7.45 (t, J = 7.5 Hz, 2H, m-Bz-H), 7.29 (d, J = 7.2 Hz, 2H, Ph-DMT), 7.16 (d, J = 8.68 Hz, 4H, PhOMe(DMT)), 7.16-7.06 (m, 3H, Ph-DMT), 6.75 (s, 1H, Im-H5), 6.71 (d, 8.6 Hz, 2H, PhOMe(DMT)), 6.68 (t, J = 8.68 Hz, 2H, PhOMe(DMT)), 6.21 (dd, J = 6.4, 6.7 Hz, 1H, H1′), 4.75 (ddd, J = 4.27, 4.27, 6.1 Hz, 1H, H3′), 4.09 (ddd, 3.8, 4.27, 5.2 Hz, 1H, H4′), 3.65 (s, 3H, -OMe), 3.64 (s, 3H, -OMe), 3.57 (m, 2H, N-CH2-), 3.47 (ddd, J = 6.1, 6.4, 13.2 Hz, 1H, H2′), 3.33 (dd, J = 3.8, 10.3 Hz, 1H, H5′), 3.24 (dd, J = 5.2, 10.3 Hz, 1H, H5′), 2.89 (t, J = 7.2 Hz, 2H, -CH_2-Im), 2.30 (ddd, J = 4.7, 6.7, 13.2 Hz, 1H, H2′) ^{13}C NMR (CD_3OD, 400 ppm): 167.96 (carbonyl), 160.98 (C-p-Ph-OMe(DMT)), 159.94 (C-p-Ph-OMe(DMT)), 155.87 (C8), 154.45 (C4), 149.04 (C2), 146.04 (C-Ph-DMT), 144.59 (C6), 137.03 (C-DMT), 136.92 (C-DMT), 136.03 (Im-C2), 135.31 (Im-C4), 133.50 (C-p-Bz), 131.24 (C-Ph-OMe(DMT)), 131.10 (C-Ph-OMe(DMT)), 129.61 (C-m-Bz), 129.30 (C-Ph-DMT), 129.13 (C-o-Bz), 128.67 (C-Ph-DMT), 127.81 (C-Ph-DMT), 124.67 (C5), 118.30 (Im-C5), 113 (C-Ph-OMe(DMT)), 87.50 (Ph_3C(DMT)), 87.44 (C4′), 85.30 (C1′), 79.42, 72.56 (C3′), 64.64 (C5′), 55.67 (-OMe), 43.63 (N-CH2-), 38.25 (C2′), 27.54 (-CH2-Im). Mass: 789($M+Na^+$), 767 (M^+).

(6N-benzoyl-8-(2-(4-Imidazolyl)ethylamino)adenyl))-3′-*O*-(2-cyanoethyl-*N,N*-diisopropylphosphoramidyl)-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxy-β-D-ribofuranose (5): Compound **4** 0.2508 g (0.327 mmol) was dried overnight by co-evaporation from a 5 mL solution of 10% pyridine in CH_2Cl_2. After dissolving the sample in 18 mL of CH_2Cl_2, adding 100 μL of N,N-diisopropylethyl amine, and cooling to −78°C, a 72.9 μL (0.327 mmol) sample of 2-Cyanoethyl-N,N-diisopropylchloro-phosphoramidite was added. The clear colorless solution was allowed to warm to room temperature after 5 min. After 12 h the reaction was quenched by the addition of 0.1 mL of MeOH, diluted in $CHCl_3$ and washed with 10% aqueous sodium bicarbonate. The organic layer was dried with sodium sulfate filtered and concentrated under reduced pressure to afford a white foam. The foam was dissolved in a minimal amount of EtOAc and precipitated by the addition of 15–20 volumes of hexane. The precipitate was centrifuged and the supernatant was removed. The further addition of approximately 5 volumes of hexane to the supernatant afforded a second crop of precipitate. The precipitates were combined and further purified by silica gel chromatography eluted with 6% MeOH/2% Et_3N in $CHCl_3$ afforded compound **5** 0.2843 g (89.9%) as a white foam. The reaction was monitored by TLC developed with 10% MeOH, ~1.5% TEA in $CHCl_3$ ($R_f = 0.36$). ^{1}H NMR ($CDCl_3$, 300 ppm): 8.67 (broad s, NH-amide), 8.42 (s, 1H, H-2), 8.03 (d, J = 7.3 Hz, 2H, o-Bz-H), 7.59 (t, J = 7.3 Hz, 1H, p-Bz-H), 7.51 (t, J = 7.3 Hz, 2H, m-Bz-H), 7.38–7.20 (9H), 6.82 (m, 4H, PhOMe(DMT)), 6.57 (m, 1H), 6.52 (s, 1H, Im-H5), 6.30 (m, 1H, H1′), 4.79 (m, 1H, H3′), 4.20 (d, J = 3.1 Hz, 0.45H, H4′), 4.15 (d, J = 3.1 Hz, 0.55H, H4′), 3.77 (s, 6H, -OMe), 3.86-3.48 (m, 7H), 3.37 (td, J = 3, 10.8 Hz, 1H), 3.1–2.78 (m, 3H), 2.60 (t, J = 6.2 Hz, 1.1 H, $-CH_2$-CN), 2.6-2.40 (m, 2H), 2.37 (t, J = 6.2 Hz, 0.9H, $-CH_2$-CN), 1.16 (d, J = 6.94 Hz, 6H), 1.15 (d, J = 6.94 Hz, 3H), 1.01 (d, J = 6.94 Hz, 3H) ^{31}P NMR ($CDCl_3$): 149.70, 149.21 Mass (ESI): 967 (M^+).

6N-Benzoyl-8-(2-(4-(1-N-(4,4′-dimethoxytrityl)-Imidazolyl)ethylamino)-2′deoxyadenosine (7): Compound **6** was refluxed with DMT-Cl in pyridine. The reaction was monitored by TLC developed with 10% MeOH in $CHCl_3$ ($R_f = 0.27$). ^{1}H NMR (400 MHz, $CDCl_3$) ppm: 9.05 (broad s, NH-amide) 8.44 (s, 1H, H2), 7.94 (d, J = 7.5 Hz, 2H, o-Bz-H), 7.49 (t, J = 7.2 Hz, 1H, p-Bz-H), 7.41 (t, J = 7.5 Hz, 2H, m-Bz-H) 7.29 (s, 1H, Im-H2), 7.24 (t, J = 3.1 Hz, 3H, Ph-DMT), 7.00 (dd, J = 5.9, 7.0 Hz, 2H, Ph-DMT), 6.94 (d, J = 8.9 Hz, 4 H, PhOMe(DMT)), 6.77 (d, J = 8.9 Hz, 4 H, PhOMe(DMT)), 6.59 (t, J = 7.5 Hz, 1H, H1′), 6.59 (s, 1H, Im-H5), 4.67 (d, J = 6.2 Hz, 1H, H3′), 3.98 (s, 1H, H4′), 3.79 (m, 2H, H5′), 3.74 (s, 6H, -OMe), 3.64 (m, 2H,-N-CH_2-), 2.89 (td, J = 5.5, 15.3 Hz, 1H, $-CH_2$-Im), 2.83 (td, J = 5.5, 15.3 Hz, 1H, $-CH_2$-Im), 2.61 (ddd, J = 13.7, 9.3, 6.4 Hz, 1H, H2′), 2.10 (dd, J = 13.1, 5.7 Hz, 1H, H2′) ^{13}C NMR (75 MHz, CD_3OD) ppm: 165.0 (carbonyl), 159.2 (C-PhOMe(DMT)), 153.0 (C-4), 152.8 (C-8), 148.3 (C-2), 143.5

(C-6), 142.6 (C-DMT), 138.7 (Im-C4), 137.9 (Im-C2), 134.5 (C-Bz), 134.4 (C-Ph-OMe(DMT)), 132.1 (C-p-Bz), 130.9 (C-PhOMe(DMT)), 129.4 (C-Ph(DMT)), 129.4 (C-Ph(DMT)), 128.6 (C-m-Bz), 128.0 (C-m-Ph(DMT)), 127.8 (C-o-Bz), 122.7 (C-5), 119.0 (Im-C5), 113.3 (C-Ph-OMe(DMT)), 87.6 (C4′), 83.8 (C1′), 74.8 (Ph_3C(DMT)), 71.9 (C3′), 61.0 (C5′), 55.3 (-OMe), 43.5 (-N-CH2-), 39.1 (C2′), 26.9 (-CH_2-Im).

ACKNOWLEDGMENT

This work was supported by UBC Start-up funds.

REFERENCES

1. Raines, R.T. Ribonuclease A. Chem. Rev. **1998**, *98*, 1045–1065.
2. Sträter, N.; Lipscomb, W.N.; Klabunde, T.; Krebs, B. Two Metal Ion Catalysis in Enzymatic Acyl and Phosphoryl-Transfer Reactions. Angew. Chem. Int. Ed. Engl. **1996**, *35*, 2024–2055.
3. Solomon, E.I.; Yan, Z. The Electronic-Structures of Active Sites in Nonheme Iron Enzymes. Acc. Chem. Res. **1992**, *25*, 343–352.
4. Pabo, C.O.; Peisach, E.; Grant, R.A. Design and Selection of Novel Cys(2)His(2) Zinc Finger Proteins. Ann. Rev. Biochem. **2001**, *70*, 313–340.
5. Pichon, C.; Goncalves, C.; Midoux, P. Histidine-Rich Peptides and Polymers for Nucleic Acids Delivery. Adv. Drug Del. Rev. **2001**, *53*, 75–94.
6. Putnam, D.; Gentry, C.A.; Pack, D.W.; Langer, R. Polymer-Based Gene Delivery with Low Cytotoxicity by a Unique Balance of Side-Chain Termini. Proc. Natl. Acad. Sci. USA **2001**, *98*, 1200–1205.
7. Midoux, P.; Monsigny, M. Efficient Gene Transfer by Histidylated Polylysine DNA Complexes. Bioconj. Chem. **1999**, *10*, 406–411.
8. Ushijima, K.; Shirakawa, M.; Kagoshima, K.; Park, W.S.; Miyano-Kurosaki, N.; Takaku, H. Anti-HIV-1 Activity of an Antisense Phosphorothioate Oligonucleotide Bearing Imidazole and Primary Amine Groups. Bioorg. & Med. Chem. **2001**, *9*, 2165–2169.
9. Konevetz, D.A.; Beck, I.E.; Beloglazova, N.G.; Sulimenkov, I.V.; Sil'nikov, V.N.; Zenkova, M.A.; Shishkin, G.V.; Vlassov, V.V. Artificial Ribonucleases: Synthesis and RNA Cleaving Properties of Cationic Conjugates Bearing Imidazole Residues. Tetrahedron **1999**, *55*, 503–512.
10. Vlassov, V.; Abramova, T.; Godovikova, T.; Giege, R.; Sil'nikov, V. Sequence-Specific Cleavage of Yeast tRNA(Phe) with Oligonucleotides Conjugated to a Diimidazole Construct. Antisense & Nucl. Acid Drug Dev. **1997**, *7*, 39–42.
11. Hovinen, J.; Guzaev, A.; Azhayeva, E.; Azhayev, A.; Lonnberg, H. Imidazole Tethered Oligonucleotides – Synthesis and RNA Cleaving Activity. J. Org. Chem. **1995**, *60*, 2205–2209.
12. Trawick, B.N.; Daniher, A.T.; Bashkin, J.K. Inorganic Mimics of Ribonucleases and Ribozymes: From Random Cleavage to Sequence-Specific Chemistry to Catalytic Antisense Drugs. Chem. Rev. **1998**, *98*, 939–960.

13. Oivanen, M.; Kuusela, S.; Lönnberg, H. Kinetics and Mechanisms for the Cleavage and Isomerization of the Phosphodiester Bonds of RNA by Bronsted Acids and Bases. Chem. Rev. **1998**, *98*, 961–990.
14. Reynolds, M.A.; Beck, T.A.; Say, P.B.; Schwartz, D.A.; Dwyer, B.P.; Daily, W.J.; Vaghefi, M.M.; Metzler, M.D.; Klem, R.E.; Arnold, L.J. Antisense Oligonucleotides Containing an Internal, Non-Nucleotide-Based Linker Promote Site-Specific Cleavage of RNA. Nucl. Acids Res. **1996**, *24*, 760–765.
15. Verbeure, B.; Lacey, C.J.; Froeyen, M.; Rozenski, J.; Herdewijn, P. Synthesis and Cleavage Experiments of Oligonucleotide Conjugates with a Diimidazole-Derived Catalytic Center. Bioconj. Chem. **2002**, *13*, 333–350.
16. Sakthivel, K; Barbas, C.F. Expanding the Potential of DNA for Binding and Catalysis: Highly Functionalized dUTP Derivatives that are Substrates for Thermostable DNA Polymerases. Angew. Chem. Int. Ed. Engl. **1998**, *37*, 2872–2875.
17. Matulic-Adamic, J.; Daniher, A.T.; Karpeisky, A.; Haeberli, P.; Sweedler, D.; Beigelman, L. Functionalized Nucleoside 5′-Triphosphates for In Vitro Selection of New Catalytic Ribonucleic Acids. Bioorg. Med. Chem. Lett. **2000**, *10*, 1299–1302.
18. Matulic-Adamic, J.; Daniher, A.T.; Karpeisky, A.; Haeberli, P.; Sweedler, D.; Beigelman, L. Synthesis of Modified Nucleoside 5′-Triphosphates for In Vitro Selection of Catalytic Nucleic Acids. Nucleosides, Nucleotides & Nucleic Acids **2001**, *20*, 1113–1115.
19. Perrin, D.M.; Garestier, T.; Helene, C. Expanding the Catalytic Repertoire of Nucleic Acid Catalysts: Simultaneous Incorporation of Two Modified Deoxyribonucleotide Triphosphates Bearing Ammonium and Imidazolyl Functionalities. Nucleosides & Nucleotides **1999**, *18*, 377–391.
20. Gourlain, T.; Sidorov, A.; Mignet, N.; Thorpe, S.J.; Lee, S.E.; Grasby, J.A.; Williams, D.M. Enhancing the Catalytic Repertoire of Nucleic Acids II: Simulataneous Incorporation of Amino and Imidazolyl Functionalities by Two Modified Triphosphates During PCR. Nucl. Acids. Res. **2001**, *29*, 1898–1905.
21. Perrin, D.M.; Garestier, T.; Helene, C. Expanding the Catalytic Repertoire of Nucleic Acid Catalysts: Simultaneous Incorporation of Two Modified Deoxyribonucleotide Triphosphates Bearing Ammonium and Imidazolyl Functionalities. Nucleosides & Nucleotides **1999**, *18*, 377–391.
22. Bashkin, J.K.; Gard, J.K.; Modak, A.S. Synthesis and Characterization of Oligonucleotide Peptides. J. Org. Chem. **1990**, *55*, 5125–5132.
23. Beban, M.; Miller, P.S. Preparation of an Imidazole-Conjugated Oligonucleotide. Bioconj. Chem. **2000**, *11*, 599–603.
24. Ramasamy, K.S.; Zounes, M.; Gonzalez, C.; Freier, S.M.; Lesnik, E.A.; Cummins, L.L.; Giffey, R.H.; Monia, B.P.; Cook, P.D. Remarkable Enhancement of Binding-Affinity of Heterocycle-Modified DNA to DNA and RNA – Synthesis, Characterization and Biophysical Evaluation of N-2-Imidazolylpropylguanine and N-2-Imidazolylpropyl-2-Aminoadenine Modified Oligonucleotides. Tet. Lett. **1994**, *35*, 215–218.
25. Singh, D.; Kumar, V.; Ganesh, K.N. Oligonucleotides, part 5+: Synthesis and Fluorescence Studies of DNA Oligomers d(AT)5 Containing Adenine Covalently Linked at C-8 with Dansyl Fluorophore. Nucl. Acids Res. **1990**, *18*, 3339–3345.

26. Wlassoff, W.A.; Dobrikov, M.I.; Safronov, I.V.; Dudkor, Y.; Bogachev, V.S.; Kandaurova, V.V.; Shishkin, G.V.; Dymshits, G.M.; Lavrik, O.I. Synthesis and Characterization of (d)NTP Derivatives Substituted with Residues of Different Photoreagents. Binconj. Chem. **1995**, *6*, 352–360.
27. Lin, X.Y.; Robins, M.J. Nucleic acid Related Compounds: Mild and Efficient Functionalization at C6 of purine 2′-Deoxynucleosides and Ribonucleosides. Org. Lett. **2000**, *2*, 3497–3499.
28. Veliz, E.A.; Beal, P.A. 6-Bromopurine Nucleosides as Reagents for Nucleoside Analogue Synthesis. J. Org. Chem. **2001**, *55*, 8592–8598.
29. Prakash, T.P.; Kumar, R.K.; Ganesh, K.N. Synthesis and Conformational Studies of D(TPA) and R(UPA) Conjugated with Histamine and Ethylenediamine. Tetrahedron **1993**, *49*, 4035–4050.
30. Perrin, D.M.; Garestier, T.; Helene, C. Bridging the Gap Between Proteins and Nucleic Acids. J. Am. Chem. Soc. **2001**, *123*, 1556–1563.
31. Aviñó, A.; Morales, J.C.; Frieden, M.; de la Torre, B.G.; Garcia, R.G.; Cubero, E.; Luque, F.J.; Orozco, M.; Azorin, F.; Eritja, R. Parallel-stranded Hairpins Containing 8-Aminopurines. Novel Efficient Probes for Triple-Helix Formation. Bioorg. Med. Chem. Lett. **2001**, *11*, 1761–1763.
32. Cubero, E.; Aviñó, A.; de la Torre, B.G.; Frieden, M.; Eritja, R.; Luque, F.J.; González, C.; Orozco, M. Hoogsteen-Based Parallel-Stranded Duplexes of DNA. Effect of 8-Amino-purine Derivatives. J. Am. Chem. Soc. **2002**, *124*, 3133–3142.
33. Ikehara, M.; Kaneko, M. Purine Cyclonucleosides – 8 Selective Sulfonylation of 8-Bromoadenosine Derivatives and an Alternate Synthesis of 8,2′- and 8,3′-S-Cyclonucleosides. Tetrahedron **1970**, *26*, 4251–4259.
34. Laayoun, A.; Decout, J.-L.; Defrancq, E.; Lhomme, J. Hydrolysis of Oligonucleotides Containing 8-Substituted Purine Nucleosides. A New Route for Preparing Abasic Oligonucleotides. Tet. Lett. **1994**, *35*, 4991–4994.
35. Singh, D.; Kumar, V.; Ganesh, K.N. Oligonucleotides, Part 5+: Synthesis and Fluorescence Studies of DNA Oligomers d(AT)5 Containing Adenine Covalently Linked at C-8 with Dansyl Fluorophore. Nucl. Acids Res. **1990**, *18*, 3339–3345.
36. Luo, C.; Krishnasamy, R.; Basu, A.K.; Zou, Y. Recognition and Incision of Site-Specifically Modified C8 Guanine Adducts Formed by 2-Aminofluorene, N-Acetyl-2-Aminofluorene and 1-Nitropyrene by UvrABC Nuclease. Nucl. Acids Res. **2002**, *28*, 3719–3724.
37. Huff, B.E.; LeTourneau, M.E.; Staszak, M.A.; Ward, J.A. Protection, Metallation, and Electrophilic Substitution of 5-methyl Tetrazole. Tet. Lett. **1996**, *37*, 3655–3658.
38. Dolensky, B.; Kirk, K.L. New Building Blocks for Fluorinated Imidazole Derivatives: Preparation of β-Fluoro- and β,β-Difluorohistamine. J. Org. Chem. **2001**, *66*, 4687–4691.
39. Bashkin, J.K.; Gard, J.K.; Modak, A.S. Synthesis and Characterization of Oligonucleotide Peptides. J. Org. Chem. **1990**, *55*, 5125–5132.
40. Santoro, S.W.; Joyce, G.F.; Sakthivel, K.; Gramatikova, S.; Barbas, C.F. RNA Cleavage by a DNA Enzyme with Extended Chemical Functionality. J. Am. Chem. Soc. **2000**, *122*, 2433–2439.

41. Perrin, D.M.; Garestier, T.; Helene, C. Expanding the Catalytic Repertoire of Nucleic Acid Catalysts: Simultaneous Incorporation of Two Modified Deoxyribonucleotide Triphosphates Bearing Ammonium and Imidazolyl Functionalities. Nucleosides & Nucleotides **1999**, *18*, 377–391.
42. Perrin, D.M.; Garestier, T.; Helene, C. Bridging the Gap Between Proteins and Nucleic Acids. J. Am. Chem. Soc. **2001**, *123*, 1556–1563.
43. Avino, A.; Morales, J.C.; Frieden, M.; de la Torre, B.G.; Garcia, R.G.; Cubero, E.; Luque, F.J.; Orozco, M.; Azorin, F.; Eritja, R. Parallel-stranded Hairpins Containing 8-Aminopurines. Novel Efficient Probes for Triple-Helix Formation. Bioorg. Med. Chem. Lett. **2001**, *11*, 1761–1763.
44. Cubero, E.; Aviñó, A.; de la Torre, B.G.; Frieden, M.; Eritja, R.; Luque, F.J.; González, C.; Orozco, M. Hoogsteen-Based Parallel-Stranded Duplexes of DNA. Effect of 8-Amino-purine Derivatives. J. Am. Chem. Soc. **2002**, *124*, 3133–3142.
45. Singh, D.; Kumar, V.; Ganesh, K.N. Oligonucleotides, Part 5+: Synthesis and Fluorescence Studies of DNA Oligomers d(AT)5 Containing Adenine Covalently Linked at C-8 with Dansyl Fluorophore. Nucl. Acids Res. **1990**, *18*, 3339–3345.
46. Ikehara, M.; Kaneko, M. Purine Cyclonucleosides – 8 Selective Sulfonylation of 8-Bromoadenosine Derivatives and an Alternate Synthesis of 8,2′- and 8,3′-S-Cyclonucleosides. Tetrahedron **1970**, *26*, 4251–4259.

Received April 15, 2002
Accepted July 5, 2002