Synthesis and Characterization of Imidazoyl-Linked Synthons and 3'-Conjugated Thymidine Derivatives

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The synthesis of the phosphoramidites and H-phosphonates of imidazoyl derivatives, including histidine and imidazoleacetic acid, blocked at the imidazole and amino groups and connected by a hexane linker arm, are described. These synthons have been used to synthesize novel 3'-conjugated thymidine derivatives using 5'-(dimethoxytrityl)thymidine. These synthons are being used to prepare oligonucleotides with terminal imidazole groups on the automatic synthesizer.

Introduction

Artificial endonucleases are chemically synthesized, sequence-specific nucleic acid cleaving reagents which provide new opportunities in nucleic acid chemistry and biology. They are comprised of a nucleic acid binding group (usually single-stranded oligonucleotide) and a cleaving reagent, which can be a metal chelate, photoactive group, or nonspecific nuclease.[1,2] Artificial or chemical endonucleases are of interest as potential activated antisense or informational drugs.[3,4] In this respect, the creation of artificial endonucleases with cleaving groups which are able to work as catalysts is especially important, since in this case even a small concentration of the antisense drug can give the desired result.

To synthesize such drugs it is reasonable to proceed from native nucleases. Interesting work in this direction was made by Schultz and colleagues, who synthesized hybrid nucleases consisting of an oligonucleotide fused to a unique site on the relatively nonspecific staphylococcal nuclease, and showed that such hybrid nucleases are able to cleave the DNA target in a sequence-specific manner.[5-7] But such complicated conjugates would be very expensive drugs, at least in the near future. It is obvious that, in terms of a pharmaceutical application, conjugates with simpler cleaving groups are preferable.

It is well known that the mechanism of action of ribonucleases is based on the catalytic action of two imidazole residues (from His-119 and His-12 in the case of RNase A) which are part of the active site of the enzyme.[8-10] Briefly, the protonated Im group of His-119 (ImH^+) activates the phosphate group of RNA by donation of a proton (general acid catalysis). The Im group of His-12 then abstracts a proton from the 2'-OH (general base catalysis) facilitating the attack of the 2'-oxygen onto phosphorus with formation of cyclic 2',3'-phosphate. Also, it was shown by Breslow and co-workers that imidazole by itself or in combination with Zn^{++} can catalyze cleavage of RNA.[9,11]

It is of interest to prepare oligonucleotide conjugates with a pendant imidazole moiety and to check their ability to cleave RNA in a sequence-specific manner.[3] Bashkin and co-workers have reported the synthesis of uridine–imidazole conjugates which are based on C-5-substituted deoxyuridine,[12] and they also reported synthesis of oligonucleotide histidines in which the histidine residue is attached by a linker arm to the C-5 of the uridine ring.[13] But, there are possible disadvantages of such derivatives containing a C-5-substituted uridine residue. Substitution of a bulky group at C-5 of the uridine ring would be expected to hinder formation of hydrogen bonds between modified uridine and the complementary adenine residue in the oligonucleotide duplex and, probably, this bulky group will hinder formation of stable complementary bonds between neighboring bases also. Hence, the stability of the duplex of modified oligonucleotide with target RNA may be decreased. Also, even with a linker arm between the imidazole residue and the uridine ring, it can still be difficult, from a spatial point of view, for the imidazole residue to reach the 2'-OH of the nearest ribose residue that is necessary for cleavage of RNA. Because of these factors we think that the introduction of an imidazole residue onto the 5'- or 3'-end of oligonucleotides is desirable.

The purpose of this work is to synthesize imidazole-containing synthons which are suitable for introducing a terminal imidazole moiety onto oligonucleotides and which is compatible with standard DNA synthesis protocols.

Results and Discussion

Phosphoramidite and H-phosphonate approaches are the most commonly used in automated solid-phase oligonucleotide synthesis. That is why we prepared and checked the capacity of both phosphoramidite and H-phosphonate imidazole-containing synthons and we proceeded from both imidazoleacetic acid and histidine as

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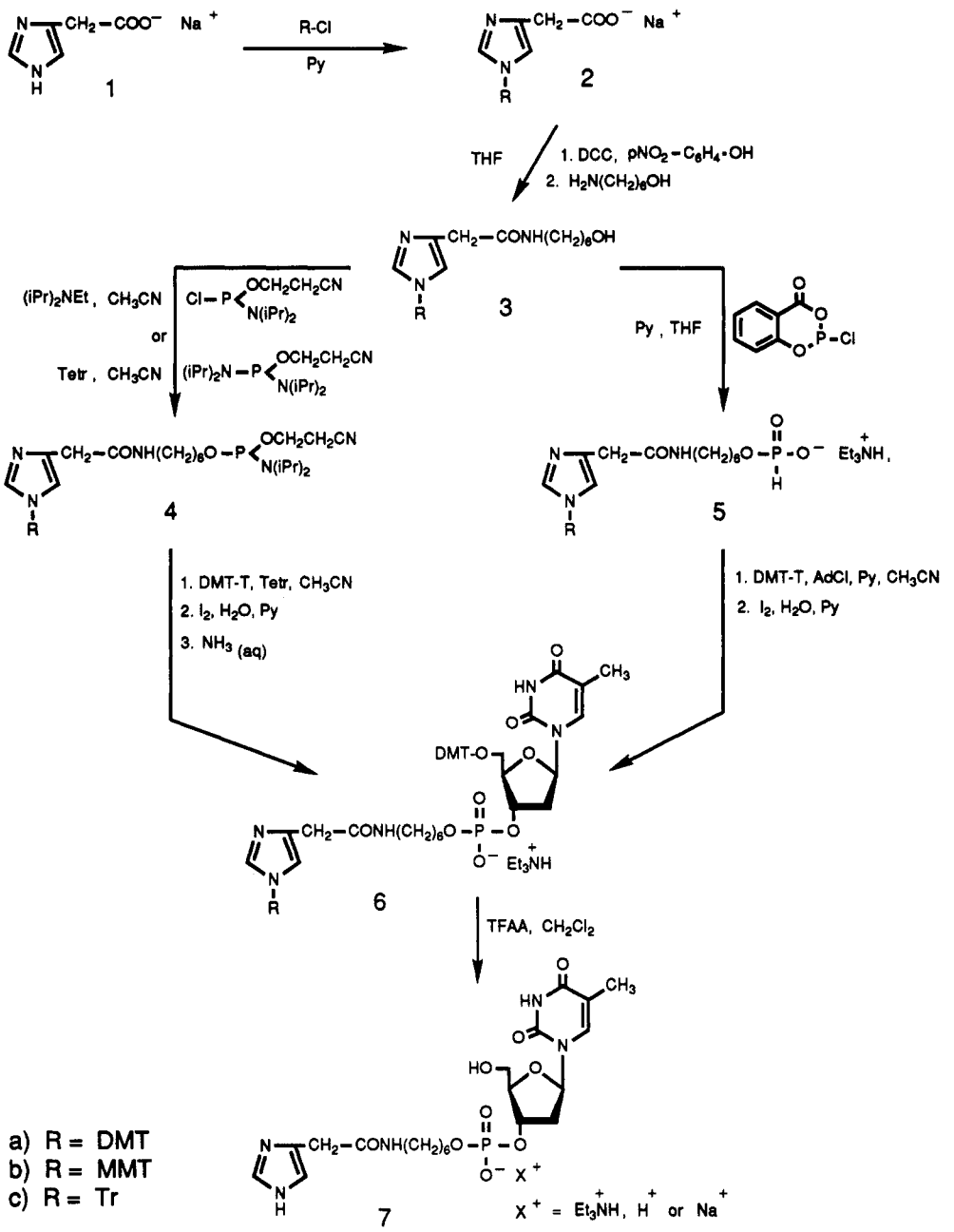
Scheme I



starting materials (see Schemes I and II). The advantage of synthons based on imidazoleacetic acid is that only one position, namely N-1 of the imidazole ring, needs to be blocked and, hence, simpler chemistry is involved. This means that the final products will be less expensive, which can be important in large-scale synthesis. By contrast, in the case of histidine derivatives we have to protect both N-1 in the imidazole ring and the α-amino group, which is a more difficult task. Fortunately, there are a number of commercially available protected histidine derivatives, some of which we successfully used in our work. Besides this, the presence of a protected amino group (especially when it is protected with an fluoren-9-ylmethoxycarbonyl (Fmoc) protecting group) improves crystallization. Thus, phosphoramidites **4b,c** are oils at room temperature, while phosphoramidites **10a–d** can be prepared as solid materials after precipitation in pentane. In any case, we thought that it was worthwhile to prepare several different kinds in order to find the most suitable imidazole-containing synthons for incorporation onto the end of oligonucleotides.

Synthons Prepared from Imidazoleacetic Acid. We investigated three acid-labile protecting groups for protection of N-1 in the imidazole ring: dimethoxytrityl (DMT), monomethoxytrityl (MMT), and trityl (Tr). These groups were introduced by treatment of the sodium salt of imidazoleacetic acid (**1**) with the corresponding chloride in anhydrous pyridine (Py)[14] (Scheme I). To introduce the hexane linker arm, protected imidazoleacetic acid, sodium salt (**2**), was coupled with *p*-nitrophenol in the presence of DCC, and the *p*-nitrophenyl ester formed was treated in situ with 6-amino-1-hexanol. Unlike acids **2a–c**, alcohols **3a–c** have good chromatographic mobility on silica gel and are suitable for investigating the stability of these protecting groups and their compatibility with the protocols of oligonucleotide synthesis. Thus, it turned out that the DMT group is extremely sensitive toward acids when it blocks N-1 in the imidazole ring. Even such

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a weak acid as tetrazole (0.25 M in CH_3CN) completely removes this group in 3–5 min at room temperature. This means that DMT, as a protecting group for the imidazole residue, is incompatible with the phosphoramidite approach, and we decided not to use it further in our work. Contrarily, Tr at the N-1 position is too stable.[15] Even by treatment with 25% CF_3COOH in CH_2Cl_2 it takes at least 3 h to completely remove the trityl group from the imidazole moiety. Although this protecting group is good enough for preparing thymidine conjugates it will definitely be a problem to use Tr in the case of oligonucleotide conjugates, as noticeable apurination will take place during severe acidic conditions which are needed for deprotection of the imidazole residue. From our experience the MMT group gives a reasonable compromise as protection for N-1 in imidazole, as it is stable enough to perform phosphoramidite ($\tau_{1/2}$ for removing MMT with 0.25 M tetrazole in CH_3CN is about 10 min) and H-phosphonate coupling reactions (even if partial deprotection of imidazole residue in the conjugate takes place, it does not affect the yield of target deprotected conjugate) and can be easily removed in mild acidic conditions.

Phosphoramidites **4b,c** and H-phosphonates **5b,c** were prepared from alcohols **3b,c** using standard phosphorylating and phosphonating reagents.[16] The activity of all synthons was checked by coupling with 5′-*O*-DMT-thymidine, which was chosen because it contains a 3′-hydroxyl group that is convenient for derivitization. This secondary alcohol is less active than the 5′-primary alcohol target for derivitization of oligodeoxyribonucleotides in the automatic synthesizer. Both in the case of phosphoramidites and in the case of H-phosphonates the isolated yields for 3′-conjugated thymidine derivatives **6** were more than 80%. It is worth pointing out that H-phosphonates **5b,c** are preferable to handle for the following reasons: (1) they can be prepared as solids, (2) they are more stable and can be kept for longer time periods and, (3) the H-phosphonate coupling reaction is less sensitive to moisture. Also, we want to stress once again that only MMT-protected synthons **4c** and **5c** can be used for preparation of oligonucleotide conjugates as the Tr group needs very strong acidic conditions for deprotection to be compatible with the protocols of oligonucleotide synthesis.

Treatment of **6b,c** with 25% CF_3COOH in CH_2Cl_2 gave the deprotected 3′-conjugated thymidine **7**, which was characterized by NMR and FAB mass spectroscopy. ^1H NMR spectra for **7** are shown in Figures 1 and 2. Using both one- and two-dimensional ^1H NMR analysis and on the basis of literature data, we were able to assign all resonance signals. It is interesting to note that the Im-$CH_2$6 group gives reduced or even no signal (Figure 1) in strong basic conditions, which reflects the exchange of these protons and the CH acidic character of this group.

Synthons Prepared from Histidine. A number of protected histidine derivatives are commercially available. In our work we used some of them (**8a–d**, Scheme II) to find the most suitable combination of protecting groups for preparing synthons which can then be used for synthesis of 5′- or 3′-conjugated oligonucleotides. The synthetic route for preparing phosphoramidites and H-phosphonates of histidine derivatives was similar to that described above

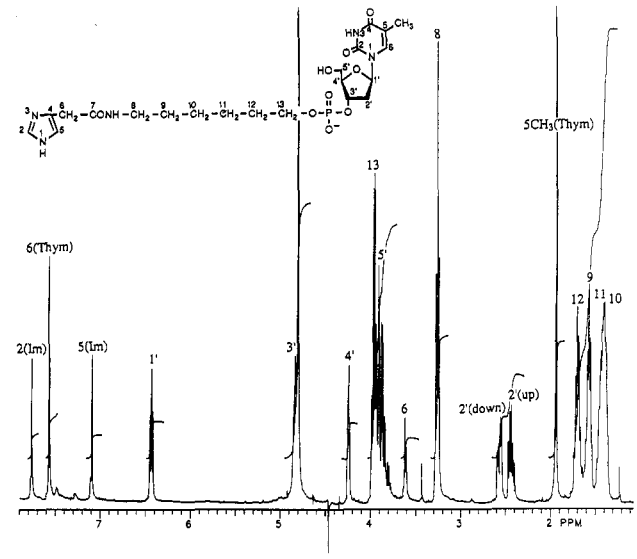

Figure 1. ^1H-NMR spectrum of the 3′-conjugated thymidine **7**. The spectrum was run at 400 MHz.

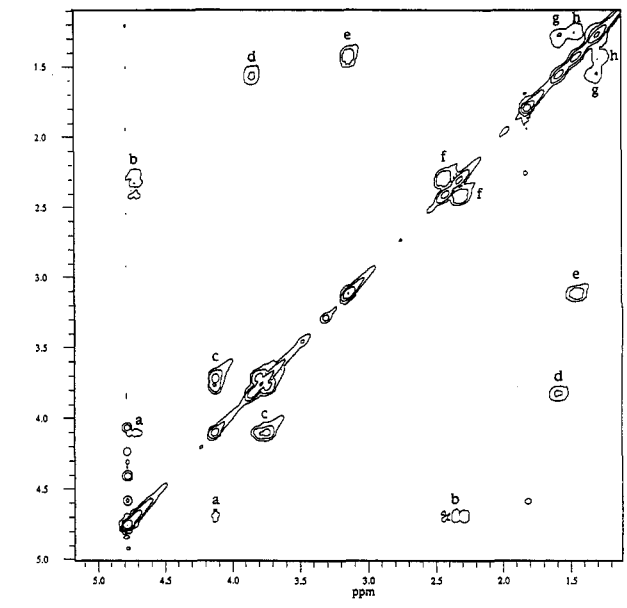

Figure 2. The expanded 1.1–5.2 ppm region of the COSY spectrum of the 3′-conjugated thymidine **7** shows scalar couplings between H3′ and H4′ (a); H3′ and two H2′ (b); H4′ and two H5′ (c); $CH_2$13 and $CH_2$12 (d); $CH_2$8 and $CH_2$9 (e); H2′ down and H2′ up (f); $CH_2$12 and $CH_2$11 (g); $CH_2$9 and $CH_2$10 (h). The spectrum was run at 400 MHz in absolute value mode, with a 512 × 1 K data matrix and 16 transients per increment.

(Scheme I) except that C_6F_5OH rather than *p*-nitrophenol was used for the preparation of alcohols **9c,d** (Scheme II). As before, the capacity of all synthons was checked by coupling with 5′-DMT-*O*-thymidine. All synthons, except **10c**, gave more than 70% isolated yield for conjugates **12**. Low yield (42%) in the case of **10c** can be explained by the instability of the Fmoc group at N-1 of the imidazole ring. We conclude that Fmoc cannot be used as protection for N-1 in the imidazole residue of synthons which are suitable for synthesis of modified oligonucleotides. Contrary to Fmoc, the trityl group, as it was mentioned above, is too stable for our purpose, even though it was successfully used for the synthesis of 3′-conjugated thymidine **13**. From our point of view Dnp and Boc are the groups of choice for N-1 protection, as they are stable enough in conditions

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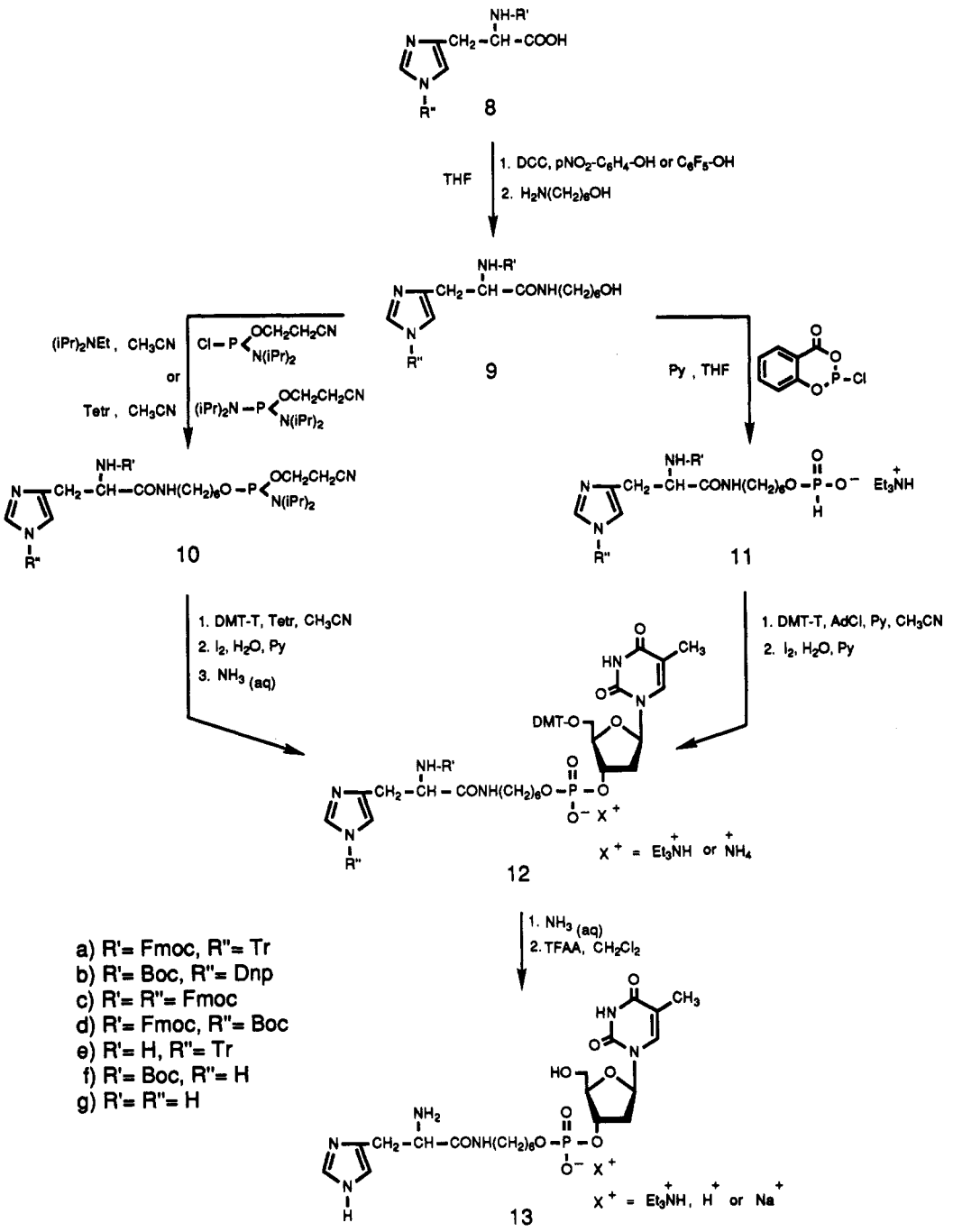
Scheme II



a) R'= Fmoc, R"= Tr
b) R'= Boc, R"= Dnp
c) R'= R"= Fmoc
d) R'= Fmoc, R"= Boc
e) R'= H, R"= Tr
f) R'= Boc, R"= H
g) R'= R"= H

of coupling reactions and can be easily removed by ammonia treatment, which is usually used for deprotection of oligonucleotides. Note that we were unable to synthesize phosphoramidite **10d** using 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite, probably because of instability of the Boc group during phosphorylation. An alternative synthesis for the phosphorylation of **9d** using milder 2-cyanoethyl *N,N,N′,N′*-tetraisopropylphosphoramidite in the presence of tetrazole[17] gave a good result (71% isolated yield). It is important to note that synthons **10b,d** and **11b,d** can be prepared as solids after precipitation in pentane which makes them more suitable to handle.

The choice for protection of the α-amino group will depend on the purification method which will be applied. If it is planned to use HPLC for purification of oligonucleotides, we recommend use of the Boc group (synthons **10b, 11b**), as this group is stable during ammonia treatment and has a hydrophobic nature. But, we should take into account that this group needs rather strong acidic conditions (25% CF_3COOH, 30 min, room temperature) to be deprotected, which can lead to noticeable apurination. If electrophoresis is to be used for purification, the Fmoc-protecting group (synthons **10d, 11d**) will be preferable.

Fully protected conjugates **12** were first deprotected with aqueous ammonia (which removes Dnp, Fmoc, and Boc from N-1 of the imidazole ring and Fmoc from the α-amino group) and then with CF_3COOH in CH_2Cl_2 (which removes Tr from N-1 of the imidazole ring, Boc from α-amino group, and DMT from 5′-OH). The structure of the fully deprotected conjugate **13** was proven by NMR and FAB mass spectroscopy. One- and two-dimensional ^1H NMR spectra are presented in Figures 3 and 4,

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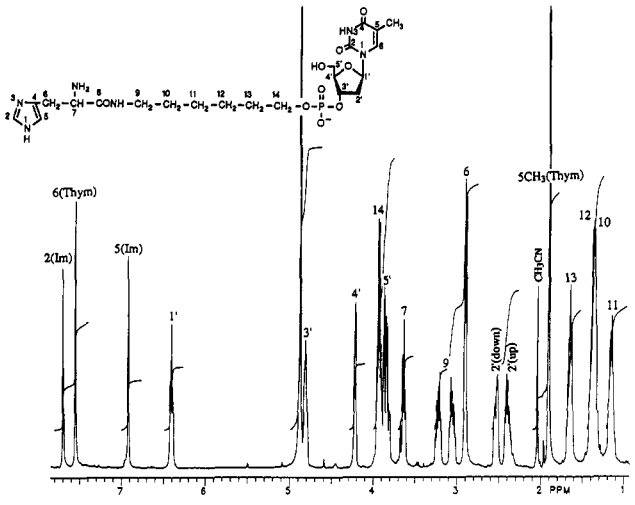

Figure 3. ^{1}H-NMR spectrum of the 3′-conjugated thymidine **13**. The spectrum was run at 400 MHz.

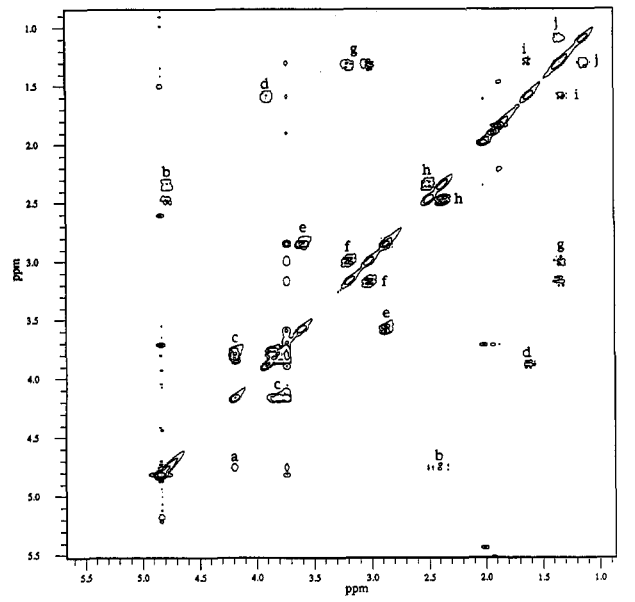

Figure 4. The expanded 0.9–5.6 ppm region of the COSY spectrum of the 3′-conjugated thymidine **13** shows scalar couplings between H3′ and H4′ (a); H3′ and two H2′ (b); H4′ and two H5′ (c); $CH_2$14 and $CH_2$13 (d); CH7 and $CH_2$6 (e); two protons of $CH_2$9 (f); $CH_2$9 and $CH_2$10 (g); H2′ down and H2′ up (h); $CH_2$13 and $CH_2$12 (i); $CH_2$10 and $CH_2$11 (j). The spectrum was run at 400 MHz in absolute value mode, with a 512 × 2 K data matrix and 16 transients per increment.

respectively. Analysis of these spectra and comparison with those for conjugate **7** allow us to assign all resonance signals.

Conclusions

We report here the synthesis of novel phosphoramidites and H-phosphonates comprised of linked imidazole residues. Some of these have protecting groups compatible with solid-phase DNA synthesis protocols and can be used for preparation of 5′- or 3′-conjugated oligonucleotides. These are of great interest as potential site-selective RNA hydrolytic catalysts. The capacity of the synthesized synthons was checked by coupling them with 5′-DMT-*O*-thymidine, and the structures of the deprotected 3′-conjugated thymidine derivatives **7** and **13** were fully characterized with extensive NMR and FAB mass spectral analysis.

Experimental Section

General. NMR chemical shifts are reported in ppm (δ units) downfield from TMS or TSP for ^{1}H and from H_3PO_4 for ^{31}P NMR spectra. Samples for FAB mass spectrometry were dissolved in 9:1,3-nitrobenzyl alcohol (NBA)/glycerol, and recorded in both negative and positive ion modes. Thin-layer chromatography was performed on Kieselgel 60 F_{254} plates with aluminum backing (Merck) and development with the following solvent systems: $CHCl_3$/MeOH = 9:1 (system A), $CHCl_3$/EtOAc/Et_3N = 45:45:10 (system B). Spots were visualized by irradiation with UV light (254 nm) and by exposing the TLC plates to vapor of concentrated hydrochloric acid (in the case of trityl-, methoxytrityl-, or dimethoxytrityl-containing compounds). Preparative TLC was carried out using silica gel plates with glass backing (Sigma). Column chromatography was performed on silica gel 60 (230–400 mesh, Merck). Reverse-phase HPLC was performed on a Hamilton PRP-1 preparative column (10 μm, 250 × 21.5 mm), using a linear gradient of 0–100% solvent B in solvent A flowing at 10 mL/min for 25 min. Solutions of samples in organic solvents were dried over Na_2SO_4.

The following compounds were obtained from commercial sources: 4-Imidazoleacetic acid, sodium salt dihydrate (Aldrich) 4,4′-dimethoxytriphenylmethyl chloride (DMT-Cl, Aldrich), 4-methoxytriphenylmethyl chloride (MMT-Cl, Aldrich), triphenylmethyl chloride (Tr-Cl, Aldrich), dicyclohexylcarbodiimide (DCC, Aldrich), 6-amino-1-hexanol (Aldrich), 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (Aldrich), 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphoramidite (Aldrich), 2-chloro-5,6-benzo-4*H*-1,3,2-dioxaphosphorin-4-one (Aldrich), 5′-*O*-(4,4′-dimethoxytriphenylmethyl)thymidine (DMT-T, Aldrich), Fmoc-L-His(Tr)-OH (Fluka), Boc-L-His(Dnp)-OH (Bachem), Fmoc-L-His(Fmoc)-OH (Bachem), Fmoc-L-His(Boc)-OH (Bachem), all solvents (Aldrich; used without any further purification). Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN.

1-(4,4′-Dimethoxytriphenylmethyl)imidazole-4-acetic Acid, Sodium Salt[18] (2a). 4-Imidazoleacetic acid, sodium salt dihydrate (**1**) (1 g, 5.4 mmol) was dissolved in MeOH (15 mL). Py (35 mL) was added to this solution and the mixture was evaporated to dryness. This procedure was repeated again. The dry residue was suspended in anhydrous Py (25 mL), and DMT-Cl (2.19 g, 6.5 mmol) was added to the suspension. The reaction mixture was stirred for 48 h at room temperature. The reaction was quenched with MeOH (5 mL) and concentrated to dryness. The residue was taken up in CH_2Cl_2 (150 mL) and washed with 0.5 M aqueous triethylammonium bicarbonate (TEAB) (2 × 150 mL). The dried organic phase was concentrated and the residue was recrystallized from methanol–acetone to yield **2a** as a white solid (1.17 g, 2.6 mmol, 48%): ^{1}H NMR ($CDCl_3$) δ 7.47 (s, 1H, H2 (Im)), 7.31–7.37 (m, 3H, Ar), 7.06–7.12 (m, 2H, Ar), 7.00–7.04 (m, 4H, Ar), 6.82–6.87 (m, 4H, Ar), 6.69 (s, 1H, H5 (Im)), 3.82 (s, 6H, OCH_3), 3.58 (s, 2H, CH_2-COO^-); FABMS *m*/*z* 427.0$^-$ (M^-), 580.3$^-$ (M^- + NBA), 303.1$^+$ (DMT^+).

1-(4-Methoxytriphenylmethyl)imidazole-4-acetic Acid, Sodium Salt (2b)[18]. **2b** was prepared from **1** exactly as **2a** except MMT-Cl was used instead of DMT-Cl and crude product was chromatographed over silica gel using a gradient of 5–15% MeOH and 1–2% Et_3N in $CHCl_3$. The product fractions were evaporated, dissolved in CH_2Cl_2 (100 mL), and washed with 0.5 M TEAB (100 mL). The dried organic phase was evaporated and decanted from pentane to yield **2b** as a white solid (1.65 g, 3.9 mmol, 73%): ^{1}H NMR ($CDCl_3$) δ 7.47 (s, 1H, H2 (Im)), 7.31–7.37 (m, 6H, Ar), 7.07–7.12 (m, 4H, Ar), 7.00–7.04 (m, 2H, Ar), 6.82–6.87 (m, 2H, Ar), 6.66 (s, 1H, H2 (Im)), 3.82 (3H, OCH_3), 3.59 (s, 2H, CH_2-COO^-); FABMS *m*/*z* 397.0$^-$ (M^-), 550.7$^-$ (M^- + NBA), 273.0$^+$ (MMT^+), 399.1$^+$ (M^- + 2H$^+$).

1-(Triphenylmethyl)imidazole-4-acetic Acid, Sodium Salt (2c). **2c** was prepared from **1** exactly as **2a** except Tr-Cl was used instead of DMT-Cl and crude product was chromatographed over silica gel using a gradient of 5–20% MeOH in $CHCl_3$. **2c** was prepared as a white solid (1.60 g, 4.1 mmol, 76%): ^{1}H NMR ($CDCl_3$) δ 7.49 (s, 1H, H2 (Im)), 7.33–7.37 (m, 9H, Ar), 7.10–7.14 (m, 6H, Ar), 6.70 (s, 1H, H5 (Im)), 3.65 (s, 2H, CH_2COO^-); FABMS *m*/*z* 367.1$^-$ (M^-), 520.2$^-$ (M^- + NBA), 243.1$^+$ (Tr^+), 369.1$^+$ (M^- + 2H$^+$).

(18) Some presence of triethylammonium salt is possible.

N^6-[[1-(4,4′-Dimethoxytriphenylmethyl)imidazol-4-yl]acetyl]-6-amino-1-hexanol (3a). The mixture of **2a** (900 mg, 2 mmol) and *p*-nitrophenol (310 mg, 2.2 mmol) were coevaporated with Py to remove traces of water. The residue was dissolved in mixture of anhydrous Py (1 mL) and anhydrous tetrahydrofuran (THF) (10 mL). Dicyclohexylcarbodiimide (DCC) (620 mg, 3 mmol) was added to the solution and the reaction mixture was stirred for 24 h at room temperature. Then dried 6-amino-1-hexanol (470 mg, 4 mmol) was added and the stirring was continued for an additional 30 min. The reaction mixture was filtered and the solid phase was washed with THF (2 × 5 mL). The combined liquid phases were concentrated to dryness, and the residue was taken up in CH_2Cl_2 (50 mL) and washed with 10% Na_2CO_3 (3 × 50 mL). The dried organic phase was concentrated and the residue was chromatographed over silica gel using a gradient of 0–5% MeOH in $CHCl_3$ (contained 0.5% of Et_3N) to yield **3a** as a hydroscopic yellow solid (410 mg, 0.78 mmol, 39%): R_f = 0.38, system A; ^1H NMR ($CDCl_3$) δ 7.40 (d, 1H, H2 (Im)), 7.30–7.34 (m, 3H, Ar), 7.07–7.11 (m, 2H, Ar), 7.00–7.04 (m, 4H, Ar), 6.82–6.87 (m, 4H, Ar), 6.70 (d, 1H, H5 (Im)), 3.82 (s, 6H, OCH_3), 3.57–3.62 (t, 2H, $1CH_2$-OH), 3.47 (s, 2H, Im-CH_2CO), 3.20–3.26 (q, 2H, CONH-$6CH_2$), 1.45–1.57 (m, 4H, $2CH_2$ + $5CH_2$), 1.26–1.42 (m, 4H, 3,4-$(CH_2)_2$); FABMS *m/z* 528.4$^+$ (M + H$^+$), 302.9$^+$ (DMT$^+$), 225.9$^+$ (M − DMT$^+$ + 2H$^+$), 526.4$^-$ (M − H$^+$), 224.0$^-$ (M − DMT$^+$).

N^6-[[1-(4-Methoxytriphenylmethyl)imidazol-4-yl]acetyl]-6-amino-1-hexanol (3b). **3b** was prepared from **2b** exactly as **3a** and yielded a hydroscopic yellow solid (74%): R_f = 0.37, system A; ^1H NMR ($CDCl_3$) δ 7.40 (d, 1H, H2 (Im)), 7.31–7.35 (m, 6H, Ar), 7.08–7.12 (m, 4H, Ar), 7.00–7.05 (m, 2H, Ar), 6.82–6.87 (m, 2H, Ar), 6.66 (d, 1H, H5 (Im)), 3.82 (s, 3H, OCH_3), 3.58–3.63 (t, 2H, $1CH_2OH$), 3.45 (s, 2H, Im-CH_2CO), 3.21–3.27 (q, 2H, CONH-$6CH_2$), 1.44–1.58 (m, 4H, $2CH_2$ + $5CH_2$), 1.26–1.42 (m, 4H, 3,4-$(CH_2)_2$); FABMS *m/z* 498.4$^+$ (M + H$^+$), 273.0$^+$ (MMT$^+$), 226.0$^+$ (M − MMT$^+$ + 2H$^+$), 496.4$^-$ (M − H$^+$), 224.1$^-$ (M − MMT$^+$). Anal. Calcd for $C_{31}H_{35}N_3O_3$: C, 74.80; H, 7.09; N, 8.44; O, 9.65. Found: C, 74.76; H, 7.12; N, 8.31; O. 9.45.

N^6-[[1-(Triphenylmethyl)imidazol-4-yl]acetyl]-6-amino-1-hexanol (3c). **3c** was prepared from **2c** exactly as **3a**, and yielded a white solid (77%): R_f = 0.35, system A; ^1H NMR ($CDCl_3$) δ 7.40 (d, 1H, H2 (Im)), 7.32–7.37 (m, 9H, Ar), 7.10–7.15 (m, 6H, Ar), 6.68 (d, 1H, H5 (Im)), 3.58–3.64 (t, 2H, $1CH_2OH$), 3.46 (s, 2H, Im-CH_2CO), 3.21–3.28 (q, 2H, CONH-$6CH_2$), 1.46–1.58 (m, 4H, $2CH_2$ + $5CH_2$), 1.29–1.42 (m, 4H, 3,4-$(CH_2)_2$); FABMS *m/z* 468.1$^+$ (M + H$^+$), 243.0$^+$ (Tr$^+$), 226.0$^+$ (M − Tr$^+$ + 2H$^+$), 466.1$^-$ (M − H$^+$), 224.1$^-$ (M − Tr$^+$). Anal. Calcd for $C_{30}H_{33}N_3O_2$: C, 77.06; H, 7.11; N, 8.99; O, 6.84. Found: C, 76.60; H, 7.11; N, 8.89; O, 6.79.

N^6-[[1-(4-Methoxytriphenylmethyl)imidazol-4-yl]acetyl]-6-aminohexyl 2-Cyanoethyl *N,N*-Diisopropylphosphoramidite (4b). **3b** (500 mg, 1 mmol) was dissolved in anhydrous CH_2Cl_2 (10 mL) under Ar atmosphere. Dry diisopropylethylamine ((iPr)$_2$NEt) (700 μL, 4 mmol) was added to this solution and the mixture was cooled on ice. 2-Cyanoethyl *N,N*-diisopropylchlorophosphoramidite (335 μL, 1.5 mmol) was added with stirring and the reaction mixture was left for 10 min at room temperature. The reaction mixture was then poured into 1 M TEAB (50 mL) and extracted with CH_2Cl_2 (50 mL). Dried organic phase was concentrated and the residue was chromatographed over silica gel using 5% Et_3N in $CHCl_3$ to yield **4b** as a pale yellow oil (450 mg, 0.65 mmol, 65%): R_f = 0.48, system B; ^{31}P NMR ($CDCl_3$) δ 144.40. FABMS *m/z* 850.0$^-$ (M + NBA − H$^+$), 866.7$^-$ (M + O + NBA − H$^+$), 719.8$^+$ (M + Na$^+$), 736.0$^+$ (M + O + Na$^+$), 273.0$^+$ (MMT$^+$).

N^6-[[1-(Triphenylmethyl)imidazol-4-yl]acetyl]-6-aminohexyl 2-Cyanoethyl *N,N*-Diisopropylphosphoramidite (4c). **3c** (0.93 g, 2 mmol) was dissolved in anhydrous CH_2Cl_2 (10 mL) under Ar atmosphere. Tetrazole (0.5 M) in anhydrous CH_3CN (4 mL, 2 mmol) and 2-cyanoethyl *N,N,N′,N′*-tetraisopropylphosphoramidite (950 μL, 3 mmol) were added one after another with stirring. The reaction mixture was left at room temperature for 30 min, poured into 1 M TEAB (150 mL), and extracted with CH_2Cl_2 (150 mL). Dried organic phase was concentrated and the residue was chromatographed over silica gel using 5% Et_3N in $CHCl_3$ to yield **4c** as a white oil (1.19 g, 1.78 mmol, 89%): R_f = 0.47, system B; ^{31}P NMR ($CDCl_3$) δ 144.40; FABMS *m/z* 820.2$^-$ (M + NBA − H$^+$), 836.9$^-$ (M + O + NBA − H$^+$), 689.7$^+$ (M + Na$^+$), 706.0$^+$ (M + O + Na$^+$), 242.9$^+$ (Tr$^+$).

N^6-[[1-(4-Methoxytriphenylmethyl)imidazol-4-yl]acetyl]-6-aminohexyl Phosphonate, Triethylammonium Salt (5b). **3b** (500 mg, 1 mmol) was dissolved in a mixture of anhydrous THF and Py (4:1,10 mL). The solution was cooled on ice and phosphonating reagent (2-chloro-5,6-benzo-4*H*-1,3,2-dioxaphosphorin-4-one) in dry THF (0.5 M, 2.4 mL, 1.2 mmol) was added with stirring. The reaction mixture was left for 15 min at room temperature. Then H_2O (1 mL) was added and the reaction mixture was kept at room temperature for another 10 min. 1 M TEAB (100 mL) was added, and the mixture was extracted with CH_2Cl_2 (100 mL). Dried organic phase was concentrated and the residue was chromatographed over silica gel using a gradient of 5–20% H_2O in acetone (contained 1% of Et_3N) to yield **5b** as a hygroscopic white solid (420 mg, 0.64 mmol, 64%): ^{31}P NMR ($CDCl_3$) δ 3.43, −0.28 (d); FABMS *m/z* 560.5$^-$ (M$^-$), 288.1$^-$ (M$^-$ − MMT$^+$ + H$^+$), 273.0$^+$ (MMT$^+$), 102.1$^+$ (Et_3NH^+).

N^6-[[1-(Triphenylmethyl)imidazol-4-yl]acetyl]-6-aminohexyl Phosphonate, Triethylammonium Salt (5c). **5c** was prepared from **3c** (467 mg, 1 mmol) exactly as **5b**, but after column chromatography and evaporation the product was dissolved in 1,2-dichloroethane (5 mL) and precipitated in pentane (200 mL) to yield a hygroscopic fluffy white solid (68%): ^{31}P NMR ($CDCl_3$) δ 3.43, −0.28 (d); FABMS *m/z* 530.3$^-$ (M$^-$), 288.1$^-$ (M$^-$ − Tr$^+$ + H$^+$), 243.0$^+$ (Tr$^+$), 102.2$^+$ (Et_3NH^+).

5′-*O*-(4,4′-Dimethoxytriphenylmethyl)thymidin-3′-yl N^6-[[1-(4-Methoxytriphenylmethyl)imidazol-4-yl]acetyl]-6-aminohexyl Phosphate, Triethylammonium Salt (6b). 1. Phosphoramidite Approach. 5′-*O*-(4,4′-Dimethoxytriphenylmethyl)thymidine (DMT-T) (110 mg, 0.2 mmol) was dried by coevaporation with CH_2Cl_2 and suspended in a solution of tetrazole in anhydrous CH_3CN (0.5 M, 1 mL, 0.5 mmol) under Ar atmosphere. To this suspension a solution of **4b** in anhydrous CH_3CN (0.1 M, 2 mL, 0.2 mmol) was added and the reaction mixture was stirred for 10 min at room temperature. Then oxidation mixture (I_2/H_2O/Py/THF = 3:2:20:85 (m/v/v/v), 3 mL) was added and stirring was continued for another 15 min. The reaction mixture was discolored with aqueous sodium hydrosulfite solution (0.5 M, 1 mL), poured into 1 M TEAB (50 mL), and extracted with CH_2Cl_2 (50 mL). Dried organic phase was concentrated to dryness and the residue was dissolved in MeOH (5 mL). To this solution was added 25% aqueous ammonium hydroxide (2 mL). The reaction mixture was left at room temperature for 15 min and was then concentrated and coevaporated with acetone to dryness. The residue was chromatographed over silica gel using a gradient of 0–10% MeOH and 0.1–1% Et_3N in $CHCl_3$. Pure fractions were pooled, concentrated to dryness, dissolved in 1,2-dichloroethane (1 mL), and precipitated in pentane (50 mL) to yield **6b** as a fluffy white solid (180 mg, 0.16 mmol, 81%).

2. H-Phosphonate Approach. DMT-T (110 mg, 0.2 mmol) and **5b** (130 mg, 0.2 mmol) were dried by coevaporation with CH_2Cl_2 and dissolved in a mixture of anhydrous Py and CH_3CN (1:1, 5 mL). 1-Adamantanecarbonyl chloride (Ad-Cl) (200 mg, 1.0 mmol) was added to this solution with stirring. The reaction mixture was stirred for 10 min at room temperature, poured into 1 M TEAB (50 mL), and extracted with CH_2Cl_2 (50 mL). Dried organic phase was concentrated to dryness and oxidation mixture (I_2/H_2O/Py = 2:2:98 (m/v/v), 3 mL) was added to the residue. The reaction mixture was left for 1 h at room temperature, discolored with aqueous sodium hydrosulfite solution (0.5 M, 1 mL), poured into 1 M TEAB (50 mL), and extracted with CH_2Cl_2 (50 mL). Further purification was performed as described above (see 1.) to yield **6b** as a fluffy white solid (190 mg, 0.18 mmol, 88%): FABMS *m/z*: 1103.4$^-$ (M$^-$), 830.3$^-$ (M$^-$ − MMT$^+$ + H$^+$), 303.0$^+$ (DMT$^+$), 273.0$^+$ (MMT$^+$), 102.1$^+$ (Et_3NH^+).

5′-*O*-(4,4′-Dimethoxytriphenylmethyl)thymidin-3′-yl N^6-[[1-(Triphenylmethyl)imidazol-4-yl]acetyl]-6-aminohexyl Phosphate, Triethylammonium Salt (6c). 1. Phosphoramidite Approach. **6c** was prepared from DMT-T and **4c** exactly as **6b** and yielded a fluffy white solid (85%).

2. H-Phosphonate Approach. **6c** was prepared from DMT-T and **5c** exactly as **6b** and yielded a fluffy white solid

(92%): FABMS m/z 1073.4$^-$ (M^-), 830.4$^-$ ($M^- - Tr^+ + H^+$), 303.0$^+$ (DMT^+), 243.0$^+$ (Tr^+), 102.2$^+$ (Et_3NH^+).

Thymidin-3′-yl N^6-(1*H*-Imidazol-4-ylacetyl)-6-aminohexyl Phosphate (7). 1. From 6b. **6b** (60–70 mg) was dissolved in CH_2Cl_2 (2 mL). 50% TFAA in CH_2Cl_2 (2 mL) was added to this solution and the reaction mixture was left for 5 min at room temperature. The orange solution was coevaporated once with toluene and twice with a mixture of H_2O and EtOH. The residue was taken up in H_2O (10 mL) and extracted with ethyl acetate (3 × 15 mL). Aqueous solution was coevaporated with acetone to dryness and the residue was decanted from acetone to yield **7** as a white solid (20–30 mg).

2. From 6c. **6c** (100–120 mg) was detritylated exactly as described above for **6b** except the deprotection time was increased to 3 h. Crude product was purified with preparative TLC (mixture $CHCl_3$/MeOH/Et_3N = 89:10:1 was used as an eluent) to yield **7** as a white solid (40–50 mg): FABMS m/z 528.2$^-$ (M^-), 530.0$^+$ ($M^- + 2H^+$).

Preparing 7 for NMR Spectra. **7** (15–20 mg), prepared as described above, was dissolved in D_2O (5 mL), the pH of this solution was adjusted to 11–12 with 2 N NaOD in D_2O, and the solution was evaporated to dryness. The residue was redissolved in D_2O (800 μL) and placed into an NMR tube: ^1H NMR δ 7.76 (s, 1H, H2 (Im)), 7.56 (s, 1H, H6 (Thym)), 7.07 (s, 1H, H5 (Im)), 6.40–6.45 (t, 1H, H1′), 4.80–4.85 (m, 1H, H3′), 4.21–4.26 (m, 1H, H4′), 3.91–3.99 (q, 2H, $CH_2$13-OP(O)═), 3.82–3.93 (m, 2H, HO-$CH_2$5′), 3.58–3.62 (d, reduced, Im-$CH_2$6), 3.22–3.28 (t, 2H, CONH-$CH_2$8), 2.52–2.60 (m, 1H, H2′ (down)), 2.38–2.47 (m, 1H, H2′ (up)), 1.93 (s, 3H, CH_3 (Thym)), 1.65–1.74 (m, 2H, $CH_2$12), 1.52–1.63 (m, 2H, $CH_2$9), 1.34–1.48 (m, 4H, $CH_2$11, $CH_2$10); ^{31}P NMR ($CDCl_3$) δ −2.85.

N^6-[N_α-(Fluoren-9-ylmethoxycarbonyl)-$N_{(im)}$-(triphenylmethy)-L-histidyl]-6-amino-1-hexanol (9a). **9a** was prepared from N_α-(fluoren-9-ylmethoxycarbonyl)-$N_{(im)}$-(triphenylmethyl)-L-histidine (**8a**) exactly as **3a** (except Et_3N was not added in $CHCl_3$ for column chromatography) and yielded a white solid (79%): R_f = 0.46, system A; ^1H NMR ($CDCl_3$) δ 7.74–7.80 (d, 2H, H1 + H8 (Fmoc)), 7.57–7.64 (d, 2H, H4 + H5 (Fmoc)), 7.24–7.44 (m, >14H, H2 (Im), Ar (Fmoc + Tr), $CHCl_3$), 7.07–7.15 (m, 6H, Ar (Tr)), 6.63–6.72 (br s, 2H, H5 (Im) + NH (aminohexanol)), 4.43–4.50 (m, 1H, OCH_2 (Fmoc)), 4.28–4.43 (m, 2H, OCH_2 (Fmoc) + CH (His)), 4.17–4.27 (t, 1H, H9 (Fmoc)), 3.54–3.63 (t, 2H, 1CH_2-OH), 3.13–3.28 (m, 2H, 6CH_2), 2.92–3.12 (m, 2H, Im-CH_2 (His)), 1.20–1.57 (m, 8H, $(CH_2)_4$); FABMS m/z 719.0$^+$ ($M + H^+$), 477.0$^+$ ($M - Tr^+ + 2H^+$), 871.2$^-$ ($M + NBA - H^+$), 628.9$^-$ ($M - Tr^+ + NBA$), 495.0$^-$ ($M - Fmoc^+$). Anal. Calcd for $C_{46}H_{46}N_4O_4$: C, 76.85; H, 6.45; N, 7.79; O, 8.90. Found: C, 76.63; H, 6.72; N, 7.42; O, 9.20.

N^6-[N_α-(*tert*-Butyloxycarbonyl)-$N_{(im)}$-(2,4-dinitrophenyl)-L-histidyl]-6-amino-1-hexanol (9b). **9b** was prepared from N_α-(*tert*-Butyloxycarbonyl)-$N_{(im)}$-(2,4-dinitrophenyl)-L-histidine (**8b**) exactly as **3a** (except Et_3N was not added in $CHCl_3$ and a stronger gradient (0–8%) of MeOH was used during column chromatography) and yielded a yellow solid (86%): R_f = 0.27, system A; ^1H NMR ($CDCl_3$) δ 8.82–8.85 (d, 1H, H3 (Dnp)), 8.55–8.61 (d/d, 1H, H5 (Dnp)), 7.67–7.73 (d, 1H, H6 (Dnp)), 7.59–7.62 (d, 1H, H2 (Im)), 6.95 (s, 1H, H5 (Im)), 6.62–6.74 (br s, 1H, NH (aminohexanol)), 6.06–6.12 (d, 1H, NH (His)), 4.38–4.50 (m, 1H, CH (His)), 3.57–3.63 (t, 2H, 1CH_2-OH), 3.14–3.25 (m, 3H, 6CH_2 + Im − CH_2 (His)), 2.94–3.02 (m, 1H, Im-CH_2 (His)), 1.75–2.00 (br s, OH), 1.20–1.56 (m, 17H, $(CH_2)_4$ + $C(CH_3)_3$); FABMS m/z 521.0$^+$ ($M + H^+$), 421.0$^+$ ($M - Boc^+ + 2H^+$), 520.0$^-$ ($M - H^+$). Anal. Calcd for $C_{23}H_{32}N_6O_8$: C, 53.07; H, 6.20; N, 16.15; O, 24.59. Found: C, 52.43; H, 6.28; N, 15.79; O, 25.72.

N^6-[N_α,$N_{(im)}$-Bis(fluoren-9-ylmethoxycarbonyl)-L-histidyl]-6-amino-1-hexanol (9c). The mixture of N_α,$N_{(im)}$-bis(fluoren-9-ylmethoxycarbonyl)-L-histidine (3.00 g, 5.0 mmol) and pentafluorophenol (1.01 g, 5.5 mmol) were coevaporated with Py to remove traces of water. The residue was dissolved in a mixture of anhydrous Py (5 mL) and anhydrous THF (30 mL). Dicyclohexylcarbodiimide (1.54 g, 7.5 mmol) was added to the solution and the reaction mixture was stirred for 1 h at room temperature. Dried 6-amino-1-hexanol (0.64 g, 5.5 mmol) was then added and the stirring was continued for 15 min. The reaction mixture was filtered, and the solid phase was washed with THF (2 × 10 mL). The combined liquid phases were concentrated to dryness, and the residue was taken up in CH_2Cl_2 (150 mL) and washed with 10% Na_2CO_3 (2 × 150 mL). The dried organic phase was concentrated and the residue was chromatographed over silica gel using a gradient of 0–5% MeOH in $CHCl_3$ to yield **9c** as a white solid (2.56 g, 3.66 mmol, 73%): R_f = 0.47, system A; ^1H NMR ($CDCl_3$) δ 8.02 (s, 1H, H2 (Im)), 7.73–7.82 (m, 4H, H1 + H8 (N_α-Fmoc, $N_{(im)}$-Fmoc)), 7.53–7.62 (m, 4H, H4 + H5 (N_α-Fmoc,$N_{(im)}$-Fmoc)), 7.27–7.46 (m, 8H, Ar (N_α-Fmoc, $N_{(im)}$-Fmoc)), 7.23 (s, 1H, H5 (Im)), 6.65–6.75 (br s, 1H, NH (aminohexanol)), 6.40–6.48 (d, 1H, NH (His)), 4.66–4.73 (d, 2H, OCH_2 ($N_{(im)}$-Fmoc)), 4.44–4.55 (m, 1H, CH (His)), 4.28–4.44 (m, 3H, OCH_2 (N_α-Fmoc) + H9 ($N_{(im)}$-Fmoc)), 4.18–4.27 (t, 1H, H9 (N_α-Fmoc)), 3.52–3.60 (t, 2H, 1CH_2OH), 3.17–3.27 (q, 2H, 6CH_2), 2.92–3.27 (m, 2H, Im-CH_2 (His)), 1.75–1.90 (br s, OH), 1.17–1.55 (m, 8H, $(CH_2)_4$); FABMS m/z 699.3$^+$ ($M + H^+$), 477.1$^+$ ($M - Fmoc^+ + 2H^+$), 629.0$^-$ ($M - Fmoc^- + NBA$), 473.0$^-$ ($M - Fmoc^+$), 253.0 ($M - 2Fmoc^+ + H^+$). Anal. Calcd for $C_{42}H_{42}N_4O_6$: C, 72.19; H, 6.06; N, 8.02; O, 13.74. Found: C, 71.02; H, 6.13; N, 7.89; O, 12.86.

N^6-[N_α-(Fluoren-9-ylmethoxycarbonyl)-$N_{(im)}$-(*tert*-butyloxycarbonyl)-L-histidyl]-6-amino-1-hexanol (9d). **9d** was prepared from N^6-[N_α-[Fluoren-9-ylmethoxycarbonyl)-$N_{(im)}$-*tert*-butyloxycarbonyl)-L-histidine (**8d**) exactly as **9c** and yielded a white solid (75%): R_f = 0.45, system A; ^1H NMR ($CDCl_3$) δ 8.02 (s, 1H, H2 (Im)), 7.74–7.80 (d, 2H, H1 + H8 (Fmoc)), 7.56–7.63 (d, 2H, H4 + H5 (Fmoc)), 7.37–7.44 (t, 2H, H2 + H7 (Fmoc)), 7.28–7.35 (t, 2H, H3 + H6 (Fmoc)), 7.20 (s, 1H, H5 (Im)), 6.75–6.83 (br s, 1H, NH (aminohexanol)), 6.44–6.53 (br d, 1H, NH (His)), 4.31–4.52 (m, 3H, OCH_2 (Fmoc) + CH (His)), 4.18–4.27 (t, 1H, H9 (Fmoc)), 3.56–3.63 (t, 2H, 1CH_2OH), 3.17–3.27 (q, 2H, 6CH_2), 2.90–3.17 (m, 2H, Im-CH_2), 1.65–1.95 (br s, OH), 1.58–1.63 (s, 9H, $C(CH_3)_3$), 1.17–1.58 (m, 8H, $(CH_2)_4$). FABMS m/z 577.3$^+$ ($M + H^+$), 477.1$^+$ ($M - Boc^+ + 2H^+$), 730.0$^-$ ($M + NBA - H^+$). Anal. Calcd for $C_{32}H_{40}N_4O_6$: C, 66.65; H, 6.99; N, 9.72; O, 16.65. Found: C, 66.64; H, 7.16; N, 9.59; O, 15.88.

N^6-[N_α-(Fluoren-9-ylmethoxycarbonyl)-$N_{(im)}$-(triphenylmethyl)-L-histidyl]-6-aminohexyl 2-Cyanoethyl *N*,*N*-Diisopropylphosphoramidite (10a). **10a** was prepared from **9a** exactly as **4b** and after precipitation in pentane yielded a white solid (73%): R_f = 0.54, system B; ^{31}P NMR ($CDCl_3$) δ 144.47; FABMS m/z 1071.7$^-$ ($M + NBA - H^+$), 1087.0$^-$ ($M + O + NBA - H^+$), 850.4$^-$ ($M - Fmoc^+ + NBA$), 940.7$^+$ ($M + Na^+$), 242.9$^+$ (Tr^+).

N^6-[N_α-(*tert*-Butyloxycarbonyl)-$N_{(im)}$-(2,4-dinitrophenyl)-L-histidyl]-6-aminohexyl 2-Cyanoethyl *N*,*N*-Diisopropylphosphoramidite (10b). **10b** was prepared from **9b** exactly as **4b** and after precipitation in pentane yielded a yellow solid (77%): R_f = 0.31, system B; ^{31}P NMR ($CDCl_3$) δ 144.36; FABMS m/z 719.8$^-$ ($M - H^+$), 736.5$^-$ ($M + O - H^+$), 707.0$^-$ ($M - Dnp^+ + NBA$), 607.8$^-$ ($M - Dnp^+ - Boc^+ + NBA + H^+$), 773.0$^-$ ($M - Boc^+ + NBA$), 758.8$^+$ ($M + O + Na^+$), 658.8$^+$ ($M + O - Boc^+ + H^+ + Na^+$).

N^6-[N_α,$N_{(im)}$-Bis(fluoren-9-ylmethoxycarbonyl)-L-histidyl]-6-aminohexyl 2-Cyanoethyl *N*,*N*-diisopropylphosphoramidite (10c). **9c** (500 mg, 0.71 mmol) was dissolved in anhydrous CH_2Cl_2 (10 mL) under Ar atmosphere. Dry $(iPr)_2$NEt (370 μL, 2.13 mmol) and 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (400 μL, 1.77 mmol) were added sequentially with stirring. The reaction mixture was left at room temperature for 10 min, poured into 1 M TEAB (70 mL), and extracted with CH_2Cl_2 (50 mL). The dried organic phase was coevaporated with toluene to dryness. The residue was dissolved in toluene (4 mL) and precipitated in pentane (160 mL) to yield a fluffy white solid (287 mg, 0.32 mmol, 45%): R_f = 0.53, system B; FABMS m/z 1052.4$^-$ ($M + NBA - H^+$), 845.1$^-$ ($M + O - Fmoc^+ + NBA$), 607.8$^-$ ($M - 2Fmoc^+ + NBA + H^+$), 714.6$^+$ ($M + O - Fmoc^+ + H^+ + Na^+$), 472.2$^+$ ($M + O - Fmoc^+ + 2H^+$).

N^6-[N_α-(Fluoren-9-ylmethoxycarbonyl)-$N_{(im)}$-(*tert*-butyloxycarbonyl)-L-histidyl]-6-aminohexyl 2-Cyanoethyl *N*,*N*-Diisopropylphosphoramidite (10d). **10d** was prepared from **9d** exactly as **4c** and after precipitation in pentane yielded a white solid (71%): R_f = 0.52, system B; ^{31}P NMR ($CDCl_3$) δ 147.28; FABMS m/z 929.0$^-$ ($M + NBA - H^+$), 944.6$^-$ ($M + O + NBA - H^+$), 708.0$^-$ ($M - Fmoc^+ + NBA$), 828.0$^-$ ($M - Boc^+ + NBA$), 793.2$^+$ ($M + O + H^+$), 692.1$^+$ ($M + O - Boc^+ + 2H^+$), 676.1$^+$ ($M - Boc^+ + 2H^+$), 799.4$^+$ ($M + O + Li^+$), 783.0$^+$ ($M +$

Li^+), 699.0$^+$ (M + O − Boc$^+$ + Li^+ + H^+), 683.2$^+$ (M − Boc$^+$ + H^+ + Li^+), 577.2$^+$ (M + O − Fmoc$^+$ + H^+ + Li^+), 561.3$^+$ (M − Fmoc$^+$ + H^+ + Li^+).

N^6-[N_α-(Fluoren-9-ylmethoxycarbonyl)-$N_{(im)}$-(triphenylmethyl)-L-histidyl]-6-aminohexyl Phosphonate, Triethylammonium Salt (11a). **11a** was prepared from **9a** exactly as **5b** and after precipitation in pentane yielded a white solid (83%): ^{31}P NMR ($CDCl_3$) δ 3.45, −0.42 (d); FABMS *m/z* 782.0$^-$ (M$^-$), 559.7$^-$ (M$^-$ − Fmoc$^+$ + H^+), 540.0$^-$ (M$^-$ − Tr$^+$ + H^+), 782.8$^+$ (M$^-$ + 2H^+), 243.0$^+$ (Tr$^+$), 102.0$^+$ (Et_3NH^+).

N^6-[N_α-(*tert*-Butyloxycarbonyl)-$N_{(im)}$-(2,4-dinitrophenyl)-L-histidyl]-6-aminohexyl Phosphonate, Triethylammonium Salt (11b). **11b** was prepared from **9b** exactly as **5b** and after precipitation in pentane yielded a hygroscopic yellow solid (55%): ^{31}P NMR ($CDCl_3$) δ 3.44, −0.32 (d). FABMS *m/z* 584.0$^-$ (M$^-$), 417.3$^-$ (M$^-$ − Dnp$^+$ + H^+), 585.0$^+$ (M$^-$ + 2H^+), 485.0$^+$ (M$^-$ − Boc$^+$ + 3H^+), 102.0$^+$ (Et_3NH^+).

N^6-[N_α-(Fluoren-9-ylmethoxycarbonyl)-$N_{(im)}$-(*tert*-butyloxycarbonyl)-L-histidyl]-6-aminohexyl Phosphonate, Triethylammonium Salt (11d). **11d** was prepared from **9d** exactly as **5b** and yielded a white oil (49%): ^{31}P NMR ($CDCl_3$) δ 3.22, −0.44 (d); FABMS *m/z* 639.8$^-$ (M$^-$), 539.0$^-$ (M$^-$ − Boc$^+$ + H^+), 417.0$^-$ (M$^-$ − Fmoc$^+$ + H^+), 640.8$^+$ (M$^-$ + 2H^+), 541.1$^+$ (M$^-$ − Boc$^+$ + 3H^+), 102.0$^+$ (Et_3NH^+).

5′-*O*-(4,4′-Dimethoxytriphenylmethyl)thymidin-3′-yl N^6-[N_α-(Fluoren-9-ylmethoxycarbonyl)-$N_{(im)}$-(triphenylmethyl)-L-histidyl]-6-aminohexyl Phosphate, Triethylammonium Salt (12a). 1. Phosphoramidite Approach. **12a** was prepared from DMT-T and **10a** exactly as **6b** (except time for ammonia treatment was reduced to 5 min) and yielded a fluffy white solid (75%).

2. H-Phosphonate Approach. **12a** was prepared from DMT-T and **11a** exactly as **6b** and yielded a fluffy white solid (90%): FABMS *m/z* 1324.7$^-$ (M$^-$), 1102.2$^-$ (M$^-$ − Fmoc$^+$ + H^+), 1081.5$^-$ (M$^-$ − Tr$^+$ + H^+), 1022.4$^-$ (M$^-$ − DMT$^+$ + H^+), 303.0$^+$ (DMT$^+$), 242.9$^+$ (Tr$^+$), 102.0$^+$ (Et_3NH^+).

5′-*O*-[4,4′-(Dimethoxytriphenylmethyl)thymidin-3′-yl N^6-[$N_{(im)}$-(triphenylmethyl)-L-histidyl]-6-aminohexyl Phosphate, Ammonium Salt (12e). **12a** (140 mg, 0.10 mmol) was dissolved in Py (2 mL), and 25% aqueous ammonium hydroxide (2 mL) was added to this solution. The reaction mixture was left at room temperature for 6 h and then concentrated to dryness. The residue was purified with preparative TLC (mixture $CHCl_3$/MeOH/Et_3N = 89:10:1 was used as an eluent) and precipitated in pentane to yield **12e** as a white fluffy solid (84 mg, 0.07 mmol, 70%): FABMS *m/z* 1101.3$^-$ (M$^-$), 859.0$^-$ (M$^-$ − Tr$^+$ + H^+), 799.2$^-$ (M$^-$ − DMT$^+$ + H^+), 303.2$^+$ (DMT$^+$), 243.1$^+$ (Tr$^+$).

5′-*O*-(4,4′-Dimethoxytriphenylmethyl)thymidin-3′-yl N^6-[N_α-(*tert*-Butyloxycarbonyl)-$N_{(im)}$-(2,4-dinitrophenyl)-L-histidyl]-6-aminohexyl Phosphate, Triethylammonium Salt (12b). 1. Phosphoramidite Approach. **12b** was prepared from DMT-T and **10b** exactly as **6b** (except time for ammonia treatment was reduced to 5 min) and yielded a fluffy yellow solid (72%).

2. H-Phosphonate Approach. **12b** was prepared from DMT-T and **11b** exactly as **6b** and yielded a fluffy yellow solid (85%); FABMS *m/z* 1126.1$^-$ (M$^-$), 823.5$^-$ (M$^-$ − DMT$^+$ + H^+), 303.0$^+$ (DMT$^+$), 102.0$^+$ (Et_3NH^+).

5′-*O*-(4,4′-Dimethoxytriphenylmethyl)thymidin-3′-yl N^6-[N_α-(*tert*-Butyloxycarbonyl)-L-histidyl]-6-aminohexyl Phosphate, Ammonium salt (12f). **12f** was prepared from **12b** exactly as **12e** from **12a** and yielded a white fluffy solid (68%): FABMS *m/z* 959.3$^-$ (M$^-$), 657.0$^-$ (M$^-$ − DMT$^+$ + H^+), 303.2$^+$ (DMT$^+$).

5′-*O*-(4,4′-Dimethoxytriphenylmethyl)thymidin-3′-yl N^6-L-Histidyl-6-aminohexyl Phosphate, Ammonium Salt (12g). 1. From 10c. A mixture of DMT-T (54 mg, 0.1 mmol) and freshly prepared **10c** (90 mg, 0.1 mmol) were dried overnight in desiccator under vacuum. To this mixture a solution of tetrazole in anhydrous CH_3CN (0.5 M, 1 mL, 0.5 mmol) was added under Ar atmosphere with stirring. After 3 min, anhydrous CH_2Cl_2 (1 mL) was added and the stirring was continued for further 6 min. The oxidation mixture (I_2/H_2O/Py/THF = 3:2:20:85 (m/v/v/v), 2 mL) was added and the reaction mixture was left at room temperature for 15 min. Residual I_2 was reduced with aqueous sodium hydrosulfite solution (0.5 M, 1 mL) and the reaction mixture was poured into 1 M TEAB (30 mL) and extracted with CH_2Cl_2 (30 mL). Dried organic phase was concentrated to dryness, the residue was dissolved in Py (2 mL), and 25% aqueous ammonium hydroxide (2 mL) was added to this solution. The reaction mixture was left at room temperature for 6 h and then concentrated to dryness. The residue was taken up in H_2O (3 mL) and extracted with ethyl acetate (3 × 4 mL). The aqueous layer was filtered and the product was purified with preparative HPLC (solvent A = 10% CH_3CN/0.1% Et_3N in H_2O, solvent B = 50% CH_3CN/0.1% Et_3N in H_2O, retention time 19.0 min) to yield **12g** as a white solid (37 mg, 0.04 mmol, 42%).

2. From 10d. **10d** (78 mg, 0.1 mmol) was condensed with DMT-T (54 mg, 0.1 mmol) and oxidized exactly as described for preparation **6b** from **4b**. The crude product was deprotected and purified exactly as described above (except time for ammonia treatment was increased to 12 h) and yielded **12g** as a white solid (77 mg, 0.08 mmol, 80%).

3. From 11d. **11d** (74 mg, 0.1 mmol) was condensed with DMT-T (54 mg, 0.1 mmol) and oxidized exactly as described for preparation **6b** from **5b**. The crude product was deprotected and purified exactly as described in item 1 (except time for ammonia treatment was increased to 12 h) and yielded **12g** as a white solid (63 mg, 0.07 mmol, 72%): FABMS *m/z* 859.3$^-$ (M$^-$), 556.5$^-$ (M$^-$ − DMT$^+$ + H^+), 303.0$^+$ (DMT$^+$).

Thymidin-3′-yl N^6-L-Histidyl-6-aminohexyl Phosphate (13). 1. From 12e. **12e** (60–70 mg) was dissolved in CH_2Cl_2 (2 mL). TFAA in CH_2Cl_2 (2 mL) was added to this solution and the reaction mixture was left for 3 h at room temperature. The orange solution was coevaporated once with toluene and twice with a mixture of H_2O and EtOH. The residue was taken up in H_2O (5 mL) and extracted with ethyl acetate (3 × 10 mL). The pH of the aqueous layer was adjusted to 10–11 with aqueous ammonium hydroxide, the solution was filtered, and the product was purified with preparative HPLC (solvent A = H_2O, solvent B = 20% CH_3CN/0.1% Et_3N in H_2O, retention time 17.5 min) to yield **13** as a white solid (15–25 mg).

2. From 12f. **13** was prepared from **12f** (60–70 mg) exactly as from **12e** (except time of acidic treatment was reduced to 30 min) and yielded a white solid (20–25 mg).

3. From 12g. **12g** (35–60 mg) was dissolved in mixture of CH_2Cl_2 and MeOH (1:1, 2 mL). 10% TFAA in CH_2Cl_2 (1 mL) was added to this solution and the reaction mixture was left for 5 min at room temperature. The orange solution was coevaporated once with toluene and twice with mixture of H_2O and EtOH. The residue was taken up in H_2O (5 mL) and extracted with ethyl acetate (3 × 10 mL). The aqueous layer was coevaporated with acetone to dryness and the residue was decanted from acetone to yield **13** as a white solid (15–30 mg): FABMS *m/z* 557.2$^-$ (M$^-$), 559.6$^+$ (M$^-$ + 2H^+).

Preparing 13 for NMR spectra. **13** was prepared for NMR spectra exactly as **7**: ^{1}H NMR δ 7.69 (s, 1H, H2 (Im)), 7.53 (s, 1H, H6 (Thym)), 6.90 (s, 1H, H5 (Im)), 6.34–6.41 (t, 1H, H1′), 4.75–4.83 (m, 1H, H3′), 4.17–4.23 (m, 1H, H4′), 3.88–3.95 (q, 2H, $CH_2$14-OP(O)), 3.77–3.90 (m, 2H, $HOCH_2$5′), 3.58–3.64 (t, 1H, CH7 (His)), 2.98–3.25 (m/d, 2H, CONH-$CH_2$9), 2.85–2.91 (d, 2H, Im-CH_2 (His)), 2.47–2.55 (m, 1H, H2′ (down)), 2.32–2.42 (m, 1H, H2′ (up)), 1.87 (s, 3H, CH_3 (Thym)), 1.58–1.67 (m, 2H, $CH_2$13), 1.26–1.43 (m, 4H, $CH_2$12 + $CH_2$10), 1.08–1.18 (m, 2H, $CH_2$11); ^{31}P NMR ($CDCl_3$) δ 2.84.

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Supplementary Material Available: Elemental analysis data for selected compounds, and copies of the ^{1}H NMR spectra for compounds **2–5**, **6c**, **9–11**, **12a**, and **12b** (24 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.