[5] M. Weidenbruch, S. Willms, W. Saak, G. Henkel, *Angew. Chem.* **1997**, *109*, 2612; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 2503.

[6] A. Sekiguchi, H. Yamazaki, C. Kabuto, H. Sakurai, S. Nagase, *J. Am. Chem. Soc.* **1995**, *117*, 8025.

[7] Crystal structure analysis of $R_4^*Si_4I_2 \cdot C_6H_6$: Siemens P4 diffractometer, $Mo_{K\alpha}$ radiation, graphite monochromator, CCD area detector, crystal dimensions $0.45 \times 0.35 \times 0.12$ mm^3, the crystal was mounted in perfluoro ether oil, $T = 183(3)$ K, $C_{54}H_{114}I_2Si_8$, $M_r = 1241.97$, red-orange platelets, monoclinic, $a = 17.9758(1)$, $b = 13.9928(1)$, $c = 26.7119(1)$ Å, $\beta = 96.709(1)^\circ$, $V = 6672.88(7)$ Å^3, $Z = 4$, space group $P2_1/c$, $\rho_{calcd} = 1.1236$ Mg m^{-3}, $\mu = 1.117$ mm^{-1}, $F(000) = 2624$. Data collection: 2θ from 2.28 to 58.08°, $-24 \le h \le 20$, $-17 \le k \le 17$, $-33 \le l \le 32$; of 36314 reflections, 10560 were independent and 8668 were considered observed ($F > 4\sigma(F)$); semi-empirical absorption correction (max./min. transmission 0.8754/0.6736), $R_1 = 0.0594$, $wR_2 = 0.1342$ ($F > 4(F)$), GOF(F^2) = 1.138; largest residual electron density 2.883 e Å^{-3}. The weighting factor was $w^{-1} = \sigma^2F_o^2 + (0.0487P)^2 + 30.7084P$ with $P = (F_o^2 + 2F_c^2)/3$.[16] The monoclinic unit cell dimensions of $R_4^*Si_4(OMe)_2 \cdot C_6D_6$: $a = 15.9308(1)$, $b = 12.8660(2)$, $c = 17.0268(1)$ Å, $\beta = 90.018(1)^\circ$, $Z = 2$, space group $P2$.[16]

[8] M. Kira, T. Maruyama, C. Kabuto, K. Ebata, H. Sakurai, *Angew. Chem.* **1994**, *106*, 1575; *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 1489.

[9] *Holleman-Wiberg*, *Lehrbuch der Anorganischen Chemie*, 101st ed., deGruyter, Berlin, **1995**.

[10] See G. Raabe, J. Michl in *The Chemistry of Organic Silicon Compounds*, *Part 2* (Eds.: S. Patai, Z. Rappoport), Wiley, Chichester, **1989**, p. 1015; T. Tsumuraya, S. A. Batcheller, S. Masamune, M. Weidenbruch, *Coord. Chem. Rev.* **1994**, *130*, 275; R. Okazaki, R. West in *Multiply Bonded Main Group Metals and Metalloids* (Eds.: R. West, F. G. A. Stone), Academic Press, San Diego, **1996**, p. 232.

[11] N. Wiberg, H. Auer, K. Polborn, M. Veith, V. Huch in *Organosilicon Chemistry* IV (Eds.: N. Auner, J. Weis), WILEY-VCH, Weinheim, **1999**, in press.

[12] A. R. Bassindale, P. G. Taylor in *The Chemistry of Organic Silicon Compounds*, *Part 1* (Eds.: S. Patai, Z. Rappoport), Wiley, Chichester, **1989**, p. 839.

[13] Crystal structure analysis of $R_4^*Si_4O \cdot 0.75\,C_6D_6$: Siemens P4 diffractometer, $Mo_{K\alpha}$ radiation, graphite monochromator, CCD area detector, crystal dimensions $0.3 \times 0.3 \times 0.2$ mm^3, the crystal was mounted within a capillary tube, $T = 173(3)$ K, $C_{52.50}H_{112.50}OSi_8$, $M_r = 984.65$, light yellow prism, monoclinic, $a = 13.3772(1)$, $b = 16.5350(2)$, $c = 30.1050(3)$ Å, $\beta = 91.202(1)^\circ$, $V = 6657.59(9)$ Å^3, $Z = 4$, space group $P2_1/c$, $\rho_{calcd} = 0.982$ Mg m^{-3}, $\mu = 0.191$ mm^{-1}, $F(000) = 2190$. Data collection: 2θ from 2.70 to 58.36°, $-17 \le h \le 17$, $-21 \le k \le 21$, $-38 \le l \le 38$; of 37866 reflections, 12890 were independent and 9598 were considered observed ($F > 4\sigma(F)$); absorption correction using the program SADABS, Sheldrick 1997 (max./min. transmission 0.9627 and 0.9448), $R_1 = 0.0753$, $wR_2 = 0.2134$ ($F > 4\sigma(F)$), GOF(F^2) = 1.128, largest residual electron density: 1.146 e Å^{-3}. The weighting factor was $w^{-1} = \sigma^2F_o^2 + (0.1119P)^2 + 9.8371P$ with $P = (F_o^2 + 2F_c^2)/3$.[16] The dimensions of the monoclinic unit cell of $2R_4^*Si_4O \cdot R_4^*Si_4O_2 \cdot 4.5\,C_6D_6$ resemble those of $R_4^*Si_4O \cdot 0.75\,C_6D_6$ ($a = 13.434(2)$, $b = 16.692(4)$, $c = 30.452(6)$ Å, $\beta = 91.17(2)^\circ$, $V = 6827(2)$ Å^3, $Z = 4$, space group $P2_1/c$).[16]

[14] J. B. Lambert, Y. Zhao, *Angew. Chem.* **1997**, *109*, 389; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 400.

[15] H. Nöth, B. Wrackmeyer in *Basic Principles and Progress*, *Vol. 14* (Eds.: P. Diehl, F. Fluck, P. Kosfeld), Springer, Heidelberg, **1978**, p. 389.

[16] The structures were solved by direct methods (SHELXS-97, **1990**; for $R_4^*Si_4O_{1.31}$, SHELXS-86 was used). All non-hydrogen atoms were refined using anisotropic thermal parameters. Hydrogen atoms were included in calculated positions and refined using fixed isotropic U_i values and a riding model. Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-101877, -101800, and -101969 ($\mathbf{3} \cdot C_6H_6$, $\mathbf{6} \cdot 0.75\,C_6D_6$, and $\mathbf{6} \cdot 0.5\,R_4^*Si_4O_2 \cdot 2.25\,C_6D_6$, respectively). Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

Expanding the Potential of DNA for Binding and Catalysis: Highly Functionalized dUTP Derivatives That Are Substrates for Thermostable DNA Polymerases**

Kandasamy Sakthivel and Carlos F. Barbas III*

Nucleic acid libraries provide tremendous opportunities for the selection of novel ligands and catalysts since the polymerase chain reaction (PCR) allows for the synthesis and selection of libraries containing more than 10^{14} different molecules. There are now many examples of nucleic acids that have been selected to bind proteins and small molecules and to catalyze a limited set of reactions.[1–3] The catalytic and mechanistic scope of nucleic acids is limited since the natural nucleotide monomers possess minimal functionality relative to the repertoire available to nature's dominant catalytic biopolymers, proteins. In recognition of this shortcoming much attention has been focused on the development of functionalized nucleotides suitable for in vitro selection with the hope of increasing the potential of nucleic acids for binding and catalysis.[4] Functionalized nucleotide triphosphates have been shown to be substrates for RNA polymerases,[4d, h] and catalytic RNAs that are dependent on the modified base[5] for their activity have been selected. In a similar manner to RNA, DNA has also been selected to bind proteins and small molecules and more recently to catalyze reactions.[1–3] While DNA possesses enhanced stability relative to RNA, the lack of a 2′-hydroxyl group, which provides for the enhanced stability of this molecule, further reduces the potential for chemical functionalization. In contrast to the success achieved in identifying modified nucleotide triphosphates for RNA libraries, there is but a single example of a deoxynucleotide triphosphate, 5-(1-pentynyl)-2′deoxyuridine triphosphate, which is a good substrate for a thermostable DNA polymerase and has been utilized in an in vitro DNA selection study.[6] Indeed, difficulties in identifying modified deoxynucleotide triphophate substrates for the thermostable polymerases required for PCR have led recently to the development of novel strategies for in vitro selection without enzymatic amplification.[7] Therefore, the major impediment to the creation of novel functionally modified DNA catalysts and binding molecules is the determination of the structures of the substrate that are accepted by the thermostable polymerases. Herein, we provide a solution to this problem through the systematic synthesis and study of deoxyuridine triphosphate derivatives and disclose the discovery of a class

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of highly functionalized derivatives suitable for in vitro selections of modified DNAs.

Studies directed towards defining base modifications that are tolerated by thermostable polymerases began with the synthesis of 5-(3-aminopropenyl)-2′deoxyuridine triphosphate from the known compound **2** (Scheme 1). We found a palladium catalyzed route starting from commercially available 5-iododeoxyuridine preferable to the earlier synthesis[8] that started with 5-chloromercuri-2′-deoxyuridine, though both provided **2** in similar yields. Optimization of the synthesis of **2** and its conversion into the corresponding 5′-triphosphate by the methodology of Kovács and Ötvös[9] followed by deprotection of the amine provided **3**, which could be isolated on a 100-mg scale with analytical purity (Scheme 1).[10]

We studied the ability of **3** to act as a substrate in place of deoxythymidine triphosphate (dTTP) for thermostable DNA polymerases under typical PCR conditions. Commercially available thermostable DNA polymerases from four organisms were studied; Taq from *Thermus aquaticus*, Vent from *Thermococcus litoralis*, Pfu from *Pyrococcus furiosus*, and rTh from *Thermus thermophilus*. PCR assays with compound **3** demonstrated its incorporation[11] into the 519 base pair product only with rTh polymerase (Figure 1). Successful PCR with this template requires incorporation of 246 modified bases including a single stretch of eight contiguous thymidine groups. Despite this limited success we derivatized **3** with three different *N*-hydroxysuccinimide esters and produced **4a**–**c** (Scheme 2).

Compound **4a**, a 4-imidazole acrylic acid derivative, proved to be an excellent substrate for all the polymerases tested, and produced a similar amount of PCR product as that obtained with dTTP. Further study of substrates **4b** and **4c** with variation in the Mg^{2+} concentration (15 to 30 mM) and analogue concentration (200 to 800 μM) failed to define conditions that allowed for incorporation of these modified bases. To study the structural feature of **4a** that allowed for it to be recognized as a good substrate for the polymerases we synthesized **4d**, a reduced analogue of **4a**. No conditions could be defined with any of the polymerases that allowed for PCR with **4d**, which suggested that the rigid and extended α,β-unsaturated arm of the 4-imidazole acrylic acid provided for its activity. To test this structure–activity relationship we

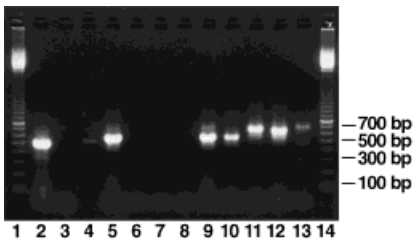
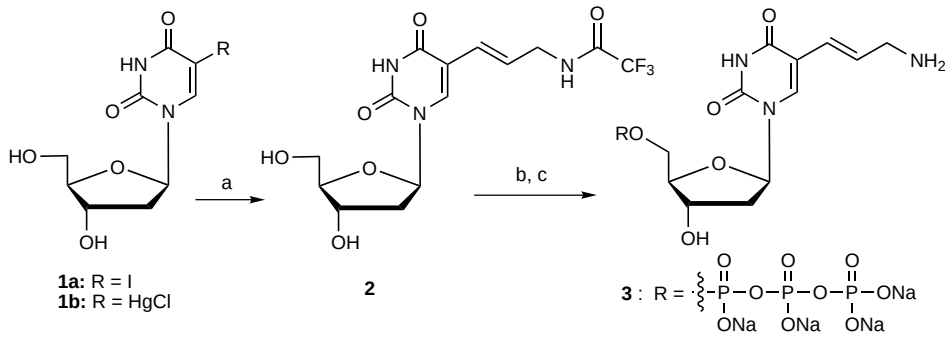


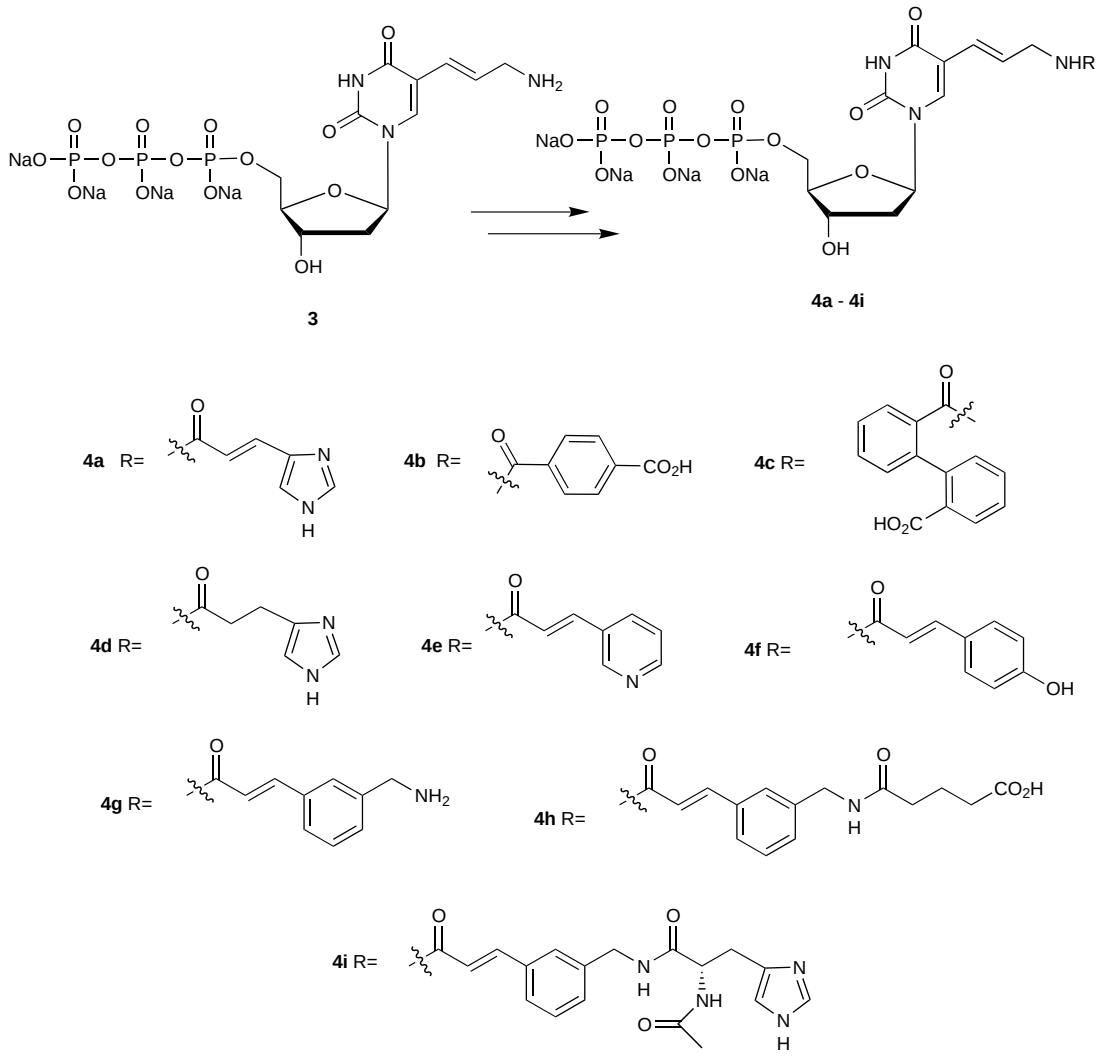
Figure 1. PCR assays of dUTP derivatives: total volume 100 μL, 1 μL (10 ng μL^{-1}) of DNA template pMal7CS2 (7030 bp), 1 μL (13 pmol) of each primer, Malseq B (GTA AAA CGA CGG CCA GTG CCA AGC) and Malseq F (GAC GCG CAG ACT AAT TCG AGC), 1 μL (5 U μL^{-1}) of Taq polymerase (AmpliTaq) (except lanes 4 and 13 where rTh polymerase was used), 8 μL of modified dNTP mix (2.5 mM of each nucleotide) or natural dNTPs (2.5 mM of each nucleotide). Conditions: 94 °C/30 s; 30 cycles of 94 °C/1 min, 56 °C/1 min, 74 °C/2 min; 74 °C/10 min. Aliquots of PCR products (10 μL each, except lane 4 where 20 μL was used) were analyzed by electrophoresis (1.5% agarose gel) and visualized by staining with ethidium bromide. Lanes 1 and 14: marker DNA (100 bp); lane 2: positive control (dTTP+dATP+dGTP+dCTP); lane 3: negative control (dATP+dGTP+dCTP); lane 4: **3**+dATP+dGTP+dCTP; lane 5: **4**+dATP+dGTP+dCTP; lane 6 : **4b**+dATP+dGTP+dCTP; lane 7: **4c**+dATP+dGTP+dCTP; lane 8: **4d**+dATP+dGTP+dCTP; lane 9: **4e**+dATP+dGTP+dCTP; lane 10: **4f**+dATP +dGTP+dCTP; lane 11: **4g**+dATP+dGTP+dCTP ; lane 12: **4h**+dATP+dGTP+dCTP; lane 13: **4i**+dATP+dGTP+dCTP.

synthesized **4e**–**4g** with the α,β-unsaturated linker arm common to **4a** maintained (Scheme 2). Compounds **4e**–**4g** were substrates for all the polymerases tested. Compound **4g** bears a free amino group suitable for further derivatization. We tested the possibility of **4g** to serve as a template for higher level modification by the synthesis and testing of **4h** and **4i**.

Analogue **4h** was a good substrate for all thermostable polymerases tested, while **4i** was a substrate only for rTh polymerase. Evidence that the modified dUTPs are indeed incorporated into the PCR products is provided by the substantial mobility shift that is obtained on electrophoresis for the modified DNA products, which is indicative of both the mass increase associated with the modified base as well as the charge of the DNA product (Figure 1). Note that DNA incorporating the analogue **4g** should possess a full positive charge at neutral pH and migrates slower than DNA incorporating **4h** that has an increased mass and negative charge at neutral pH (Figure 1). The role of the extended linker arm is apparent in comparison of the efficiency of incorporation of **3** with **4g**, where both carry a primary amine group. The larger derivative **4g** bears the extended linker arm and is a robust substrate for PCR relative to **3**. PCR products that incorporate modified bases were found to be resistant to cleavage by the restriction enzymes *Sac* I and *Xba* I that recognize the sequences GAGCTC and TCTAGA, respectively, and cleave the natural DNA product. PCR products obtained with modified dUTPs



Scheme 1. Synthesis of 5-(3-aminopropenyl)-2′-deoxyuridine triphosphate: a) *N*-allyltrifluroacetamide, $Na_2[PdCl_4]$ in sodium acetate (0.1M, pH 5.2), 24 h; b) 1. $POCl_3$ in trimethylphosphate, 1.8-bis(dimethylamino)naphthalene (proton sponge), tri-*n*-butylammonium pyrophosphate, tri-*n*-butylamine; 2. NH_3 (aq.); c) sodium perchlorate in acetone.



Scheme 2. Synthesis of highly functionalized dUTPS.

were cloned and sequenced.[12] The fidelity of the incorporation of the modified dUTPs was similar to that observed with dTTP incorporation in the control reactions. The key criteria for the use of a modified dNTP in an in vitro selection methodology is its ability to serve as a substrate for thermostable polymerases and the resulting product to serve as a template for multiple cycles of PCR amplification. These criteria are met with **3**, **4a**, and **4e**–**i**. Reverse transcriptases may also be utilized in conjunction with PCR for in vitro selection schemes of DNA enzymes. We tested the ability of **3** and **4**–**i** to act as substrates of a reverse transcriptase (Superscript II, Gibco/BRL) and determined that all but **4c** and **4h** were substrates in template directed synthesis assays (see supporting information). The specificity of this reverse transcriptase is broader and not predictive of the structural requirements of the thermostable polymerases. In our design of analogues we have attempted to provide functional groups that are lacking in DNA in order to expand its catalytic scope. The functional groups of the natural nucleic acids have pK_as far removed from neutral pH and are therefore not suited for general acid–base catalysis in this pH range.[13] The analogues described here address this limitation as well as provide potential for covalent binding, electrostatic interactions, and particularly metal ion catalysis. Analogues **4g** and **4h** provide the first examples of the incorporation of cationic and anionic nucleotide analogues into DNA by PCR, which dramatically alter its electrostatic properties. With the exception of **4e**, the analogues may be regarded as functional equivalents of the amino acids lysine: **3** and **4g**, histidine: **4** and **4i**, tyrosine: **4f**, and aspartic and glutamic acid: **4h**. The successful incorporation of L-histidine in analogue **4i** supports the possibility of incorporating other natural amino acids as well as small peptides. All the analogues provide new potential for hydrophobic binding interactions that are essential for folding and pocket formation in protein enzymes, as well as for pK_a perturbations of functional groups.[14]

In summary, we have defined a class of highly functionalized C5-substituted dUTP derivatives that are substrates for thermostable DNA polymerases and reverse transcriptase, and are suitable for in vitro selection studies and the enzymatic synthesis of combinatorial DNA libraries. While we have defined here only substrates that replace the natural DNA base thymidine, the tremendous breadth of substrate analogues accepted by the polymerases we have studied, provided certain structural guidelines are followed, suggests the likelihood that guanine, cytosine, and adenine analogues

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could be identified, which would then allow four coded substitutions into modified DNA libraries. While this falls short of the 20 coded amino acids available in protein libraries, higher order coding of derivatives may be obtained by treating synthesized libraries with designed alkylating or acylating reagents to introduce additional functionality wherein the chemical reactivity of the targeted deoxynucleotide is encoded by its structural context. Given the size of the libraries accessible with nucleic acids and PCR, and the ease with which they may be evolved through many generations in the laboratory, we anticipate that modified DNA will make significant inroads into areas of catalysis that were previously believed to be the sole purview of protein catalysts.

Experimental Section

2: This compound was first synthesized by Cook and his co-workers[8] from 5-chloromercuri-2′-deoxyuridine **1b**. We synthesized **2** from commercially available 5-iodo-2′-deoxyuridine by a similar procedure: A suspension of 5-iodo-2′-deoxyuridine **1a** (3.5 g, 10 mmol) in sodium acetate buffer (0.1 M, pH 5.2) was treated with *N*-allyltrifluroacetylamide (13 g, 88 mmol) followed by a solution of sodium tetrachloropalladate (2.5 g in 5 mL water). The mixture was stirred at room temperature for 18 h and then filtered through celite. The filtrate was concentrated and extracted several times with ethyl acetate. The combined organic layers was dried over anhydrous magnesium sulfate. The solvent was evaporated to dryness and the product purified by column chromatography on silica gel with ethyl acetate as eluent to give **2** (1.7 g, 44 %). ^{1}H NMR (CD_3OD): $\delta = 8.11$ (s, br, 1H; C-6H), 6.45 (m, 1H) and 6.16 (m, 2H; H_{vinyl} and H-1′), 4.32 (m, 1H; H-3′), 3.8, (m, 3H; H_{allyl}, H-4′), 3.75 – 3.60 (m, 2H; H-5′, H-5″), 2.17 (m, 2H; H-2′, H-2″).

3:[9]: Compound **2** (126 mg, 0.33 mmol) was stirred in dry trimethylphosphate (0.75 mL) with 1,8-bis(dimethylamino)naphthalene (proton sponge, 100 mg, 0.47 mmol) at 0 °C. Phosphorous oxychloride (35 µl, 99.9 % Aldrich) was added and the mixture was stirred at 0 – 4 °C. After 2.5 h a solution of tri-*n*-butylammonium pyrophosphate in anhydrous DMF (0.5 M, 3 mL) and tri-*n*-butylamine (0.3 mL) was added quickly to the reaction mixture at 0 °C. After 1 min an aqueous solution of triethylammonium bicarbonate (0.2 M) was poured into the mixture. After evaporation the residue was treated with aqueous ammonia (2 mL), and stirred overnight at room temperature. After evaporation of the ammonia, the residue was purified by column chromatography on DEAE-Sephadex A-25 with triethylammonium bicarbonate buffer (0.2 – 0.5 M, pH 7.5). The final purification was achieved by reversed-phase HPLC with the gradient 0 – 3 % acetonitrile in 50 mM triethylammonium bicarbonate buffer over 30 min to give **3** (110 mg, 54 %). ^{1}H NMR (D_2O): $\delta = 8.17$ (s, br, 1H; C-6H), 6.58 – 6.54 (d, 1H), 6.48 – 6.40 (m, 1H), and 6.35 (t, 1H; H_{vinyl} and H-1′), 4.66 (m, 1H; H-3′), 4.26 – 4.2 (m, 3H; 5′, 5″, H-4′), 3.70 (d, 2H; H_{allyl}), 2.41 (m, 2H; H – 2′,2″); ^{13}C NMR (D_2O): $\delta = 166.67$, 153.35, 141.0, 128.74, 124.7, 114.00, 88.27, 87.90, 72.7, 67.66, 43.60, 41.98; ^{31}P NMR (D_2O): $\delta = -9.42$ (d), -10.73 (d), -21.38 (t); electrospray MS: *m/z*: 589 [M+H$^+$], 611 [M+Na$^+$], 633 [M+2Na$^+ -$ 2H$^+$]; elemental analysis calcd for $C_{12}H_{16}N_3Na_4O_{14}P_3$: C 23.58, H 2.64, N 6.88; found: C 23.75, H 2.81, N 7.00.

General synthesis of **4**, **4d**, **4e**, and **4f**: Compound **3** (30 mg, 50 µmol) was treated with a slight excess of the corresponding *N*-hydroxysuccinimide ester in 0.1 M sodium borate buffer/DMF (1/1) at room temperature and stirred for 10 – 20 h. After completion of the reaction (as monitored by TLC with ammonia/water/isopropanol, 2/1/1), the mixture was evaporated to dryness. The triphosphates were purified by reversed-phase HPLC. For complete physical data and synthetic procedures for all compounds see supporting information.

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[1] Ligand-binding nucleic acids: a) S. E. Osborne, A. D. Ellington, *Chem. Rev.* **1997**, *97*, 349 – 370; b) M. Famulok, J. W. Szostak, *Angew. Chem.* **1992**, *104*, 1001 – 11; *Angew. Chem. Int. Ed. Engl.* **1992**, *31*, 979 – 88; c) L. Gold, B. Polisky, O. Uhlenbeck, M. Yarus, *Annu. Rev. Biochem.* **1995**, *64*, 763 – 97.

[2] Catalytic nucleic acids: a) R. R. Breaker, *Chem. Rev.* **1997**, *97*, 371 – 390; b) R. R. Breaker, *Curr. Opin. Chem. Biol.* **1997**, *1*, 26 – 31; c) J. R. Lorsch, J. W. Szostak, *Acc. Chem. Res.* **1996**, *29*, 103 – 110.

[3] Catalytic DNA: a) R. R. Breaker, G. F. Joyce, *Chem. Biol.* **1994**, *1*, 223 – 9; b) B. Cuenoud, J. W. Szostak, *Nature* **1995**, *375*, 611 – 14; c) R. R. Breaker, G. F. Joyce, *Chem. Biol.* **1995**, *2*, 655 – 60; d) C. R. Geyer, D. Sen, *Chem. Biol.* **1997**, *4*, 579 – 593; e) S. W. Santoro, G. F. Joyce, *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 4262 – 4266; f) P. Burgstaller, M. Famulok, *Angew. Chem.* **1995**, *107*, 1303 – 1306; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 1189 – 1192; g) D. Faulhammer, M. Famulok, *Angew. Chem.* **1996**, *108*, 2984 – 88; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2837 – 2841; h) D. Faulhammer, M. Famulok, *J. Mol. Biol.* **1997**, *269*, 188 – 202; i) Y. Li, D. Sen, *Nat. Struct. Biol.* **1996**, *3*, 743 – 747; j) J. Burmeister, G. von Kiedrowski, A. D. Ellington, *Angew. Chem.* **1997**, *109*, 1379 – 81; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 1321 – 1324; k) N. Carmi, L. A. Shultz, R. R. Breaker, *Chem. Biol.* **1996**, *3*, 1039 – 1046; l) N. Carmi, H. R. Balkhi, R. R. Breaker, *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 2233 – 2237.

[4] a) B. E. Eaton, W. A. Pieken, *Annu. Rev. Biochem.* **1995**, *64*, 837 – 63; b) B. E. Eaton, *Curr. Opin. Chem. Biol.* **1997**, *1*, 10 – 16; c) G. J. Crouch, B. E. Eaton, *Nucleosides Nucleotides* **1994**, *13* 939 – 44; d) T. M. Dewey, A. Mundt, G. J. Crouch, M. C. Zyzniewski, B. E. Eaton, *J. Am. Chem. Soc.* **1995**, *117*, 8474 – 5; e) T. M. Dewey, M. C. Zyzniewski, B. E. Eaton, *Nucleosides Nucleotides* **1996**, *15*, 1611 – 1617; f) C. Tu, C. Keane, B. E. Eaton, *Nucleosides Nucleotides* **1995**, *14*, 1631 – 8; g) P. A. Limbach, P. F. Crain, J. A. McCloskey, *Nucleic Acids Res.* **1994**, *22*, 2183 – 96; h) H. Aurup, D. M. Williams, F. Eckstein, *Biochemistry* **1992**, *31*, 9636 – 41.

[5] a) T. W. Wiegand, R. C. Janssen, B. E. Eaton, *Chem. Biol.* **1997**, 4, 675 – 683; b) T. M. Tarasow, S. L. Tarasow, B. E. Eaton, *Nature* **1997**, *389*, 54 – 57.

[6] J. A. Latham, R. Johnson, J. J. Toole, *Nucleic Acids Res.* **1994**, *22*, 2817 – 22.

[7] J. Smith, E. V. Anslyn, *Angew. Chem.* **1997**, *109*, 1956 – 58; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 1879 – 1881. These authors state that "when modified oligomers are incorporated, the selection procedure is terminated after just one round of amplification since polymerases will not tolerate most modified mononucleotides".

[8] A. F. Cook, E. Vuocolo, C. L. Brakel, *Nucleic Acids Res.* **1988**, *16*, 4077 – 95.

[9] T. Kovács, L. Ötvös, *Tetrahedron Lett.* **1988**, *29*, 4525 – 8.

[10] Compound **3** was synthesized previously from deoxyuridine triphosphate (dUTP), however, problems with by-products and the expense of dUTP as a starting material posed serious obstacles for this synthetic route. P. R. Langer, A. A. Waldrop, D. C. Ward, *Proc. Natl. Acad. Sci.U.S.A.* **1981**, *78*, 6633 – 7.

[11] Several derivatives of **3** have been shown to be substrates for *E. coli* DNA polymerase and useful in nick translation and random primed synthesis when they replace dTTP. Homogeneous incorporation of these derivatives in PCR is not possible because of chain termination, see ref. [11c] for a discussion of derivatives in PCR. a) M. Shimkus, J. Levy, T. Herman, *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 2593 – 7; b) B. L. Iverson, P. B. Dervan, *J. Am. Chem. Soc.* **1987**, *109*, 1241 – 3. and ref. [10]; c) H. Yu, J. Chao, D. Patek, R. Mujumdar, A. S. Waggoner, *Nucleic Acids Res.* **1994**, *22*, 3226 – 32.

[12] PCR products with incorporated modified nucleotide analogues were cloned into the vector pCR2.1TOPO with the topoisomerase-activated vector provided from the manufacturer (Invitrogen).

[13] S. Nesbitt, L. A. Hegg, M. J. Fedor, *Chem. Biol.* **1997**, *4*, 619 – 630.

[14] Reviews of the structures of nucleic acids that were selected to bind ligands detail the differences between the ligand-binding pockets of nucleic acids and proteins, see a) K. A. Marshall, M. P. Robertson, A. D. Ellington, *Structure* **1997**, *5*, 729 – 734; b) M. Egli, *Angew. Chem.* **1997**, *109*, 494 – 497; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 480 – 482; c) D. J. Patel, *Curr. Opin. Chem. Biol.* **1997**, *1*, 32 – 46; d) J. Feigon, T. Dieckmann, F. W. Smith, *Chem. Biol.* **1996**, *3*, 611 – 617.

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