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Ketone-DNA: A Versatile Postsynthetic DNA Decoration Platform

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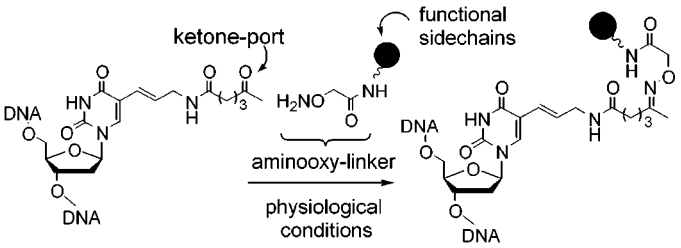
ABSTRACT



A general strategy for the functional diversification of DNA oligonucleotides under physiological conditions was developed. We describe the synthesis of DNA molecules bearing ketone ports (*ketone*-DNA) and the efficient postsynthetic decoration of *ketone*-DNA with structurally diverse aminooxy compounds.

Chemical oligodeoxynucleotide (ODN) synthesis by the phosphoramidite approach enables the introduction of diverse modified nucleotides, labeling reagents, and biomolecular structures at precise sites within DNA sequences.[1] The traditional protocol requires the synthesis of a phosphoramidite building block for the introduction of each structural modification. Modified ODNs have served as essential elements in DNA diagnostic,[2] sequencing technologies,[3] and as tools for the in vitro evolution of catalytic DNA molecules (deoxyribozymes).[4]

An alternative approach to nucleic acid modification involves the construction of a single "universal" nucleoside, which bears a unique functional group or "port". Once incorporated into DNA, the nucleotide "port" may be conjugated to any desired molecule by a selective chemical reaction. In general, postsynthetic DNA-modification strategies must address the following criteria: (i) the modifiable port must support reaction with diverse chemical groups through formation of a stable covalent bond; (ii) modification reactions should be rapid and proceed under physiological conditions; (iii) postsynthetic purification of the modified DNA should be minimal; and (iv) introduction of the port and/or modification should not interfere with DNA hybridization properties or subsequent molecular biology operations. Several elegant postsynthetic DNA modification technologies currently exist that meet several of these design criteria.[5]

Our DNA decoration approach involves the synthesis of ketone-modified DNA (*ketone*-DNA) and its subsequent chemical modification with aminooxy derivatives through oxime formation (Figure 1). Ketones and aldehydes have been used for selective biomolecular modification.[6] Specif-

(1) For a review, see: Verma, S.; Eckstein, F. *Annu. Rev. Biochem.* **1998**, *67*, 99−134.

(2) For a recent example, see: Taton, T. A.; Mirkin, C. A.; Letsinger, R. L. *Science* **2000**, *289*, 1757−1760.

(3) Prober, J. M.; Trainor, G. L.; Dam, R. J.; Hobbs, F. W.; Robertson, C. W.; Zagursky, R. J.; Cocuzza, A. J.; Jensen, M. A.; Baumeister, K. *Science* **1987**, *238*, 336−341.

(4) For reviews, see: (a) Breaker, R. R. *Nature Biotechnol.* **1997**, *15*, 427−431. (b) Sen, D.; Geyer, C. R. *Curr. Opin. Chem. Biol.* **1998**, *2*, 680−687. (c) Jäschke, A. *Curr. Opin. Struct. Biol.* **2001**, *11*, 321−326.

(5) (a) Hwang, J. -T.; Greenberg, M. M. *J. Org. Chem.* **2001**, *66*, 363−369. (b) Kahl, J. D.; Greenberg, M. M. *J. Am. Chem. Soc.* **1999**, *121*, 597−604. (c) Xu, Y, -Z.; Zheng, Q.; Swann, P. F. *J. Org. Chem.* **1992**, *57*, 3839−3845. (d) Ferentz, A. E.; Verdine, G. L. *J. Am. Chem. Soc.* **1991**, *113*, 4000−4002.

(6) Sugar ligation: Peri, F.; Cipolla, L.; La Ferla, B.; Dumy, P.; Nicotra, F. *Glycoconjugate J.* **1999**, *16*, 399−404. Aldehyde groups for DNA ligation: Trévisiol, E.; Renard, A.; Defranq, E.; Lhomme, J. *Tetrahedron Lett.* **1997**, *38*, 8687−8690. Aldehyde-containing RNA: Berthod, T.;

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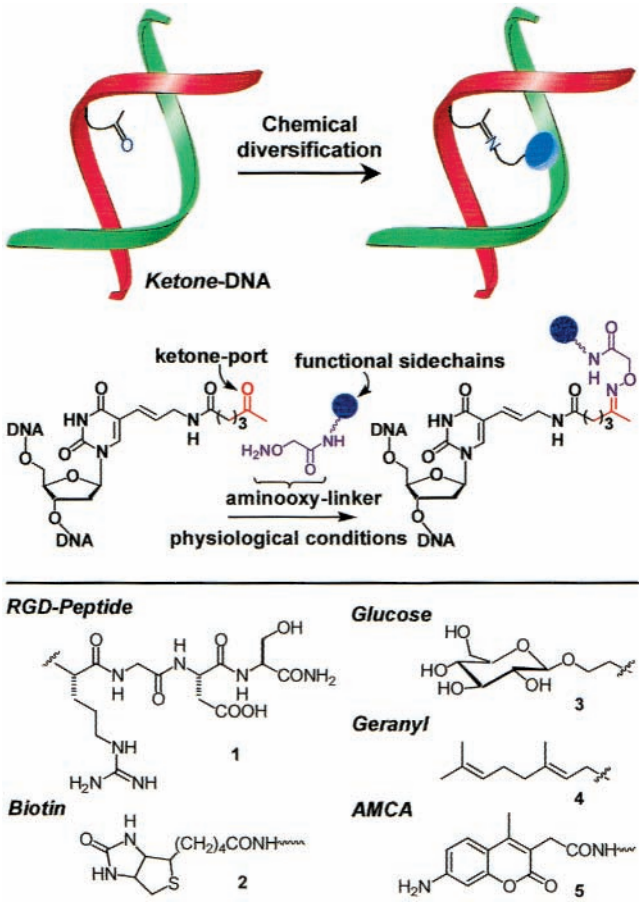
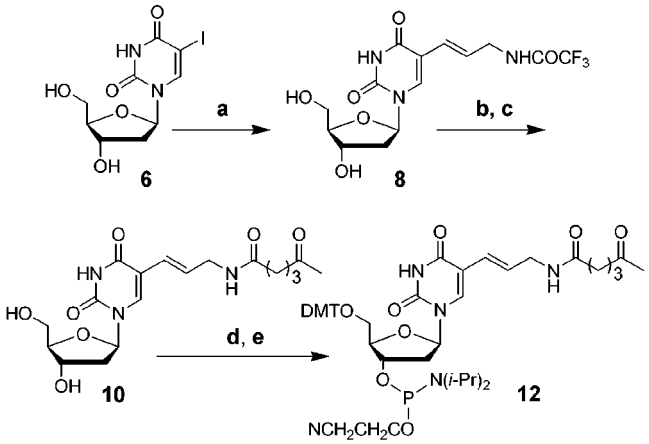


Figure 1. *Ketone*-DNA decoration strategy.

ically, ketone groups have been employed for the modification of peptides,[7] proteins,[8] and cell surfaces[9] and as a tool for RNA labeling.[10] Herein we report the synthesis and characterization of *ketone*-DNA and the postsynthetic decoration of these DNA strands with diverse structural elements.

Our approach to *ketone*-DNA required the synthesis of a universal methyl ketone-containing phosphoramidite derivative (**12**, Scheme 1). Phosphoramidite **12** was chosen on the basis of previous studies, which indicated that C-5 pyrimidine substitutions provide an accessible DNA modification site that does not interfere with duplex stability[11] or DNA polymerase function.[3,12,13]

Scheme 1. Synthesis of *Ketone*-DNA Phosphoramidite[a]



[a] (a) $CH_2{=}CHCH_2NHCOCF_3$ (**7**), $Na_2[PdCl_4]$, DMF−NaOAc buffer, 70 °C, 2h (**8**, 76%); (b) NH_4OH (**9**, 100%); (c) 5-oxohexanoic acid anhydride, DIPEA (**10**, 92%); (d) DMTCl, DMAP, pyridine (**11**, 89%); (e) $[CHN_4]\cdot[i\text{-}Pr_2EtNH_2]$, $[i\text{-}Pr_2N]_2PO(CH_2)_2CN$ (**12**, 85%).

Our synthetic route to **12** is detailed in Scheme 1. To introduce the required C-5 allylamine side chain, 5-iododeoxyuridine (**6**) was coupled to *N*-allyltrifluoroacetamide **7**[14] using a $Na_2[PdCl_4]$-mediated Heck reaction.[15] High yields of **8** were obtained using a modification of the procedure reported by Sakthivel and Barbas.[13] In our experience, the coupling yield was limited by the insolubility of **6** in the aqueous reaction medium. However, using DMF as a cosolvent and increasing the reaction temperature to 70−75 °C improved the yield from the reported 44% to 76% while decreasing the reaction time from 18 to 2 h. The trifluoroacetamide group was removed quantitatively from **8** within 8 h by treatment with 28% aqueous ammonia. The *ketone*-nucleoside **10** was prepared by coupling aminonucleoside **9** with the ketohexanoic acid anhydride. Subsequent tritylation and conversion to phosphoramidite **12** proceeded smoothly.

Phosphoramidite **12** was used to synthesize three *ketone*-DNA sequences, employing standard phosphoramidite coupling procedures.[16] *Ketone*-DNAs **13** and **14** contain a single *ketone* nucleotide, positioned at a terminal or central sequence site, respectively, while **15** incorporates two modifications (Figure 2). Phosphoramidite **12** displayed an average cou-

Petillot, Y.; Guy, A.; Cadet. A.; Forest, E.; Molko, D. *Nucleosides Nucleotides* **1996**, *15*, 1287−1306. Biomolecular aminooxy ligations−oligonucleotides: (a) Defrancq, E.; Lhomme, J. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 931−933. (b) Trévisiol, E.; Defranq, E.; Lhomme, J.; Layoun, A.; Cros, P. *Eur. J. Org. Chem.* **2000**, 211−217. (c) Salo, H.; Virta, P.; Hakala, H.; Prakash, T. P.; Kawasaki, A. M.; Manoharan, M.; Lönnberg, H. *Bioconjugate Chem.* **1999**, *10*, 815−823.

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(9) Mahal, L. K.; Yarema, K. J.; Bertozzi, C. R. *Science* **1997**, *276*, 1125−1128.

(10) Trévisiol, E.; Defrancq, E.; Lhomme, J.; Laayoun, A.; Cros, P. *Tetrahedron* **2000**, *56*, 6501−6510.

(11) Ahmadian, M.; Zhang, P.; Bergstorm, D. E. *Nucleic Acids Res.* **1998**, *26*, 3127−3135.

(12) Sági, J.; Nowak, R.; Zmudzka, B.; Szemzö, A.; Ötvös, L. *Biochim. Biophy. Acta* **1980**, *606*, 196−201.

(13) Sakthivel, K.; Barbas, C. F., III. *Angew. Chem., Int. Ed.* **1998**, *37*, 2872−2875.

(14) Trifluoroacetamide **7** was synthesized according to the following: Cook, A. F.; Vuocolo, E.; Brakel, C. L. *Nucleic Acids Res.* **1988**, *16*, 4077−4095. We point out that elimination of the aqueous workup increased the yield of highly water-soluble **7** from 35% to 95%.

(15) (a) Hobbs, F. W., Jr. *J. Org. Chem.* **1989**, *54*, 3420−3422. (b) Bergstrom, D. E.; Ruth, J. L.; Warwick, P. *J. Org. Chem.* **1981**, *46*, 1432−1441.

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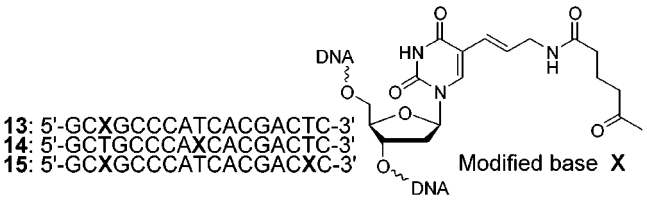

Figure 2. *Ketone*-DNA sequences (**13**, **14**, **15**).

pling yield (>99%) equal to the natural nucleotides, as judged by trityl cation assay. Following synthesis, ODNs were deprotected and cleaved from the solid support by concentrated aqueous ammonia to produce *ketone*-DNAs in excellent yield and purity. *Ketone*-DNAs were further purified by reverse phase HPLC (RP-HPLC), and their identities were verified by matrix-assisted laser desorption time-of-flight mass spectroscopy (MALDI-TOF MS).[17]

Thermal denaturation and circular dichroism analysis of duplexes composed of *ketone*-DNAs and complementary natural DNA sequence **16** revealed that C-5 ketone modifications did not perturb DNA duplex stability or structure. As shown in Figure 3A, the melting temperatures for all three *ketone*-DNA·**16** duplexes were similar to their natural DNA sequence counterpart (T_m = 63−64 °C). The CD spectra of *ketone*-DNA·**16** hybrid duplexes were superimposable with that of the corresponding natural DNA duplex (Figure 3B), indicating that duplexes containing *ketone*-nucleotides adopt standard B-form helices.

A series of structurally diverse aminooxy tagging groups for the decoration of *ketone*-DNA were prepared: RGD-peptide (**1**), biotin (**2**), glucose (**3**), geranylamine (**4**), and fluorescent AMCA (**5**) derivatives (Figure 1). The aminooxy derivatives of these compounds were synthesized by amide bond coupling of the amino precursor to Fmoc-aminooxy-glycine and subsequent deprotection (Supporting Information).

Ketone-DNAs were selectively modified with aminooxy derivatives **1**−**5** under simulated physiological conditions. In phosphate-buffered saline (PBS), DNA conjugation reactions were complete within 24 h at 37 °C.[18]

Reactions between RGD-peptide **1** and single-stranded *ketone* DNAs **13** or **14** were analyzed by denaturing polyacrylamide gel electrophoresis (PAGE), as shown in Figure 4. As illustrated in panel **A**, quantitative modification of single-stranded **13** (lanes 1−5) and **14** (lanes 6 and 7) with peptide **1** was observed over a 24 h time course, giving **13-1** and **14-1**, respectively. In contrast, DNA lacking a methyl ketone (**17**) remained unmodified under the same conditions (lane 9). The pH-dependence of the modification

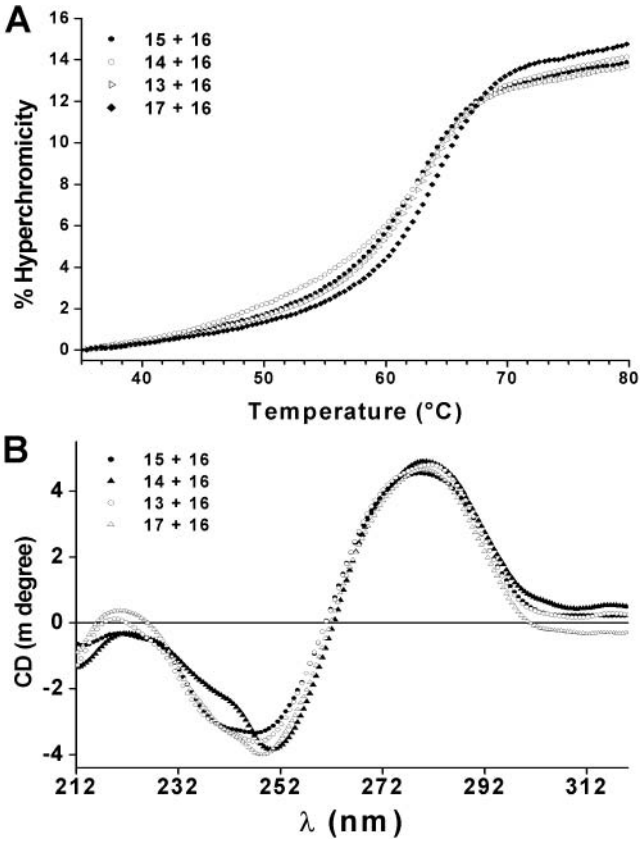

Figure 3. Thermal denaturation (**A**) and CD spectral (**B**) analysis of *ketone*-DNA·**16** duplexes. Single strand concentration = 2 μM. Buffer: 25 mM PO_4^{3-}, ionic strength (adjusted with NaCl) = 150 mM, 0.1 mM EDTA, pH = 7.00. Complement **16**: 5′-GAGTCGT-GATGGGCAGC-3′. **17**: 5′-GCTGCCCATCACGACTC-3′.

reaction rate also was measured. As expected,[19] decreasing the pH of the reaction enhanced the rate of oxime formation. For example, at pH 6.24, ligation of **1** and **13** was essentially complete within 6 h at 37 °C (Supporting Information). Ligations of **13** with other aminooxy modifiers (**2**−**5**) proceeded with similar efficiencies.

The modification of double-stranded DNA was equally efficient, as demonstrated in Figure 4B. Duplex **13**·**16** was incubated with **1** under PBS conditions, and the reaction mixture was analyzed by nondenaturing gel electrophoresis. As shown in lane 4 (Figure 4B), aminooxy modification of duplex *ketone*-DNA proceeded smoothly to produce double-stranded adduct, **13-1**·**16**.

Ketone-DNA modification by aminooxy derivatives **1**−**5** was analyzed further by RP-HPLC and MALDI-TOF MS. For each modifier, clean conversion to a new ODN product was observed within 24 h in PBS buffer.[20] For example, Figure 5A shows the RP-HPLC profile of a representative reaction between **13** and aminooxy-biotin conjugate **2**. After

(17) MALDI-TOF MS. **13**: $[M]^-$ calcd, 5244.49; found, 5245.79. **14**: $[M]^-$ calcd, 5244.49; found, 5245.70. **15**: $[M]^-$ calcd, 5397.67; found, 5397.8. See Supporting Information for details.

(18) *Ketone*-DNAs (single strand concentration = 5 μM) were incubated at 37 °C with a 100-fold excess of the aminooxy modifier in PBS buffer (10 mM PO_4^{3-}, ionic strength (adjusted with NaCl) = 150 mM, pH = 6.96 at 37 °C).

(19) Jencks, W. P. *Catalysis in Chemistry and Enzymology*; Dover: New York, 1969; pp 463−496.

(20) Following ligation reactions, excess modifiers were removed from the product by filtration through a Sephadex-G10 gel cartridge.

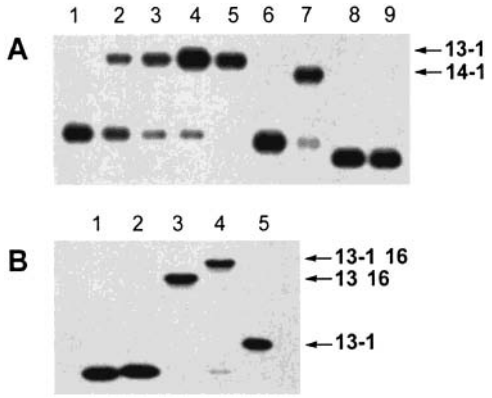

Figure 4. Efficient modification of single- and double-stranded *ketone*-DNAs with RGD-peptide **1**. (**A**) Single-stranded DNA decoration. *Ketone*-DNAs were modified with RGD-peptide under standard conditions at 37 °C in PBS buffer, and products were analyzed by 20% denaturing PAGE. Lane 1: **13** only. Lanes 2, 3, 4 and 5: **13** + **1** for 3, 6, 12 and 24 h, respectively. Lane 6: **14** only. Lane 7: **14** + **1** for 24 h. Lane 8: standard DNA **17** only. Lane 9: control, **17** + **1** for 24 h. (**B**) Double-stranded DNA decoration. Duplex DNAs were constituted by heating single-stranded DNA (5 μM each strand) to 95 °C and cooling to rt in 25 mM PO_4^{3-}, ionic strength (adjusted with NaCl) = 150 mM, 0.1 mM EDTA, pH = 6.87. After annealing, duplex was incubated with **1** (100 equiv) for 26 h at 37 °C. Products were analyzed by 20% nondenaturing PAGE. Lane 1: **13** only. Lane 2: **16** only. Lane 3: duplex **13·16**. Lane 4: duplex **13·16** + **1**. Lane 5: **13** + **1**.

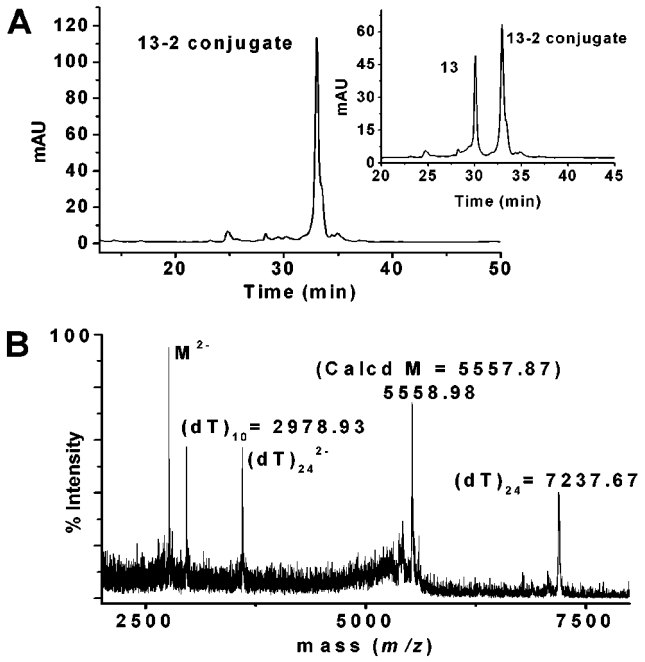

Figure 5. RP-HPLC and MALDI-TOF MS analysis of the **13-2** ligation reaction in PBS buffer buffer for 24 h. (**A**) RP-HPLC analysis of **13** + **2** ligation reaction. Inset: coinjection of **13** with **13** + **2** reaction products. (**B**) Negative mode MALDI-TOF analysis of **13-2**. See Supporting Information for detail.

24 h, a single new product was formed, which displayed a retention time different (t_R = 33.0 min) from that of the starting **13** (t_R = 30.0 min). The new material was identified as conjugate **13-2** by MALDI-TOF MS analysis (Figure 5B, **13-2**: $[M]^-$ calcd, 5557.87; found, 5558.98). The reaction products of **13** and each modifier (**1**−**5**) were identified as the expected oxime products by RP-HPLC and MALDI-TOF MS.[21]

In conclusion, we have described a highly efficient method for the selective, postsynthetic decoration of DNA oligonucleotides. Chemical routes to *ketone*-nucleosides and *ketone*-DNAs were developed. A series of aminooxy derivatives were synthesized and shown to quantitatively modify *ketone*-DNA under simulated physiological conditions. The decorated DNA was readily purified within minutes by a simple size exclusion column. Furthermore, ketone modifications did not alter DNA duplex structure or stability. *Ketone*-DNA decoration by oxime formation offers a versatile and convergent method for the chemoselective modification of DNA with virtually any functional side chain under mild aqueous conditions. Thus, our approach may have broad utility for a range of DNA applications, including DNA catalysis, antisense therapeutics, DNA detection and diagnostics, and DNA-based material synthesis.

(21) MALDI-TOF MS ligation reaction analysis. **13-1**: $[M]^-$ calcd, 5731.96; found, 5731.95. **13-3**: $[M]^-$ calcd, 5522.75; found, 5523.75. **13-4**: $[M]^-$ calcd, 5452.79; found, 5452.65. **13-5**: $[M]^-$ calcd, 5546.77; found, 5547.35.

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Supporting Information Available: Detailed experimental protocols and spectral data for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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