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p-Cyano Substituted 5-Benzoyldeoxyuridine as a Novel Electron-Accepting Nucleobase for One-Electron Oxidation of DNA

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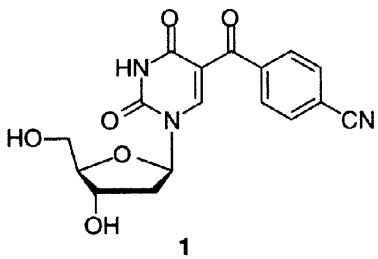
Abstract: *p*-Cyano substituted 5-benzoyl-2'-deoxyuridine **1** was synthesized as a novel electron-accepting nucleobase. DNA cleavage by **1** under photoirradiation conditions occurred selectively at the 5'-G of 5'GG3' sequences after hot piperidine treatment. Photoirradiation of **1** in the presence of dG produced imidazolone as a major product. The electron-accepting nucleobase **1** was successfully incorporated into DNA oligomer by automated DNA synthesis using phosporamidite **2**.

There has been much current interest in long range electron transfer (ET) through DNA duplex.[1] DNA oligomers containing various types of electron acceptors such as Rh(III) intercalator,[1,2] anthraquinone derivatives,[3] stilbene dicarboxamide,[4] and acridine[5] have been utilized for studying such photoinduced long range ET from electron donating guanine base (G). However, the lack of information on the precise location of electron acceptor in a duplex DNA has sometime become a serious problem to accurately calculate β value for distance dependent ET rate constants. To incorporate an electron-accepting group into DNA duplex with a well-defined position and geometry, the design of a new nucleobase that is directly attached to electron acceptor via a non-flexible linker is highly desirable. Recently, we have demonstrated that *p*-cyano substituted benzophenone is an excellent photophore for one-electron oxidation of DNA.[6] Taking these into account for our design of novel electron-accepting nucleobases, we synthesized *p*-cyano substituted 5-



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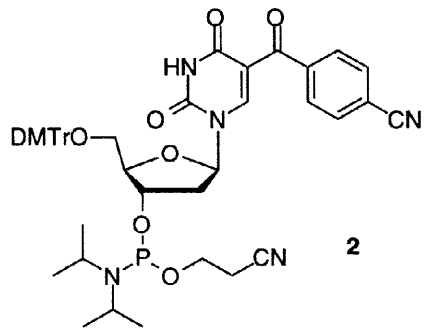

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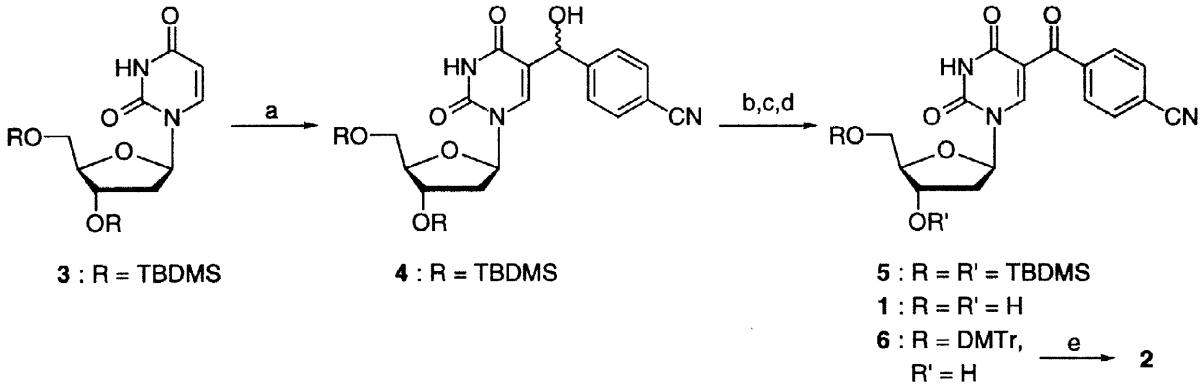
PII: S0040-4039(98)01231-3

benzoyl-2'-deoxyuridine **1** ($d^{pCNBz}U$). We herein report that **1** is an excellent electron-accepting nucleobase for one-electron oxidation of DNA and could be easily incorporated into DNA oligomer via phosphoramidite **2**.

Scheme 1[a]



[a] Reagents and conditions: (a) i) *sec*-BuLi (2.2 eq), TMEDA, THF, –78 °C, 90 min; ii) 4-cycanobenzaldehyde, 38%; (b) Dess-Martin periodinane, CH_2Cl_2, rt, 3 h, 90%; (c) TBAF, THF, 94%; (d) DMTrCl, Et_3N, DMAP, pyridine; (e) 2-cyanoethyl tetraisopropylphosphorodiamidite, tetrazole, CH_3CN.

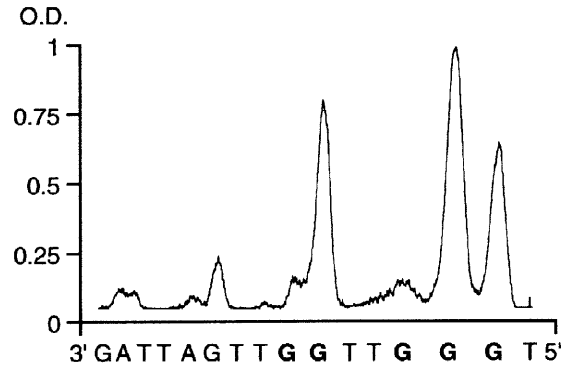
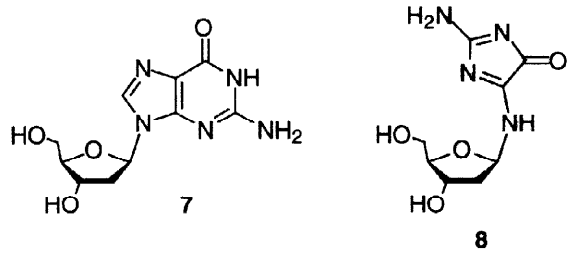


Figure 1. Base sequence selectivity for photoinduced cleavage of 41-mer ODN in the presence of **1** analyzed by densitometry. ^{32}P-5'-end-labeled ODN was annealed with a complementary strand in a buffer (Na cacodylate, 10 mM, pH 7.0) and the solution of the duplex containing sonicated calf thymus DNA was irradiated at 312 nm (Funakoshi, TFX-20M Transilluminator, 6x15W) for 1 h in the presence of **1** (50 μM) at 0 °C. Photoirradiated ODN recovered by ethanol precipitation was heated in 10% piperidine at 90 °C for 20 min and analyzed by electrophoresis on a sequencing gel containing 12% polyacrylamide and 7 M urea. The gel was exposed to X-ray film and cleavage bands were quantified by densitometer. Only partial sequence of the ODN was shown in the figure.



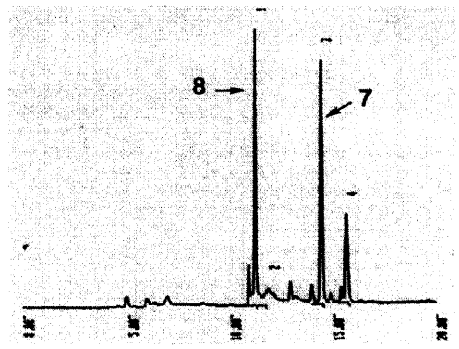


Figure 2. HPLC profile of the crude reaction mixture produced by photoirradiation of **1** (100 μM) in the presence of **7** (100 μM) in sodium cacodylate (10 mM, pH 7.0) at 312 nm for 3 h at 0 °C. HPLC analysis was carried out on a CHEMCOBOND 5-ODS-H column (10 X 150 mm) eluted with water containing 0–20% acetonitrile linear gradient over 20 min at a flow rate of 1.0 mL/min, detected at 254 nm.

Synthesis of **1** and **2** was outlined in Scheme 1. Lithiation of 3',5'-bis(*tert*-butyldimethylsilyloxy)-2'-deoxyuridine (**3**) with *sec*-butyl lithium followed by trapping the resulting dianion with 4-cyanobenzaldehyde produced a diastereomeric mixture of adduct **4**,[7] which was oxidized with Dess-Martin periodinane to give **5**. Deprotection of **5** afforded $d^{pCNBz}U$ **1**,[8] which was further transformed to phosphoramidite **2** via the protection of the primary alcohol with dimethoxytrityl chloride (DMTrCl) followed by treating with cyanoethyl tetraisopropylphosphordiamidite.

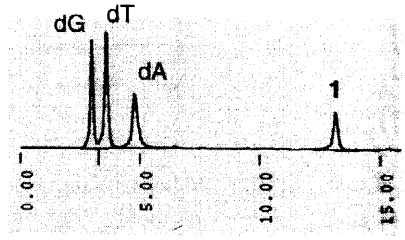


Figure 3. HPLC profile for product analysis of a mixture obtained by enzymatic digestion of 10-mer d(TA^{pCNBz}UTATGGTT) with snake venom phosphodiesterase, calf intestine alkaline phosphatase, and nuclease P1 giving dG, dT, dA, and $d^{pCNBz}U$ **1**. Each HPLC peak was identified by comigration with an authentic sample.

Photoinduced DNA cleavage by **1** was examined by relaxation assay of supercoiled plasmid DNA, showing that $d^{pCNBz}U$ **1** cleaved DNA much more efficiently than unsubstituted 5-benzoyldeoxyuridine[7] under photoirradiation at 312 nm. Base sequence selectivity for DNA cleavage by **1** was examined using 41-mer oligodeoxynucleotide (ODN) d(CGT GCT TCA TTG GGT TGG TTG ATT AGT TCG TTG TTT ACT CT) containing 5'GG3' doublet and 5'GGG3' triplet and the cleavage bands were determined by densitometric analysis (Figure 1). As clear from figure 1, the G cleavage occurred selectively at the 5'-G of GG doublet with a very weak cleavage at GA sequence. In the case of GGG triplet, the cleavage occurred most strongly at the middle G.[9] Photoirradiation of **1** in the presence of dG (**7**) predominantly produced a product which comigrated on HPLC with an authentic sample of imidazolone **8**, which was already identified in benzophenone-photosensitized oxidation of **7** via one-electron transfer from dG to triplet excited benzophenone (Figure 2).[10] Selective cleavage at the 5'-G of GG doublet and a predominant formation of imidazolone **8** strongly support that photoinduced DNA cleavage by $d^{pCNBz}U$ **1** proceeded via an electron transfer process.[6,9]

Having established the electron-accepting property, incorporation of **1** into DNA oligomer was next examined. Since it was found that **1** could not tolerate under standard base-deprotecting conditions (25% ammonium hydroxide, 55 °C, 8 h), 4-isopropylphenoxyacetyl-dG- and phenoxyacetyl-dA-cyanoethyl phosphoramidites were used for the automated DNA synthesis and deprotection was carried out with 25% ammonium hydroxide at 37 °C for 5 h. HPLC analysis of a nucleoside mixture obtained by enzymatic digestion of 10-mer d(TA^{pCNBz}UTATGGTT) indicated the incorporation of electron-accepting base **1** into the ODN (Figure 3). MALDI-TOF mass spectrum showed a molecular weight of 3162.88, which was in good agreement with calculated mass of 3163.16. Melting temperature for oligomer duplex d(TA^{pCNBz}UTATGGTT/AACCATAATA) measured in 250 mM sodium chloride and 5 mM sodium cacodylate buffer (pH 7.0) at 100 μM base concentration was 27.8 °C, being destabilized by 4.5 °C than the corresponding normal 10-mer duplex containing dT-dA base pair instead of d^{pCNBz}U-dA. These results showed that the bulky *p*-cyano substituted benzoyl group directly attached to C5 of dU destabilizes the modified duplex and suggest that dU derivatives tethering to an electron acceptor via a less bulky and rigid linker such as an acetylenic unit would improve the duplex stability.

In summary, the present studies demonstrated that *p*-cyano substituted 5-benzoyldeoxyuridine has a unique property as an electron-accepting nucleobase and can serve as a photophore for one-electron oxidation of DNA. Furthermore, DNA oligomers containing $d^{pCNBz}U$ can be easily synthesized by automated DNA synthesis.

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(8) **1**: ^{1}H NMR (CD_3OD, 400 MHz) δ 2.28 (dt, 1H, J = 13.7, 6.4 Hz), 2.40 (ddd, 1H, J = 13.7, 6.2, 4.0 Hz), 3.65 (dd, 1H, J = 12.0, 3.4 Hz), 3.70 (dd, 1H, J = 12.0, 3.2 Hz), 3.95 (q, 1H, J = 3.3 Hz), 4.37 (m, 1H), 6.27 (t, 1H, J = 6.3 Hz), 7.81 and 7.85 (each d, 2H×2, J = 8.8 Hz), 8.72 (s, 1H); ^{13}C NMR (CD_3OD, 100 MHz) δ 42.1, 62.4, 72.0, 87.8, 89.5, 113.5, 116.7, 119.2, 130.8, 133.2, 143.3, 149.4, 151.3, 162.9, 191.5; HRFABMS calcd for $C_{17}H_{16}O_6N_3$ $(M+H)^+$ 358.1038, found 358.1054.

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