Tetrahedron Letters, Vol. 33, No. 30, pp. 4265-4268, 1992
Printed in Great Britain

0040-4039/92 $5.00 + .00
Pergamon Press Ltd

Synthesis of Photoactive DNA: Incorporation of 8-Bromo-2'-Deoxyadenosine into Synthetic Oligodeoxynucleotides

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Key Words: DNA oligonucleotide; automated synthesis; 8-bromo-2'-deoxyadenosine; phenoxyacetyl protecting group; DNA duplex stability

Abstract: 8-Bromo-N^6-phenoxyacetyl-5'-*O*-(4,4'-dimethoxytrityl)-2'-deoxyadenosine-3'-*O*-(2-cyanoethyl-*N*,*N*-diisopropyl) phosphoramidite was synthesized and used to introduce 8-bromo-2'-deoxyadenosine (8-Br-dA) into oligodeoxynucleotides by means of automated synthesis. 8-Br-dA was found not to affect adversely the stability of duplex DNA, as judged by the melting behavior of several 8-Br-dA-containing duplexes.

Chemically modified oligodeoxynucleotides have found numerous applications in biological and medicinal chemistry.[1] We are interested in their use as probes of macromolecular recognition and catalysis in sequence-specific protein-DNA complexes,[2] and particularly in the use of photoactive nucleoside analogues to identify DNA base ↔ amino acid contact pairs in protein-DNA complexes, as a means toward understanding the structural basis for sequence discrimination. The power of such a photocross-linking strategy has recently been demonstrated by Matthews *et al.* in the determination of specific contacts between *lac* repressor protein and its DNA operator.[3]

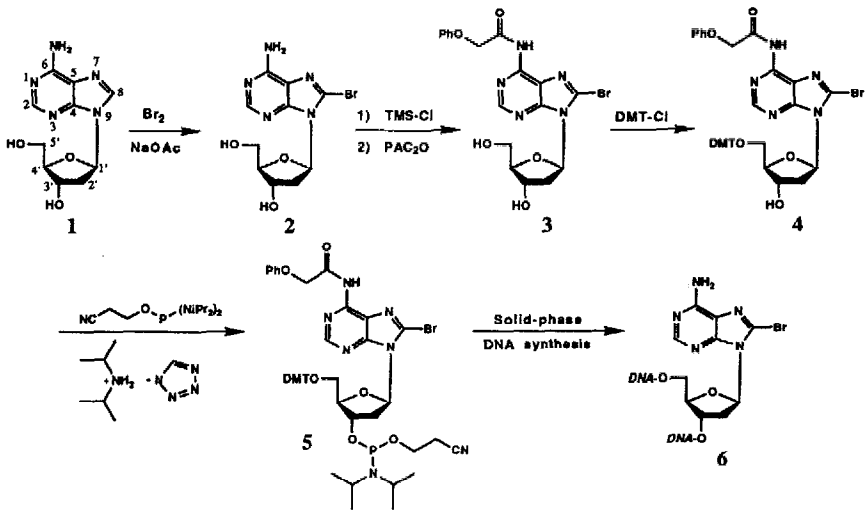
In order for the photocross-linking technique to be generally useful, photoactive analogues of all four nucleosides present in DNA are needed. Although the phosphoramidites of 5-bromo-2'-deoxyuridine[3] and 5-bromo-2'-deoxycytidine are commercially available and can thus be easily incorporated into DNA by standard automated synthesis, corresponding methods for the introduction of brominated (or iodinated) purines into DNA have not been developed. X-ray analyses of protein-DNA complexes have shown purines to be involved frequently in direct amino acid contacts;[4] therefore, much useful information could be gained from access to synthetic oligonucleotides bearing photoactivatable dA or dG analogs. In this communication we report the synthesis of an 8-bromo-2'-deoxyadenosine (8-Br-dA) phosphoramidite and its use in the site-specific introduction of this photoactive dA analog into DNA via solid-phase methods.

In preliminary experiments, we noted that 8-Br-dA (**2**) is susceptible to nucleophilic attack by ammonia to yield 8-amino-dA under conditions of elevated temperature or prolonged exposure. Since the final step of standard DNA synthesis ordinarily involves deprotection with ammonia under such conditions, we turned to an alternative protecting group strategy, which would allow for milder deprotection using ammonia. The phenoxyacetyl (PAC) amine-protecting group was chosen for this purpose, since it is well-known to undergo deprotection under less harsh conditions (conc. NH_4OH, several hr, room temperature),[5] as compared with the traditional benzoyl protecting group (8-16 hr, 55 °C).[6]

8-Bromo-2'-deoxyadenosine (**2**) was prepared from 2'-deoxyadenosine (**1**) by a known procedure (Scheme I).[7] The PAC amine-protecting group was then introduced by a modification of the transient protection method.[8] Compound **2** (330 mg, 1 mmol) was silylated with trimethylsilylchloride (5 mmol) in pyridine at 0 °C (30 min),

followed by treatment of phenoxyacetic anhydride (5 mmol) at room temperature (4 hr). After quenching and extraction to remove phenoxyacetic acid,[8] the 3'- and 5'- silyl groups were removed by treatment with triethylamine-pyridine-water. The reaction mixture was allowed to stand at room temperature for only 5 minutes, since longer reaction times resulted in loss of the PAC group. This increased lability of the PAC protecting group on 8-Br-dA as compared with PAC-dA probably results from the presence of the electron-withdrawing bromo substitent. The crude product from the desilylation reaction was purified by flash column chromatography (methanol/chloroform, 2% to 5%) to afford pure 8-bromo-N^6-phenoxyacetyl-2'-deoxyadenosine (**3**) (208 mg, 0.45 mmol; 45% from **2**).[9]

Scheme I



The N^6-phenoxyacetylated 8-bromo-2'-deoxyadenosine **3** (401 mg, 0.86 mmol) was tritylated according to a standard procedure[10] (2 hr, r.t.), and purified by flash chromatography (ethyl acetate/hexanes, 40/60 to 70/30%) to give 8-bromo-N^6-phenoxyacetyl-5'-*O*-(4,4'-dimethoxytrityl)-2'-deoxyadenosine (**4**) (345 mg, 0.45 mmol; 52%).[11] The silica column was pre-treated with triethylamine (3% in hexanes) in order to avoid detritylation and depurination of **4**, which occurred when the column was used directly without pre-treatment. Triethylamine was not used in the eluting solvents, however, because its presence led to significant loss of the PAC protecting group from **4**.

Protected nucleoside **4** (345 mg, 0.45 mmol) was then treated with 2-cyanoethyl-*N*,*N*,*N*',*N*'-tetraisopropylphosphordiamidite (0.50 mmol) and diisopropylammonium tetrazolide (0.23 mmol) in CH_2Cl_2 (5 ml) according to the published procedure (2 hr, r.t.).[12] The solvent was removed in vacuo, and the crude product was purified by flash chromatography (ethyl acetate/hexanes, 25/75 to 50/50%) using silica gel that had been pre-treated with triethylamine. The 8-Br-dA phosphoramidite (**5**) thus obtained (375 mg, 0.39 mmol; 86%) was pumped extensively before being used in DNA synthesis. ^{31}P NMR of **5** showed the characteristic pair of signals, one from each of the two diastereomers at phosphorus.[13]

Phosphoramidite **5**, together with the phosphormidites of the four naturally occurring nucleosides (PAC-dA, PAC-dG, isobutyryl-dC, and dT; Pharmacia), was used in standard automated syntheses of three oligodeoxynucleotides, each possessing one strand of a specific binding site for the transcription factor NF-κB:[14] 5'-d(AGGGCTGGGGATTCCCCATCTCC) (top strand); and 5'-d(GGAGATGGGGAATCCCCAGCCCT) and 5'-d(GGAGATGGGGAATCCCCAGCCCT) (bottom strand) (underlined dA = PAC-8-Br-dA). During the syntheses, phenoxyacetic anhydride was used instead of acetic anhydride as capping reagent, so as to ensure that only PAC substituents (and not acetyl) were present on the DNA bases.[15] The modified oligodeoxynucleotides

were simultaneously cleaved from the solid support and deprotected by treatment with concentrated ammonium hydroxide solution (2 h, r.t.). The oligodeoxynucleotides were then purified by gel electrophoresis.

The purified oligodeoxynucleotides were treated with snake venom phosphordiesterase and alkaline phosphatase and the digested material was analyzed by HPLC. Five major peaks were observed (Figure 1), which by comparison with authentic standards were identified as dC, dT, dG, dA and 8-Br-dA (**2**). A small impurity peak (about 10% of **2**) was also observed, which was identified to be 8-oxo-7*H*-2'-deoxyadenosine.[16]

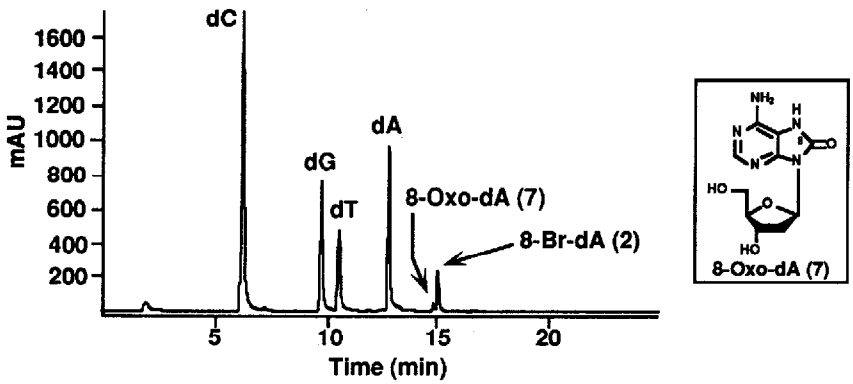


Figure 1. HLPC profile of enzymatically digested 5'-d(GGAGATGGGGA**6**TCCCCAGCCCT)

Notable in these digests was the lack of 8-amino-dA, the product of displacement of the 8-bromo substituent. Upon extension of the deprotection time to 10 h, or increasing the temperature to 55 °C, significant amounts of 8-amino-dA were observed. Hence as indicated by the nucleoside model studies it is necessary to use base-labile protecting groups in the synthesis of 8-Br-dA-containing oligodeoxynucleotides.

Unmodified control strands were also synthesized and base-paired with the complementary **6**-containing strands to afford the three singly modified duplexes shown in Table 1. An unmodified duplex was also prepared as a reference. The stability of duplex structure in these molecules was examined in thermal denaturation experiments. For all samples, the same buffer (10 mM KH_2PO_4, pH 7.0, 1M NaCl) and concentration of duplex DNA (1.4 μM based on single strands) was used.[17] All the duplex molecules exhibited sharp helix-to-coil

Table 1. Melting Temperatures (T_m) of DNA Duplexes (**6**= 8-BrdA).

DNA Duplex	T_m (°C)
5'-AGGGCTGGGGATTCCCCATCTCC-3' 3'-TCCCGACCCCTAAGGGGTAGAGG-5'	73.2
5'-AGGGCTGGGG**6**TTCCCCATCTCC-3' 3'-TCCCGACCCCTAAGGGGTAGAGG-5'	71.9
5'-AGGGCTGGGGATTCCCCATCTCC-3' 3'-TCCCGACCCCT**6**AGGGGTAGAGG-5'	73.0
5'-AGGGCTGGGGATTCCCCATCTCC-3' 3'-TCCCGACCCCTA**6**GGGGTAGAGG-5'	72.0

transitions; moreover, the differences in melting half-transition temperatures (T_m) among the four were within the limit of experimental error (Table 1). These observations suggest that substitution of bromine for hydrogen does not lead to substantial distortion of duplex DNA structure, which in turn suggests that 8-Br-dA should not perturb protein binding for conformational reasons alone. This point was tested directly by electrophoretic mobility

assays using the p50 subunit of NF-κB, which bound the modified duplex molecules in Table 1 only 1.5-2.5-fold more weakly than the unmodified control.

In conclusion, we have successfully introduced the photoactive probe 8-bromo-2'-deoxyadenosine into oligonucleotides and have shown that it causes little perturbation of DNA stability and function as a protein receptor. The 8-position of dA is close to a site that is frequently involved in specific contacts with proteins (*N*7-dA); however, since 8-Br-dA is close to the edge of the major groove surface, it is unlikely to cause steric interference of binding. We are currently examining the use of 8-Br-dA-containing oligodeoxynucleotides in probing specific contacts in protein-DNA complexes.[18]

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9. Spectroscopic data of compound **3**: ^{1}H NMR ($CDCl_3$, δ 7.24 ppm): δ 9.42 (s, 1H, NH), 8.70 (s, 1H, H2), 7.33 (m, 2H, PhH), 7.03 (m, 3H, PhH), 6.51 (m, 1H, H1'), 5.83 (bs, 2H, OH), 4.81 (s, 3H, CH_2OPh, H3'), 4.22 (m, 1H, H4'), 3.95 (m, 1H, H5'), 3.79 (m, 1H, H5'), 3.04 (m, 1H, H2'), 2.33 (m, 1H, H2') ppm. HRMS: calcd for $C_{18}H_{18}N_5O_5Br$ (M+Na), 486.0388; found 486.0390.
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11. Spectroscopic data of compound **4**: ^{1}H NMR ($CDCl_3$, δ 7.24 ppm): δ 9.28 (bs, 1H, NH), 8.51 (s, 1H,H2), 7.36-6.72 (m, 18H, PhH), 6.43 (m, 1H, H1'), 4.94 (m, 1H, H3'), 4.80 (s, 2H, CH_2OPh), 4.10 (m, 1H, H4'), 3.76 (s, 3H, OCH_3), 3.75 (s, 3H, OCH_3), 3.53 (m, 1H, H2'), 3.37 (m, 2H, H5'), 2.40 (m, 1H, H2') ppm. HRMS: calcd for $C_{39}H_{36}N_5O_7Br$ (M+Na), 788.1694; found 788.1696.
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13. Spectroscopic data of compound **5**: ^{1}H NMR ($CDCl_3$, δ 7.24 ppm): δ 9.28 (bs, 1H, NH), 8.51 and 8.49 (2s, 1H, H2), 7.36-6.69 (m, 18H, PhH), 6.43 (m, 1H, H1'), 5.00-4.93 (m, 1H, H3'), 4.81 (s, 2H, CH_2OPh), 4.28 (m, 1H, H4'), 3.75 (d, 6H, OCH_3), 3.86-3.67 (m, 2H, Me_2**CH**), 3.64-3.60 (m, 3H, $POCH_2$ and H2'), 3.40 (m, 1H, H5'), 3.28 (m, 1H, H5'), 2.61-2.40 (m, 3H, H2' and $CNCH_2$), 1.18 (m, 12H, N-CH(**CH$_3$**)$_2$) ppm. ^{31}P NMR ($CDCl_3$, external ref. 85% phosphoric acid δ 0.0 ppm): δ 149.3, 149.0 ppm. HRMS: calcd for $C_{48}H_{53}N_7O_8BrP$ (M+Na), 988.2771; found 988.2775.
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15. We thank Dr. Chuck Brush of Pharmacia P-L Biochemicals for providing information on the usage of phenoxyacetic anhydride as a capping reagent. For reference, see Chaix, C.; Molko, D.; Teoule, R. *Tetrahedron Lett.* **1989**, *30*, 71-74.
16. For comparison, 8-oxo-7*H*-2'-deoxyadenosine was prepared from 8-bromo-2'-deoxyadenosine by treatment with 0.4 N NaOH aqueous solution. The product was characterized by ^{1}H NMR, ^{13}C NMR, UV and HPLC analysis.
17. We are grateful to Professor L.W. McLaughlin of Boston College for use of his T_m instrument. For experimental details, see: Casale, R.; McLaughlin, L.W. *J. Am. Chem. Soc.* **1990**, *112*, 5264-5271.
18. We thank Christopher J. Larson and Dr. Mikiko Sodeoka for providing samples of NF-κB p50. This work was supported by a grant from Hoffmann-La Roche. GLV is a Presidential Young Investigator, a Searle Scholar, a Lilly Grantee, and a Sloan Fellow.

(Received in USA 23 March 1992)