Pergamon

Tetrahedron Letters 39 (1998) 6803–6806

TETRAHEDRON
LETTERS

Synthesis and adduction of fully deprotected oligodeoxynucleotides containing 6-chloropurine

Hye-Young Kim, Lubomir Nechev, Liang Zhou, Pamela Tamura,
Constance M. Harris* and Thomas M. Harris*

Chemistry Department and Center in Molecular Toxicology
Vanderbilt University, Nashville, Tennessee 37235

Received 9 March 1998; accepted 6 July 1998

Abstract

Fully deprotected oligodeoxynucleotides containing 6-chloropurine have been synthesized and used in solution phase reactions with amine nucleophiles to prepare oligonucleotides containing substituted adenine residues. This strategy was used for the preparation of a double-stranded oligonucleotide crosslinked by a 4-carbon tether between N^6 positions of deoxyadenosines in the two strands. © 1998 Elsevier Science Ltd. All rights reserved.

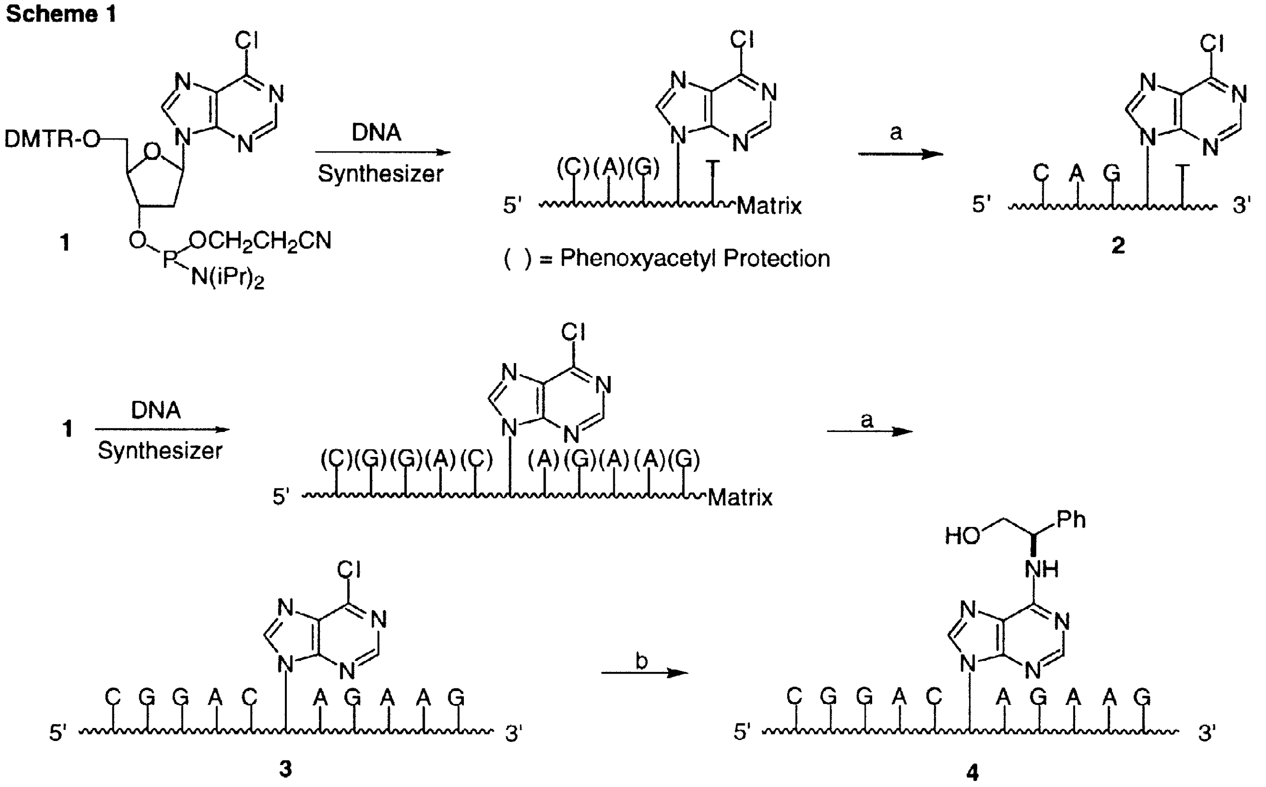
Keywords nucleosides, nucleic acids, purines

Over the past decade our laboratory and others have been interested in the synthesis of oligonucleotides containing bases which bear substituents capable of undergoing further transformations after oligonucleotide synthesis [1-19]. These transformations can be carried out while the oligonucleotide is still attached to the matrix or concomitant with removal from the matrix and deprotection of the other bases. Our studies have focused on the use of halopurines for the preparation of oligonucleotides bearing adducts of certain carcinogens [5,12,14,17,18]. For preparation of adenine adducts we have incorporated 6-chloro- or 6-fluoropurine deoxyriboside into oligonucleotides followed by reaction of the matrix-bound oligonucleotide with amine nucleophiles (such as the triol amine formed by opening of a PAH diol epoxide with anhydrous NH_3). The adducted oligonucleotide is then removed from the matrix and deprotected with ammonium hydroxide in the usual manner. This strategy suffers from the problems inherent in working in an inhomogeneous system such as insufficient mixing and slow reaction. In addition, when N^4-benzoyl-protected cytosines are present a side reaction may occur in which the amine can displace the acylamino group at C4 [4,20]. Furthermore, it is cumbersome to follow the course of reactions that are carried out on the beads. We previously developed methodology for preparing purified deprotected oligonucleotides containing a suitable guanine precursor (2-fluoro-O^6-(trimethylsilylethyl)-deoxyinosine) and showed that both mono-adducted and interstrand crosslinked oligonucleotides could be synthesized from them [12]. The strategy employed commercially available supports and PAC-type protection for the exocyclic amino groups on the bases [21-23]; room temperature treatment with 0.1 M NaOH was sufficient for detachment of the oligonucleotides from the matrix and removal of all protecting groups. We now report

0040-4039/98/$ - see front matter
PII: S0040-4039(98)01462-2

extension of this strategy to the preparation of fully deprotected oligonucleotides containing 6-chloropurine and the use of these oligonucleotides for the synthesis of both N^6 dA monoadducts and N^6-N^6 dA crosslinked oligomers.

Scheme 1



a, 0.1 M NaOH, rt; Sephadex G-25; HPLC
b, (R)-2-amino-2-phenylethanol, DMSO, 65 °C; HPLC

Initially, 6-chloropurine deoxyriboside was treated under the deprotection conditions to test stability inasmuch as halogen substituents at the 6 position of purines are more reactive than at the 2 position. Treatment of 6-chloropurine nucleoside with 0.1 M NaOH for 86 h at room temperature caused only ~5% decomposition. As a preliminary study, 5'-$\text{CAGPu}^{\text{Cl}}\text{T}$-3' was prepared (Expedite™ phosphoramidites; Expedite™ 8909 DNA synthesizer, PerSeptive Biosystems) (Scheme 1). Deprotection (1 mL of 0.1 M NaOH/40 mg of solid support, rt) was followed by UV and HPLC. Release from the beads was complete within 1 h and removal of all protecting groups within 9-10 h. The reaction mixture was neutralized with 0.2 M HOAc and purified by HPLC.[1] Purified oligonucleotide **2** was characterized by electrospray mass spectrometry (ESMS: calcd $M_r = 1506$; measured mass based on 752.1 (M-2H)/2z, 501.2 (M-3H)/3z = 1506.4) and enzymatic hydrolysis to deoxynucleosides which showed the chlorine was still in place on the modified nucleoside and that no deamination of cytosine had occurred. NMR spectroscopy showed only the expected signals in the aromatic and anomeric regions, confirming that deprotection was complete and no degradation of the chloronucleoside had occurred; the aromatic protons of chloropurine are significantly downfield, 8.59 and 8.75 ppm, relative to those of adenine, 8.27-7.95 ppm,

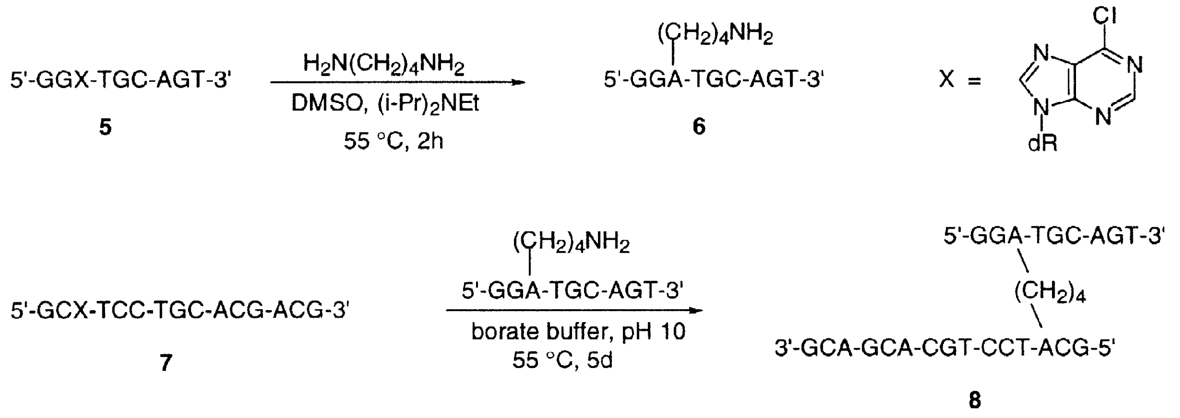
---

[1] Experimental conditions for HPLC and enzymatic hydrolysis to deoxynucleosides have previously been described [14,18].

in this 5-mer. The strategy was extended to the synthesis of an 11-mer containing a styrene oxide adduct which had previously been prepared by the matrix-bound strategy (Scheme 1) [5]. Fully deprotected 5'-CGGACPu$^{\mathrm{Cl}}$AGAAG-3' (**3**) was prepared as for the 5-mer except that a reaction period of 48 h was required for complete deprotection. In the course of this study, it was observed that the time required for complete deprotection is dependent on the size of the oligonucleotide and number of guanines present; deprotection for as long as 72 h at 25 °C did not cause significant hydrolysis of the chloropurine. Higher temperatures were not explored. Purified deprotected **3** (0.40 µmoles, 40% yield, ESMS: calcd M_r = 3418; measured mass based on 1138.0 (M-3H)/3z, 853.7 (M-4H)/4z = 3417.9) was treated with (R)-2-amino-2-phenylethanol (10 mg) in anhydrous DMSO (0.5 mL) at 65 °C until HPLC indicated that the reaction was complete (<12 h compared to 48 h for the solid phase reaction). Adducted oligonucleotide **4** was obtained in 50% yield after purification and was identical by HPLC and enzymatic digestion to the material prepared by solid-phase synthesis.

The method described herein for preparing oligonucleotides with substituents on N^6 of adenine has significant advantages over similar methods previously described by Verdine and coworkers which employed oligonucleotides containing 6-phenoxy- [7], or 6-(4-chlorophenoxy)purine [24]. Namely, 6-chloropurine deoxyriboside is more reactive and easier to prepare [25-28]. The low reactivity of the phenoxy synthons necessitated use of high concentrations of nucleophiles even with unhindered amines. As an example of the utility of the chloropurine method, we have prepared a 9-mer/15-mer duplex containing a 4-carbon interchain crosslink (Scheme 2). The high molecular weights of the two oligonucleotides employed in the crosslinking reaction necessitated using them in only millimolar concentrations. It is unlikely that this crosslinking reaction could be achieved with the less reactive phenoxy reagents.

Scheme 2



Fully deprotected 9-mer **5** (0.33 µmoles) was prepared as above and treated with 2 equiv of 1,4-diaminobutane (0.35 ml DMSO, 50 °C, 2 h) to give modified oligonucleotide **6** which was purified by HPLC and ion-exchange chromatography (Econo-Pac™ High Q (Bio-Rad) cartridge, gradient with 0.1 M NaOH/0.1 M NaOH containing 0.6 M NaCl), neutralization and desalting (Sephadex G-25) to give a 70% yield of monoadduct **6** (ESMS: calcd M_r = 2849; measured mass based on 949.0 (M-3H)/3z, 711.2 (M-4H)/4z, 568.9 (M-5H)/5z, 474.1 (M-6H)/6z = 2849.7). The ion-exchange step is necessary to ensure complete removal of excess diamine. Monoadduct **6** (0.11 µmoles, 0.36 mM) and purified 6-chloropurine-containing oligonucleotide **7** (0.33 µmoles, 1.0 mM) were reacted in sodium borate buffer (0.05 M, pH 10, 0.30 ml, 55 °C) until HPLC analysis showed no **6** remained (5 d). Under these conditions significant conversion of the chloronucleoside to inosine was observed, although little hydrolysis was seen at room temperature. The reaction was neutralized (5% HOAc) and the product was isolated by HPLC to give ~50% of crosslinked oligonucleotide **8** which was characterized by enzyme digestion,[2] capillary gel electrophoresis and MS (ESMS: calcd $M_r = 7367$; measured mass based on 817.9 (M-9H)/9z, 735.7 (M-10H)/10z, 668.8 (M-11H)/11z, 612.8 (M-12H)/12z = 7367.6).

The strategy described herein has advantages over the matrix-bound route: the starting halooligonucleotide can be purified and characterized, the reaction can be followed by HPLC, the reaction times are shorter and reactions are cleaner and easier to purify. It is particularly valuable for crosslinking reactions and reactions in which the nucleophile is in short supply or too unstable for conditions used in the matrix-bound strategies.

Acknowledgements We wish to acknowledge the generous financial support from the National Institute for Environmental Health Sciences (ES05355, ES07781, and ES00267).

1] Matteucci MD, Webb TR Tetrahedron Letters 1987;28:2469-2472.
2] Gibson KJ, Benkovic SJ Nucleic Acids Res. 1987;15:6455-6467.
3] Haralambidis J, Chai M, Tregear GW Nucleic Acids Res. 1987;15:4857-4876.
4] MacMillan AM, Verdine GL J. Org. Chem. 1990;55:5931-5933.
5] Harris CM, Zhou L, Strand EA, Harris TM J. Am. Chem. Soc. 1991;113:4328-4329.
6] MacMillan AM, Verdine GL Tetrahedron 1991;47:2603-2616.
7] Ferentz AE, Verdine GL J. Am. Chem. Soc. 1991;113:4000-4002.
8] Gao H, Fathi R, Gaffney BL, Goswami B, Kung P-P, Rhee Y, Jin R, Jones RA J. Org. Chem. 1992;57:6954-6959.
9] Eritja R, Robles J, Avino A, Albericio F, Pedroso E Tetrahedron 1992;48:4171-4182.
10] Ferentz AE, Keating TA, Verdine GL J. Am. Chem. Soc. 1993;115:9006-9014.
11] Ramasamy K, Zounes M, Gonzalez C, Freier SM, Lesnik EA, Cummins LL, Griffey RH, Monia BP, Cook PD Tetrahedron Lett. 1994;35:215-218.
12] Tsarouhtsis D, Kuchimanchi S, DeCorte BL, Harris CM, Harris TM J. Am. Chem. Soc. 1995;117:11013-11014.
13] Eritja R, Acedo M, Avino A, Fabrega C Nucleosides & Nucleotides 1995;14:821-824.
14] Kim SJ, Jajoo HK, Kim H-Y, Zhou L, Horton P, Harris CM, Harris TM Bioorg. Med. Chem. 1995;3:811-822.
15] Schmid N, Behr J-P Tetrahedron Lett. 1995;36:1447-1450.
16] Avino A, Garcia RG, Marquez VE, Eritja R Bioorg. Med. Chem. Lett. 1995;5:2331-2336.
17] Kim SJ, Stone MP, Harris CM, Harris TM J. Am. Chem. Soc. 1992;114:5480-5481.
18] DeCorte BL, Tsarouhtsis D, Kuchimanchi S, Cooper MD, Horton P, Harris CM, Harris TM Chem. Res. Toxicol. 1996;9:630-637.
19] Diaz AR, Eritja R, Garcia RG Nucleosides & Nucleotides 1997;16:2035-2051.
20] Weber H, Khorana HG J. Mol. Biol. 1972;72:219-249.
21] Koster H, Kulikowski K, Liese T, Heikens W, Kohli V Tetrahedron Letters 1981;37:363-369.
22] Chaix C, Molko D, Teoule R Tetrahedron Letters 1989;30:71-74.
23] Sinha ND, Davis P, Usman N, Perez J, Hodge R, Kremsky J, Casale R biochimie 1993;75:13-23.
24] Allerson CR, Chen SL, Verdine GL J. Am. Chem. Soc. 1997;119:7423-7433.
25] Robins MJ, Basom GL Nucleic Acid Chemistry 1978;601-606.
26] Nair V, Richardson SG J. Org. Chem. 1980;45:3969-3974.
27] Gao X, Jones RA J. Am. Chem. Soc. 1987;109:1275-1278.
28] Acedo M, Fabrega C, Avino A, Goodman M, Fagan P, Wemmer D, Eritja R Nucleic Acids Res. 1994;22:2982-2989.
29] Ferentz AE, Verdine GL Nucleosides & Nucleotides 1992;11:1749-1763.

---

[2] Nucleoside standards for enzymatic hydrolysis: ***N*^{6}-(4-aminobutyl)deoxyadenosine** [29] was prepared by treatment of 6-chloropurine deoxyriboside with 2-fold excess of diaminobutane (DMSO, diisopropylethylamine, 60 °C, 5 h). ^{1}H NMR (DMSO-d_6 + D_2O, 45 °C) δ 8.25 (s, 1H), 8.16 (s, 1H), 6.34 (m, 1H), 4.41 (m, 1H), 3.89 (m, 1H), 3.58 (m, 4H), 2.87 (m, 2H), 2.71 (m, 1H), 2.28 (m, 1H), 1.63 (m, 2H), 1.45 (m, 2H). +FABMS: calcd. for $C_{14}H_{23}N_6O_3$ $[M+H]^+$ 323.1831; found 323.1828. **1,4-bis(deoxyadenosine-*N*^{6}-yl)butane** was prepared similarly except that the chloronucleoside was used in 2-fold excess. ^{1}H NMR (DMSO-d_6, 45 °C) δ 8.25 (s, 2H), 8.16 (s, 2H), 7.54 (t, J=6Hz, 2H), 6.33 (m, 2H), 5.11 (d, J=4Hz, 2H, 3'-OH), 4.98 (dd, J=6Hz and 5Hz, 2H, 5'-OH), 4.41 (m, 2H), 3.87 (m, 2H), 3.59 (m, 8H), 2.69 (m, 2H), 2.27 (m, 2H), 1.67 (m, 4H). +FABMS: calcd. for $C_{24}H_{33}N_{10}O_6$ $[M+H]^+$ 557.2584; found 557.2593.