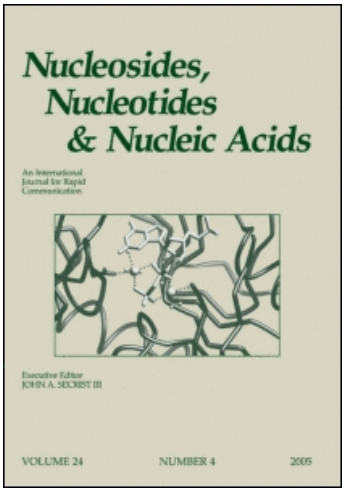

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Oligonucleotides Containing N^7-Cyanoborane-2'-deoxyguanosine

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To cite this Article Hasan, Ahmad and Shaw, Barbara Ramsay(1997) 'Oligonucleotides Containing N^7-Cyanoborane-2'-deoxyguanosine', Nucleosides, Nucleotides and Nucleic Acids, 16: 7, 1539 — 1542
To link to this Article: DOI: 10.1080/07328319708006224
URL: http://dx.doi.org/10.1080/07328319708006224

NUCLEOSIDES & NUCLEOTIDES, 16(7-9), 1539-1542 (1997)

OLIGONUCLEOTIDES CONTAINING N^7-CYANOBORANE-2'-DEOXYGUANOSINE

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ABSTRACT: For synthesis of N^7-cyanoborane-containing oligonucleotides, the 5'-DMT protecting group is not a suitable precursor because the boronated nucleoside is incompatible with DMT cations released during deprotection of the oligonucleotide. As an alternative to DMT, we have investigated use of the 5'-Fmoc protecting group. We found that the cyanoborane group is stable during synthesis and deprotection conditions used with Fmoc derivatives.

INTRODUCTION: Boronated-nucleic acids are a class of compounds that mimic natural and synthetic congeners in many ways. For example, a base-boronated nucleoside[1], wherein one of the endocyclic nitrogens is coordinated with the cyanoborane (BH_2CN) moiety as in N^7-cyanoborane-dG, resembles the alkylated N^7-methyl-2'-deoxynucleoside (Fig. 1). Furthermore, N^7-cyanoborane-dG also resembles the N-7-deazanucleoside, since both compounds preclude hydrogen bonding at position 7 of guanine. Sood et al. have reported that nucleosides containing the cyanoborane moiety at the endocyclic nitrogen base exhibit potent anti-tumor activity in mammalian cell lines, and anti-inflammatory and hypolipidemic activity in mice.[1-3]

The N^7-boronated-2'-deoxyguanosine (^{7b}dG) is of particular interest because, like the N^7-deaza analogue, it permits Watson-Crick base pairing but cannot undergo Hoogsteen-type base pairing as might be found in triplex DNA.[4] Further, ^{7b}dG is more stable at acidic pH to depurination than is normal dG, and is far more stable (decomposing much slower in water) than the corresponding N-alkylated derivatives.[5] More recently, we reported that the ^{7b}dG-5'-triphosphate is an excellent substrate for DNA polymerases including the thermostable Vent® and Taq® polymerases, and it is incorporated within an M13mp2 DNA duplex efficiently.[3,6] The unique properties exhibited by ^{7b}dG and the ability to enzymatically prepare long base-boronated DNA polymers of good stability[6,7] have led us to explore the chemical methods for the synthesis of monomers and oligomers

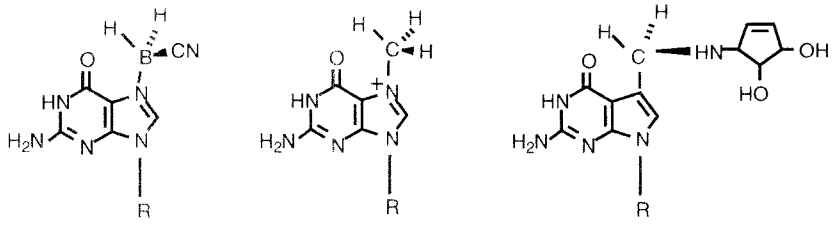
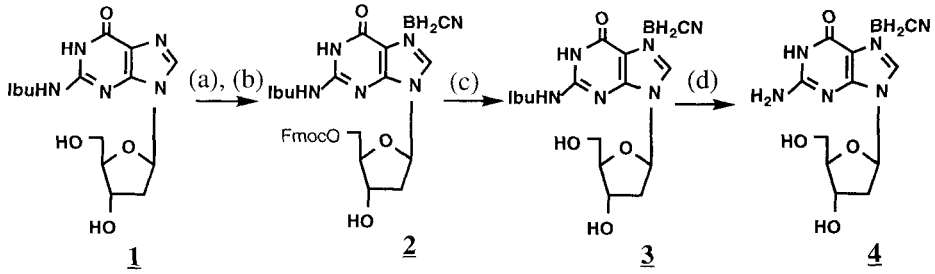


FIGURE 1

containing the cyanoborane moiety. In this report we describe studies on the compatibility of the cyanoborane moiety of N^2-Ibu-5'-Fmoc-^{7b}dG with oligonucleotide deprotection conditions.

RESULTS AND DISCUSSION

Chemistry: We found that the 5'-DMT cation released during deprotection from the 5'-DMT-dN monomer is incompatible with cyanoborane-nucleosides.[7] Therefore, we investigated the use of Fmoc instead of DMT as the 5'-protecting group for the synthesis of boronated-oligonucleotides.[8] In order to know whether the cyanoborane moiety is stable during the 5'-deprotection and whether 5'-Fmoc has selectivity over exocyclic amine deprotection conditions, we carried out a systematic investigation with compound **2** under conditions used in oligonucleotide synthesis. Compound **2**[8] (Scheme 1) was treated with 0.1 M DBU in acetonitrile and the deprotection reaction was monitored with TLC. A 30-second treatment of compound **2** resulted in selective cleavage of the 5'-Fmoc group, yielding compound **3**, without affecting the N^7-cyanoborane moiety. Moreover, hydrolysis of the Ibu group from the exocyclic amine was not observed, indicating that the 5'-Fmoc group exhibits selectivity with respect to Ibu. Furthermore, when compound **3** was treated with conc. ammonium hydroxide at room temperature there was no deboronation even after 15 h. At 50 °C a minor amount (~2%) of deboronation was observed after 15 h, but none was observed after 4 h when the Ibu was fully hydrolyzed. This showed that the 5'-Fmoc-protected nucleoside is a suitable precursor for the synthesis of base-boronated oligonucleotides.



(a), FmocCl, pyridine, 0°C; (b), Triphenylphosphine cyanoborane, THF, reflux; (c), 0.1 M DBU in acetonitrile, rt; (d), NH_4OH, rt, and/or 55 °C

SCHEME 1

EXPERIMENTAL PROCEDURES

General: All solvents, chemicals, and reagents were of analytical grade and used without further purification unless otherwise indicated. Baker analyzed silica gel (60-200 mesh) was used for flash column chromatography. Thin layer chromatography (TLC) was performed using 250 micron layers of silica gel GF precoated glass plates (Analtech, Inc.). Spots on the TLC plates were detected by visualization under short wave ultraviolet (UV) light by heating the chromatogram at 100 °C after spraying with 5% sulfuric acid in methanol.

5'-Fmoc-deprotection conditions for N^7-boronated-2'-deoxyguanosine

N^7-Cyanoboranyl-N^2-Ibu-2'-deoxyguanosine (**3**): A solution of 0.1 M DBU in acetonitrile (2 mL) was added to 5'-Fmoc-N^7-cyanoboranyl-N^2-Ibu-2'-deoxyguanosine[8] (**2**, 60 mg, 100 μmol). The TLC (solvent methanol:methylene chloride: 1:10 v/v) of the reaction mixture after 5 min showed complete conversion of the starting material to a more polar compound identical to the standard N^2-Ibu-N^7-deoxyguanosine. The solvent was removed under reduced pressure and the residue was adsorbed on silica gel and loaded on the column packed with silica gel. Elution of the column with 1-3% methanol in methylene chloride gave 29 mg (77% yield) of the pure compound.

N^2-Ibu-deprotection conditions for N^7-boronated-2'-deoxyguanosine

N^7-Cyanoboranyl-2'-deoxyguanosine (**4**)**:** Two reactions were carried out at the same time: (a) N^2-Ibu-dG (10 mg) and ammonium hydroxide (1 mL) were kept at room

temperature and another set was placed at 50°C. (b) Similarly, N^7-cyanoborane-dG (10 mg) was treated with ammonium hydroxide (1 mL) under identical conditions. TLC ($iPrOH:NH_4OH:H_2O$ 15:1:4) after 15 h showed that reaction (a) at room temperature hydrolyzed more than 80% and at 50°C complete hydrolysis was observed. Similarly, in the case of reaction (b) there were no changes after 4 h and 15 h at room temperature but after 15 h at 50°C we did notice minor (~2%) decomposition of cyanoborane.

CONCLUSION: DBU selectively removes the 5'-Fmoc group without deprotection of the nucleoside exocyclic amine and without affecting the cyanoborane moiety. The stability of cyanoborane under conditions effecting complete ammonia deprotection of the exocyclic amine has facilitated synthesis of base-boronated dinucleotides.[8]

ACKNOWLEDGMENT: This work was supported by grant NP-741 from the American Cancer Society to B.R.S.

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