Automated Solid-Phase Synthesis of Site-Specifically Platinated Oligodeoxyribonucleotides

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Cisplatin, *cis*-diamminedichloroplatinum(II), has been effectively used to treat testicular and other cancers.[1] DNA is a principal target for this drug,[2] which forms covalent adducts preferentially with the N7 atoms of purine bases. 1,2-Intrastrand Pt−N7(purine) cross-links comprise the major species.[3] Since these adducts are nested within naturally occurring variable DNA sequences, it is desirable to prepare similarly platinated oligonucleotides for studies in vitro. Up to now, oligonucleotides site-specifically modified with cisplatin have usually limited the purine content of the platinated strand to only the target nucleotides, in order to eliminate unwanted side products.[4] This requirement severely restricts investigations of the effects of sequence context on the properties of a given adduct. Here we report a protocol by which cisplatin can be introduced at any desired position into synthetic DNA by using existing automated solid phase techniques.

Solid phase DNA syntheses typically introduce nucleosides activated at their 3′-terminus.[5−7] We therefore attempted to platinate a protected deoxyguanosine 3′-phosphoramidite (**2**) with *cis*-$[Pt(NH_3)_2(DMF)Cl]NO_3$ (**1**) for use in an automated synthesizer. Analysis revealed initial formation of the desired monofunctional adduct at N7 of guanine, but within a few hours multiple signals in the H8 region of the ^{1}H NMR spectrum indicated decomposition.[8] An alternative strategy was therefore devised in which **1** was allowed to react with the triethylammonium salt of fully protected deoxyguanosine 3′-H-phosphonate (**3**, Scheme 1). The platinated monomer, Pt-dG H-phosphonate (**4**), thus obtained was sufficiently stable to be added to the 5′-OH of DNAs synthesized by conventional phosphoramidite methodology.

In a typical synthesis, DNA was prepared by using phosphoramidites on an automated synthesizer up to the nucleotide positioned directly to the 3′-side of the desired cisplatin adduct, which was added manually as the H-phosphonate (Scheme 1). Automated solid phase phosphoramidite synthesis was then continued, although it was necessary to modify certain procedures in order to accommodate the Pt−N7(dG H-phosphonate) moiety. The monofunctional adduct formed by using **4** was transformed into a 1,2-intrastrand d(GpG) cross-link involving an adjacent guanosine nucleoside base by incubation for 18 h at 37 °C in deionized water[9] prior to cleavage from the resin and deprotection with 12 M ammonium hydroxide and triethylamine (80 °C, 30 min).[10] Additional details are provided in the supporting information.

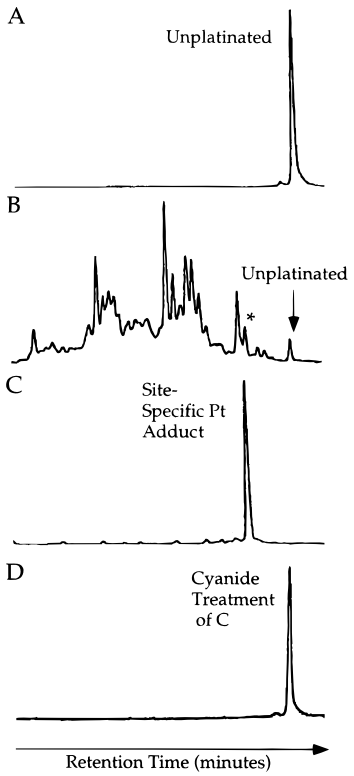

Figure 1. Ion-exchange high-pressure liquid chromatography (HPLC) traces of (A) unplatinated 20-mer with a retention time of 27.5 min and (B) reaction mixture of (A) and 1.0 equiv of **1a** in deionized water. The peak marked with an asterisk has an identical retention time to the major peak in (C). (C) Site-specifically platinated 20-mer (20-G^{Pt}) with a retention time of 26.0 min; lower molecular weight failure sequences were removed by filtration through a Centricon 3 concentrator prior to an injection. (D) treatment of 20-G^{Pt} with 0.3 M NaCN at pH 9 and 37 °C for 18 h. The retention time of the major peak in (D) is identical to that in (A), indicating that removal of platinum from 20-G^{Pt} leads to the formation of the control 20-mer. HPLC conditions are described in ref 11.

The validity of this procedure was tested for the synthetic 20-mer oligonucleotide, 5′-$T_1C_2A_3G_4G_5T_6A_7G_8$*G_9*$A_{10}C_{11}$-$T_{12}T_{13}G_{14}G_{15}T_{16}G_{17}T_{18}C_{19}T_{20}$-3′, where the asterisks indicate the target nucleotides for platination, designed to contain several unplatinated purine sites. As shown in Figure 1A,B, reaction of the unplatinated 20-mer with $[Pt(NH_3)_2(H_2O)Cl]NO_3$ (**1a**) afforded multiple peaks in the ion-exchange HPLC trace arising no doubt from different adducts formed with purine bases.[11] It was impossible to assign the individual peaks in this chromatogram to their corresponding platinum adducts, obviating purification of any desired 1,2-intrastrand cross-linked species. In contrast, a single major peak was observed (Figure 1C) when **4** was manually coupled to A_{10}, and automated coupling was resumed to complete the sequence to form the platinated 20-mer, 20-G^{Pt} (Scheme 1, 0.2 μmol). Treatment of 20-G^{Pt} with cyanide to remove platinum as $[Pt(CN)_4]^{2-}$ yielded material with a retention time identical to that of the unplatinated DNA (Figure 1D), consistent with formation of the desired 20-mer.

This platinated oligonucleotide was further characterized in the following manner. Atomic absorption spectroscopic analysis revealed the presence of 0.9 (± 0.1) Pt atoms per strand for

(1) Frei, E. *Cancer Res.* **1985**, *45*, 6523−6537.
(2) Lippard, S. J. In *The Robert Welch Foundation 37th Conference on Chemical Research*; Houston, Texas, 1993; pp 49−63.
(3) Sherman, S. E.; Lippard, S. J. *Chem. Rev.* **1987**, *87*, 1153−1181.
(4) See, for example: Takahara, P. M.; Rosenzweig, A. C.; Frederick, C. A.; Lippard, S. J. *Nature* **1995**, *377*, 649−652.
(5) Mackie, H.; Hogrefe, R. I. H-Phosphonate Chemistry-A Review. *Glen Research*, 1990.
(6) Beaucage, S. L.; Iyer, R. P. *Tetrahedron Lett.* **1993**, *49*, 6123−6194.
(7) *Oligonucleotide Synthesis-A Practical Approach*; Gait, M. J., Ed.; IRL Press: Oxford, 1984.
(8) Dunham, S. U.; Lippard, S. J. Unpublished results.
(9) Gonnet, F.; Kozelka, J.; Chottard, J.-C. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 1483−1484.
(10) Cruachem, User Manual Version 4.0, 1992.

(11) High-pressure liquid chromatography was conducted on a Waters 484 system equipped with a Dionex nucleopac PA-100 column (4 × 25 mm). Typically, an ion-exchange gradient from 75% 25 mM NH_4OAc in 10% aqueous CH_3CN (buffer A) and 25% 1 M NaCl, 25 mM NH_4OAc in 10% aqueous CH_3CN (buffer B) to 50% buffer A and 50% buffer B over 30 min at a flow rate of 1 mL/min was employed; the peaks were detected at 260 nm.

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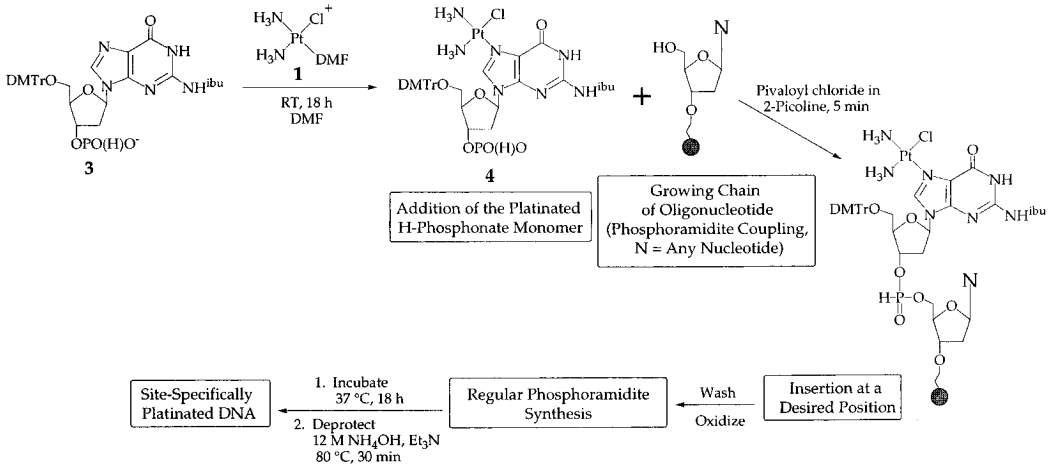
Scheme 1



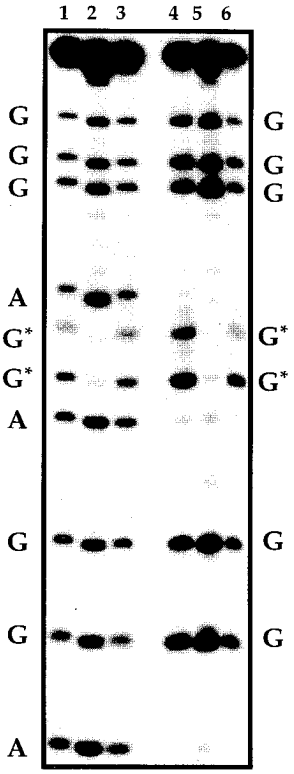


Figure 2. Maxam−Gilbert sequencing of 5′-end radiolabeled unplatinated 20-mer, site-specific Pt-adduct (20-G^{Pt}), and cyanide-reversed 20-G^{Pt}. Lane 1: formic acid (A + G) reaction of the unplatinated 20-mer showing cleavage sites at all purine residues. Lane 2: a similar reaction of 20-G^{Pt} after which bound platinum was removed from the fragments by treatment with 0.3 M NaCN at pH 9.0 and 37 °C before loading the gel. Lane 3: formic acid reaction as in Lane 1 but preceded by removal of Pt from 20-G^{Pt} through treatment with 0.3 M NaCN at pH 9 and 37 °C for 18 h. Lane 4: same as Lane 3 but using dimethyl sulfate (DMS) to reveal the G bases. Lane 5: same as Lane 4 but without the cyanide treatment. Lane 6: same as Lane 1 but using DMS rather than formic acid.

20-G^{Pt}, as expected for a single Pt−DNA adduct.[12] In addition, Maxam−Gilbert sequencing of the unplatinated 20-mer (control) and 20-G^{Pt} was undertaken to determine the nature and site of the platinum cross-link. Figure 2 shows the results of (A + G) and G sequencing reaction. Lanes 1 and 6 depict controls with the unplatinated 20-mer showing all purine and guanine residues, respectively. Lanes 2 and 5 display the 20-G^{Pt} cleavage products, clearly demonstrating the presence of platinum bound to N7 of the desired guanine bases, which blocks the protonation or methylation step required for sequencing. In lanes 3 and 4, the previously Pt-blocked guanine residues in 20-G^{Pt} were observed following deplatination (0.3 M NaCN, pH 9) prior to treatment with formic acid or dimethyl sulfate (see figure caption). These results demonstrate that the desired 1,2-intrastrand Pt cross-link was formed between the N7(G_8) and the N7(G_9) of the 20-mer. The position of the Pt adduct was controlled by the site-specific insertion of **4**, affording N7-platinated G_9 in the sequence despite the presence of other purine nucleosides in the strand. Subsequent closure to form N7-platinated G_8 yielded the desired intrastrand cross-link upon incubation as described in Scheme 1 (overall yield of purified product, 15−35%; quantity, 70 nmol, 0.5 mg). We caution that the protocol has not yet been fully optimized and that the isolated yields have been variable.

The methodology described here offers flexibility in designing different sequences and can be applied to existing automated synthesizers. The desired product is easily purified to homogeneity. Although in this first-generation synthesis the yields are modest, sufficient material can be obtained for a variety of studies in which it is of interest to examine the effects of sequence context on the properties of platinated DNA. Moreover, the procedure should be easily extended to prepare additional cisplatin adducts as well as oligodeoxyribonucleotides that require site-specific incorporation of a variety of other heavy metals.

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Supporting Information Available: Details of the syntheses of *cis*-[Pt(NH_3)$_2$(solv)Cl]NO_3 (**1**, solv = DMF; **1a**, solv = H_2O), synthesis and characterization of Pt-dG^{ibu} H-phosphonate (**4**), and coupling protocol insertion of **4** into the growing oligonucleotide chain (2 pages). Ordering information is given on any current masthead page.

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(12) Platinum atomic absorption was measured at 266 nm on a Perkin-Elmer atomic absorption spectrophotometer model AA-1495 equipped with a GTA-95 graphite tube atomizer; concentration of the oligonucleotides was measured on a Cary 1 spectrophotometer at 260 nm with an extinction coefficient of 191 000 M^{-1} cm^{-1}.