ChemComm

A novel approach to site-specifically platinated oligonucleotides applying combinations of nucleobase protecting groups

Robert J. Heetebrij, Reynier A. Tromp, Gijs A. van der Marel, Jacques H. van Boom and Jan Reedijk*

Leiden Institute of Chemistry, Gorlaeus Laboratories, Leiden University, PO Box 9502, 2300 RA Leiden, The Netherlands. E-mail: reedijk@chem.leidenuniv.nl

Received (in Cambridge, UK) 1st June 1999, Accepted 14th July 1999

Platination of a properly protected oligonucleotide followed by deprotection represents a new method for the synthesis of site-specific platinum-modified nucleic acids; N7 platination of 2′-deoxyguanosine can be circumvented by introducing a bulky protective group at the O6 position.

Since the discovery of the cytotoxic activity of cisplatin, *cis*-$[Pt(NH_3)_2Cl_2]$, much effort has been directed towards elucidating the mechanism of action of this drug. At present it is generally accepted[1] that intracellular damage occurs by coordination of cisplatin to DNA, causing a profound spatial disturbance and destabilisation of the double-helix leading to inhibition of DNA replication. It is well-established now that the intrastrand 1,2-GG (G = guanine) crosslink is the predominant platinum–DNA adduct. Moreover, other adducts such as 1,2-AG and 1,3-GXG adducts, as well as interstrand GG crosslinks, have also been observed.[2]

The molecular mechanistic aspects of platinum antitumour drugs are commonly investigated by elucidation of the 3D structures of platinum adducts of oligonucleotides.[3] In addition, selectively platinated oligonucleotides are required in fairly large amounts for applications in new therapeutic strategies like gene modulation *via* antisense and antigene methodologies.[4] The construction of selectively platinated DNA adducts by modification of an unprotected oligonucleotide with a platinum complex is often hampered by lack of selectivity of the platination reaction. Consequently the available range of DNA–Pt crosslinks to be studied is rather limited.

One approach to overcome these problems entails the use of platinum-bound mononucleotides[5] in the synthesis of platinum adducts by automated DNA synthesis following an H-phosphonate coupling approach. However, the recovery of site-specifically platinated oligonucleotides was rather disappointing. Recently, an automated DNA synthesis based on phosphoramidite chemistry of Ru^{II} and Os^{II} modified oligonucleotides was published.[6] This methodology leads to higher yields, but has been limited to coordinatively saturated complexes covalently linked to the nucleobase *via* polypyridine ligands.

Lippert *et al.*[7] showed that coordination of M^{II}(dien) (M = Pd,Pt) towards N6′,N6′,N9-trimethyladenine solely occurs at N3. The latter finding implies that platination at the N7 position of guanosine can be prevented by the introduction of a sterically demanding protective group at the O6 position of the guanine moiety. Consequently, it was to be expected that platination would be inhibited by N2,O6 protection, while N7 platination would be allowed in the case of N2 protection.

In order to substantiate the viability of the aforementioned hypothesis, we first examined the influence of the N2 protecting group on the course of the platination (Fig. 1). To this end we compared the traditionally used[8a] diphenylacetyl (DPA) group in **1a** with the more mildly removable[8b,c] di-*n*-butylaminomethylene (DNB) group in **1b**. For reasons of solubility the platination experiments were carried out in DMF-d_7–D_2O (80:20) mixtures and using [Pt(dien)Cl]Cl.† The reaction between [Pt(dien)Cl]Cl and **1a** and **1b** at 37 °C, monitored by ^{1}H NMR (see Fig. 2), clearly reveals that N7 platination occurs. Surprisingly the 2-*N*-DNB protected guanine derivative **1b** proved to be roughly two-fold more reactive towards $[Pt(dien)]^{2+}$ than the 2-*N*-DPA protected derivative **1a**. The latter observation might be explained by electronic effects of the amidine group in **1b**, as well as by stacking of the diphenyl group in **1a**, thereby reducing the accessibility of N7. A detailed study on these effects is currently being performed.

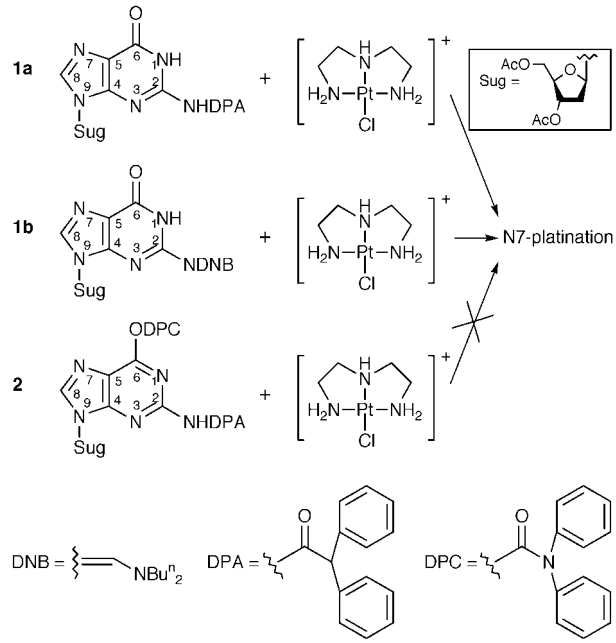


Fig. 1 Structures of 2′-deoxy-3′,5′-*O*-acetylated guanosine model compounds differing in base-protection and their (absence of) reactivity towards [Pt(dien)Cl]Cl.

The diphenylcarbamoyl (DPC) group[9] was selected to protect O6 of the guanine base giving, in combination with a diphenylacetyl (DPA) group at the N2 position, compound **2**. It can be seen in Fig. 2 that N7 platination of the N2,O6 protected derivative **2** is completely prevented.

Additional evidence for retardation of N7 platination was found by comparing the reactivity of *trans*-$[Pt(NH_3)_2Cl(DMF\text{-}d_7)]BF_4$ towards 2-*N*-(isobutyryl)-2′-deoxyguanosine (2-*N*-Bu^i-dG)‡ and 6-*O*-(diphenylcarbamoyl)-2-*N*-(diphenylacetyl)-2'-deoxyguanosine (6-*O*-DPC-2-*N*-DPA-dG). At room

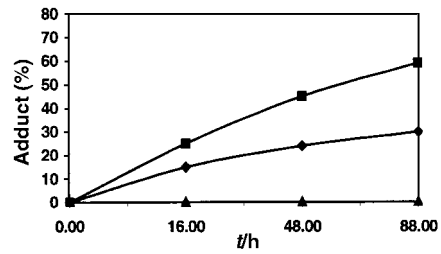
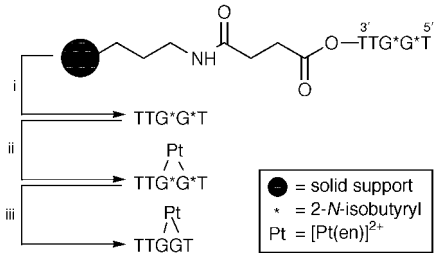


Fig. 2 Observed product formation during the reaction between [Pt(dien)Cl]Cl and (◆) **1a**, (■) **1b** and (▲) **2** in a 1:1 ratio at 4 mM concentration in DMF-d_7–D_2O (8:2 v/v) at 37 °C.



Scheme 1 *Reagents and conditions* i, NH_3–H_2O, (room temp., 15 min; ii, $[Pt(en)Cl(H_2O)]NO_3$, pH 6.0, 50 °C, 16 h; iii, NH_3–H_2O, 50 °C, 16 h.

temperature in DMF-d_7 with a slight excess of the platinum species, platination of 2-*N*-Bu^i-dG at N7, as gauged by ^{1}H NMR, went to completion within 1 h. No significant amount of platinated 6-*O*-DPC-2-*N*-DPA-dG could be observed under the same conditions. In the reaction of a 1 : 1 mixture of 2-*N*-Bu^i-dG and 6-*O*-DPC-2-*N*-DPA-dG with 1.1 equiv. of *trans*-$[Pt(NH_3)_2Cl(DMF\text{-}d_7)]BF_4$, only the downfield shifted H8 and H1′ resonances of the 2-*N*-Bu^i-dG derivative are observed, implying selective N7 platination for the 2-*N*-protected guanosine derivative in this competitive system.

The additional role of the electronic and/or steric effect of the protecting group at the exocyclic amine upon the reaction kinetics of N7 platination would allow a synergistic effect in our approach of protecting group directed selectivity enhancement.

Implementation of the latter results in the synthesis of selectively platinated oligonucleotides requires an effective platination of a N2-protected oligonucleotide. Therefore, the fully protected and immobilised pentamer d(TTGGT), having 2-*N*-Bu^i protection of the guanine bases and a β–cyanoethyl protection of the phosphate, which lacks strong competing nucleobase binding sites besides the 1,2-GG motif, was selected as a test system for maximisation of the product yield. The protected pentamer was obtained *via* routine automated DNA synthesis using controlled-pore glass (CPG) as the solid support. The platination of this immobilised pentamer by $[Pt(en)]^{2+}$ to form the 1,2-GG adduct was investigated. Platination experiments were performed under various conditions. Anion-exchange analysis on the platinated and deprotected sequence reveals that only 50–60% platinated pentanucleotide was obtained in this heterogeneous $[Pt(en)]^{2+}$/oligo-support system (data not shown). To increase the yield of platinated oligonucleotide, an alternative approach was followed (see Scheme 1) in which the oligonucleotide was cleaved from the support leaving the isobutyryl groups at the N2 position of the guanines intact.§ Subsequent platination of the base-protected pentamer by $[Pt(en)Cl(H_2O)]^+$, followed by removal of 2-*N*-Bu^i groups by aminolysis, provided a platination efficiency which was nearly quantitative. This can be attributed to both the homogeneity of this system, and loss of β-cyanoethyl phosphate protection (by the ammonia treatment) affording a negatively-charged backbone, thus allowing nucleophilic attack by the N7 and also electrostatic interaction between Pt and the target, as well as an increased hydrophilicity. Moreover, the observed stability of the [Pt(en){d(TTGGT)-N7(3),N7(4)}] adduct under prolonged ammonia exposure allows the use of more base-stable protecting groups like DPA and Bu^i at N2 within this, protective-group forced, platination strategy.

In summary, a promising new synthetic strategy utilising G building blocks with O6 protecting groups to prevent platination and G building blocks bearing amidine and acyl type N2 protection at the predetermined site for the bifunctional Pt-lesion has been presented. The general applicability of our approach to the synthesis of site-specifically platinated oligonucleotides is currently under investigation.

The authors wish to thank Professor Dr B. Lippert, Fachbereich Chemie Universität Dortmund, Germany, for scientific contributions and helpful discussions on the manuscript. This work was performed in the frame of the BIOMED project BMHA-CT97-2485. The authors wish to thank Johnson & Matthey (Reading, UK) for their generous loan of K_2PtCl_4.

Notes and references

† [Pt(dien)Cl]Cl, $[Pt(en)Cl_2]$ and *trans*-$[Pt(NH_3)_2Cl_2]$ were synthesized according to literature procedures, (ref. 10); the latter two were converted into the reactive species $[Pt(en)Cl(DMF)]NO_3$ and *trans*-$[Pt(NH_3)_2Cl(DMF\text{-}d_7)]BF_4$ by treatment with 1 equiv. of $AgNO_3$ or $AgBF_4$, respectively, in DMF and DMF-d_7. The protected mononucleosides **1a** and **1b** and 2-*N*-(isobutyryl)-2′-deoxyguanosine were synthesized according to procedures adapted from the literature (ref. 8), 6-*O*-protected nucleoside **2** was synthesized by a procedure analogous to Robbins *et al.* (ref. 9). ^{1}H and ^{195}Pt NMR measurements were performed on a Bruker WM 300 spectrometer. Internal calibration was based on the methyl groups of DMF in the case of ^{1}H NMR measurements and externally on K_2PtCl_6 for ^{195}Pt NMR measurements. Platination reactions of the protected mononucleosides and [Pt(dien)Cl]Cl were performed at a 4 mM concentration of both reactants in a DMF-d_7–D_2O mixture (80 : 20 v/v) at 37 °C. Calculations were performed by relative integration (estimated error 10%) of H8 and H1′ proton signals of both reaction products and starting materials during the reaction.

‡ Reaction of 2-*N*-(isobutyryl)-2′-deoxyguanosine at 4 mM concentration with 1.1 equiv. of *trans*-$[Pt(NH_3)_2Cl(DMF\text{-}d_7)]BF_4$ in DMF-d_7 showed a downfield shift of the H8 proton from δ 8.29 to δ 9.01 and from δ 6.30 to δ 6.38 for the H1′ proton.

§ The model oligonucleotide d(TTGGT) was assembled using an automated Gene Assembler (Pharmacia). Stepwise elongation of thymidine, immobilized by a 3′-*O*-succinyl bond to controlled pore glass (CPG), was carried out using standard synthesis protocols. The immobilised pentamer was washed thoroughly with MeCN and dried *in vacuo*. Cleavage conditions, for removal of the protected oligonucleotide from CPG matrix (*i.e.* hydrolysis of the succinyl linker) by treatment with NH_3–H_2O (25%) at room temperature, were established by analytical reversed phase HPLC (Lichrosphere® C_{18} column, starting buffer of 5% MeCN in TEAA (pH 7), 100 mM, to 75% MeCN in Et_4NOAc buffer). Deprotected species started to appear upon exposure times exceeding 20 min. For platination experiments a 1 mM stock solution of protected pentamer in H_2O was prepared and used immediately in reactions with $[Pt(en)Cl(H_2O)]NO_3$ (0.5 mM at pH 6.0). After reaction was allowed to proceed for 16 h at 50 °C, excess NH_3–H_2O (25%) was added and deprotection performed for 16 h at 50 °C, the reaction mixture was concentrated *in vacuo* and redissolved for analysis by anion exchange FPLC (APB MonoQ® HR 5/5 column, gradient from starting eluent 0.01 M NaOH to 1.2 M NaCl in 0.01 M NaOH).

1 J. Reedijk, *Chem. Commun.*, 1996, 801.
2 A. M. J. Fichtinger-Schepman, J. L. Van der Veer, J. H. J. Den Hartog, P. H. M. Lohman and J. Reedijk, *Biochemistry*, 1985, **24**, 707.
3 P. M. Takahara, A. C. Rosenzweig, C. A. Frederik and S. J. Lippard, *Nature*, 1995, **377**, 649; D. Yang, S. S. G. E. van Boom, J. Reedijk, J. H. van Boom and A. H.-J. Wang, *Biochemistry*, 1995, **34**, 12 912; A. Gelasco and S. J. Lippard, *Biochemistry*, 1998, **37**, 9230; J. M. Teuben, C. Bauer, A. J.-H. Wang and J. Reedijk, *Biochemistry*, in the press.
4 R. Dalbiès, D. Payet and M. Leng, *Proc. Natl. Acad. Sci. U.S.A.*, 1994, **91**, 8147; C. Colombier, B. Lippert and M. Leng, *Nucleic Acids Res.*, 1996, **24**, 4519; E. Bernal-Méndez, J.-S. Sun, F. Gonzalez-Vilchez and M. Leng, *New. J. Chem.*, 1998, 1479.
5 J. Schliepe, U. Berghoff, B. Lippert and D. Cech, *Angew. Chem., Int. Ed. Engl.*, 1996, **35**, 646; R. Manchanda, S. U. Dunham and S. J. Lippard, *J. Am. Chem. Soc.*, 1996, **118**, 5144; U. Berghoff, K. Schmidt, M. Janik, G. Schröder and B. Lippert, *Inorg. Chim. Acta*, 1998, **269**, 135.
6 D. J. Hurley and Y. Tor, *J. Am. Chem. Soc.*, 1998, **120**, 2194; S. I. Khan, A. E. Beilstein and M. W. Grinstaff, *Inorg. Chem.*, 1999, **38**, 418.
7 C. Meiser, B. Song, E. Freisinger, M. Peilert, H. Sigel and B. Lippert, *Chem. Eur. J.*, 1997, **3**, 388.
8 For some examples of synthetic approaches to the 2-*N*-protected 2′-deoxyguanosines, see: (*a*) C. H. M. Verdegaal, P. L. Jansse, J. F. M. de Rooij, G. A. van der Marel and J. H. van Boom, *Recl. Trav. Chim. Pays-Bas*, 1981, **100**, 200; (*b*) L. J. McBride, R. Kierzek, S. L. Beaucage and M. H. Carruthers, *J. Am. Chem. Soc.*, 1986, **108**, 2040; (*c*) B. C. Froehler and M. D. Matteucci, *Nucleic Acids Res.*, 1983, **11**, 8031.
9 R. Zou and M. J. Robbins, *Can. J. Chem.*, 1987, **65**, 1436; M. J. Robbins, R. Zou, Z. Guo and S. F. Wnuk, *J. Org. Chem.*, 1996, **61**, 9207.
10 G. W. Watt and W. A. Cude, *Inorg. Chem.*, 1968, **7**, 335; A. Pasini, C. Caldirola, S. Spinelli and M. Valsecchi, *Synth. React. Inorg. Met.-Org.Chem.*, 1993, **23**, 1021; G. B. Kauffman and D. O. Cowan, *Inorg. Synth.*, 1963, **7**, 239.

Communication 9/04365I