Synthesis and Optical Properties of the C-8 Adduct of Benzo[*a*]pyrene and Deoxyguanosine

Linda Valis, Hans-Achim Wagenknecht*

Chemistry Department, Technical University of Munich, Lichtenbergstr. 4, 85747 Garching, Germany
Fax +49(89)28913210; E-mail: Wagenknecht@ch.tum.de

Received 10 June 2005

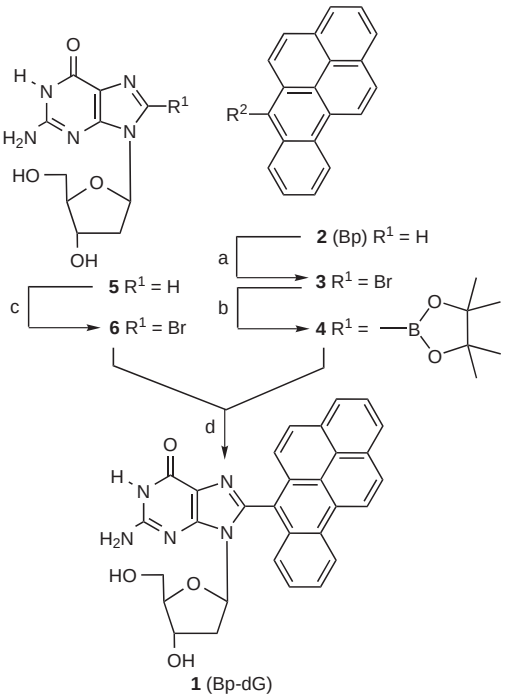
Abstract: 8-(Benzo[*a*]pyren-6-yl)-2′-deoxyguanosine (Bp-dG) was prepared via a palladium-catalyzed Suzuki–Miyaura-type cross-coupling reaction from the pinacol ester of 6-benzo[*a*]pyrenyl boronic acid and the corresponding brominated deoxyguanosine precursor. The absorption and steady-state fluorescence properties of Bp-dG were characterized and compared with that of 6-benzo[*a*]pyrene. The modified nucleoside Bp-dG exhibits an unexpected high stability towards nucleosidic hydrolysis even under irradiation with UV light.

Key words: DNA damage, nucleoside, cross-coupling, palladium, pyrene

Among the polycyclic aromatic hydrocarbons (PAH), benzo[*a*]pyrene (Bp) is one of the most potent with respect to the formation of carcinogenic chemical induced DNA damages.[1] The first critical step in the multistage process leading to tumor development[2] is the formation of covalent adducts between Bp and DNA. The preceeding metabolic activation of Bp can occur by two major pathways:[3] (i) mono-oxygenation to produce diol epoxides of Bp and (ii) one-electron oxidation to yield the Bp radical cation. Both products represent electrophilic species with a high reactivity towards DNA bases yielding covalent adducts preferably with the electron-rich purines. In case of guanosine and deoxyguanosine (dG), Bp adducts have been identified at the nitrogens, mainly in positions 2 and 7, or at the carbon atoms, mainly in position 8.[4] For the identification of these adducts in model experiments, Bp was oxidized by electrochemical methods or by horseradish peroxidase-catalyzed reactions.[5]

It was shown that the tumor initiation of adducts between Bp and dG correlates with the level of their depurinating properties.[6] The C-8 adduct of Bp and dG, 8-(6-benzo[*a*]pyrenyl)-2′-deoxyguanosine (**1**), is of special interest for the understanding of carcinogenesis since it seems to exhibit a high tendency for nucleosidic hydrolysis.[5,6] This chemical-induced depurination of **1** in duplex DNA yields an abasic site that represents another major mutagenic lesion in DNA.[7] In order to study the mutagenic and carcinogenic properties of the C-8 adduct **1** in DNA on a molecular level, it is necessary to work out a preparative route by methods of organic synthesis, which allows the later incorporation into oligonucleotides via automated phosphoramidite chemistry. In this preliminary communication, we want to present the chemical synthesis of **1**, the characterization of its optical properties by absorption and fluorescence spectroscopy, and preliminary experiments about its tendency for nucleosidic hydrolysis.

Recently, we[8] and others[3,9–13] have developed methods for the Pd-catalyzed C–C or C–N bond formation of chromophore-modified nucleosides via Suzuki–Miyaura-type or Buchwald-type reactions, respectively. Our approach to synthesize the pyrene-modified nucleosides was to apply the palladium-catalyzed Suzuki–Miyaura-type cross coupling of 1-pyrenyl boronic acid to the corresponding halogenated nucleosides.[8] In general, Suzuki–Miyaura-type couplings can be conducted easily because they work in moist or even aqueous solutions, and they tolerate the presence of unprotected functional groups. Many boronic acids are commercially available and inexpensive, and many of them are non-toxic.



Scheme 1 Synthesis of the Bp-dG adduct **1**. *Reagents and conditions*: a) NBS (1.0 equiv), CCl_4, reflux, 3 h, 86%; b) 4,4,5,5-tetramethyl-1,3,2-dioxaborolan (3.1 equiv), Et_3N (3.1 equiv), $PdCl_2dppf$ (0.03 equiv), dioxane, 100 °C, 28 h, 25%; c) NBS (1.1 equiv), H_2O, r.t., 2 h, 85%; d) NaOH (20 equiv), $Pd(PPh_3)_4$ (0.1 equiv), THF–H_2O–MeOH = 2:1:2, reflux, 24 h, 25%.

SYNLETT 2005, No. 15, pp 2281–2284
Advanced online publication: 03.08.2005
DOI: 10.1055/s-2005-872262; Art ID: G20405ST

The synthesis of Bp-dG (**1**, Scheme 1) started with the bromination of Bp (**2**) using NBS according to the literature.[14] In analogy to our previously reported synthesis of pyrene-modified nucleosides[8] we attempted to convert the 6-bromobenz[*a*]pyrene (**3**) to the corresponding boronic acid by lithiation using *n*-BuLi, treatment with trimethylborate and subsequent acidic work-up. Unfortunately, it was impossible to isolate the desired benz[*a*]pyren-6-yl boronic acid using this way. Alternatively, we coupled 4,4,5,5-tetramethyl-1,3,2-dioxaborolan to **3** in analogy to described procedures[15] and obtained the pinacol ester **4** of the benz[*a*]pyren-6-yl boronic acid in moderate yield (25–30%).[16] For this coupling reaction, it was optimal to apply $PdCl_2$dppf as the catalyst together with Et_3N in dioxane. Compound **4** was characterized by NMR spectroscopy and identified by high-resolution EI mass spectrometry.[16] Bromination of 2′-deoxyguanosine (**5**) using NBS in H_2O gave 8-bromo-2′-deoxyguanosine (**6**) in good yield (85%).[17] Subsequently, **4** and **6** have been coupled in a Suzuki–Miyaura-type reaction. We applied our optimized conditions for this reaction including $Pd(PPh_3)_4$ as the catalyst, and NaOH (20 equiv) as the base, in THF–MeOH–H_2O = 2:1:2 as the solvent mixture.[8] In contrast to the good yield for the preparation of 8-(pyren-1-yl)-2′-deoxyguanosine,[8] it was not possible to obtain a yield of Bp-dG (**1**), which was higher than 25%. The Bp-dG adduct **1** was characterized by NMR spectroscopy and identified by FTICR-ESI mass spectrometry.[18]

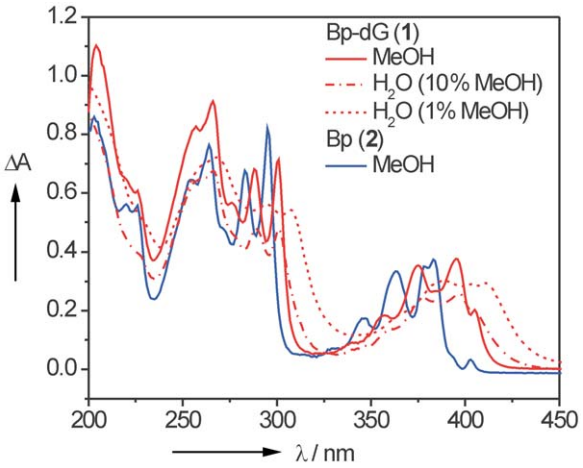


Figure 1 Absorption spectra of Bp-dG (**1**) in MeOH and MeOH–H_2O in comparison to Bp (**2**) in MeOH.

Subsequently, we characterized the electronic properties of the Bp-dG adduct **1** by methods of the optical spectroscopy.[19] Compared to the unmodified Bp (**2**) in MeOH, the absorption spectra of **1** show a significant red-shift in the range between 330 nm and 420 nm which clearly shows the electronic influence of the guanine moiety on the benz[*a*]pyrene chromophore (Figure 1). We additionally measured the absorption spectra in H_2O to come closer to physiological conditions; for solubility reasons at least 1% MeOH was necessary for these measurements. The corresponding spectra show an additional red-shift compared to that of **1** in MeOH and the absorption bands are slightly broader in H_2O. Based on our previous experiments with pyrenyl-modified DNA it is important to point out that the electronic behavior of these chromophore-modified nucleosides in DNA is much more similar to MeOH than to aqueous solutions.[20]

For the interpretation of the fluorescence spectra of **1** compared to the well-known fluorescence properties of Bp (**2**), it is important to note that in the nucleoside **1** the chromophore is linked covalently to the nucleoside by a single C–C bond yielding a strong electronic coupling between them. This can be clearly seen from the emission of the Bp-dG adduct **1** compared to that of Bp (**2**, Figure 2). The emission profile of Bp-dG (**1**) shows a broad band that does not exhibit the fine structure as it is the case for the unmodified Bp (**2**).

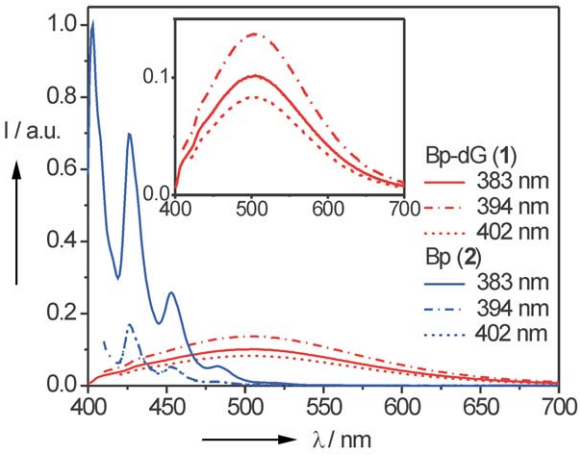


Figure 2 Fluorescence spectra of Bp-dG (**1**) in comparison to Bp (**2**) in MeOH, at the three different excitation wavelengths.

These results clearly show that the C-8 adduct **1** between Bp and dG must be interpreted as a single chromophore that consists of two aromatic systems strongly interacting which each other electronically. Excitation of Bp-dG (**1**) leads to a photoinduced intramolecular exciplex containing both excited state (Bp-dG)* and charge separated state Bp^{-}-dG^{+} and exhibiting intense, unstructured fluorescence bands with solvent-dependent maxima.[20,21] The charge transfer assignment has been shown previously by picosecond transient absorption measurements using benzo[*a*]pyren-6-y1-2′-deoxyguanosine conjugates which are electronically very similar to **1**.[22] Additionally, it can be rationalized by the thermodynamic situation including the potentials E_{00} = 3.25 eV for Bp*, $E_0(Bp/Bp^{\bullet -})$ = –1.65 V vs. NHE[23] and $E_0(dG^{\bullet +}/dG)$ = 1.3 V[24] that yield a driving force of ca. –0.3 eV. This assignment indicates that Bp-dG potentially has an additional mutagenic and carcinogenic effect in DNA since photo-induced electron hole transport may cause oxidative damage at nearby or even distant guanines.

The electronic situation in Bp-dG (**1**) could give reason for its high depurinating tendency especially in combination with exposure to light. Hence, we investigated the

stability of the nucleosidic bond in **1** under irradiation with a Xe lamp (75 W) together with a cut-off filter (>305 nm). The nucleosidic hydrolysis was analyzed by HPLC-MS (ESI). As a control reaction complete depurination of **1** was observed after addition of 1% trifluoroacetic acid in MeOH within several minutes. In more detailed experiments, MeOH–H_2O = 1:1 mixtures were used at four different pH values (0.5 M citrate buffer, adjusted to pH 5.0, 4.0, 3.5 and 2.5). Remarkably, at pH values above 2.5 we could not observe any nucleosidic hydrolysis of **1** in these samples, not in the dark (up to 20 h) and not under irradiation (up to 5 h). Only at pH 2.5 about 30% depurination (after 30 h) occurred in the sample; this reaction was not light-dependent.

In conclusion, the modified nucleoside Bp-dG exhibits an unexpected high stability towards nucleosidic hydrolysis that occurs only at low pH values and seems not to be supported by the exposure to light. Thus, the Bp-dG adduct **1** needs further investigation inside the DNA helix. Therefore, we currently incoporate Bp-dG (**1**) into oligonucleotides via automated phosphoramidite chemistry in order to explore the potentially carcinogenic photoinduced processes in duplex DNA.

Acknowledgment

We are grateful to Dr. Roderich Süßmuth (University of Tübingen) who measured the HRMS of **1**. We thank Andreas Enthart (Technical University of Munich) for performing the HSQC experiments. This work was supported by the Deutsche Forschungsgemeinschaft, the Volkswagen Foundation and by the Fonds der Chemischen Industrie. We are grateful to Professor Horst Kessler, Technical University of Munich, for the generous support.

References

(1) Luch, A. *Chem. Unserer Zeit* **2001**, *35*, 294.
(2) Lowe, S. W.; Lin, A. W. *Carcinogenesis* **2000**, *21*, 485.
(3) Lakshman, M. K.; Ngassa, F. N.; Bae, S.; Buchanan, D. G.; Hahn, H.-G.; Mah, H. *J. Org. Chem.* **2003**, *68*, 6020.
(4) Rama Krishna, N. V. S.; Fao, G.; Padmavathi, N. S.; Cavalieri, E. L.; Rogan, E. G.; Cerny, R. L.; Gross, M. L. *Chem. Res. Toxicol.* **1992**, *5*, 293.
(5) (a) Rogan, E. G.; Cavalieri, E. L.; Tibbels, S. R.; Cremonesi, P.; Warner, C. D.; Nagel, D. L.; Tomer, K. B.; Cerny, R. L.; Gross, M. L. *J. Am. Chem. Soc.* **1988**, *110*, 4023. (b) Cremonesi, P.; Cavalieri, E. L.; Rogan, E. G. *J. Org. Chem.* **1989**, *54*, 3561.
(6) (a) Chen, L.; Devanesan, P. D.; Higginbotham, S.; Ariese, F.; Jankowiak, R.; Small, G. J.; Rogan, E. G.; Cavalieri, E. L. *Chem. Res. Toxicol.* **1996**, *9*, 987. (b) Devanesan, P. D.; Higginbotham, S.; Ariese, F.; Jankowiak, R.; Suh, M.; Small, G. J.; Cavalieri, E. L.; Rogan, E. G. *Chem. Res. Toxicol.* **1996**, *9*, 1113.
(7) Lhomme, J.; Constant, J.-F.; Demeunynck, M. *Biopolymers* **1999**, *52*, 65.
(8) (a) Amann, N.; Wagenknecht, H.-A. *Synlett* **2002**, 687. (b) Mayer, E.; Valis, L.; Huber, R.; Amann, N.; Wagenknecht, H.-A. *Synthesis* **2003**, 2335.
(9) See reviews: (a) Lakshman, M. K. *J. Organomet. Chem.* **2002**, *653*, 234. (b) Agrofoglio, L. A.; Gillaizeau, I.; Saito, Y. *Chem. Rev.* **2003**, *103*, 1875. (c) Hocek, M. *Eur. J. Org. Chem.* **2003**, 245.
(10) (a) Lakshman, M. K.; Hilmer, J. H.; Martin, J. Q.; Keeler, J. C.; Dinh, Y. Q. V.; Ngassa, F. N.; Russon, L. M. *J. Am. Chem. Soc.* **2001**, *123*, 7779. (b) Lakshman, M. K.; Gunda, P. *Org. Lett.* **2003**, *5*, 39.
(11) Western, E. C.; Daft, J. R.; Johnson, E. M. II; Gannett, P. M.; Shaugnessy, K. H. *J. Org. Chem.* **2003**, *68*, 6767.
(12) Havelková, M.; Dvorák, D.; Hocek, M. *Synthesis* **2001**, 1704.
(13) Schoffers, E.; Olsen, P. D.; Means, J. C. *Org. Lett.* **2001**, *3*, 4221.
(14) The crude product was purified by column chromatography on silica gel(hexane) and recrystallized from acetone yielding a yellow solid (86%). R_f = 0.23 (hexane). All spectroscopic data of **3** were in agreement with the published data: (a) Dewhurst, F.; Kitchen, D. A. *J. Chem. Soc., Perkin Trans. 1* **1972**, 710. (b) Cho, B. P.; Harvey, R. G. *J. Org. Chem.* **1987**, *52*, 5668.
(15) (a) Muramata, M. *J. Org. Chem.* **2000**, *65*, 164. (b) Krämer, C. S.; Zimmermann, T. J.; Sailer, M.; Müller, T. J. J. *Synthesis* **2002**, 1163.
(16) The crude product was purified by column chromatography on silica gel (hexane–toluene = 1:1) yielding a yellow solid (25%). R_f = 0.23 (hexane–toluene = 1:1). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 9.08 (m, 2 H), 8.67 (m, 1 H), 8.44 (d, *J* = 9.0 Hz, 1 H), 8.35 (d, *J* = 9.0 Hz, 1 H), 8.24 (d, *J* = 7.5 Hz, 1 H), 8.10 (d, *J* = 7.5 Hz, 1 H), 7.97 (m, 2 H), 7.81 (m, 2 H), 1.66 (s, 12 H). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 135.17, 134.82, 131.72, 131.58, 129.48, 129.14, 128.80, 128.65, 128.49, 128.16, 126.55, 126.36, 126.11, 126.0, 125.76, 125.42, 123.62, 123.57, 122.59, 85.03, 25.68. HRMS (EI): *m/z* calcd for $C_{26}H_{23}O_2B$: 378.17911; found: 378.17896.
(17) All spectroscopic data of **6** were in agreement with the published data: Gannett, P.; Sura, T. P. *Synth. Commun.* **1993**, *23*, 1611.
(18) The crude product was purified by column chromatography on silica gel (CH_2Cl_2–acetone = 4:1, then EtOAc–MeOH = 10:1, then EtOAc–MeOH–H_2O = 10:1:0.5) yielding a yellow solid (25%). Analytical HPLC (RP-18 column, gradient A:B = 10:90 to 90:10 over 45 min, A = MeCN, B = H_2O) was performed to ensure the purity of **1** of >99.5%. R_f = 0.40 (EtOAc–MeOH–H_2O = 10:1:0.5). NMR signals were assigned based on 2D NMR measurements (HSQC). ^{1}H NMR (500 MHz, DMSO-d_6): δ = 10.90 (br s, 1 H, NH), 9.34 (m, 2 H, H-10, H-11), 8.57 (d, *J* = 9.2 Hz, 1 H, H-12), 8.45 (d, *J* = 7.9 Hz, 1 H, H-1), 8.27 (d, *J* = 7.4 Hz, 1 H, H-3), 8.08 (m, 2 H, H-2, H-4), 7.91 (m, 1 H, H-9), 7.83 (m, 1.5 H, H-8, H-7), 7.61 (d, *J* = 8.3 Hz, 0.5 H, H-7), 7.55 (d, *J* = 9.5 Hz, 0.5 H, H-5), 7.40 (d, *J* = 9.5 Hz, 0.5 H, H-5), 6.53 (br s, 2 H, NH_2), 5.47 (m, 1 H, H-1′), 4.36 (m, 1 H, H-3′), 3.76 (m, 1 H, H-4′), 3.58 (m, 1 H, H-5′), 3.46 (m, 1 H, H-5′), 2.81 (m, 1 H, H-2′), 1.70 (m, 1 H, H-2′). ^{13}C NMR (125 MHz, DMSO-d_6): δ = 157.49, 156.30, 154.27, 132.58 (C-7), 130.02 (C-12), 130.33 and 127.83 (C-4 and C-2), 128.05, 127.75 (C-1), 127.08 (C-3), 127.66 (C-9), 126.05, 125.93 (C-5), 123.43 and 124.94 (C-10 and C-11), 121.35, 88.80 (C-4′), 85.97 (C-1′), 71.93 (C-3′), 62.94 (C-5′), 37.39 (C-2′). HRMS (ESI/FTICR): *m/z* calcd for $C_{30}H_{23}N_5O_4$ [M^+ + H]: 518.18228; found: 518.18207.
(19) Steady-state fluorescence spectroscopy was performed at r.t. on a Spex Fluoromax III spectrometer. The emission spectra are corrected according to detection system variation with wavelength. UV/Vis absorbance spectroscopy was performed at r.t. on a Varian Cary 100 photometer. Dry solvents (Fluka puriss. over molecular sieve, H_2O <0.01%) were used for the measurements.

(20) (a) Fiebig, T.; Wagenknecht, H.-A. In *Charge Transfer: From Mechanism to Applications*; Wiley-VCH: Weinheim, **2005**, 195–223. (b) Trifonov, A.; Buchvarov, I.; Wagenknecht, H.-A.; Fiebig, T. *Chem. Phys. Lett.* **2005**, *409*, 277.

(21) (a) See review: Grabowksi, Z. R.; Rotkiewicz, K.; Rettig, W. *Chem. Rev.* **2003**, *103*, 3899. (b) For an example, see: Fiebig, T.; Stock, K.; Lochbrunner, S.; Riedle, E. *Chem. Phys. Lett.* **2001**, *345*, 81.

(22) (a) O'Connor, D.; Shafirovich, V. Y.; Geacintov, N. E. *J. Phys. Chem.* **1994**, *98*, 9831. (b) Shafirovich, V. Y.; Courtney, S. H.; Ya, N.; Geacintov, N. E. *J. Am. Chem. Soc.* **1995**, *117*, 4920.

(23) Kubota, T.; Kano, J.; Uno, B.; Konse, T. *Bull. Chem. Soc. Jpn.* **1987**, *60*, 3865.

(24) Steenken, S.; Jovanovic, S. V. *J. Am. Chem. Soc.* **1997**, *119*, 617.