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Chemical Synthesis of Cross-Linked Purine Nucleosides

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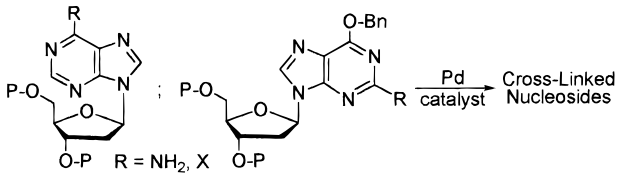
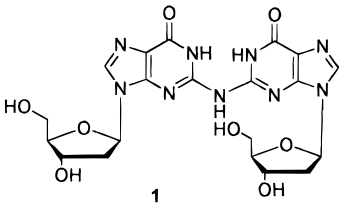
ABSTRACT



An efficient route to all of the possible cross-linked 2′-deoxypurines 1–3 has been developed by means of the Pd-mediated C–N bond formation in the key step. Utilizing this protocol, the synthesis of the first unnatural protected purine trimeric adduct 4 has been accomplished.

Active carcinogenic agents often are strong electrophiles reactive enough to interact with the nucleophilic DNA residues.[1] It is known that sodium nitrite, widely used as an additive for cured meats,[2] and nitric oxide, a physiological bioregulator,[3] in the presence of acids[4] or oxygen,[5] respectively, can induce the formation of reactive nitrosating species such as N_2O and N_2O_4. These oxides attack DNA—particularly guanines—converting the amino groups to diazonium ions.[6] Some of the latter may undergo nucleophilic attack by water, giving rise to a carbonyl group, whereas others are attacked[7] by the amino group of a neighboring nucleoside giving inter- or intrastrand or interhelical DNA cross-links.[6−8] In 1977, Shapiro reported the isolation and partial characterization of the first coupled nucleosides **1** and **2**, from nitrous acid treated DNA,[8a] whereas in 1992 Hopkins showed that **1** was formed via an interstrand cross-linking process.[8b,c]



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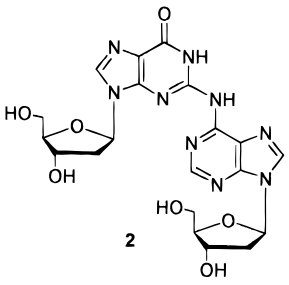

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(1) Miller, E. C.; Miller, J. A. *Cancer* **1981**, *10*, 2327−2345.

(2) *Handbook of Food Additives*, 2nd ed.; Furia, T. E., Ed.; CRC Press: Cleveland, 1972; pp 150−155.

(3) (a) Garthwaite, J., Charles, S. L.; Chess-Williams, R. *Nature* **1988**, *336*, 385−388. (b) Bredt, D. S.; Hwang, P. M.; Snyder, S. H. *Ibid.* **1990**, *347*, 768−770.

(4) (a) Committee on Nitrite and Alternative Curing Agents in Food. *The Health Effects of Nitrate, Nitrite, and N−Nitroso Compounds*; National Academy Press: Washington, DC, 1981. (b) Zimmermann, F. K. *Mutat. Res.* **1977**, *39*, 127−148.

(5) (a) Wink, D. A.; Kasprzac, K. S., Maragos, C. M.; Elespuru, R. K., Misra, M.; Dunams, T. M., Cebula, T. A.; Koch, W. H.; Andrews, A. W.; Allen, J. S.; Keefer, L. K. *Science* **1991**, *254*, 1001−1003. (b) Tannenbaum, S. R.; Tamir, S.; Rojas-Walker, T. D.; Wishnok, J. S. DNA Damage and Cytotoxicity Caused by Nitric Oxide. In *Nitrosamine and Related N-Nitroso Compounds − Chemistry and Biochemistry*; Loeppky, R. N., Michejda, C. L., Eds.; ACS Symp. Ser. No. 553; American Chemical Society: Washington, DC, 1994; Chapter 10, pp 120−135. (c) Caulfield, J. L.; Wishnok, J. S.; Tannenbaum, S. R. *J. Biol. Chem.* **1998**, *273*, 12689−12695.

(6) Glaser, R.; Rayat, S.; Lewis, M.; Son, M.-S.; Meyer, S. *J. Am. Chem. Soc.* **1999**, *121*, 6108−6119 and references therein.

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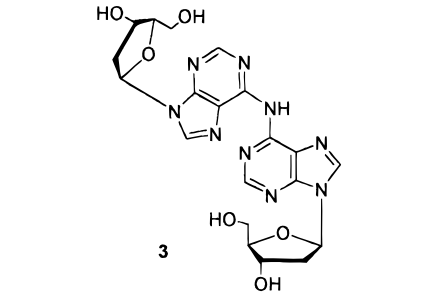
In this paper, we report our results on the chemical synthesis of all of the possible N^2- and N^6-cross-linked 2′-deoxypurines **1**−**3** and the fortuitous formation, during the synthesis of **2**, of the unique unnatural fully protected trimeric adduct **4**. Our results represent a remarkable extension of our work on the synthesis of the N^2- and N^6-(*o*-aminoaryl) derivatives of 2′-deoxyguanosine (dG) and 2′-deoxyadenosine (dA)[9] using the versatile palladium-catalyzed amination reaction.[10−12]



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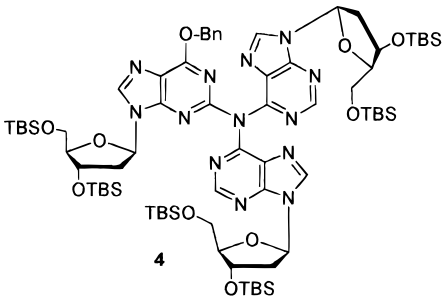

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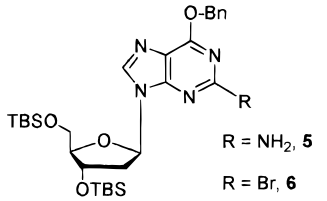
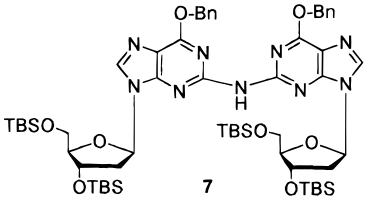
While our synthetic studies were underway, Hopkins and his associates reported the first synthesis of **1** using a similar procedure.[11] However, the key step of their protocol—the palladium-catalyzed amination reaction—gave only a moderate yield (40%), probably because of the use of sodium *tert*-butoxide, which is known to be detrimental in the case of silylated nucleosides.[12]





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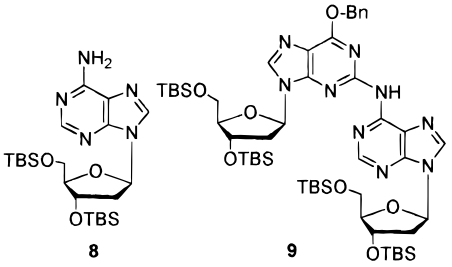
In our previous work, we had found that a catalyst system composed of 10 mol % $Pd(OAc)_2$, 15 mol % (±)-BINAP, and Cs_2CO_3, as the base, was efficient for the coupling of labile biomolecules, such as nucleosides. On the basis of these studies, we decided to evaluate the efficiency of this protocol in the coupling between 3′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-2′-β-deoxyguanosine (**5**) and 2-bromo-6-benzyloxy-9-[2-deoxy-3,5-bis-*O*-(*tert*-butyldimethylsilyl)-β-D-*erythro*-pentofuranosyl]purine (**6**). We were delighted to find that the previously reported[11] **7**[13] was formed in excellent yield (90%). The ^{1}H NMR spectrum of the purified material showed resonance peaks that were identical to those reported previously.[11]

In his 1977 communication, Shapiro reported the isolation of the nonsymmetrical adduct **2**.[8a] Unfortunately, the extremely low yields obtained did not allow full spectroscopic characterization of the product (only two UV spectra at different pH values and a mass spectrum of a derivative were reported). In 1991, a reinvestigation by Hopkins et al.[8b,c] on the DNA interstrand cross-linked adducts, formed by treating calf thymus DNA with nitrous acid, failed to give **2**.

Described herein is the first chemical synthesis of **2**, which conclusively demonstrates that the covalent DNA lesion found in trace amounts by Shapiro has this structure.

In our first synthetic attempt to couple 3′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyadenosine (**8**) with **6**, we used the same reaction conditions followed for the successful synthesis of **7**. Unfortunately, from the reaction mixture we



8 **9**

obtained only low yields of **9** (24%) plus unreacted starting material. In an endeavor to enhance the rate of the reaction, we planned the synthesis of the more reactive 2-iodo-6-benzyloxy-9-[2-deoxy-3,5-bis-*O*-(*tert*-butyldimethylsilyl)-β-D-*erythro*-pentofuranosyl]purine (**10**) following the reaction conditions described by Matsuda et al.[14] and Nair et al.[15] Unfortunately both procedures led to low yields of a highly

(7) Burnotte, J.; Verly, W. G. *J. Biol. Chem.* **1971**, *246*, 5914−5918 and references therein.

(8) (a) Shapiro, R.; Dubelman, S.; Feinberg, A. M.; Crain, P. F.; McCloskey, J. A. *J. Am. Chem. Soc.* **1977**, *99*, 302−303. (b) Kirchner, J. J.; Hopkins, P. B. *J. Am. Chem. Soc.* **1991**, *113*, 4681−4682. (c) Kirchner, J. J.; Sigurdsson, S. Th., Hopkins, P. B. *J. Am. Chem. Soc.* **1992**, *114*, 4021−4027.

(9) De Riccardis, F.; Bonala, R. R.; Johnson, F.*J. Am. Chem. Soc.* **1999**, *121*, 10453−10460.

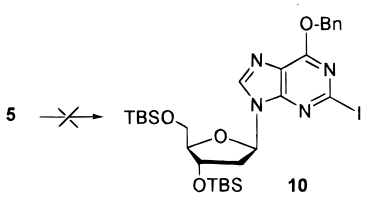
(10) (a) Hartwig, J. F. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 2046−2067. (b) Wolfe, J. P.; Wagaw, S.; Marcoux, J.-F.; Buchwald, S. L. *Acc. Chem. Res.* **1998**, *31*, 805−818. (c) Hartwig, J. F. *Acc. Chem. Res.* **1998**, *31*, 852−860. (c) Frost, C. G.; Mendonça, P. *J. Chem. Soc., Perkin Trans. 1* **1998**, 2615−2623. (d) Hartwig, J. F. *Synlett* **1997**, 329−340.

(11) Harwood, E. A.; Sigurdsson, S. Th.; Edfeldt, N. B. F.; Reid, B. R.; Hopkins, P. B. *J. Am. Chem. Soc.* **1999**, *121*, 5081−5082.

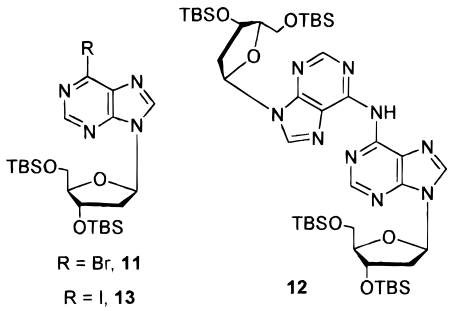
(12) Lakshman, M. K.; Keeler, J. C.; Hilmer, J. H.; Martin, J. Q. *J. Am. Chem. Soc.* **1999**, *121*, 6090−6091.

(13) Satisfactory analytical data (^{1}H and ^{13}C NMR, FAB-MS spectra and elemental analyses) were obtained for all the new compounds (see the Supporting Information)

photosensitive compound, which decomposed within few hours. An alternative to these unsuccessful approaches was



the palladium-catalyzed nucleophilic displacement of the 6-bromo-9-[2-deoxy-3,5-bis-*O*-(*tert*-butyldimethylsilyl)-β-D-*erythro*-pentofuranosyl]purine (**11**) [easily synthesized through light-catalyzed bromination of 3′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyadenosine (**8**)[12,16a]] with the more reactive N^2-amino group of the protected dG, **5**. A similar coupling, using compound **11**, has been reported by the Lakshman group for the synthesis of the simpler N^6-aryl-2′-deoxyadenosine analogues.[12] The nucleophilic displacement of the 6-bromo group occurred with considerable ease and gave the nonsymmetrical adduct **9** in 60% yield (75% yield based on unrecovered starting material).

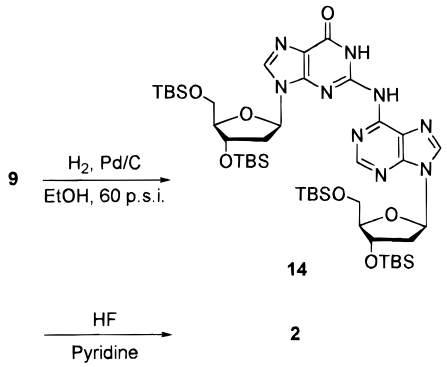


In view of this success, we decided, prior to deprotecting **9**, to investigate the potential of this method for the synthesis of the symmetrical, adduct **3**, which has never been reported. Thus, a reaction between the less nucleophilic **8** and the bromonucleoside **11** was attempted under the usual reaction conditions. As expected, this reaction gave only a low yield of **12** (21%), contaminated with a minor but inseparable impurity. Increasing the temperature (120 °C), the reaction times (24 h, 48 h), and the amount of $Pd(OAc)_2$ and ligand (20 mol % and 30% mol, respectively) or replacing the Pd species ($Pd_2(dba)_3$) and the base (K_3PO_4)[17] did not induce any increase in the yield or of the rate of conversion. Finally, using our standard amination conditions, but replacing the bromopurine **11** with the more reactive, light-stable 6-iodo-9-[2-deoxy-3,5-bis-*O*-(*tert*-butyldimethylsilyl)-β-D-*erythro*-pentofuranosyl]purine (**13**), conveniently available through the procedure of Richardson,[16b] we were able to raise the yield of this coupling step to a satisfying 51%.

Having the iodonucleoside **13** available, we reinvestigated the coupling reaction with the protected dG (**5**), in an attempt to increase further the yield of adduct **9**. To our surprise, the cross-coupling reaction gave a lower yield (45%). Careful investigation of the reaction products led to the isolation of a less polar compound. Its spectral data (particularly ^{1}H and ^{13}C NMR) showed the presence of two different resonance patterns in a 2:1 ratio. The preeminent one was assigned to a 2′-deoxyadenosine residue, the other to a 2′-deoxyguanosine. The absence, in the ^{1}H NMR, of an N-*H* signal and a molecular weight of 1510 Da (FAB-MS spectrum) suggests structure **4** for the new compound (formed in 17% yield).

The ultimate acquisition of **2** and **3** required only standard deprotection chemistry (Scheme 1). Compound **9** was

Scheme 1



subjected to hydrogenolysis using palladium-on-charcoal catalyst, at 60 psi, to give **14** in quantitative yield. We were also gratified to observe that **14** was easily desilylated with HF and pyridine[18] to give the cross-linked nucleoside **2** in 97% yield.[19] Desilylation of compound **12** was similarly accomplished to give the symmetrical so far nonnatural adduct **3** in quantitative yield.

The further extension of this type of coupling reaction to other nucleosides including both purine and pyrimidine classes is under investigation.

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Supporting Information Available: Experimental procedures and full characterization for compounds **2**−**4**, **7**, **9**, **12,** and **14**; ^{1}H and ^{13}C NMR spectra of **2**−**4**, **7**, **9**, **12,** and **14**; UV spectra (pH = 5.7 and 10.5) of **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(14) Matsuda, A.; Shinozaki, M.; Yamaguchi, T.; Homma, H.; Nomoto, R.; Miyasaka, T.; Watanabe, Y.; Abiru, T. *J. Med. Chem.* **1992**, *35*, 241−252.

(15) Nair, V.; Young, D. A.; DeSilva, R. *J. Org. Chem.* **1987**, *52*, 1344−1347.

(16) (a) Nair, V.; Richardson, S. G. *J. Org. Chem.* **1980**, *45*, 3969−3974. (b) Cosstick, R.; Douglas, M. E. *J. Chem. Soc., Perkin Trans. 1* **1991**, 1035−1040.

(17) Old, D. W.; Wolfe, J. P., Buchwald, S. L. *J. Am. Chem. Soc.* **1998**, *120*, 9722−9723.

(18) Nicolaou, K. C., Webber, S. E. *Synthesis* **1986**, 453−461.

(19) The UV spectra of this product were almost identical to those reported by Shapiro et al.[8a] in 1977 (see the Supporting Information).