Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 5, No. 24, pp. 3033-3038, 1995

0960-894X/95 $9.50+0.00

0960-894X(95)00521-8

THE SYNTHESIS AND INVESTIGATION OF THE DNA BINDING PROPERTIES OF DIELECTROPHILES INCORPORATING BIS-VICINAL TRICARBONYLS.

Harry H. Wasserman* and Carmen M. Baldino
Department of Chemistry, Yale University, New Haven, Connecticut 06520-8107, USA

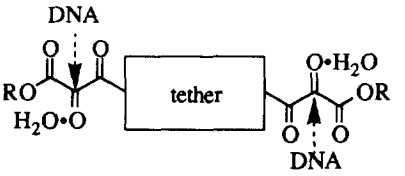
Abstract: A concise and versatile synthesis of bis-vicinal tricarbonyls was developed from readily available diacids or their corresponding diacid chlorides. The bis-tricarbonyls prepared by this method were shown to be effective interstrand DNA cross-linking agents.

It is well known that the high levels of *in vitro* and *in vivo* cytotoxicity shown by many systems can be attributed to their effectiveness in producing interstrand DNA cross-links.[1] Natural products such as mitomycin,[2] FR 900482,[3] psoralen,[4] the pyrrolizidine alkaloids,[5] carzinophilin,[6] and isochrysohermidin[7] have all been identified as DNA cross-linking agents. Based on the structures and probable modes of action of these naturally occurring DNA alkylating agents, a large number of synthetic bifunctional DNA binding molecules have been studied in recent years.[8] These synthetic derivatives include the nitrogen mustards,[9] diepoxybutane,[10] U-77,779,[11] seco-C_2BI-CDPI$_2$,[12] cis-platin,[13] dimeric anthramycin analogs,[14] diaziridinylbenzoquinones,[15] nitrous acid,[16] and formaldehyde.[17] The electrophilic sites in these natural and synthetic compounds are present in the parent systems or are incorporated in a latent form, and unmasked through protocols such as oxidative[2,3] or photochemical[4] activation. The goal of most of these studies is to bring about sequence-selective binding to duplex DNA by dual alkylation, generating specific DNA cross-links as exemplified in the work with anthramycin- and CC-1065-based agents.

During our studies on the chemistry of vicinal tricarbonyls, we have shown that this reactive species may serve as a versatile electrophilic intermediate for the synthetic chemist, particularly in the preparation of heterocyclic systems.[18] This unique functionality has played a key role in our syntheses of a number of natural products.[19] Recently, we have also synthesized a group of di- and tripeptides terminating in vicinal tricarbonyl hydrates which have proven to be potent serine protease inhibitors.[20] These results along with the finding that a related tricarbonyl subunit is contained in the immunosuppressant FK-506[21] and the related agents, rapamycin and 29-demethoxyrapamycin,[22] have prompted us to investigate the role of this powerfully electrophilic functionality in other biological systems.

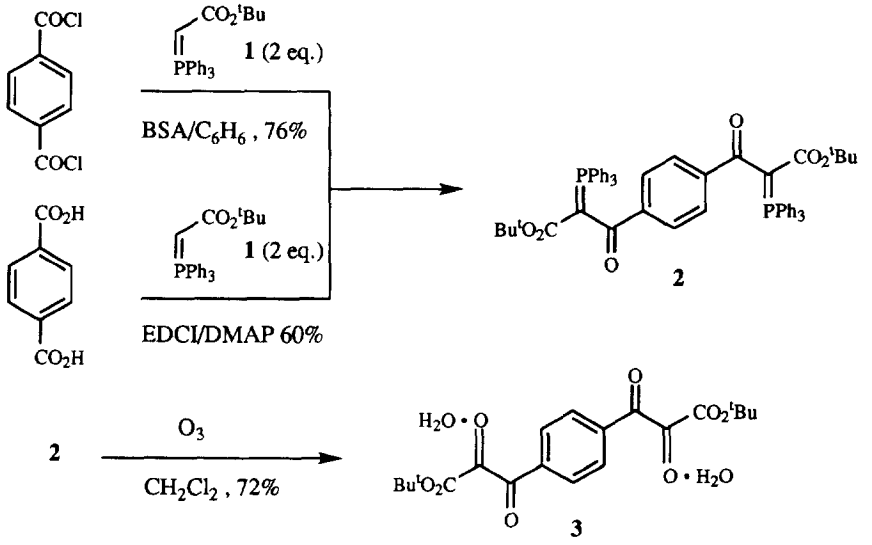
We now report the concise and versatile synthesis of a series of bis-vicinal tricarbonyls and a preliminary evaluation of their DNA binding properties. Our products incorporate two reactive electrophilic centers in a single molecule, separated by a tether which can be easily varied (Figure 1). The method of preparation of these substances offers an opportunity for design of interstrand DNA cross-linking agents by controlling spacial factors associated with the interactions between the nucleophilic sites housed within the DNA duplex and the reactive tricarbonyl moieties.

Figure 1.



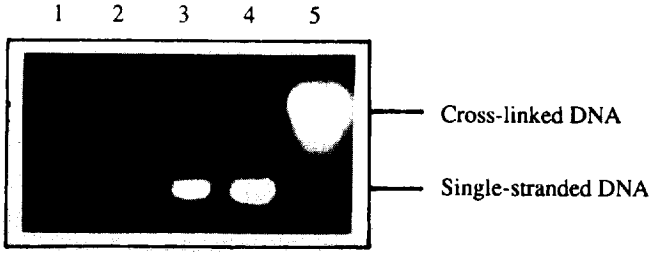
The bis-tricarbonyl derivatives were prepared from readily available dicarboxylic acids or their diacid chloride analogs (Scheme 1). The coupling of the diacids or diacid chlorides with *t*-butyl (triphenylphoshoranylidine)acetate (**1**) was accomplished using standard methods.[23] Oxidative cleavage of the carbon-phosphorous double bonds in **2** was conducted utilizing either ozone[24] or Oxone®[25] to provide the bis-tricarbonyls **3**. The agents thus generated contain benzene (**3** and **4**), naphthalene (**5**) and decane (**6**) as the tethers (Scheme 2).

Scheme 1.



With the bis-tricarbonyls in hand, we sought to determine their effectiveness in generating interstrand DNA cross-links. The agents were each incubated with ΦX 174 *Pst* 1 linear double-stranded DNA (pH 7.1, 5386 base pairs, 1 x 10^{-8} M) for 24 h at 37 °C. The reaction samples were then denatured (100 °C, 3 min) and loaded onto a denaturing agarose gel (0.7 % agarose with 0.03 M NaOH, 5 h, 50 v).[26] Cross-linking was detected by fluorescence visualization of a substantially slower moving band, compared to the fast moving band associated with the lower molecular weight single-stranded DNA (Figure 2).

Figure 2. Cross-linking Assay.[27]

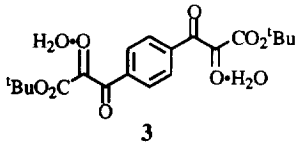


Lane 1, 5 mM **3**; Lane 2, 1 mM **3**; Lane 3, 0.5 mM **3**;
Lane 4, Untreated DNA; Lane 5, 10 mM Psoralen.

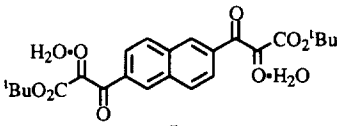
As shown in Figure 2, the 1,4-bis-tricarbonyl benzene derivative (**3**) is effective at generating cross-linked DNA at 5mM and 1mM (lanes 1 and 2). This activity is not apparent at lower concentrations of **3** (0.5 mM, lane 3). Lane 4 shows a faster moving band (single-stranded DNA) corresponding to the incubation of the DNA without added agent. For a positive cross-linking comparison (lane 5), a control experiment was carried out employing psoralen-induced DNA cross-links [28] (10 mM, 1 h, 365 nm).

Although the 1,4-benzene derivative **3** was the most effective of the bis-dielectrophiles used, each of the bis-tricarbonyls shown in Scheme 2 was able to effect cross-links in the DNA at agent concentrations of 5 mM. The derivatives **4** and **5** were slightly less effective, while the products **6** and **7** from dodecanedioic and phthalic acids provided the lowest levels of cross-linking.

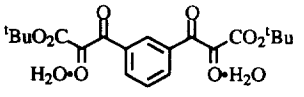
Scheme 2



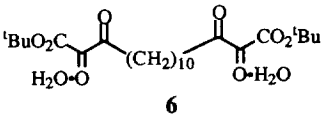
3



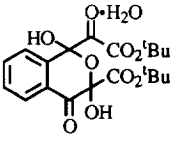
5



4



6

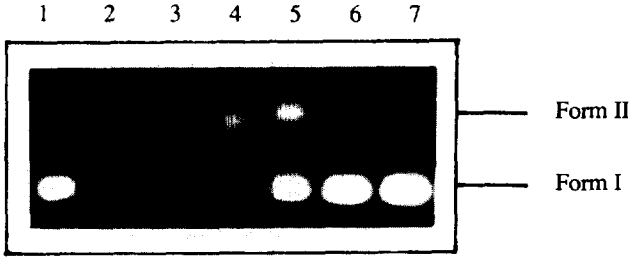


7

We also studied the interaction of the bis-tricarbonyl derivative **3** with supercoiled ΦX 174 DNA (pH 8, 5386 base pairs, 1 x 10^{-8} M) leading to DNA cleavage. The agent was incubated with the supercoiled DNA at

55 °C for 2.5 h followed by non-denaturing gel electrophoresis[29] (1% agarose, 2 h, 100 v) and fluorescence visualization of the various bands, as shown in Figure 3. Supercoiled DNA (Form I, lane 1, fastest moving band) underwent cleavage to generate circular DNA (Form II, slower moving band). The cleaved or circular DNA (Form II) was generated at 25-0.5 mM- concentrations (lanes 3-7).

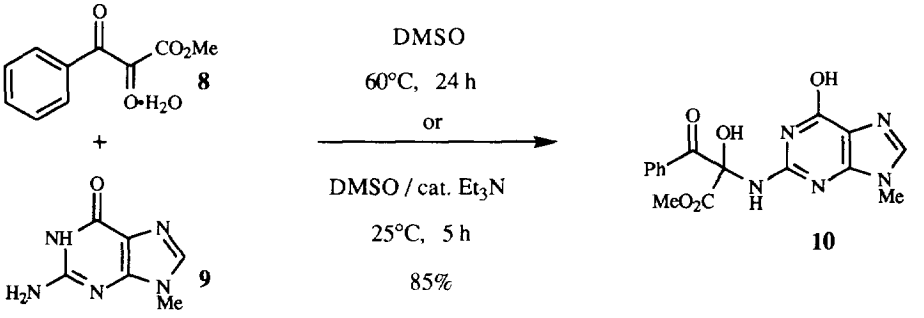
Figure 3. Cleavage of Super-coiled DNA.[30]



Lane 1, untreated DNA; Lane 2, 50 mM **3**; Lane 3, 25 mM **3**;
Lane 4, 10 mM **3**; Lane 5, 5 mM **3**; Lane 6, 1 mM **3**; Lane 7, 0.5 mM **3**.

In studying the mode of interaction between our agents and the DNA, we investigated the reaction of a model mono-tricarbonyl **8** with 9-methylguanine (**9**). A one-to-one adduct, the structure of which is completely consistent with **10**,[31] was isolated (85%) either by mixing the reagents at 60 °C for 24 h or at 25 °C for 5 h in the presence of Et_3N (Scheme 3). A rationale for this type of reactivity in our cross-linking studies was provided by a molecular modeling study,[32] in which a 1,4-bis-tricarbonyl benzene derivative is bound in a segment of the minor groove of DNA (5'-GGTCC-3'). This analysis showed that the central carbonyls of the bis tricarbonyl unit may be positioned in close proximity to the amino groups of guanine residues separated by three base-pairs.

Scheme 3



In conclusion, a versatile synthesis of bis-vicinal tricarbonyls has been developed from bis-dicarboxylic acids separated by an appropriate tether. These agents have been shown to generate interstrand DNA cross-links and also to cleave supercoiled DNA.

Acknowledgment: This work was funded by grants from the NIH and the NSF. We would like to thank Professors Donald Crothers and Alanna Schepartz for many helpful discussions and for allowing us the use of laboratory equipment to conduct the biological studies. We would also like to thank Dr. Matthew Hayward, David Paolella and Leslie Sloan for technical assistance while conducting the biological studies. We are indebted to Dr. Carol Lamberson of Sandoz Corporation for the molecular modeling study.

References and Notes:

1. Kohn, K. W. In *Development of Target-Oriented Anticancer Drugs*; Cheng, Y.-C.; Goz, B.; Minkoff, M. Eds.; Raven Press: New York, 1983; pp 181-188.
2. Teng, S. P.; Woodson, S. A.; Crothers, D. M. *Biochemistry* **1989**, *28*, 3901. Kumar, S.; Lipman, R.; Tomasz, M. *Biochemistry* **1992**, *31*, 1399. Li, V. S.; Kohn, H. *J. Am. Chem. Soc.* **1991**, *113*, 275.
3. Williams, R. M.; Rajski, S. R. *Tetrahedron Lett.* **1992**, *33*, 2929. Woo, J.; Sigurdsson, S. T.; Hopkins, P. B. *J. Am. Chem. Soc.* **1993**, *115*, 1199.
4. Gia, O.; Magno, S. M.; Garbes, A.; Colonna, F. P.; Palumbo, M. *Biochemistry* **1992**, *31*, 11818.
5. Woo, J.; Sigurdsson, S. T.; Hopkins, P. B. *J. Am. Chem. Soc.* **1993**, *115*, 3407.
6. Armstrong, R.W.; Salvati, M. E.; Nguyen, M. *J. Am. Chem. Soc.* **1992**, *114*, 3144.
7. Boger, D.L.; Baldino, C. M. *J. Am. Chem. Soc.* **1993**, *115*, 11418.
8. Reviews of cross-linking agents: Sinden, R. R.; Hoepfner, R. W.; Ussery, D. W. *Methods in Enzymology* **1992**, *212*, 242. Hopkins, P. B.; Millard, J. T.; Woo, J.; Weidner, M. F.; Kirchner, J. J.; Sigurdsson, S. T.; Raucher, S. *Tetrahedron* **1991**, *47*, 1415.
9. Rink. S. M.; Solomon, M. S.; Taylor, M. J.; Rajur, S.B.; McLaughlin, L. W.; Hopkins, P. B. *J. Am. Chem. Soc.* **1993**, *115*, 2551.
10. Millard, J. T.; White, M. M. *Biochemistry* **1993**, *32*, 2120.
11. Lee, C.S.; Gibson, N.W. *Biochemistry* **1993**, *32*, 2592. Mitchell, M. A.; Kelly, R. C.; Wichienski, N. A.; Hatznenbuhler, N. T.; Williams, M. G.; Petzold, G. L.; Slightom, J. L.; Siemieniak, D. R. *J. Am. Chem. Soc.* **1991**, *113*, 8994.
12. Boger, D. L.; Palanki, M. S. S. *J. Am. Chem. Soc.* **1992**, *114*, 9318. Boger, D. L.; Johnson, D. S.; Palanki, M. S. S.; Kitos, P. A.; Chang, J.; Dowell, P. *Bioorg. Med. Chem.* **1993**, *1*, 27.
13. Szymkowski, D. E.; Yarema, K.; Essigmann, J. M.; Lippard, S. J.; Wood, R.D. *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 10772. Bradley, L. J. N.; Yarema, K. J.; Lippard, S. J.; Essigmann, J. M. *Biochemistry* **1993**, *32*, 982.
14. Bose, D. S.; Thompson, A. S.; Ching, J.; Hartley, J.A.; Berardini, M. D.; Jenkins, T. C.; Neidle, S.; Hurley, L. H.; Thurston, D. E. *J. Am. Chem. Soc.* **1992**, *114*, 4939.
15. Berardini, M. D.; Souhami, R. L.; Lee, C.S.; Gibson, N. W.; Butler, J.; Hartley, J.A. *Biochemistry* **1993**, *32*, 3306.
16. Kirchner, J. J.; Sigurdsson, S. T.; Hopkins, P. B. *J. Am. Chem. Soc.* **1992**, *114*, 4021.
17. Huang, H.; Solomon, M. S.; Hopkins, P. B. *J. Am. Chem. Soc.* **1992**, *114*, 9240.
18. Wasserman, H. H.; Cook, J. D.; Fukuyama, J. M.; Rotello, V. M. *Tetrahedron Lett.* **1989**, *30*, 1721. Wasserman, H. H.; Frechette, R.; Oida, T.; van Duzer, J. H. *J. Org. Chem.* **1989**, *54*, 6012. Wasserman, H. H.; Cook, J. D.; Vu, C. B. *J. Org. Chem.* **1990**, *55*, 1701. Wasserman, H. H.; Vu, C. B.; Cook, J. D. *Tetrahedron* **1992**, *48*, 2101. Wasserman, H. H.; Cook, J. D.; Vu, C. B. *Tetrahedron Lett.* **1990**, *31*, 4945. Wasserman, H. H.; Han, W. T. *J. Am. Chem. Soc.* **1985**, *107*, 1444. Wasserman, H. H.; Han, W. T. *Tetrahedron Lett.* **1984**, *25*, 3743. Wasserman, H. H.; Han, W. T. *Tetrahedron Lett.* **1984**, *25*, 3747. Wasserman, H. H.; Niu, C. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 2437. Wasserman, H. H.; Henke, S. L.; Luce, P.; Nakanishi, E. *J. Org. Chem.* **1990**, *55*, 5821. Wasserman, H. H.; Henke, S. L.; Nakanishi, E. *J. Org. Chem.* **1992**, *57*, 2641.
19. Wasserman, H. H. *Aldrichimica Acta* **1987**, *20*, 63 and references cited therein. Wasserman, H. H.; Lombardo, L. J. *Tetrahedron Lett.* **1989**, *30*, 1725. Wasserman, H. H.; Rotello, V. M.; Krause, G. B. *Tetrahedron Lett.* **1992**, *33*, 5419. Wasserman, H. H.; Kuo, G. H. *Tetrahedron Lett.* **1991**, *32*, 7131. Wasserman, H. H.; Kuo, G. H. *Tetrahedron* **1992**, *48*, 7071. Wasserman, H. H.; Amici, R.; Frechette, R.; van Duzer, J. H. *Tetrahedron Lett.* **1989**, *30*, 869. Wasserman, H. H.; Kuo, G. H. *Tetrahedron Lett.* **1989**, *30*, 873. Wasserman, H. H.; Amici, R. M. *J. Org. Chem.* **1989**, *54*, 5843. Wasserman, H. H.; Kelly, T. A. *Tetrahedron Lett.* **1989**, *30*, 7117.
20. Wasserman, H. H.; Ennis, D. S.; Power, P. L.; Ross, M. J.; Gomes, B. *J. Org. Chem.* **1993**, *58*, 4785.
21. Tanaka, H.; Kuroda, A.; Marusawa, H.; Hatanaka, H.; Kino, T.; Goto, T.; Hashimoto, M.; Taga, T. *J. Am. Chem. Soc.* **1987**, *109*, 5031.
22. Findlay, J. A.; Radics, L. *Can. J. Chem.* **1980**, *58*, 579. Swindells, D. C. N.; White, P. S.; Findlay, J. A. *Can. J. Chem.* **1978**, *56*, 2491. Vézina, C.; Kudelski, A.; Sehgal, S. N. *J. Antibiot.* **1975**, *28*, 721. (d) Sehgal, S. N.; Baker, H.; Vézina, C. *J. Antibiot.* **1975**, *28*, 727.

23. Wasserman, H. H.; Ennis, D. S.; Blum, C. A.; Rotello, V. M. *Tetrahedron Lett.* **1992**, *33*, 6003. Cooke, M. P., Jr.; Burman, D. L. *J. Org. Chem.* **1982**, *47*, 4955.
24. Wasserman, H. H.; Fukuyama, J.; Murugesan, N.; van Duzer, J.; Lombardo, L.; Rotello, V.; McCarthy, K. *J. Am. Chem. Soc.* **1989**, *111*, 371.
25. Wasserman, H. H.; Vu, C. B. *Tetrahedron Lett.* **1990**, *31*, 5205.
26. Cech, T. R. *Biochemistry* **1981**, *20*, 1431.
27. DNA Cross-Linking Study: An Eppendorf tubecontaining the *Pst* I digest of double-stranded ΦX174 DNA (18μL, TE buffer pH 7.1) was treated with the bis-tricarbonyl **3** in a solution of DMSO (2μL, 50 mM; 5mM final concentration). The solution was mixed by vortexing and brief centrifugation and subsequently incubated (37 °C, 24 h). The buffer solution was treated with bromophenol blue dye solution (2.5 μL) and 30 mM NaOH (2.5 μL) warmed at 100 °C for 3 min, placed in an ice bath and the supernatant (20 μL) was loaded onto an alkaline agarose gel. Gel electrophoresis was conducted at 60 v (5 h) at 4 °C on a 0.7 % agarose gel containing 30 mM NaOH and 0.1 μg/μL ethidium bromide. Electrophoresis running buffer (Keller, 4 °C) contained Tris base (0.4 mM), NaOAc (0.05 mM), $Na_2EDTA \bullet 2H_2O$ (10 mM), and NaOH (30 mM) was dissolved in H_2O.
28. Psoralen Cross-Linking Study: An Eppendorf tube containing the *Pst* I digest of double stranded ΦX174 DNA (18μL, TE buffer pH 7.1) was treated with psoralen in a DMSO solution (2 μL, 100 mM, 10 mM final concentration). The reaction was mixed by vortexing and brief centrifugation and subsequently incubated (25 °C, 30 min) and then irradiated (365 nm, 1 h). The buffer solution of cross-linked DNA were treated with bromophenol blue dye solution (2.5 μL), 30 mM NaOH (2.5 μL) and warmed at 100 °C for 3 min. The gel electrophoresis was conducted as described in reference 27.
29. Boger, D. L.; Menezes, R. F.; Dang, Q.; Yang, W. *Bioorg. Med. Chem.. Lett.* **1992**, *2*, 261.
30. Supercoiled DNA Cleavage Studies: An Eppendorf tube containing the ΦX174 supercoiled DNA (18 μL, TE buffer pH 8.0) were treated with the bis-tricarbonyl **3** in a solution of DMSO (2 μL, 100 mM, 10 mM final concentration). The solution was mixed by vortexing and brief centrifugation and subsequently incubated (55 °C, 2.5 h). The buffer solution of DNA was treated with bromophenol blue dye solution (3 μL) and loaded onto an agarose gel. Gel electrophoresis was conducted at 100 v (2.5 h) at 25 °C on a 1% agarose gel containing 0.1 μg/μL ethidium bromide. Electrophoresis running buffer (Keller, 25 °C) containing Tris base (0.2 mM), NaOAc (0.05 mM), $Na_2EDTA \bullet 2H_2O$ (10 mM) was dissolved in H_2O.
31. **10**: ^1H NMR (DMSO-d_6) δ 3.30 (s, H), 3.64 (s, 3 H), 3.79 (s, 3 H), 7.65 (t, 3 H, J = 7.57 Hz), 7.76 (t, 2 H, J = 7.32 Hz), 8.13 (s, 1 H), 8.24 (s, 1 H); ^{13}C NMR (DMSO-d_6) δ 37.3, 56.1, 93.6, 123.0, 129.8, 131.9, 132.0, 135.7, 145.2, 152.3, 155.5, 158.1, 167.0, 180.5; IR (KBr) ν max 3436, 2961, 2924, 1762, 1678, 1540, 1160, 1112 cm^{-1}; FABMS (glycerol + TFA) 358 ($M+H^+$, 25), 340 ([$M+H^+$]-H_2O, 100); FAB HRMS (NBA +TFA) $C_{16}H_{15}N_5O_5$+H^+-H_2O requires 340.1059, found 340.1053.
32. The molecular modeling study was conducted using the Sybyl 6.0 program by Tripos and the Tripos molecular mechanics force field was used for all calculations. The Tripos-Biopolymer module was used to generate the double-stranded B-form DNA. The model dielectrophile is an analog of **3** (dimethyl ester of the unhydrated form).

(*Received in USA* 5 *October* 1995; *accepted* 10 *November* 1995)