# Synthesis of Oligonucleotides Containing Interchain Cross-Links of Bifunctional Pyrroles

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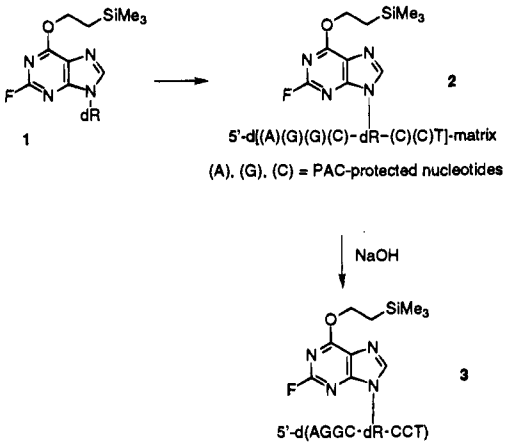
A solid-phase, postoligomerization strategy has been developed in this laboratory for preparing oligonucleotides bearing adducts of mutagens on exocyclic amino sites of guanine and adenine in which structural specificity is achieved by reversing the normal electrophile−nucleophile relationship of mutagen and purine. Amine analogs of the mutagens are condensed with halopurine analogs of guanine and adenine; these reactions are carried out after assembly of the oligonucleotides.[1,2] The condensation reactions have been carried out prior to removal of protective groups or cleavage from the solid supports used in automated DNA synthesis in order to avoid problems with hydrolysis of the halopurine during the deprotection step.

We now report an extension of this strategy to the synthesis of DNA duplexes containing interchain cross-links. Homogeneous samples of DNA containing interchain cross-links have hitherto been relatively inaccessible. Direct reaction of bis-electrophiles with DNA has been limited to situations in which the reaction gives inherently high yields and only a single target site is present in the DNA duplex.[3]

In order to prepare interchain cross-links, procedures first had to be developed for freeing the oligonucleotides from the solid matrix since the prospects were poor for efficient cross-linking if the reaction were carried out on the solid matrix. Fluoronucleoside **1**,[4] which is the synthetic equivalent of deoxyguanosine in the electrophile−nucleophile reversal system, was converted to the cyanoethyl dimethoxytrityl phosphoramidite, and the phosphoramidite was incorporated into oligonucleotide **2** by automated solid-phase synthesis (Scheme 1). PAC-phosphoramidites[5] were used for introducing the other nucleosides; these phosphoramidite reagents employ labile acyl groups to protect the exocyclic amino groups so that deprotection can be achieved under mild conditions. A careful study of deprotection conditions led to the discovery that complete deprotection could be achieved, i.e., removal of the acyl groups from exocyclic amines, cyanoethyl groups from phosphate, and the oligonucleotide from the solid support, without disturbing the fluoro substituent or TMSE group from **1** by using 0.1 M NaOH (6 h, 25 °C).[6] The constitution of oligonucleotide **3** was rigorously established by electrospray mass spectroscopy on the oligonucleotide and by enzymatic degradation to give fluoronucleoside **1**.[7,8]

**Scheme 1**




IPP (**4**) is a bifunctional pyrrole which has been investigated as a chemotherapeutic agent.[9] **4** forms interstrand cross-links in B DNA by cross-linking $N^2$ positions of guanines at CpG sites, e.g., reaction with duplex **5** would form cross-linked duplex **6**, with the alkylations occurring by two stages of elimination−addition[10] (Scheme 2). Only small amounts of cross-link, <4%, are generated by direct reaction of **2** with duplexed DNA, with most reactions aborting at the monoadduction stage. It is likely, however, that the cross-linked species is the pharmacologically important adduct.

The IPP cross-link was synthesized by reaction of oligonucleotide **3** with diamine **7** in a stepwise fashion. The first condensation was carried out using 10 equiv of **7** (pH 9.6, 20 °C, 4 days). Isomeric monoadducts **8** and **9** were formed in a ~1:3 ratio and isolated in net ~40% yield. Structures of the adducts were assigned on steric grounds. The $O^6$-TMSE cleaved spontaneously from the adducts; the TMSE group is relatively stable in 2-fluoropurine derivatives but becomes labile when the fluoro substituent is replaced by an amine. The mixture of **8** and **9** was treated with 1.5 equiv of oligonucleotide **3** (pH 9.6, 20 °C, 8 days). Monoadduct **9** reacted efficiently with **7** to give cross-linked oligonucleotide duplex **6**, but **8** reacted only lethargically. We hypothesize that steric hindrance to reaction at the 6-aminomethyl group accounts for the difference in reactivity of **8** and **9**. Electrospray mass spectroscopic data were fully consistent with the assigned structure of **6**.[11] Additional

(1) (a) Harris, C. M.; Zhou, L.; Strand, E. A.; Harris, T. M. *J. Am. Chem. Soc.* **1991**, *113*, 4328−4329. (b) Kim, S. J.; Stone, M. P.; Harris, C. M.; Harris, T. M. *J. Am. Chem. Soc.* **1992**, *114*, 5480−5481. (c) Kim, S. J.; Jajoo, H. K.; Kim, H.-Y.; Zhou, L.; Horton, P.; Harris, C. M.; Harris, T. M. *Bioorg. Med. Chem.* **1995**, *3*, 811−822.

(2) See also: (a) MacMillan, A. M.; Verdine, G. L. *Tetrahedron* **1991**, *47*, 2603−2616. (b) Webb, T. R.; Matteucci, M. D. *Nucleic Acids Res.* **1986**, *14*, 7661−7674.

(3) Borowy-Borowski, H.; Lipman, R.; Chowdary, D.; Tomasz, M. *Biochemistry* **1990**, *29*, 2992−2999. Tomasz, M. In *Advances in DNA Sequence Specific Agents*; Hurley, L. H., Ed.; JAI Press: London, 1992; Vol. 1, pp 247−261. Lown, W. J. *Chemtracts: Org. Chem.* **1993**, *6*, 205−237. Spielmann, H. P.; Sastry, S. S.; Hearst, J. E. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 4514−4518.

(4) DeCorte, B. L.; Tsarouhtsis, D.; Kuchimanchi, S.; Cooper, M. D.; Harris, C. M.; Harris, T. M. Unpublished results.

(5) PAC-phosphoramidite reagents (Pharmacia P-L Laboratories) have phenoxyacetyl, *p*-isopropylphenoxyacetyl, and isobutyryl protection on the exocyclic positions of dA, dG, and dC, respectively, making them more hydrolytically labile that the normal phosphoramidites.

(6) We will report elsewhere a related procedure in which oligonucleotides containing nucleoside **1** are prepared using ordinary phosphoramidite reagents. The oligonucleotides can be removed from the solid matrix by NaOH but are incompletely deprotected. Purification of the resulting mixture of oligonucleotides is not possible; nevertheless, the mixed oligonucleotides containing **1** are useful for preparation of monoadducted oligonucleotides in that a troublesome side reaction of nucleophiles with *N*-acylcytosine[2a] is avoided.

(7) **3**: Electrospray MS (negative ion mode) ions ($m - 2H)/2z$ 1255.6 and ($m - 3H)/3z$ 837.0 represent a molecular weight of 2513.60. Calcd for **3**: 2513.84.

(8) Enzymatic hydrolysis with nuclease P1 (pH 7.0) followed by snake venom phospho-diesterase and alkaline phosphatase (pH 9.0) gave nucleoside **1** plus the four normal nucleosides in the expected ratios. Enzymatic hydrolysis of cross-linked duplexes **6** and **13** were carried out similarly giving dinucleosides **10** and **14**.

(9) Anderson, W. K.; Corey, P. F. *J. Med. Chem.* **1977**, *20*, 812−818. Anderson, W. K.; Corey, P. F. *J. Med. Chem.* **1977**, *20*, 1691−1694.

(10) (a) Woo, J.; Sigurdsson, S. T.; Hopkins, P. B. *J. Am. Chem. Soc.* **1993**, *115*, 3407−3415. (b) Weidner, M. F.; Millard, J. T.; Hopkins, P. B. *J. Am. Chem. Soc.* **1989**, *111*, 9270−9272. (c) Weidner, M. F.; Sigurdsson, S. T.; Hopkins, P. B. *Biochemistry* **1990**, *29*, 9225−9233. (d) Millard, J. T.; Weidner, M. F.; Raucher, S.; Hopkins, P. B. *J. Am. Chem. Soc.* **1990**, *112*, 3637−3641.

(11) **6**: Electrospray MS (negative ion mode) 2548.8 ($m - 2H)/2z$, 1698.4 ($m - 3H)/3z$), and 1273.5 ($m - 2H)/2z$ representing a MW of 5098.6. Calcd for **6**: 5097.41.

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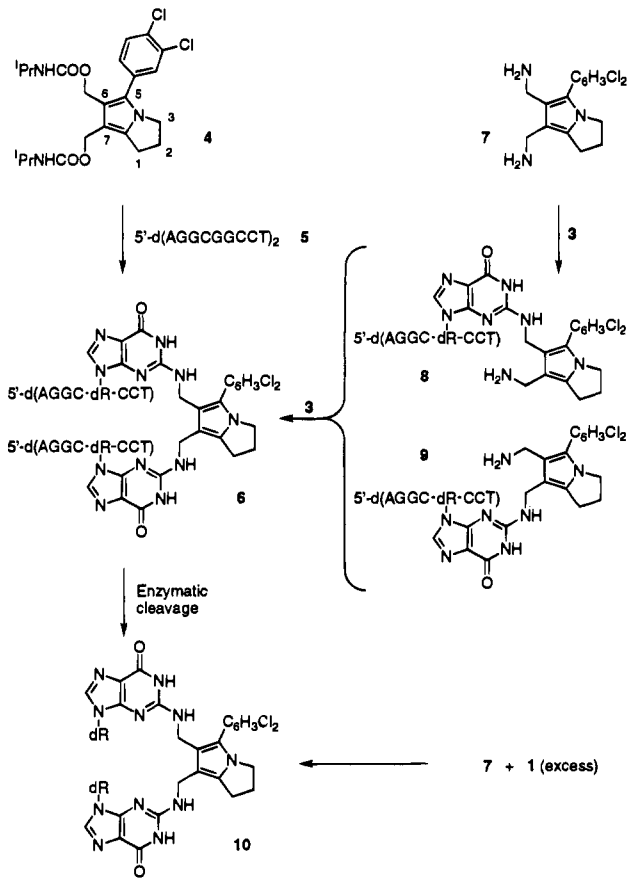
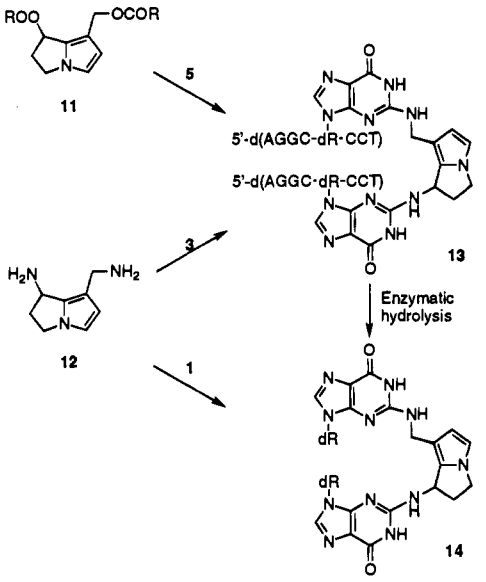
**Scheme 2**




**Scheme 3**




proof of the structure was obtained by enzymatic degradation to form bis(nucleoside) **10**,[12] which was identical to bis(nucleoside) formed by reaction of nucleoside **1** with diamine **7**.[8]

Retronecine alkaloids are pyrrolines which undergo metabolic dehydrogenation; the resulting pyrroles, dehydroretronecine diesters **11**, form mono- and bis-adducts in DNA analogous to those formed by IPP.[13] Likewise, direct adduction by **11** gives low yields of cross-linked DNA.[10b] DNA containing the dehydroretronecine cross-link has been constructed by a procedure (Scheme 3) directly analogous to that described above for preparation of **6**. Diamine **12**[14] reacted with fluorooligonucleotide **3** to form monoadducts, which on further reaction with oligonucleotide **3** gave cross-linked oligonucleotide **13**. The structure of **13** was established by enzymatic degradation to bis(nucleoside) **14**,[7] the structure of which was established by independent synthesis from diamine **12** and fluoronucleoside **1**.[15]

The real value of the method may actually lie in its structural specificity relative to the direct reactions of bis-electrophiles with DNA where one's ability to prepare homogeneous samples is significantly limited by the presence of multiple reactive sites. A self-complementary duplex containing a single (CpG):(CpG) cross-linking site was chosen for the initial demonstration of the new methodology. However, the method should be equally applicable for creating a single cross-link in oligonucleotides containing multiple (CpG):(CpG) sites. It should also be applicable to generation of less-favored cross-links, for example, in d(GpC):(GpC) sequences, and for creation of regiospecific cross-links in non-self-complementary duplexes.

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**Supporting Information Available:** Experimental details including general methods, preparation of **7**, **10**, **3**, **6**, **13**, and **14**, and enzymatic hydrolysis of oligonucleotides (6 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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(12) **10**: $^1$H NMR (MeOH-$d_4$, 57 °C) $\delta$ 7.93 (s, 1 H), 7.90 (s, 1 H), 7.49 (d, $J$ = 2 Hz, 1 H), 7.45 (d, $J$ = 8 Hz, 1 H), 7.29 (dd, $J$ = 2 and 8 Hz, 1 H1), 6.22 (m, 2 H), 4.48 (m, 12 H), 3.93 (m, 4 H), 3.73 (m, 2 H, H5″), 3.65 (m, 2 H), 2.82 (t, $J$ = 7 Hz, 2 H), 2.67 (m, 2 H), 2.45 (q, $J$ = 7 Hz, 4 H), 2.28 (m, 2 H), 1.13 (m, 4 H), 0.01 (s, 9 H), −0.03 (s, 9 H); FAB-MS (TEA−DMSO−PEG matrix) calcd for $C_{45}H_{60}O_8N_{11}C_{12}Si_2$ 1008.3541 (M − H)$^-$, found 1008.3536. The dinucleoside has previously been prepared, but NMR spectral data were not reported.[10a]

(13) Furuya, T.; Asada, Y.; Mori, H. Pyrrolizidine Alkaloids. In *Naturally Occurring Carcinogens of Plant Origin*; Hirono, I., Ed.; Elsevier: New York, 1987; Vol. 2, pp 25−52. Reed, R. L.; Ahern, K. G.; Pearson, G. D.; Buhler, D. R. *Carcinogenesis* **1988**, *9*, 1355−1361.

(14) Culvenor, C. J.; Edgar, J. A.; Smith, L. W.; Tweedale, H. J. *Aust. J. Chem.* **1970**, *23*, 1853−1867.

(15) **14**: UV $\lambda_{max}$ 254 and 282; FAB-HRMS (glycerol−DMSO−3-nitrobenzoic acid) 650.2848, calcd for $C_{28}H_{32}N_{11}O_8$ 650.2450 (M − H)$^-$.