Synthesis of DNA duplexes containing site-specific interstrand cross-links via sequential reductive amination reactions involving diamine linkers and abasic sites on complementary oligodeoxynucleotides

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Abstract: Interstrand DNA cross-links are important in biology, medicinal chemistry, and materials science. Methods for the targeted installation of interstrand cross-links in DNA duplexes may be useful in diverse fields including studies of DNA repair, materials science, and structural biology. Here a simple procedure is reported for the preparation of DNA duplexes containing site-specific, chemically-defined interstrand cross-links. The approach involves sequential reductive amination reactions between diamine linkers and two abasic (apurinic/apyrimidinic, AP) sites on complementary oligodeoxynucleotides. Use of the symmetrical triamine, tris(2-aminoethyl)amine, in this reaction sequence enabled preparation of a cross-linked DNA duplex bearing a derivatizable aminoethyl group.

■ INTRODUCTION

Interstrand cross-links (ICLs) are an important type of DNA modification because they block and read-out and replication of the genetic code. ICLs generated by anticancer drugs exert therapeutic effects through their ability to block DNA replication in rapidly dividing cancer cells.[1, 2] Unavoidable, endogenous ICLs may contribute to cancer, accelerated aging (progeria), and neurodegeneration.[3-7] The mechanisms by which ICLs are removed from or bypassed in genomic DNA are important in biology and medicine but are not yet completely understood.[1, 8-18]

Some studies aimed at elucidating the mechanisms of cellular ICL repair require DNA substrates containing a single, site-specific cross-link.[19-25] The preparation of such cross-linked DNA duplexes can be challenging. Single-step procedures involving treatment of a DNA duplex with a cross-linking agent often give complex mixtures and low ICL yields.[2, 26, 27] Alternatively, high yield preparation of some ICLs has been accomplished through multi-step organic synthesis.[28-31] Here we describe a concise method for the synthesis of cross-linked DNA duplexes from commercially-available reagents. Our approach employs sequential reductive amination reactions between diamine linkers and a pair of abasic (apurinic/apyrimidinic, AP) sites on complementary oligodeoxynucleotides to achieve good yields of site-specific, alkylamine ICLs in duplex DNA. This procedure was extended to the preparation of an ICL bearing a derivatizable amino group.

■ EXPERIMENTAL PROCEDURES

Materials and Methods. Oligonucleotides were purchased from Integrated DNA Technologies (IDT, Coralville, IA, USA), [γ-^{32}P]-ATP (6000 C_i/mmol) was purchased from Perkin-Elmer, C-18 Sep-Pak cartridges were purchased from Waters (Milford, MA, USA), and BS Poly-prep columns were obtained from BioRad (Hercules, CA, USA), acrylamide/bis-acrylamide 19:1 (40% solution, electrophoresis grade) was purchased from Fisher Scientific (Waltham, MA, USA). Uracil DNA glycosylase (UDG) and streptavidin were purchased from New England Biolabs (Ipswich, MA, USA). The compounds 1,4-diaminobutane, 1,5-diaminopentane, 1,6-diaminohexane, tris(2-aminoethyl)amine, $NaBH_3CN$, buffers, and other chemical reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA). Quantification of radioactivity in polyacrylamide gels was carried out using a Personal Molecular Imager (BioRad) with Quantity One software (v.4.6.5). DMBAA (dimethylbutylammonium acetate) solutions used in the ESI-MS experiments was prepared as follows: a stock solution of *N*,*N*-dimethylbutyl amine (7.125 M) was diluted to 100 mM with water and adjusted to pH 7.1 with glacial acetic acid.

Representative Cross-linking Protocol. The single-stranded 2'-deoxyuridine (dU)-containing oligonucleotides were purified on a 20% polyacrylamide preparative gel prior to 5'-^{32}P-radiolabeling.[32] The oligonucleotides were labeled with a 5'-^{32}P-phosophoryl group using standard methods.[32] The labeled dU-containing oligonucleotide was treated with the enzyme uracil DNA glycosylase (UDG, final concentration of 200 units/mL) in Tris-HCl buffer (20 mM, pH 8) containing dithiothreitol (DTT, 1 mM) and EDTA (1 mM). After incubation at 37 °C for 2 h, the UDG enzyme was removed by phenol-chloroform extraction and the DNA ethanol precipitated.[32] The resulting AP-containing

oligonucleotide **1** was re-dissolved and incubated in HEPES (50 mM, pH 7.4) containing NaCl (100 mM), amine linker (10 mM) and $NaBH_3CN$ (250 mM) at 37 °C for 12 h. The DNA was ethanol precipitated. The unlabeled dU-containing oligonucleotide was treated with UDG (200 units/mL, final concentration) in Tris-HCl buffer (20 mM, pH 8) containing DTT (1 mM), EDTA (1 mM). After incubation for 2 h at 37 °C, the UDG enzyme was removed by phenol-chloroform extraction and the DNA ethanol precipitated. The labeled oligonucleotide **1** with the pendent amino group was then incubated with the unlabeled, AP-containing oligonucleotide **2** in HEPES (50 mM, pH 7.4) containing NaCl (100 mM), and $NaBH_3CN$ (250 mM) at 37 °C for 24 h. The reaction mixture was combined with formamide loading buffer, and loaded onto a 0.4 mm thick, 20% denaturing polyacrylamide gel (containing 6 M urea).[32] The gel was electrophoresed for 4 h at 1600 V. The gel images obtained and the amount of radiolabeled DNA in each band on the gel quantitatively measured by storage phosphor autoradiography.[33]

Conjugation of Biotin to NH_2-ICL. The NH_2-ICL was prepared as described above except using tris(2-aminoethyl)amine linker. The resulting NH_2-ICL was treated with the biotin-*N*-hydroxysuccinimidyl ester (**12**, 3 mM) for 12 h at 4 °C in sodium bicarbonate buffer (12.5 mM, pH 8.0). The DNA was then ethanol precipitated and dissolved in sodium phosphate buffer (8 mM, pH 7.4, with 140 mM NaCl, 2 mM potassium phosphate, and 10 mM KCl) containing streptavidin (1 mg/mL) and incubated for 12 h at 37 °C. The mixture was combined with formamide loading buffer and the resulting sample loaded onto a 10% non-denaturing polyacrylamide gel (2 mm thick) and electrophoresed for 2 h at 200 V. Gel

images and quantitative measurement of the amount of radiolabeled DNA in each band were obtained by storage phosphor autoradiography.[33]

Mass spectrometric analysis of the cross-linked duplexes. LC-MS data were acquired on an Agilent Technologies 6520A Accurate Mass QTOF. Samples were analyzed according to the protocol of Studzinska and Buszewski,[34] with slight modifications as outlined. Sample was injected onto a C8 trap column (Michrom Bioresources Captrap) at a flow rate of 5 μL/min of 10 mM DMBAA, pH 7.1 over 4 min. and separated by isocratic elution (either 80% or 42.5% methanol, 15 mM DMBAA, pH 7.1) at a flow rate of 0.4 μL/min on a 10 cm x 75 μm C8 analytical column (fused silica packed with Michrom Bioresources C8, 3.5 μm particles). Following the 4 min trap load, separation on the trap/analytical columns continued for 16 min, under isocratic elution conditions. Total run time was 20 min. Mass spectra were acquired using the following parameters: negative-ion mode; VCap 2500 V; mass range 290-3200 m/z; 0.63 spectra/second; fragmentor at 300 V (250 V for IDT oligo); internal MS recalibration was achieved using the K/Na adducted Hexakis 1221 Chip Cube High Mass Reference compound (m/z 1279.99). Samples were loaded in sequence as follows: blank (10 mM DMBAA), sample, and blank. Multiply-charged DNA peaks were deconvoluted using the maximum entropy algorithm in Qualitative Analysis software (version B.07.00 Agilent Technologies) with the following parameters: adduct = proton-loss; m/z range = 600-1500 m/z; mass range = expected mass ±2 kDa; peak height to calculate mass = 25%. The m/z values reported are neutral deconvoluted masses. The cross-linking reactions were carried out as described above. After a 72 h incubation, 20 vol of ammonium acetate (2.5 M) was added. After 30

min at room temperature the mixture was desalted using a C18 Sep-Pak column eluted with HPLC grade methanol and ultrapure, deionized water.

■ RESULTS AND DISCUSSION

Background: Reductive Amination Reactions Involving AP sites in DNA. Our approach involves the use of complementary AP-containing oligodeoxynucleotides, diamines, and two sequential reductive amination reactions to generate DNA duplexes containing site-specific ICLs. AP residues in DNA exist as an equilibrium mixture of the ring-closed hemiacetal and the ring-opened aldehyde.[35] The ring-opened aldehyde form of an AP site can engage in reductive amination reactions. In this process, reaction of the AP aldehyde with an amine yields an iminium ion intermediate that is selectively reduced by a hydride reagent to yield a chemically stable alkylamine or arylamine attachment to the deoxyribose backbone (Scheme 1).[20, 36-39] This reaction has been widely employed in mechanistic studies of amine-catalyzed strand cleavage at AP sites in DNA[40-45] but, perhaps surprisingly, has seen limited use for derivatization or targeted modification of DNA.[19, 20, 36-38, 46-50] In the present studies, reductive amination reactions involving the AP aldehyde residues in DNA were carried out by incubation of the AP-containing oligodeoxynucleotides with amine-containing linkers in the presence of the water-compatible hydride reducing agent $NaBH_3CN$.[51, 52]

Generation of a Site-Specific ICL By Two Sequential Reductive Amination Reactions Involving Diamines and AP Sites On Complementary Oligodeoxynucleotides. AP sites were installed in the oligodeoxynucleotides by the treatment of the corresponding uracil-containing DNA oligomer with uracil DNA glycosylase (UDG).[37, 53-55] One of the

AP-containing strands in each cross-linking reaction was labeled with a 5'-^{32}P-phosphoryl group to enable detection of the reaction products. The ^{32}P-labeled products were analyzed by electrophoresis on denaturing 20% polyacrylamide gels and the radioactivity in each DNA band quantitatively measured by storage phosphor autoradiography.[33] To confirm installation of the AP site, piperidine work-up[56, 57] was employed to convert the full-length AP-containing oligodeoxynucleotide (displaying intermediate mobility in the gel shown in Figure 1, lane 1) to a faster-migrating, small fragment that appears near the bottom of the gel. The results showed that the UDG reaction produced the desired AP-containing oligonucleotide in nearly quantitative yield (Figure 1, lane 2).

In the first step of the cross-linking procedure, the ^{32}P-labeled, AP-containing oligonucleotide **1** was incubated in HEPES buffer (50 mM, pH 7.4, containing 100 mM NaCl) with 1,4-diaminobutane (10 mM) and $NaBH_3CN$ (250 mM) at 37 °C for 12 h, followed by ethanol precipitation of the DNA. The resulting product displayed slightly decreased gel mobility relative to the parent AP-containing oligonucleotide, consistent with the expected effects of increased mass and the partially protonated amino groups associated with the pendent diaminobutane linker (Figure 1, lane 3). Importantly, no strand cleavage product was observed, despite the fact that amines and diamines can catalyze β-elimination of the 3'-phosphoryl group from AP sites in DNA.[56-58] Evidently, under these conditions, reduction of the iminium ion intermediate by the $NaBH_3CN$ reagent is much faster than the competing β-elimination reaction that leads to strand cleavage.

In the second step of the cross-linking procedure, the single-stranded, ^{32}P-labeled oligodeoxynucleotide containing the pendent diaminobutane linker was combined with the complementary, unlabeled AP-containing oligonucleotide **2**, followed by incubation at 37

°C in HEPES buffer (50 mM, pH 7.4, containing 100 mM NaCl) with $NaBH_3CN$ (250 mM) for 12 h. This reaction provided a high yield (86±2%) of a product that migrated slowly in the denaturing gel, as expected for a cross-linked DNA duplex (Figure 1, lane 4).[37, 59, 60] The major signal observed in ESI-TOF mass spectrometric analysis of the cross-linked DNA corresponded to a deconvoluted mass of 21,311.82 amu, in close agreement with the calculated mass for the expected diaminobutane-ICL (Table 1 and Scheme 2).

A weak band (marked with a "#" symbol in lane 4 of Figure 1) can be seen in the gel migrating slightly faster than the major ICL product. We ascribe this minor product to a lower molecular weight cross-link (diamino-LMW-ICL, **3** in Figure 5) arising from a reductive amination reaction between the pendent amine linker and the 3'-α,β-unsaturated aldehyde residue generated by strand cleavage of the unlabeled AP-oligonucleotide in the second step of the cross-linking protocol (for background regarding strand cleavage via β-elimination at AP sites in DNA, see references [40, 56, 61-65] and for an analogous reductive amination involving reaction of an unsaturated aldehyde with the amino group of lysine, see reference [66]). Indeed, a relatively weak signal consistent with this low molecular weight ICL attachment was observed in the mass spectrometric analyses (Table 1, Figure S1). It is interesting that no strand cleavage of the ^{32}P-labeled oligonucleotide **1** was detected during the first step of the cross-linking protocol (lane 3, Figure 1), while formation of the low molecular weight ICL indicates that small amounts of strand cleavage can compete with reduction of the iminium ion in the unlabeled oligonucleotide during the second step of the process.

The cross-link in duplex **1**/**2** described above involves connection of AP sites that are offset by one nucleotide (nt) in the DNA duplex. We examined the effects of AP

spacing on the yield of ICL in this procedure. We found that the highest yields were obtained with 3'-offset spacings of 1 or 2 nt between the two AP sites (Figure 2). Somewhat lower yields of ICL were obtained with AP/AP offsets of 0 and 3 nt (Figure 2). A larger offset spacing of 10 nt produced a very low yield of slow-migrating ICL band (Figure 2). In general, DNA duplexes containing two AP sites on opposing strands retain an overall, B-DNA-like structure with some minor distortions near the AP residues.[67, 68] Presumably bridging distant AP sites with the 1,4-diaminobutane linker would require substantial, energetically costly distortions of the duplex structure.

We next investigated the ability of various commercially available diamines to function in the cross-linking procedure. We found that the cross-linking protocol using the duplex sequence in which AP sites are offset by 1 nt was insensitive to increases in linker length, performing equally well with 1,4-diaminobutane, 1,5-diaminopentane, and 1,6-diaminohexane (Figure 3).

Use of the Double-Reductive Amination Protocol to Generate a Cross-Linked DNA Duplex Containing a Derivatizable Amino Group. Derivatizable DNA ICLs may be useful in DNA repair studies. For example, Evison *et al*. prepared an alkynyl psoralen that generates DNA cross-links that can be derivatized using a Huisgen alkyne-azide 1,3-dipolar cycloaddition ("click") reaction.[69] To prepare a DNA duplex containing a derivatizable ICL using our sequential reductive amination protocol, we employed a symmetrical triamine reagent, tris(2-aminoethyl)amine. Use of this triamine linker in the two-step protocol gave a slow-migrating ICL band in high yield (77±3%), analogous to the ICL derived from cross-linking of duplex **1**/**2** by 1,4-diaminobutane (Figure S2). The

major signal observed in ESI-TOF mass spectrometric analysis of the cross-linked material (Table 1, Figure S1) corresponded to the deconvoluted neutral mass expected for duplex **1**/**2** containing two reductively-aminated AP sites bridged by the tris(2-aminoethyl)amine linker (depicted schematically as NH_2-ICL in Figure 4).

To demonstrate the utility of the derivatizable aminoethyl group in the NH_2-ICL, the cross-linked DNA duplex was treated with a commercial biotin *N*-hydroxysuccinimidyl (NHS) ester reagent **12** (Figure 5). Conjugation of the cross-linked duplex with the biotin-linker ensemble did not cause a significant shift in gel mobility (Figure 4, lane 3) but efficient biotinylation of the cross-link was revealed by a dramatic gel shift induced by incubation of the biotinylated ICL with the biotin-binding protein streptavidin[70] to generate the duplex-biotin-streptavidin complex (Figure 4, lane 4). In contrast, a control experiment showed that incubation of the underivatized NH_2-ICL (lacking the pendent biotin group) did not induce a gel shift (Figure 4, lane 2). Mass spectrometric analysis was consistent with the expected biotin-ICL structure (Table 1). As described above in the context of the diaminobutane ICL, we assigned the relatively weak band migrating near the ICL (marked with an "#" symbol in Figure 4) to a lower molecular weight cross-link (NH_2-LMW) analogous to **3**, arising from strand cleavage of the unlabeled AP-oligonucleotide in the second step of the cross-linking protocol. Consistent with this view, a signal consistent with this low molecular weight ICL attachment was observed in the mass spectrometric analysis (Table 1, Figure S1).

■ CONCLUSIONS

In this work, we developed a concise method for the preparation of cross-linked

DNA duplexes involving simple manipulations of deprotected, commercially available oligodeoxynucleotides. This facile, site-specific installation of cross-links into duplex DNA may be useful in diverse fields including DNA repair, materials science,[71] diagnostic medicine,[72, 73] and structural biology.[74] Our two-step reaction sequence (four steps if one counts the enzymatic generation of an AP site in each of the complementary oligonucleotides) evades multistep organic synthesis of non-canonical nucleobases that is often required for the construction of cross-linked DNA duplexes.[21, 28-31]

Our protocol employs sequential reductive amination reactions between diamine linkers and two complementary AP-containing oligodeoxynucleotides and provides good yields of cross-linked duplexes. This strategy is conceptually similar to approaches described previously involving cross-linking of aldehyde residues on non-native nucleobases by diamine linkers.[21, 22] Similarly, this approach mirrors the cross-linking of AP in duplex DNA residues with bis-aminooxy linkers via reversible oxime formation.[75] Multiple examples of interstrand cross-link formation involving reductive amination reactions between a single AP site and an NH_2-group on the opposing strand of a DNA duplex have been reported.[19, 20, 37, 47, 76]

We extended our procedure to the preparation of a cross-linked duplex bearing a derivatizable amino group. This was accomplished using the symmetrical triamine, tris(2-aminoethyl)amine. The resulting pendent aminoethyl group in the cross-link is uniquely reactive amongst all of the NH_2 groups in the duplex and can be selectively derivatized by NHS-ester or isothiocyanate reagents.[77-80] We demonstrated that the NH_2-ICL can be biotinylated using a commercially-available NHS-ester derivatizing reagent.

DNA duplexes containing a derivatizable ICL might find a variety of applications

in the identification and characterization of proteins involved in cross-link repair. For example, a cross-linked duplex derivatized with biotin could be used in biotin-streptavidin pull-down experiments[81] that identify proteins in cell extracts that associate with a cross-link or a replication fork that has been stalled by a cross-link. Similarly, conjugation of the ICL with a photoaffinity labeling agent introduced via an NHS-ester such as **13** (Figure 5) may enable the capture of proteins involved in the recognition and repair of DNA cross-links.[82-84] Finally, DNA cross-links derivatized with fluorophores could be used in Förster resonance energy transfer (FRET)[85] or microscale thermophoresis (MST)[86] experiments designed to characterize ICL-binding proteins and protein complexes.

■ Supporting Information

The Supporting Information is available free of charge via the Internet at http://pubs.acs.org.

Mass spectrometric analysis of AP-AP cross-linked duplexes and gel electrophoretic analysis of the reactions generating the tris(2-aminoethyl)amine ICL.

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Notes.

The authors declare no conflicts of interest

■ ACKNOWLEDGEMENTS

We are grateful to the National Institutes of Health (ES021007) and the National Science Foundation (NSF-CHE 1808672) for support of this work. In addition, we thank Amanda Wallace for assistance with data analysis and Jay S. Jha for critical review of the manuscript.

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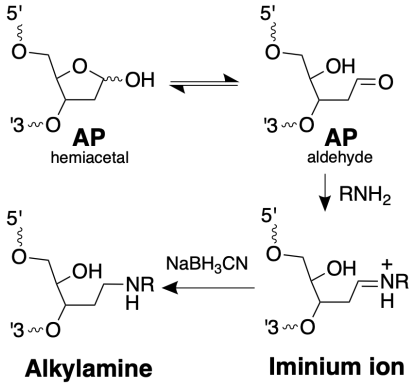
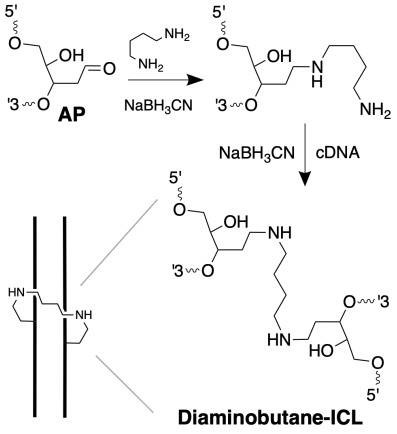
Scheme 1



Scheme 1. Reductive amination reactions involving an amine and a DNA abasic (AP) site yield a chemically stable alkylamine attachment to the deoxyribose backbone.

Scheme 2



Scheme 2. Sequential reductive amination reactions involving 1,4-diaminobutane and complementary AP-containing oligodeoxyribonucleotides produce high yields of a site-specific AP-AP ICL in duplex DNA. cDNA = complementary AP-containing strand.

Figure 1

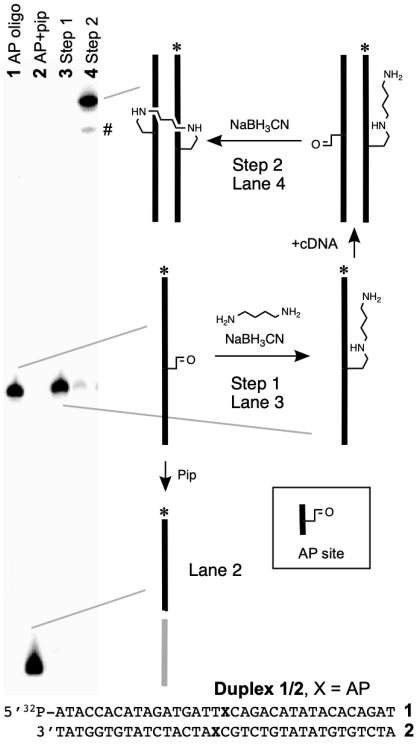


Figure 1. Synthesis of a DNA duplex containing a site-specific interstrand cross-link via sequential reductive amination reactions involving a 1,4-diaminobutane linker and complementary AP-containing oligodeoxynucleotides. The 5'-^{32}P-labeled oligonucleotides were resolved by electrophoresis on a 20% denaturing polyacrylamide gel and the radioactivity in each band visualized and quantitatively measured by storage phosphor autoradiography.[33] Lane 1: 5'-^{32}P-labeled AP-containing oligodeoxynucleotide **1** as a size marker. Lane 2: Treatment of the AP-containing oligodeoxynucleotide **1** with piperidine (1 M, 95 °C, 25 min) to generate the strand cleavage product containing a 3'-phosphoryl group.[56, 58] Lane 3: Reaction of the 5'-^{32}P-labeled AP-containing oligodeoxynucleotide **1** with 1,4-diaminobutane and $NaBH_3CN$ (250 mM) at 37 °C for 12 h in HEPES buffer (50 mM, pH 7.4) containing NaCl (100 mM). Lane 4: Generation of the cross-linked duplex **1**/**2** by reaction of the 5'-^{32}P-labeled oligodeoxynucleotide bearing the pendent amino group (the product from lane 3) and the complementary AP-containing oligodeoxynucleotide **2** with $NaBH_3CN$ (250 mM) at 37 °C for 24 h in HEPES buffer (50 mM, pH 7.4) containing NaCl (100 mM). The asterisk (*) indicates a 5'-^{32}P-phosphoryl group. The minor band marked by the "#" symbol was assigned to a small amount of a lower molecular weight ICL (**3**) resulting from cross-linking of the pendent amine produced in the first step to a 3'-α,β-unsaturated sugar remnant generated by a small amount of strand cleavage of the unlabeled AP-containing oligonucleotide **2**.

Figure 2

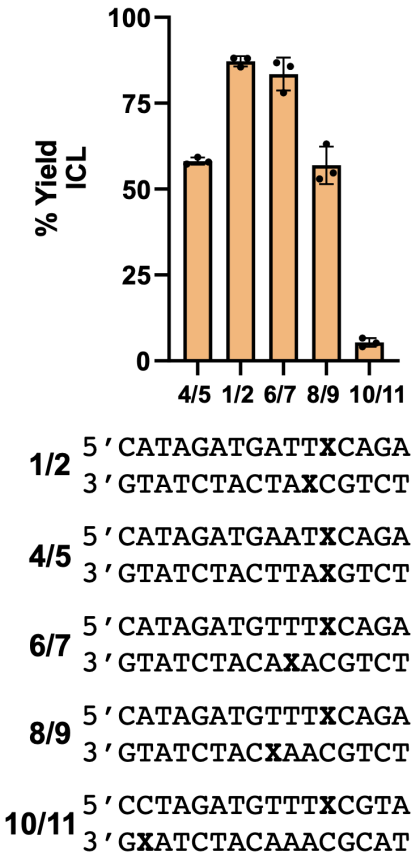


Figure 2. Effects of AP/AP spacing in duplex DNA on the yields of AP-AP ICL formation with the 1,4-diaminobutane linker. X = AP in the sequences shown. The 16 nucleotide, AP-containing sequences shown above are part of 35 nucleotide duplexes with the same flanking sequences shown for duplex **1**/**2** at the bottom of Figure 1. The error bars represent the standard deviation calculated from at least three separate measurements.

Figure 3

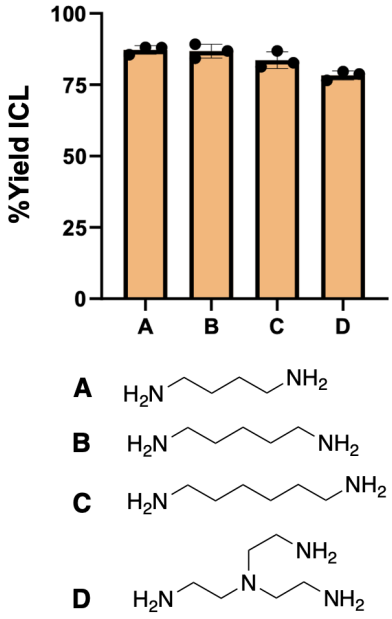


Figure 3. Various linkers function effectively in the AP/AP cross-linking protocol using duplex **1**/**2** (shown above in Figure 1). The linkers from top to bottom are 1,4-diaminobutane (**A**), 1,5-diaminopentane (**B**), 1,6-diaminohexane (**C**), and tris(2-aminoethyl)amine (**D**). The error bars represent the standard deviation calculated from at least three separate measurements.

Figure 4

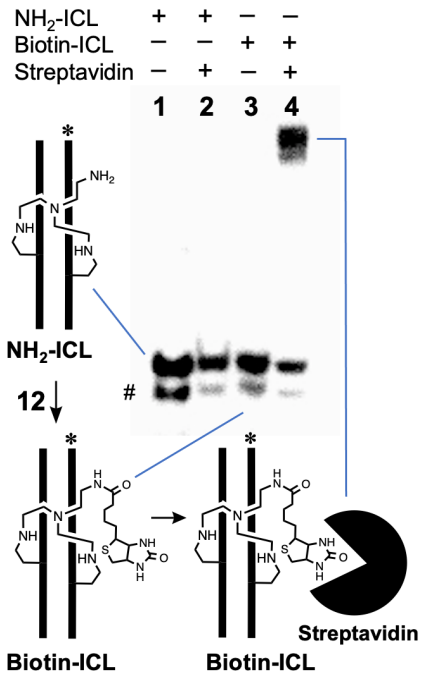


Figure 4. Conjugation of biotin to the derivatizable NH_2-ICL. In these experiments, the 5'-^{32}P-labeled NH_2-ICL (prepared using the tris(2-aminoethyl)amine linker) was analyzed by electrophoresis on a 20% denaturing polyacrylamide gel and the radioactivity in each band visualized and quantitatively measured by storage phosphor autoradiography.[33] The schematic diagram simplifies the chemical structure of the biotin-linker conjugate. The actual structure of the biotin-NHS reagent **12** showing the complete linker is shown below. The asterisk (*) indicates a 5'-^{32}P-phosphoryl group. Lane 1: 5'-^{32}P-labeled NH_2-ICL as a marker lane. Lane 2: Control experiment in which NH_2-ICL was incubated with the biotin-binding protein streptavidin for 12 h at 37 °C. Lane 3: Reaction of NH_2-ICL with the biotin-NHS reagent **12** in sodium bicarbonate buffer (12.5 mM, pH 8) for 12 h at 4 °C. Lane 4: Incubation of the biotin-NHS-treated NH_2-ICL with streptavidin for 12 h at 37 °C, to generate the gel-shifted NH_2-ICL-biotin•streptavidin complex. The minor band marked by the "#" symbol was assigned to a small amount of a lower molecular weight ICL resulting from cross-linking of the pendent diamine produced in the first step to the 3'-α,β-unsaturated sugar remnant generated by a small amount of strand cleavage of the unlabeled AP-containing oligonucleotide **2**.

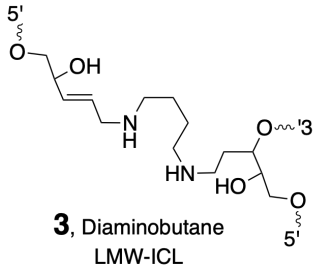
Table 1. Mass spectrometric characterization of cross-linked duplexes.

ICL-Containing Duplex[a,b]	Observed Mass	Calculated Mass
Diaminobutane-ICL	21311.82	21,312.03
NH_2-ICL	21369.78	21,370.11
Biotin-ICL	21,843.85	21,843.70
Diaminobutane-LMW[c]	16,062.35	16062.61
NH_2-LMW	16,120.06	16,120.70

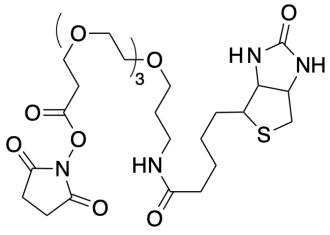
[a] Cross-linked duplexes for mass spectrometric analyses were prepared using the protocol described in the Experimental Procedures except without 5'-^{32}P labels.

[b] Cross-links were prepared in duplex **1**/**2** (see Figure 1 for sequence).

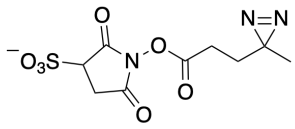
[c] LMW-ICLs appear as weaker signals in the MS analyses.



3, Diaminobutane LMW-ICL



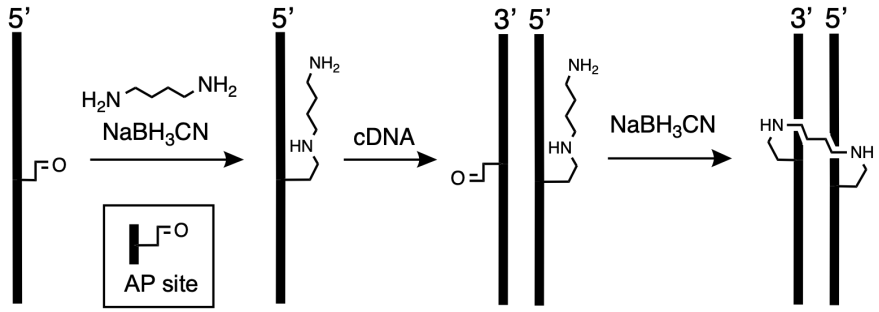
12, NHS-Biotin



13, NHS-Diazirine

Figure 5. Structures discussed in this work.

TOC Graphic


5'
O
H2N
NH2
NaBH3CN
AP site
5'
NH2
HN
cDNA
3' 5'
O
NH2
HN
NaBH3CN
3' 5'
HN
NH