Supporting Information to Accompany Manuscript ja036105.pdf

Long Range 1,4 and 1,6-Interstrand Cross-Links Formed by a Trinuclear Platinum complex. Minor Groove Pre-association Affects Kinetics and Mechanism of Cross-Link Formation as well as Adduct Structure

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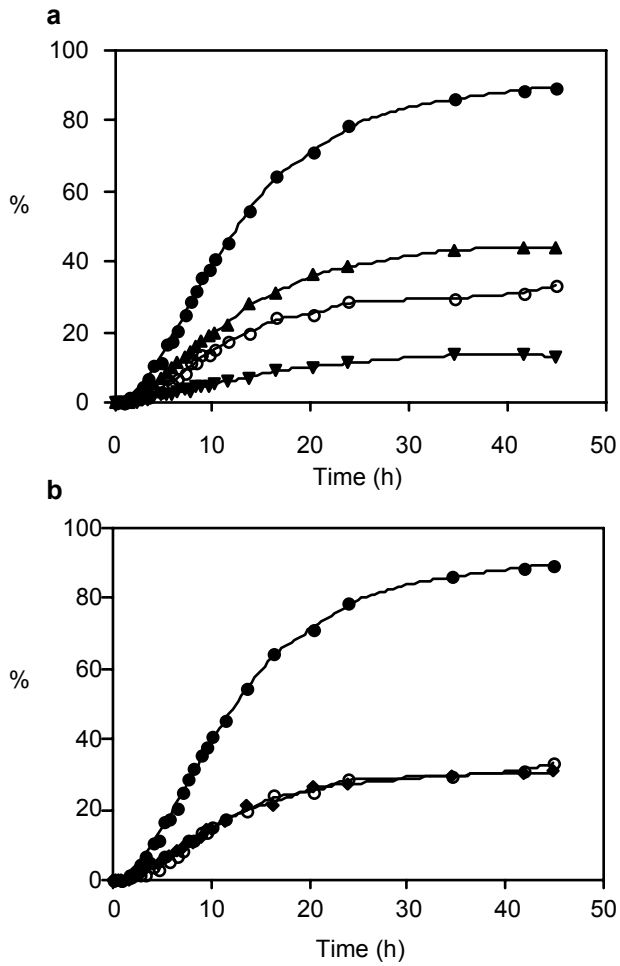

Figure S1. a) Plot of the relative peak volumes of peaks in the Pt-NH_3 (end) region of the [^{1}H,^{15}N] NMR spectra during the reaction between the 1,4-GG sequence (**I**) and ^{15}N-**1**. Only peaks assignable to the two conformers **X** and **Y** of the bifunctional adduct (**5**) are shown. Labels: ▲ sum of peaks at δ 4.37/-60.6 and 4.35/-60.9 (**X** and **Y** Pt-NH_3 groups B_1 and B_2), ○ sum of broad peaks at δ ca 4.28-4.31/-60.9 (**X** Pt-NH_3 groups A_1 and A_2), ▼ sum of peaks at δ 4.57/-59.7 and 4.51/-59.9 (**Y** Pt-NH_3 groups A_1 and A_2), ● total of Pt-NH_3 (end) peaks assignable to conformers **X** and **Y**. In b) the volume of the peaks at δ 4.57/-59.7 and 4.51/-59.9 (i.e. ▼ in plot (a)) has been subtracted from those at δ 4.37/-60.6 and 4.35/-60.9 (▲ in plot (a)) to give the new curve with label ◆. The close correspondence between the two curves ◆ and ○ (i.e. conformer **X** Pt-NH_3 groups B_1/B_2 and A_1/A_2, respectively) provides good evidence that the B_1 and B_2 groups of **5X** and **5Y** are indistinguishable and both contribute to the same two peaks at δ 4.37/-60.6 and 4.35/-

S2 Scientist Kinetic Model 1,0,1/t,t,t + 1,4-GG MODEL 1

```
// MicroMath Scientist Model File
// 101ttt + 12-mer 1,4-interstrand duplex
// MODEL 2 - includes "other products"
IndVars: T
DepVars: A, B, C, D,  L, E, F, G
Params: KAB, KBC, KCD, KCE
A'=-KAB*A
B'=KAB*A-KBC*B*L
C'=KBC*B*L-KCD*C-KCE*C
D'=KCD*C
E'=KCE*C
L'=-KBC*B*L
// A=1,0,1/t,t,t B=aqua/Cl, C=G/Cl, D= sum prod, E = other prod, F= conformer Y, G = conformer X
//L=12-mer 1,4-interstrand duplex
// Initial conditions
T=0.0
A=0.0016
B=0.0
C=0.0
D=0.0
E=0.0
F=0.0
G=0.0
L=0.00296
***
```

S3 Scientist Kinetic Model 1,0,1/t,t,t + 1,4-GG MODEL 2

```
// MicroMath Scientist Model File
// 101ttt + 12-mer 1,4-interstrand duplex
// MODEL 3 - includes conformers and other products
IndVars: T
DepVars: A, B, C, D,  L, E, F, G
Params: KAB, KBC, KCF, KCG, KCE
A'=-KAB*A
B'=KAB*A-KBC*B*L
C'=KBC*B*L-KCF*C-KCG*C-KCE*C
E'=KCE*C
F'=KCF*C
G'=KCG*C
L'=-KBC*B*L
// A=1,0,1/t,t,t B=aqua/Cl, C=G/Cl, D= sum prod, E = other prod, F= conformer Y, G = conformer X
//L=12-mer 1,4-interstrand duplex
// Initial conditions
T=0.0
A=0.0016
B=0.0
C=0.0
D=0.0
E=0.0
F=0.0
G=0.0
L=0.00296
***
```

S4 Scientist Kinetic Model 1,0,1/t,t,t + 1,6-GG MODEL 1

```
// MicroMath Scientist Model File
// 101ttt + 12-mer 1,6-interstrand duplex
// MODEL 2 - both monfunctionals and products
// minor mf leads to major prod
// OLIGO Conc. given by Kallansrud conversion
IndVars: T
DepVars: A, B, C, D,  L, E, F
Params: KAB, KBC, KCD, KCF, KBE, KED
A'=-KAB*A
B'=KAB*A-KBC*B*L-KBE*B*L
C'=KBC*B*L-KCD*C-KCF*C
D'=KCD*C+KED*E
E'=KBE*B*L-KED*E
F'=KCF*C
L'=-KBC*B*L-KBE*B*L
// A=1,0,1/t,t,, B=aqua/Cl, C=G/Cl, D= G/G, E = other mf, F= other prod
//L=12-mer 1,6-interstrand duplex
// Initial conditions
T=0.0
A=0.0019
B=0.0
C=0.0
D=0.0
E=0.0
F=0.0
G=0.0
L=0.00262
***
```

S5. Effect of Temperature on the 1,4 and 1,6 interstrand cross-links. In order to study the effect of temperature on the 1,4- and 1,6-interstrand cross-links ^{1}H and [^{1}H,^{15}N] spectra were recorded between 288 and 308 K (see Figures S6, S7 and S8). In the [^{1}H,^{15}N] NMR spectra no significant differences were found for the adducts between 288 and 298 K. The ^{1}H shifts of the Pt-NH_3 end and linker groups are generally more deshielded at the lower temperature consistent with H-bonding influencing the shifts in the ^{1}H dimension. In case of the 1,4-cross-link, no evidence is found for inter-conversion of the major and minor conformers over the entire temperature range, although a series of changes in ^{1}H and ^{15}N shifts is indicative of changes in environment. Changes in the H8 resonances for the two conformers provide a similar interpretation (Figure S8).

Minor changes are observed in the Pt-NH_3 region for the 1,6 cross-link (Figure S7), particularly at the highest temperature (308 K) suggesting less conformational flexibility for the longer cross-link. The major difference is the presence of strongly deshielded peaks for the linker NH_3 and NH_2 groups. The Pt-NH_3 peak shows a temperature dependence consistent with H-bonding but the shift of the Pt-NH_2 peak is insensitive to changes in temperature over this range.

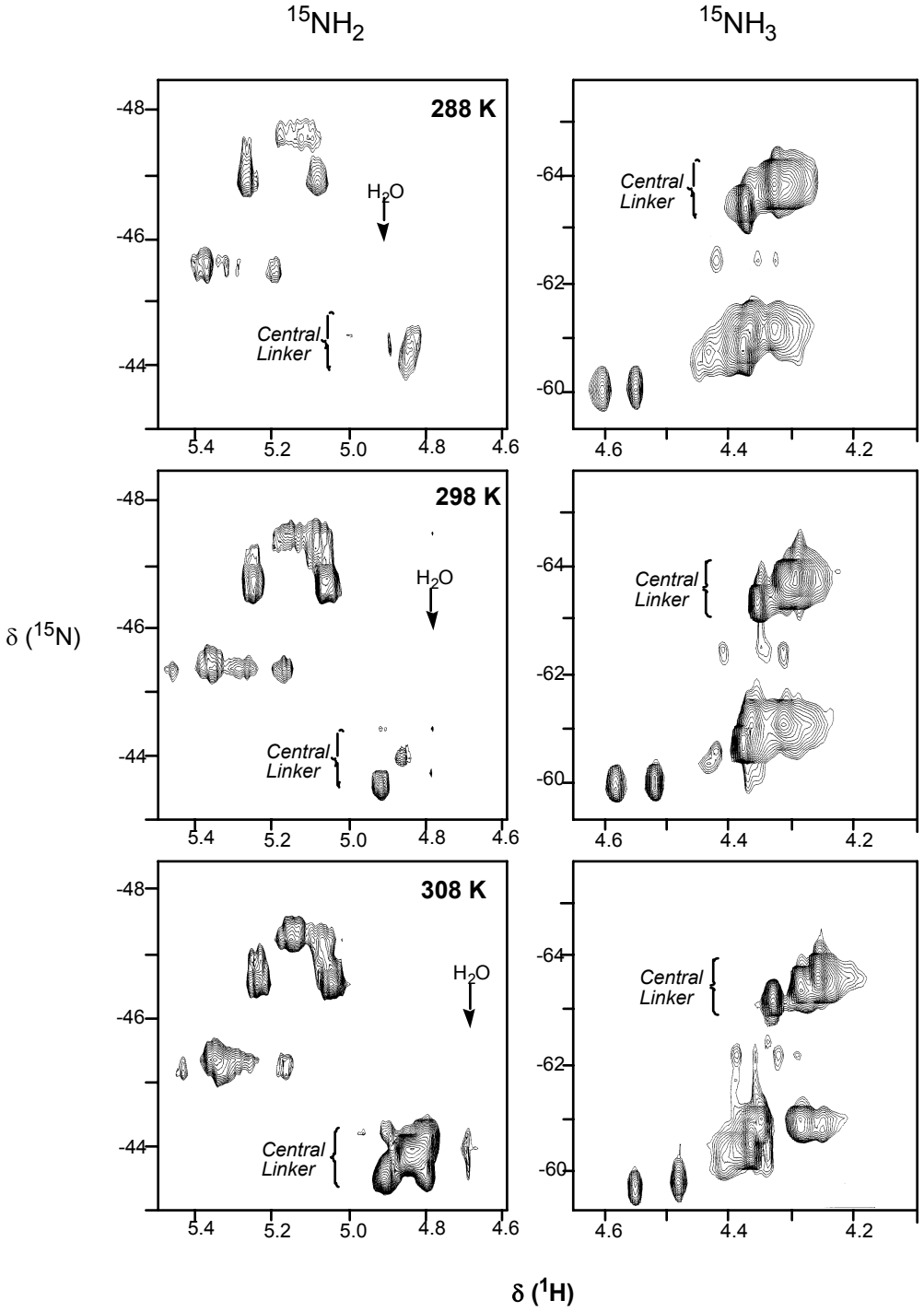

Figure S6. [^{1}H,^{15}N] HSQC NMR (600 MHz) spectra of the final product of the reaction of the 1,4-GG sequence (**I**) and ^{15}N-**1** at 288, 298 and 308 K. The only significant change is for the peaks assigned to the Pt-NH_3 A_1/A_2 environments of the major conformer (**5X**) at 308 K (see Figure 1a). There is no evidence for interconversion between the two conformers. The apparent change in the Pt-NH_2 linker region at the higher temperature is largely due to a shift in the position of the $^{1}H_2O$ peak and the appearance of peaks that had previously been suppressed. It is evident that the linker amine protons are in a very different environment to those in the 1,6-interstrand cross-link (see Figures1b and S7).

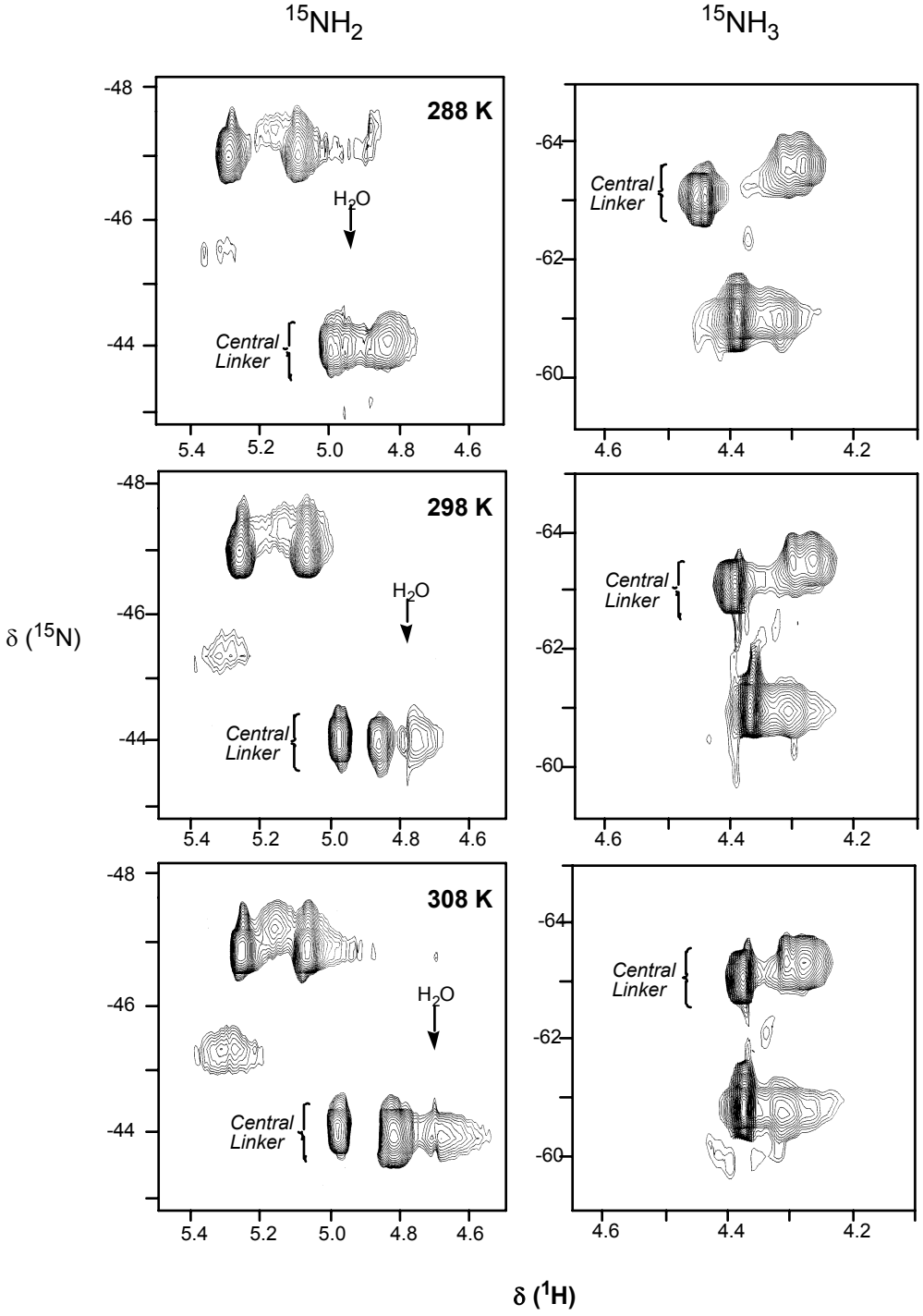

Figure S7. [^{1}H,^{15}N] HSQC NMR (600 MHz) spectra of the final product of the reaction of the 1,6-GG duplex (**II**) and ^{15}N-**1** at 288, 298 and 308 K.

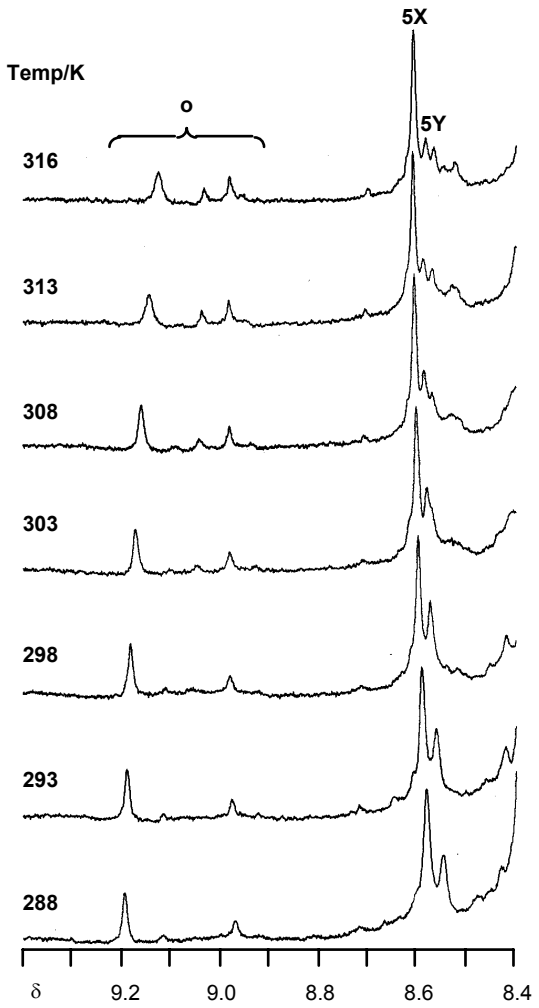

Figure S8. Aromatic region of the ^{1}H NMR spectra (600 MHz) of the final product of the reaction of the 1,4-GG duplex (**I**) and ^{15}N-**1** at temperatures between 288 and 316 K. The peaks are assigned to the H8 resonances of the G5 and G5' bases coordinated to platinum in the the two conformers (**X** and **Y)** of the 1,4-interstrand cross-link (**5**). As found for the [^{1}H,^{15}N] spectra there is no evidence for interconversion between the two conformers with temperature. However at temperatures above 300 K the minor peak (δ 8.58) splits into two new peaks (δ 8.59, 8.57), suggesting that the minor conformer undergoes a structural change that influences the environment of the two coordinated guanine residues (G5 and G5'). The process is reversible on decreasing the temperature.

S9 HPLC Gradient Profile used for the elution of the DNA adducts in the reactions of 1 with I and II

	Time (min)	Flow Rate (mL min^{-1})	%A	%B
Analytical Column	0	1	93	7
	20	1	87	13
	25	1	93	7
	30	1	93	7
Preparative Column	0	6	93	7
	20	6	87	13
	25	6	93	7
	40	6	93	7

Solvent A is 50mM NH_4OAc (pH=5.4) and Solvent B is HPLC grade MeCN. Both solvents were filtered through 0.2 μm filtres and degassed (sparged) using Helium.

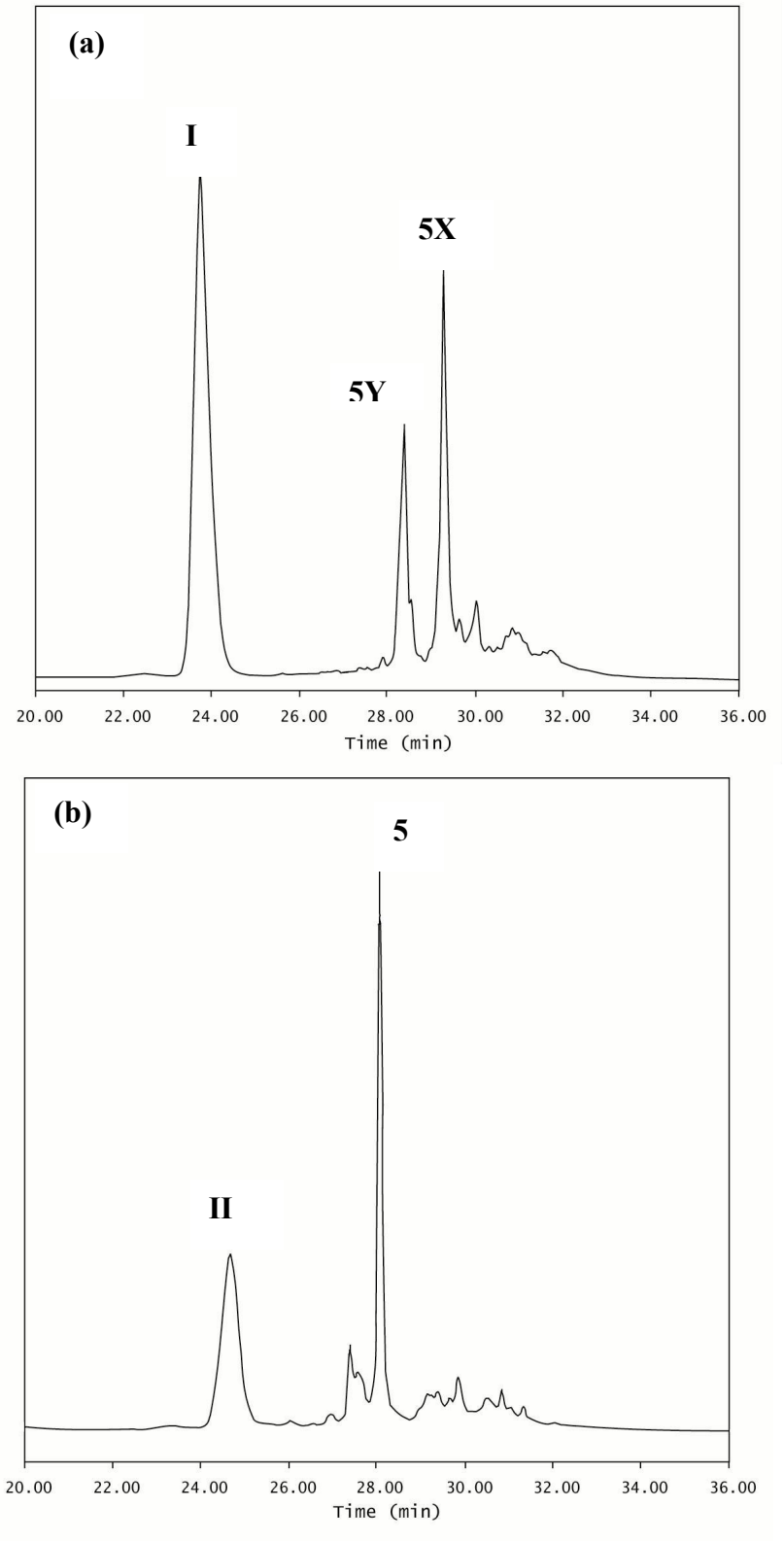

Figure S10: HPLC chromatograms (preparative column) of the final products of the reactions of **1** with duplex **I** (a) and **II** (b) (detection wavelength 254 nm). In (b) the sample is the same as used for the NMR experiment (^{15}N-**1**) whereas in (a) the reaction was repeated under identical conditions to the NMR experiment, but with unlabeled **1**. Peaks for unplatinated **I** and **II** are consistent with the excess DNA used in the two reactions. In (a) the two major adduct peaks (**5X**) and (**5Y**) are assumed to correspond to the two conformers of the bifunctional adduct (**5**). The two fractions were separated and in both cases the ESI mass spectrum obtained was consistent with a cross-linked adduct of **1** and the duplex **I** (calcd molecular weight 8203.1 a.m.u.) as the major species. In (b) there is only one major adduct peak consistent with the observation of only one major bifunctional product in the NMR experiment. The ESI mass spectrum of the purified adduct was consistent with a cross-linked adduct of ^{15}N-**1** and the duplex **II** (calcd molecular weight 8213.1 a.m.u.). (See text).

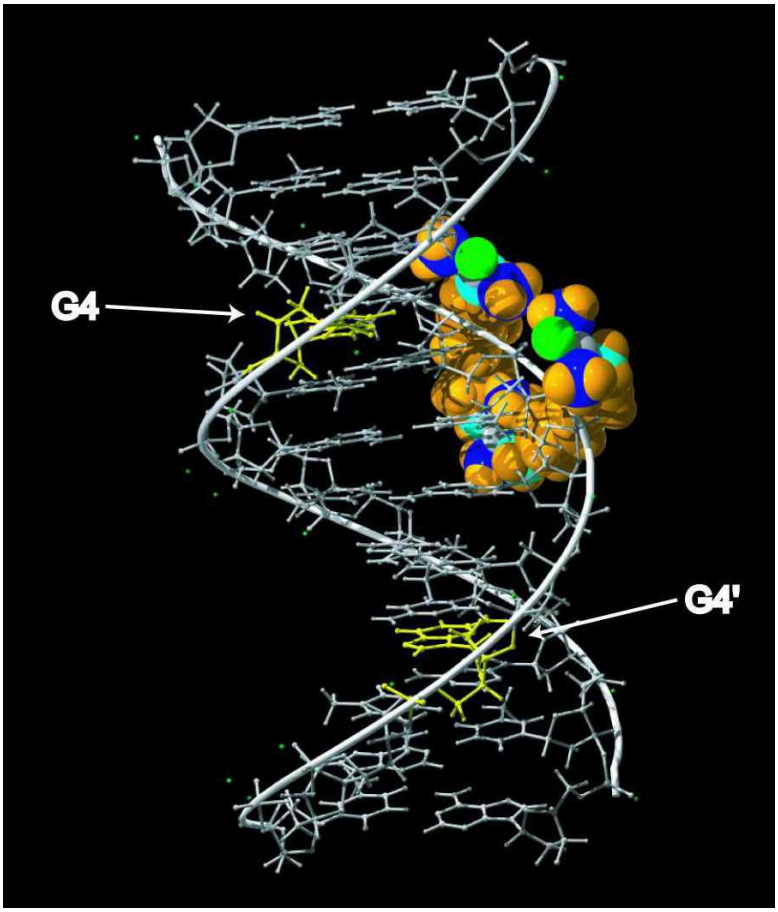

Figure S11. Molecular model illustrating the pre-association of **1** with duplex **II.** The greater distance between the two G bases compared to duplex **I** dictates that the linker cannot remain in the initial position in the minor groove on formation of the 1,6-interstrand cross-link. The reorientation of the central linker between the pre-associated and monofunctionally bound forms may be the key factor that determines that only one conformer is formed in this case.