Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Observation of the Reversibility of a Covalent Pyrrolobenzodiazepine (PBD) DNA Adduct by HPLC/MS and CD Spectroscopy

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Supporting Information

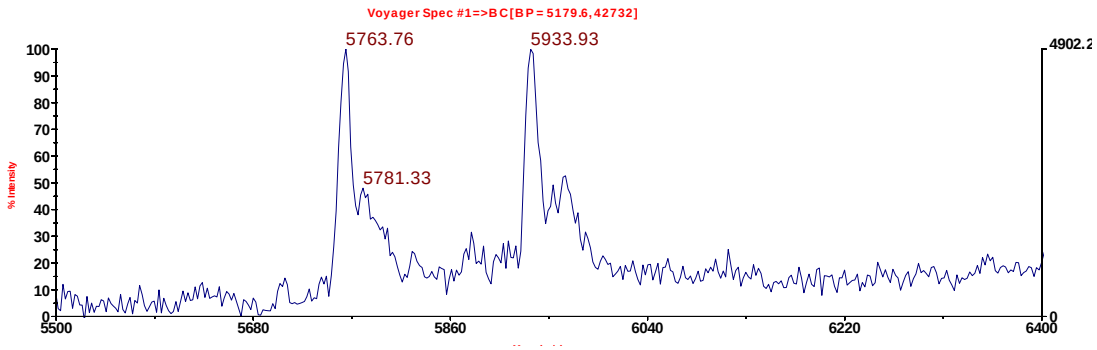

Figure S1: MALDI-TOF MS spectrum showing **1**/*Seq-1* adduct mass. Expected mass 5764.12, observed mass 5763.76

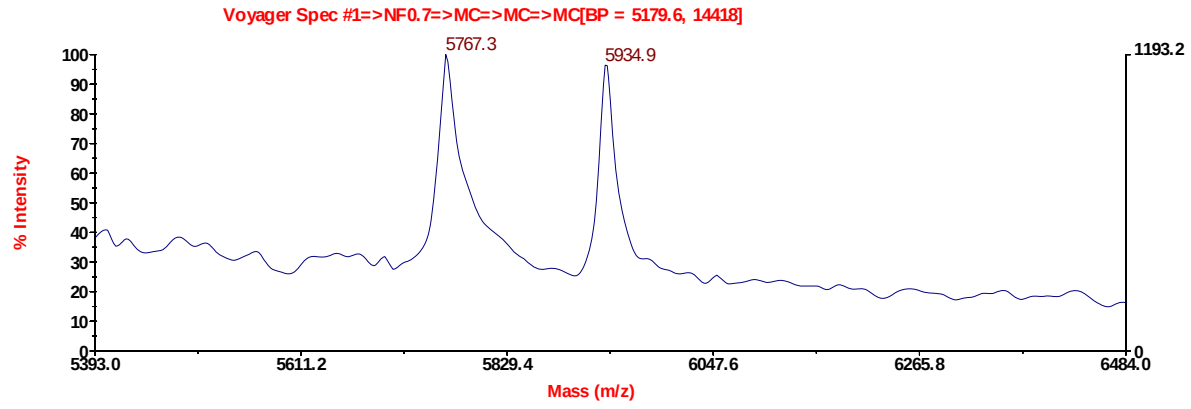

Figure S2: MALDI-TOF MS spectrum showing **1**/*Seq-2* adduct mass. Expected mass 5768.04, observed mass 5767.3

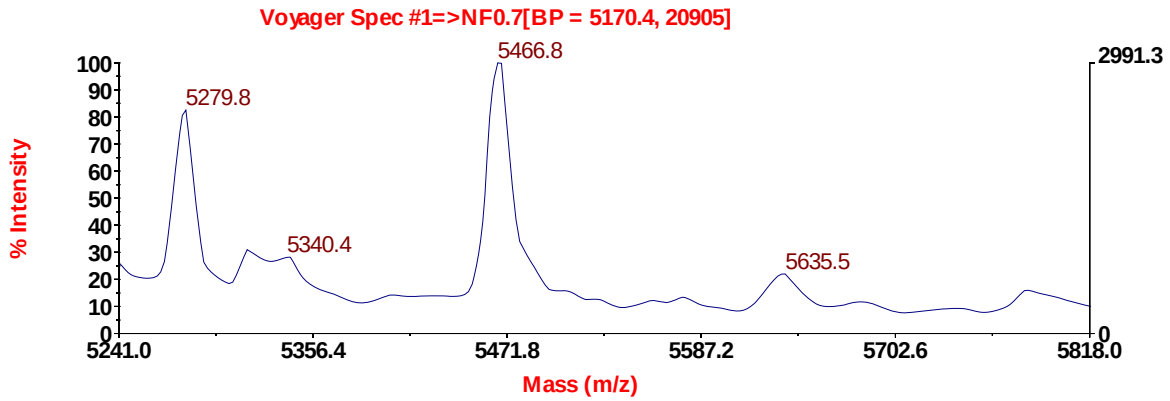

Figure S3: MALDI-TOF MS spectrum showing **2**/*Seq-1* adduct mass. Expected mass 5470.8, observed mass 5466.8

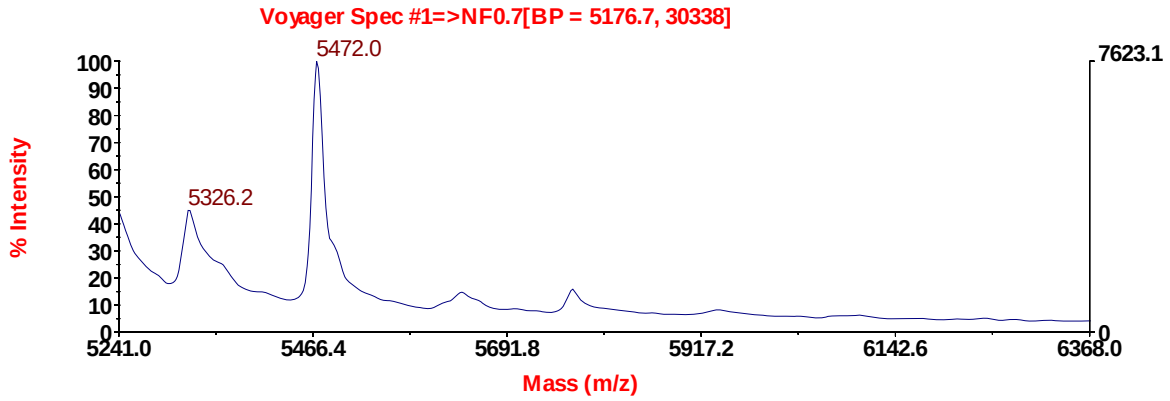

Figure S4: MALDI-TOF MS spectrum showing **2**/*Seq-2* adduct mass. Expected mass 5474.72, observed mass 5472.0

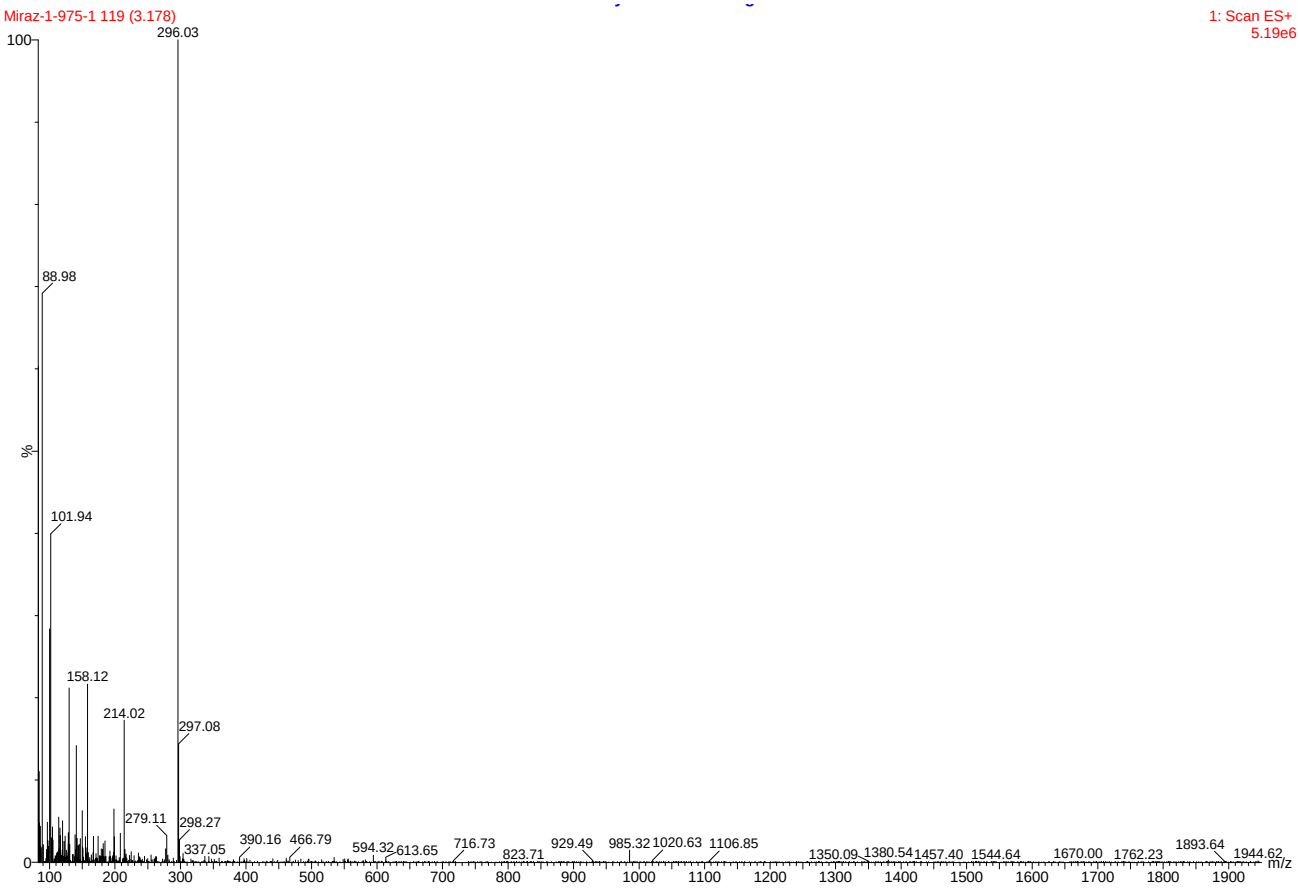

Figure S5: EIMS spectrum of RT 29.9 mins peak (Figure 5D, main manuscript) showing m/z 296.03 which corresponds to [M+H$^+$] of C11a-C1 dehydro analogue of anthramycin N10-C11 imine.

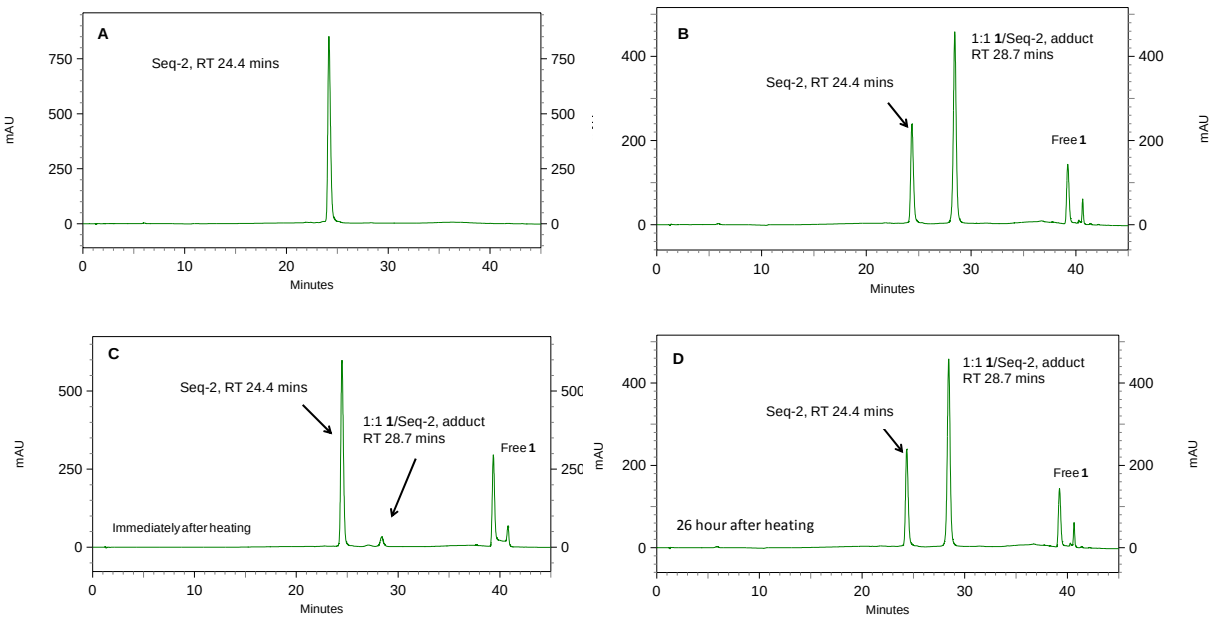

Figure S6: HPLC chromatograms: **A**, *Seq-2*, RT at 24.4 mins; **B**, *Seq-2* 24 hours after incubating with **1** showing 1:1 **1**/*Seq-2* adduct at 28.7 mins, the reaction was not complete even after 24 hours. **C,** 1:1 **1**/*Seq-2* adduct immediately after heating period showing almost all adduct has reverted back to *Seq-2*. **D**, Chromatogram obtained 26 hours post-heating period showing slowness of adduct re-formation.

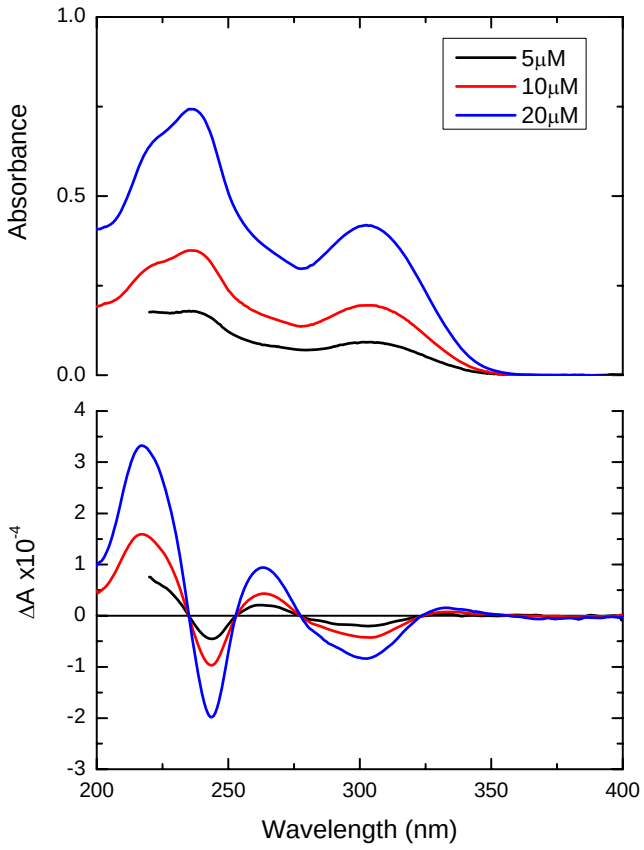

Figure S7: UV and CD spectra of GWL-78 in phosphate buffer

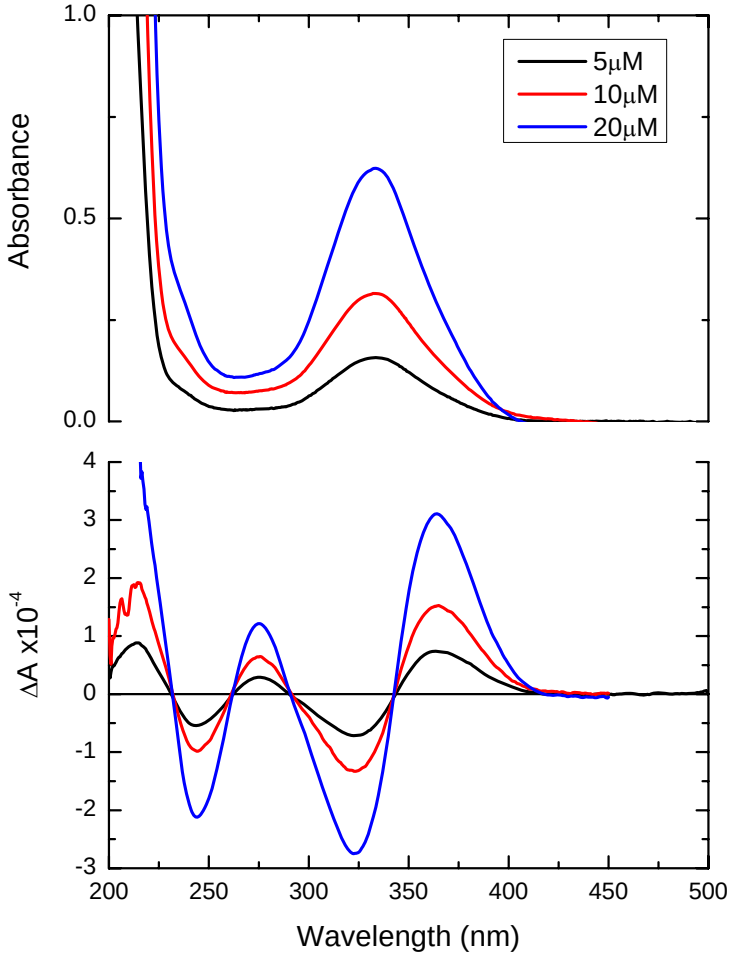

Figure S8: UV and CD spectra of anthramycin in phosphate buffer

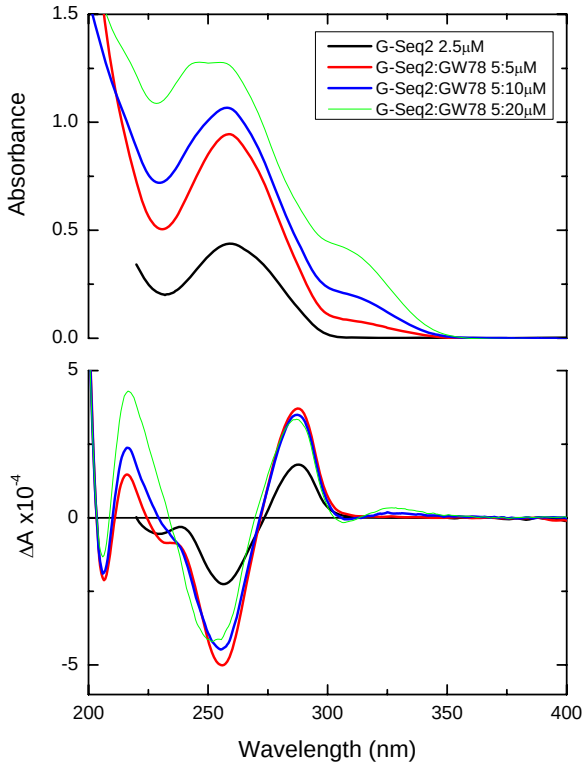

Figure S9: UV and CD spectra of Seq-2 as a function of GWL-78 (**1**) in phosphate buffer

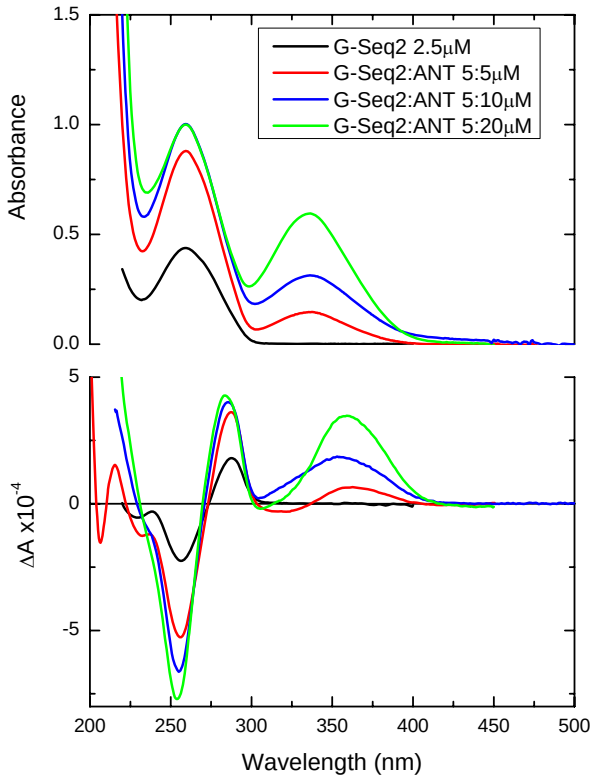

Figure S10: UV and CD spectra of Seq-2 as a function of anthramycin (**2**) in phosphate buffer

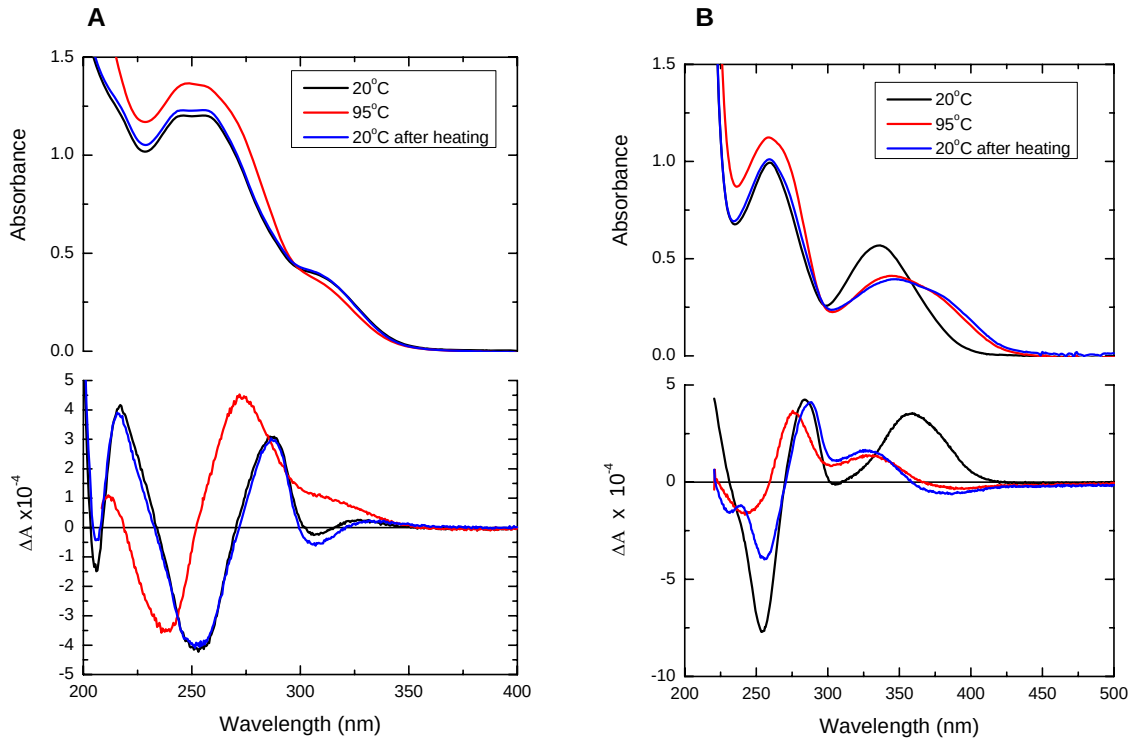

Figure S11. **A**, UV and CD spectrum of the 4:1 **1**:*Seq-2* adduct at 20°C (black), after heating to 90°C (red) and then cooling to 20°C (blue); **B**, UV and CD spectrum of the 4:1 **2**:*Seq-2* adduct at 20°C (black), after heating to 90°C (red) and then cooling to 20°C (blue). Note that drug-induced negative CD signal at 305 nm re-appeared after cooling for **1,** but the drug-induced CD signal at 360 nm for **2** did not re-appear post-heating thus confirming permanent detachment of **2** from the DNA.

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