Synthesis and Purification of DHU-14. DHU-14 was synthesized on an ABI synthesizer, deprotected using 50 mM K_2CO_3 in anhydrous methanol for 5-7 hours at room temperature, and lyophilized to dryness. After purification by BioRad reverse phase HPLC (buffer A = 50 mM TEA-acetate, buffer B = 50% (V/V) 35 mM TEA-acetate/CH_3CN; 10-70% B in 120 min), the 5'-trityl **DHU-14** was detritylated using 5% acetic acid at room temperature for 15 min. **DHU-14** was lyophilized and purified a second time by reverse phase HPLC and desalted on a Pharmacia FPLC using Hi-Trap desalting columns and deionized water. Further purification by ion exchange chromatography (Pharmacia Mono Q HR 16/10 FPLC; buffer A = deionized water, buffer B = 2M NaCl pH 7.0) was necessary to remove organic and ionic impurities. **DHU-14** was lyophilized to dryness then dissolved in 0.5 mL 90%H_2O/10%D_2O pH 6.5 or 99.98% D_2O pH 6.5 for NMR experiments.

Mass Spectrometry. MALDI mass spectrometry was performed on a Bruker Reflex II instrument (reflectron MALDI-TOF) on trityl-on and trityl-off **DHU-14** at the Centers for Disease Control and Prevention Biotechnology Facility (Atlanta, GA).

NMR Spectroscopy. All one dimensional ^1H NMR experiments were performed on a GE GN-Omega 600 MHz spectrometer. All reported two dimensional NMR experiments were performed on a Varian 800 MHz spectrometer at the Complex Carbohydrates Research Center at the University of Georgia (purchased by the Georgia Research Alliance). Spectra were processed using Felix 2.3 from Biosym, Inc. on a Silicon Graphics Personal Iris or INDY workstation. All NMR spectra were collected at 5 °C unless otherwise noted. Exchangeable proton 1D NMR spectra of **DHU-14** and **C-14** in 0.5 mL 90% H_2O/10% D_2O at pH 6.5 were recorded using a 1331 solvent suppression sequence.[1] The 1D NOE experiments used a 2 s presaturation before the 11 solvent suppression sequence.[2] WATERGATE[3] Nuclear Overhauser Effect Spectroscopy (NOESY)[4] experiments were recorded in phase-sensitive mode[5] (150 ms mixing time). NOESY experiments collected in 99.98% D_2O were recorded in phase-sensitive mode (250 mixing time). The Correlation Spectroscopy (COSY)[6] experiment and the Total Correlation Spectroscopy (TOCSY)[7] experiment were collected in D_2O at pH 6.5.

References

(1) Hore, P. J. *J. Magn. Reson.* **1983**, *55*, 283-300.
(2) Plateau, P.; Gueron, M. *J. Am. Chem. Soc.* **1982**, *104*, 7310-7311.
(3) Piotto, M.; Saudek, V.; Sklenar, V. *J. Biomol. NMR* **1992**, *2*, 661-665.
(4) Macura, S.; Huang, Y.; Suter, D.; Ernst, R. R. *J. Magn. Reson.* **1981**, *43*, 259-281.
(5) Bax, A.; Subramanian, S. *J. Magn. Reson.* **1986**, *67*, 565-569.
(6) Bax, A.; Freeman, R.; Morris, G. *J. Magn. Reson.* **1981**, *42*, 164-168.
(7) Braunschweiler, L.; R., E. R. *J. Magn. Reson.* **1983**, *53*, 521-528.

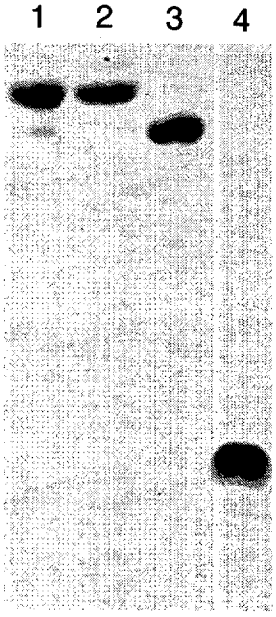


Figure S1. UV-shadowed non-denaturing 20% polyacrylamide gel with the self-complementary duplexes **C-14** (lane 1), **DHU-14** (lane 2), **G3-D** (5'-d(ATGGGTACCCAT)-3', lane 3), and the hairpin **G3-H** (5'-d(ATGGGTTCCCAT)-3', lane 4).

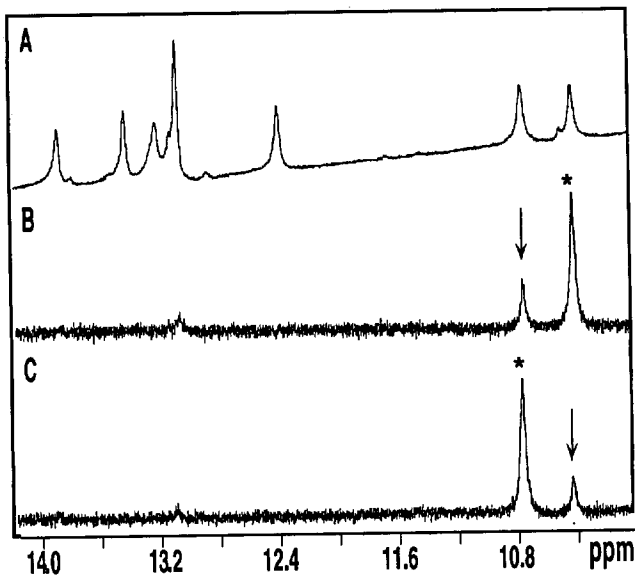


Figure S2. 1D NOE experiments of the imino proton region 2 mM (strands) **DHU-14** at pH 6.5 at 5 °C. The A spectrum shows the entire imino proton region. B and C are the difference spectra. Arrows in B and C indicate the magnetization transfer observed, and an asterisk marks the frequencies of presaturation.

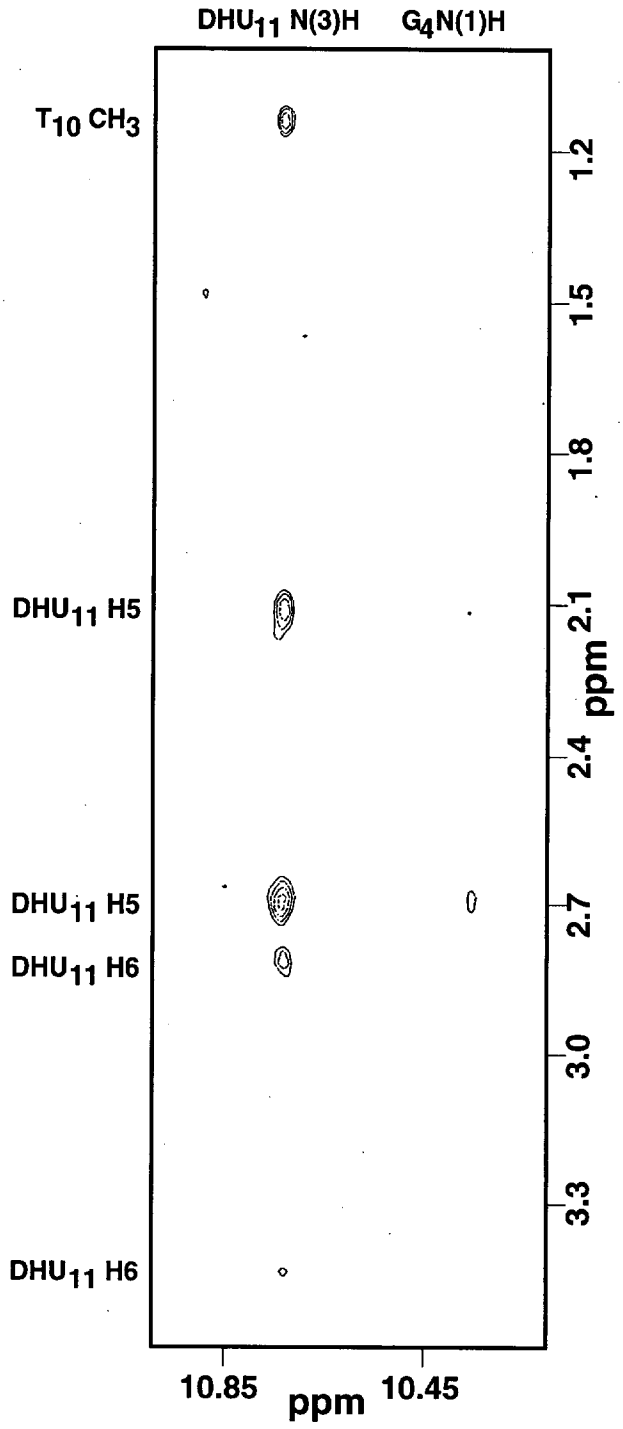


Figure S3. DHU_{11} and G_4 imino signals/$T_{10}CH3$ and $DHU_{11}H5/H6$ signal NOE cross-peaks found in the WATERGATE NOESY (150 ms mixing time) of 2 mM (strands) DHU-14 at pH 6.5 at 5 °C.

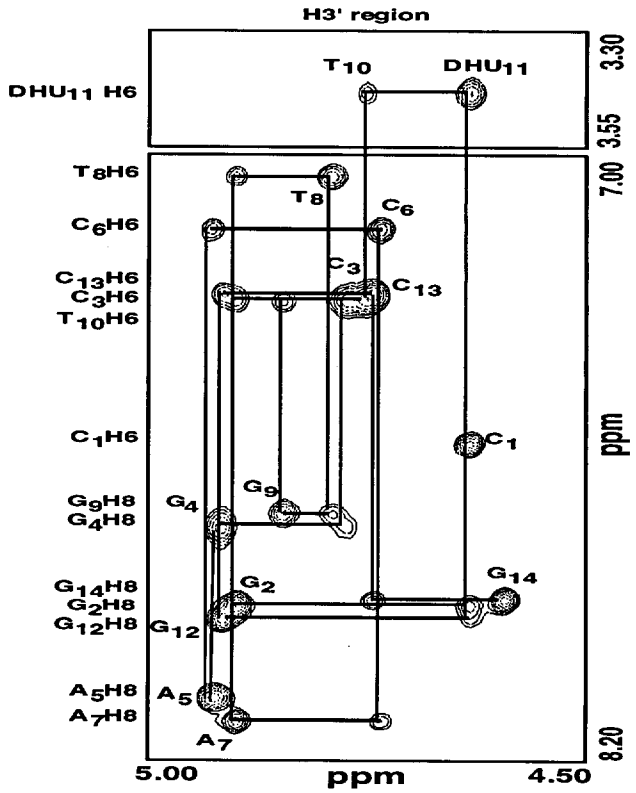


Figure S4. Base/H3' region of the NOESY spectrum (250 ms mixing time) of **DHU-14** in 99.98% D_2O at pH 6.5 at 5 °C.

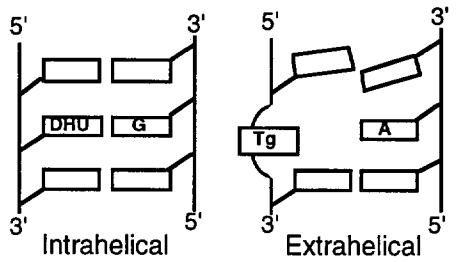


Figure S5. Schematic representation of the intrahelical DHU-G wobble base pair in **DHU-14** vs. the extrahelical Tg found previously by Kung et. al.[16]

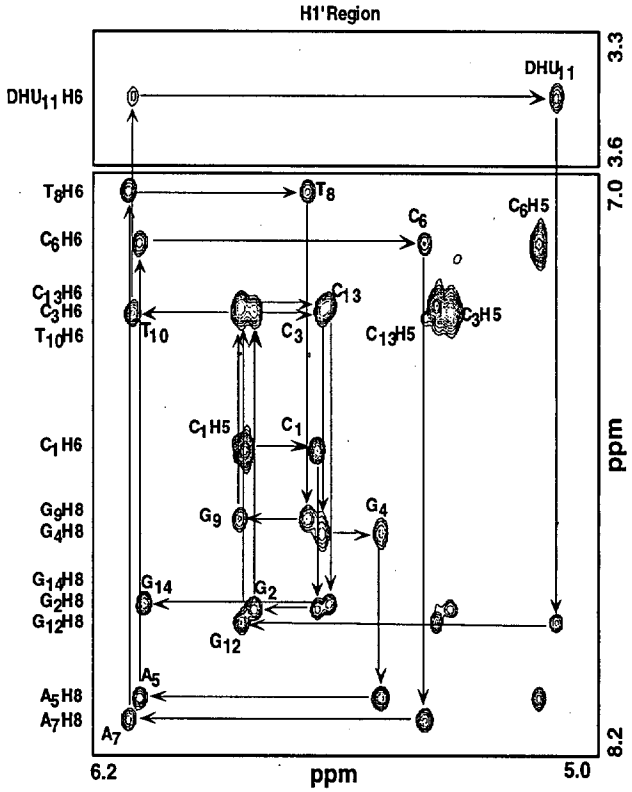
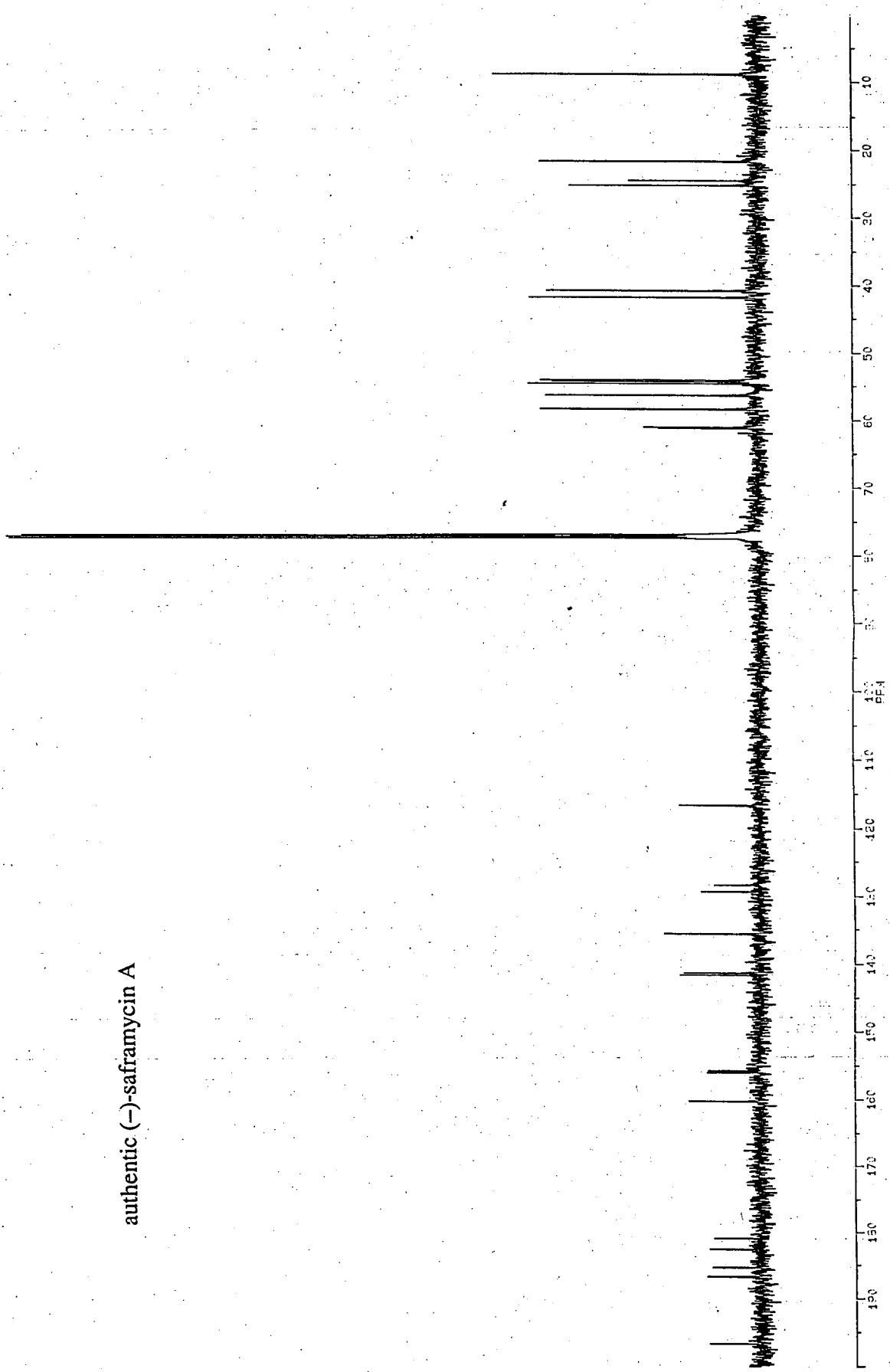
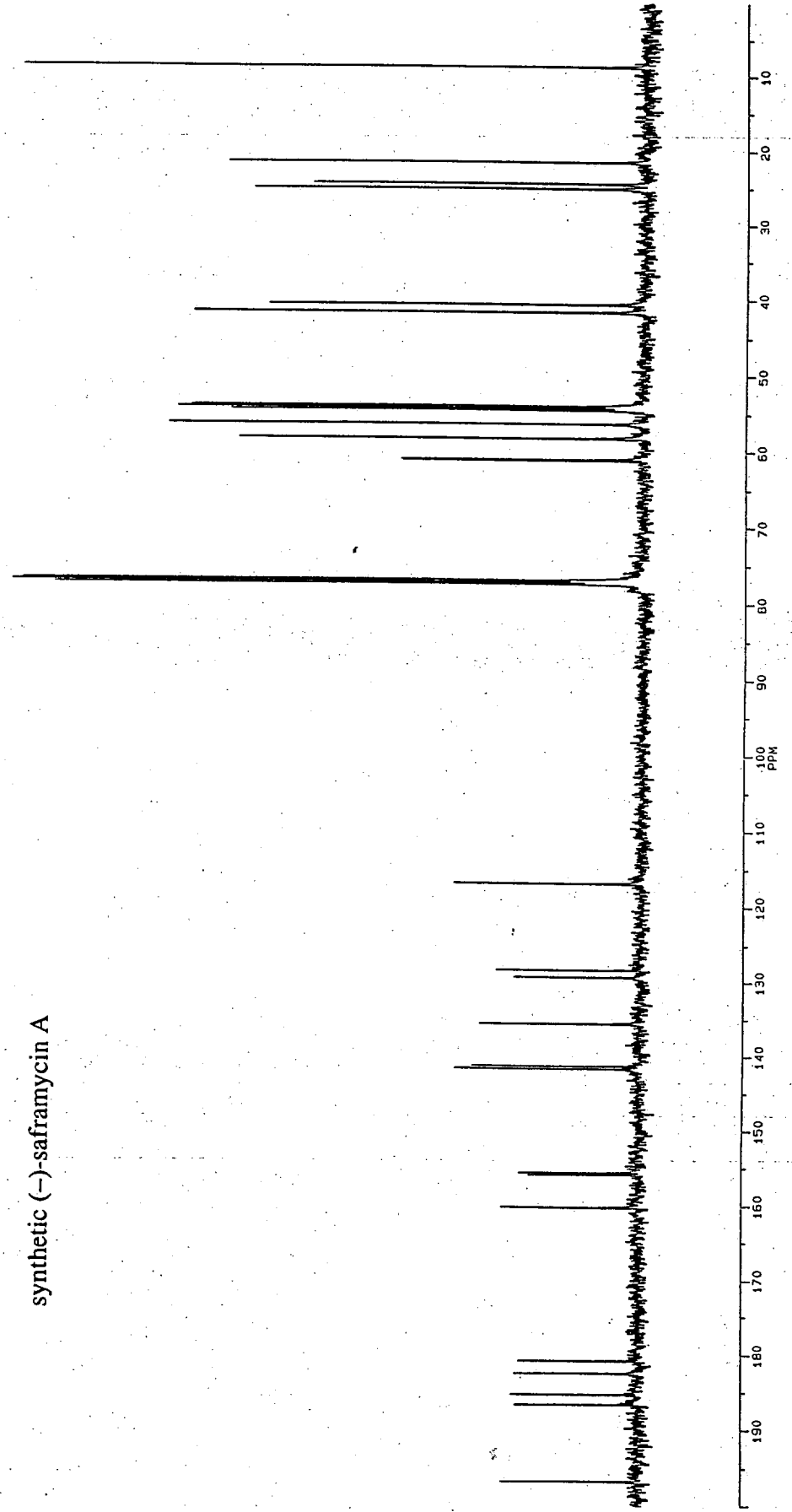


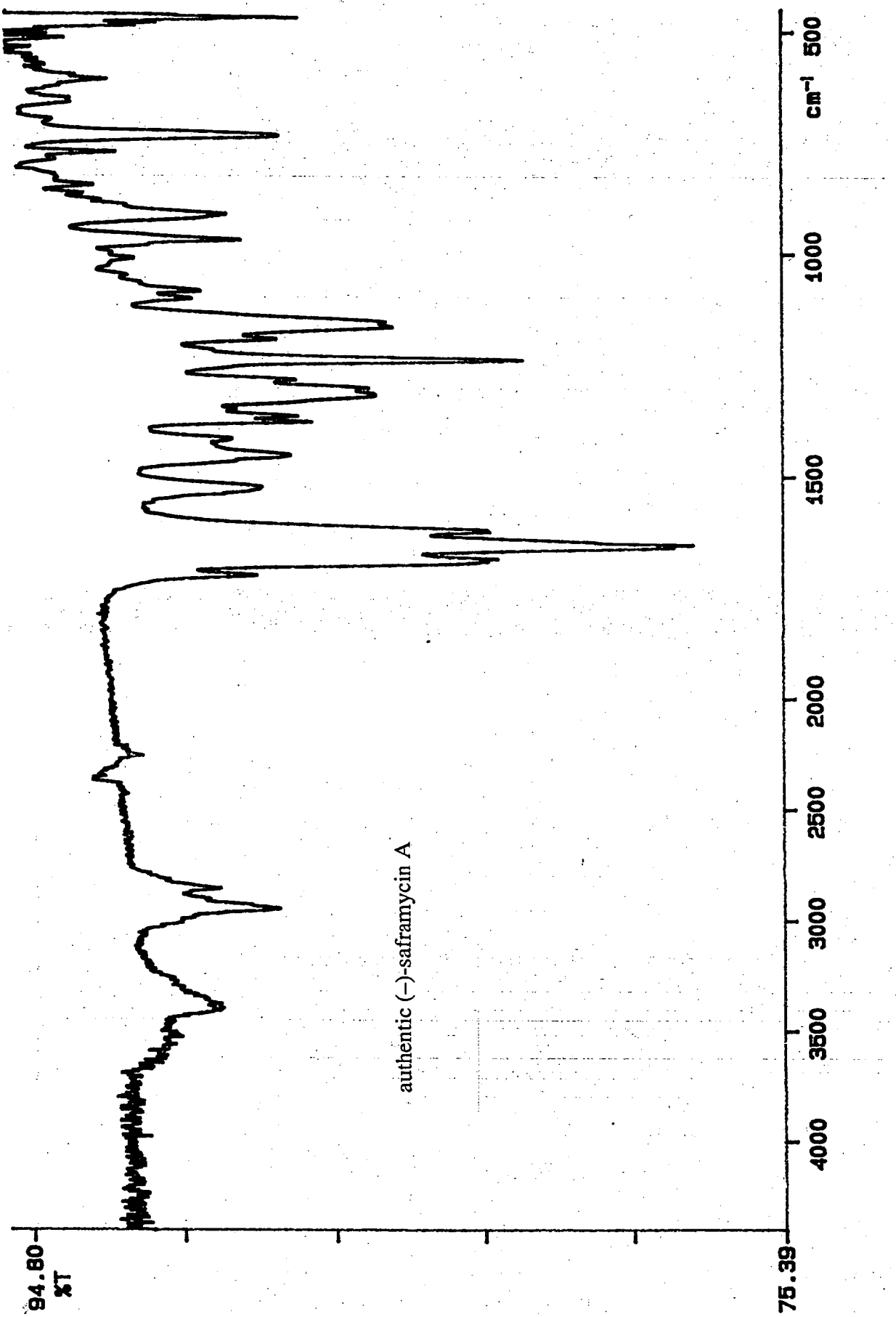
Figure S6. Base/H1' region of the NOESY spectrum (250 ms mixing time) of 2 mM (strands) **DHU-14** in 99.98% D_2O at pH 6.5 and 5 °C.

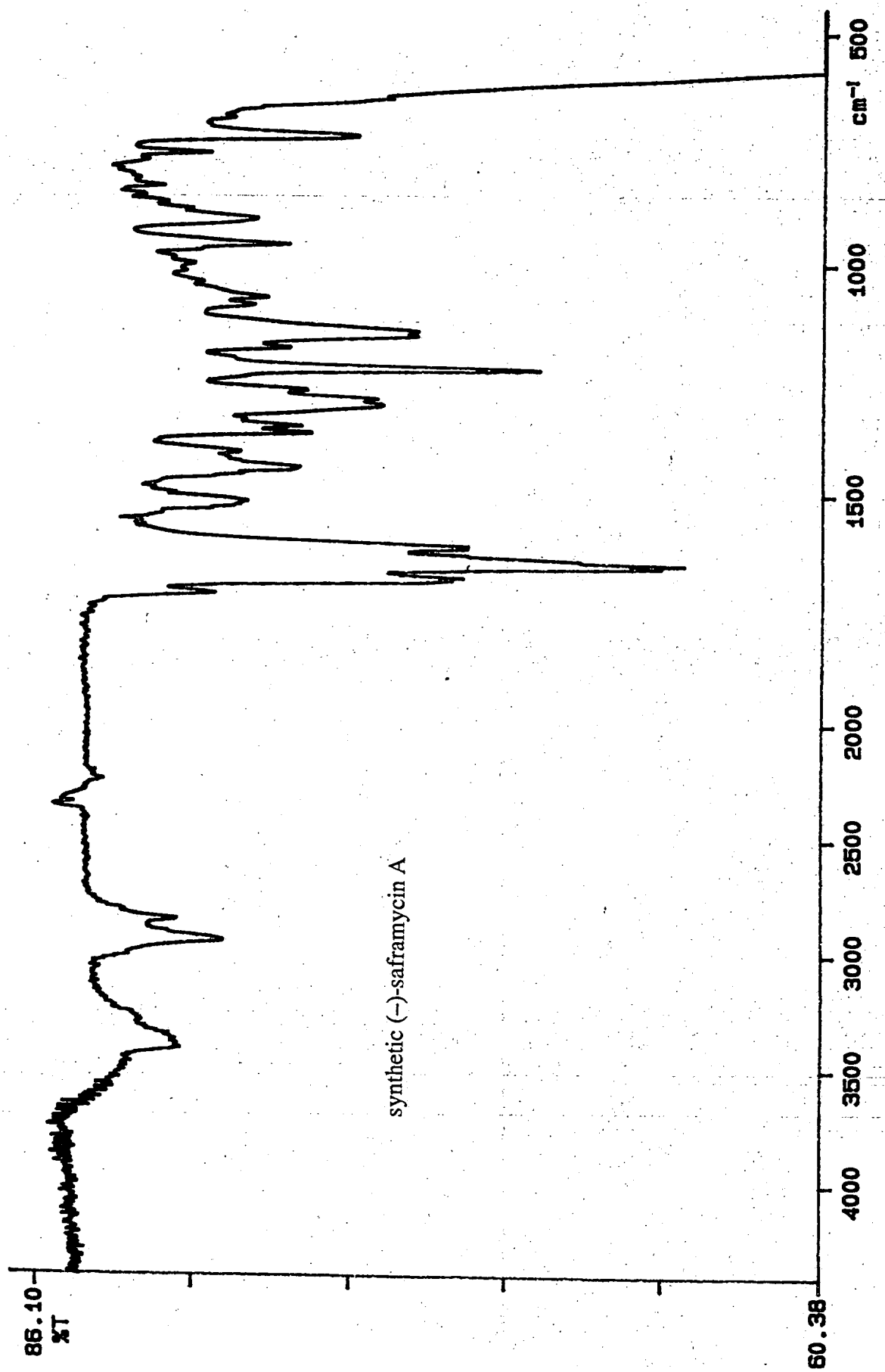


authentic (−)-saframycin A



5 of





synthetic (–)-saframycin A