

Supplementary Material

Constrained bisantrene derivatives as G-quadruplex binders

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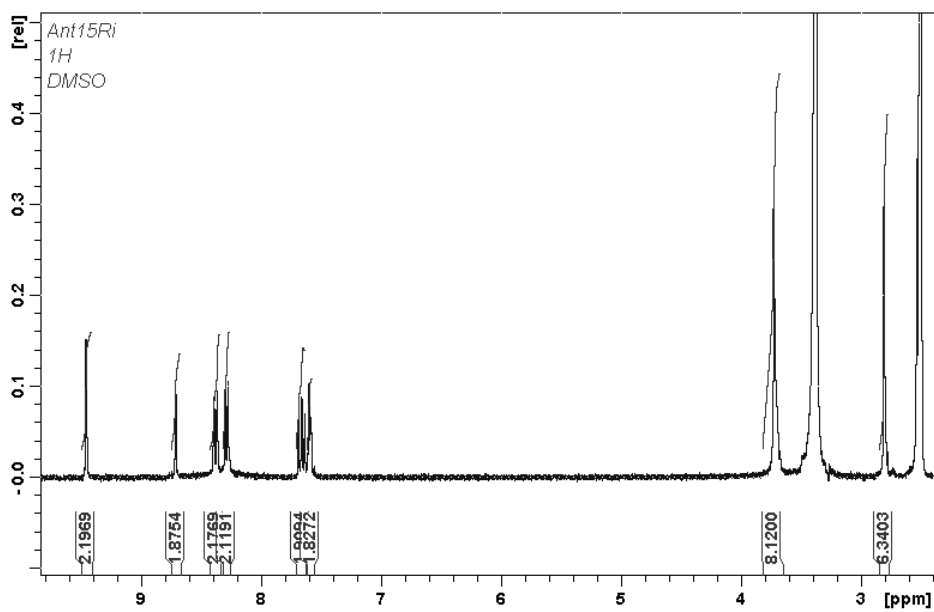
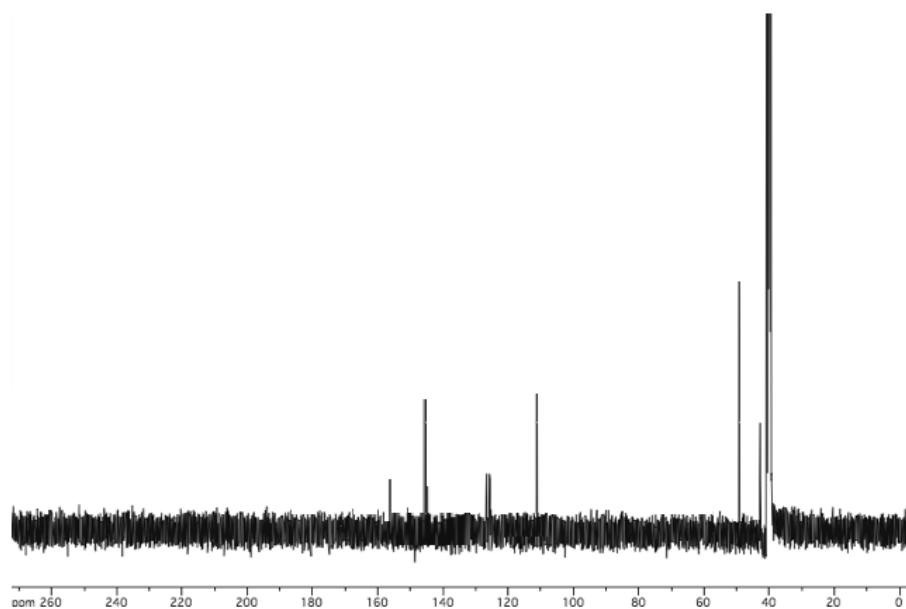
CONTENT:

- S1-S5 Compounds characterization (NMR Spectra, HRMS, tandem Mass Spectrometry):**
- Ant1,5-Ri: ¹H, ¹³C
 - Ant1,5-Ro: ¹H, ¹³C
 - Detailed assignation of ¹H NMR signals
 - Compounds characterization by HRMS and tandem Mass Spectrometry
- S6-S9 Mass Spectra of G-quadruplex DNA and ligand interaction studies:**
- Preparation of DNA samples for Mass Spectrometry (MS) analysis
 - Mass Spectrometry (MS) binding studies
- S10 In silico conformational search**
- S11 References**

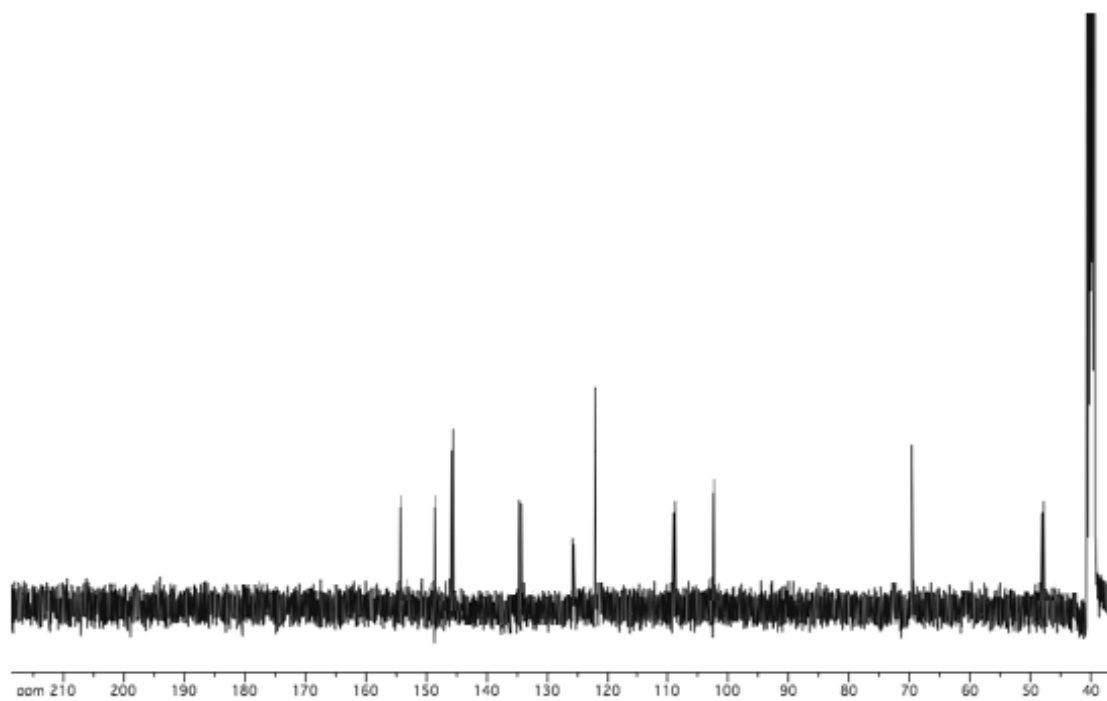
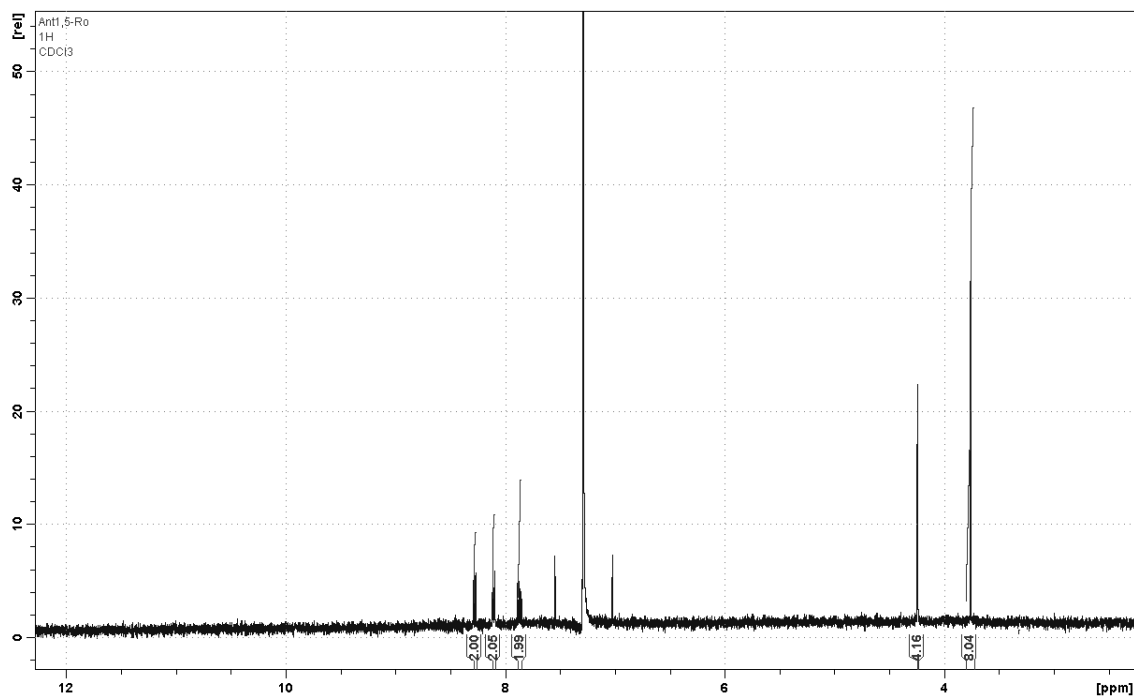
S1

COMPOUNDS CHARACTERIZATION

NMR Spectra

Ant1,5-RiFigure S1. ¹H-NMR (DMSO)Figure S2. ¹³C-NMR (DMSO)

S2

Anti,5-Ro

S3

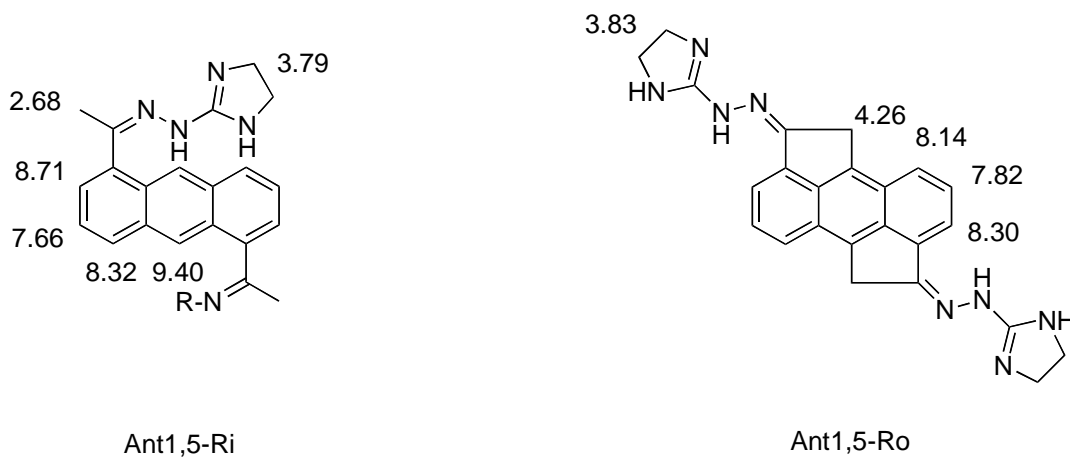
Detailed assignment of ^1H NMR signals

Figure S5. NMR assignment

S4

HRMS

Experiments were carried out on a Mariner ESI-TOF (Perceptive Biosystems Inc.) in positive ionization mode.

Ant1,5-Ri [(M+2H⁺)/2]⁺

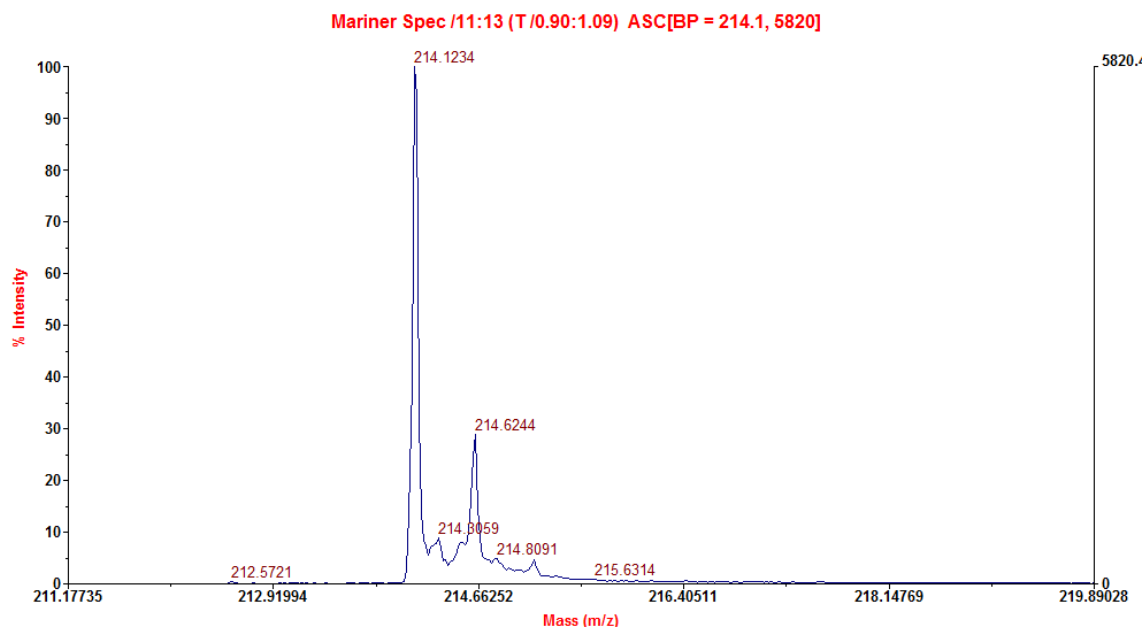
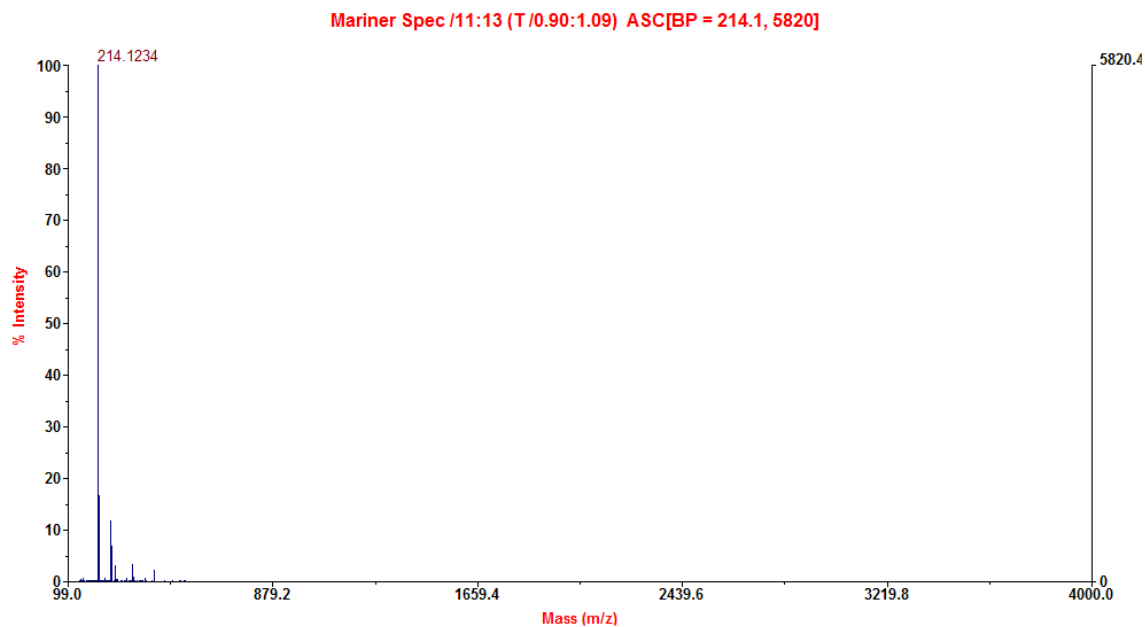


Figure S6. HRMS spectra

S5

Tandem Mass Spectrometry

Experiments were carried out on a Thermo Orbitrap LTQ Velos in positive ionization mode. HCD fragmentation experiments (13-16 eV) were performed using a 10 μ M solution of the compound of interest in 150 mM ammonium acetate.

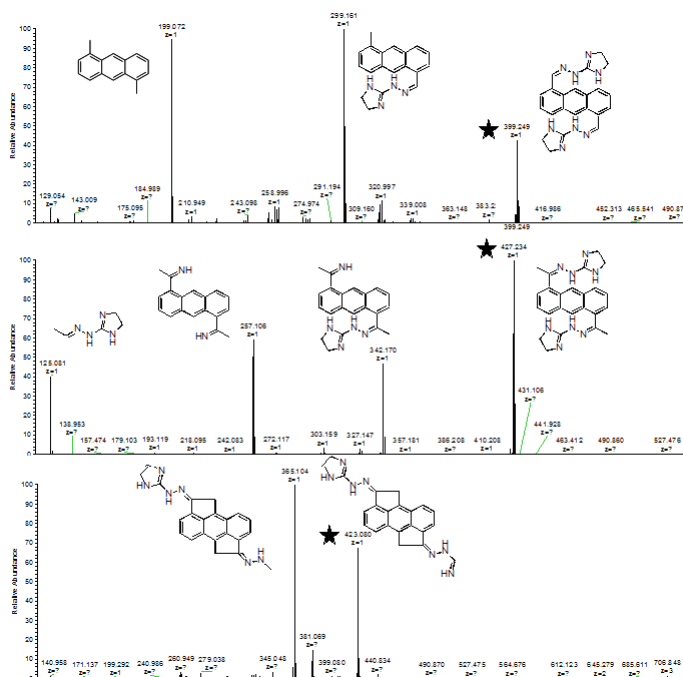


Figure S7. HRMS fragmentation spectra

Parent peak is marked with a star and the structures of possible fragments are reported next to their relative peaks.

S6

MASS SPECTRA OF G-QUADRUPLEX AND LIGAND INTERACTION STUDIES**Preparation of DNA samples for Mass Spectrometry (MS) analysis**

Oligonucleotides were purchased from IDT, USA. The telomeric sequence “GQ” (5' - AGG GTT AGG GTT AGG GTT AGG GT - 3', average mass 7270.774) was dissolved in water and allowed to stand in an ice bath for 2 hours. The obtained solutions were poured in two 3000 Da cut-off centrifuge tube and 4 mL of 150 mM ammonium acetate were added. The tubes were centrifuged for 1 hour at 4°C and the cleaning procedure was repeated another time with fresh ammonium acetate solution and for the third time with water. The final concentration of the cleaned solution obtained was measured with NanoDrop 2000c UV-Vis spectrophotometer (Thermo). The samples were then diluted to 100 μ M with ammonium acetate (150 mM) and stored in a 4°C fridge for three weeks. Samples for the experiments were prepared just before the analysis from opportune dilutions of stock solutions. The presence of the G-quadruplex structure in solution without any ligand was verified through direct analysis (negative ionization mode) of these samples after a dilution to 5 μ M in 150 mM ammonium acetate.

S7

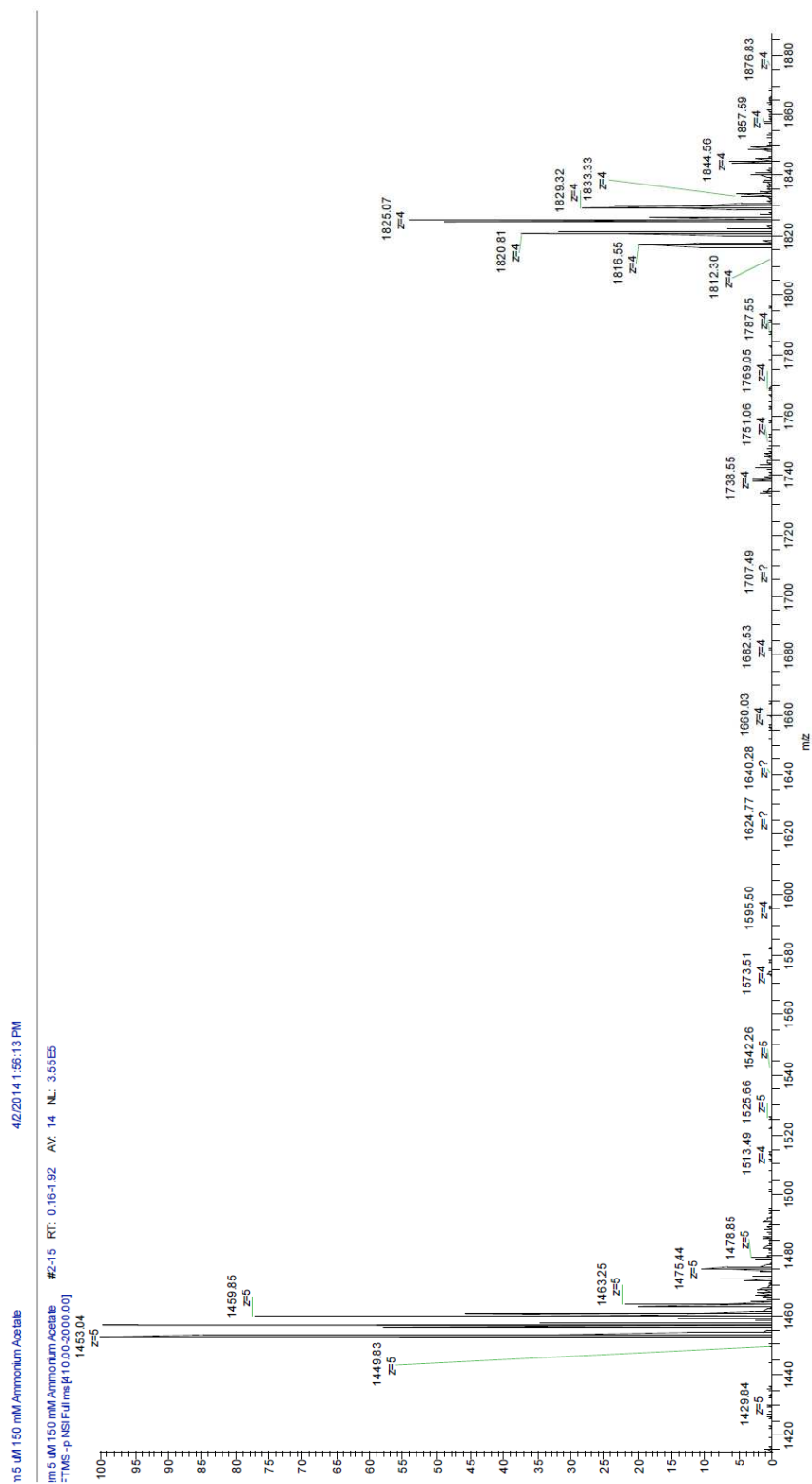


Figure S8. In the spectrum above naked oligonucleotide (1453 and 1816, charge state - 5 and -4 respectively), mono (1456 and 1820, charge state -5 and -4 respectively) and di

S8

ammonium (1459 and 1825, charge state -5 and -4 respectively) adducts are clearly detectable. Considering the -4 charge state the $(GQ + 2NH_4^+)^-$ peak (1825), that can be assumed as the one representative for the quadruplex structure, is the most intense of the group.

S9

Mass Spectrometry (MS) binding studies

Experiments were performed on a Thermo Orbitrap LTQ Velos. Samples were prepared just before the analysis from opportune dilutions of stock solutions. The final concentration of the oligonucleotide was 3 μM in 150 mM ammonium acetate, with a ratio compound/oligo of 5:1 if a binding study was being performed. Samples were prepared just before the analysis and experiments were recorded in negative ionization mode.

Compounds were tested singularly towards GQ DNA (spectrum b reported below, example for Ant1,5) and then a more comprehensive experiment was designed in order to evaluate at the same time the competitive binding capability of the three molecules; the three molecules were mixed together with the GQ DNA sequence before the analysis and the resulting mixture was then analyzed (spectrum a).

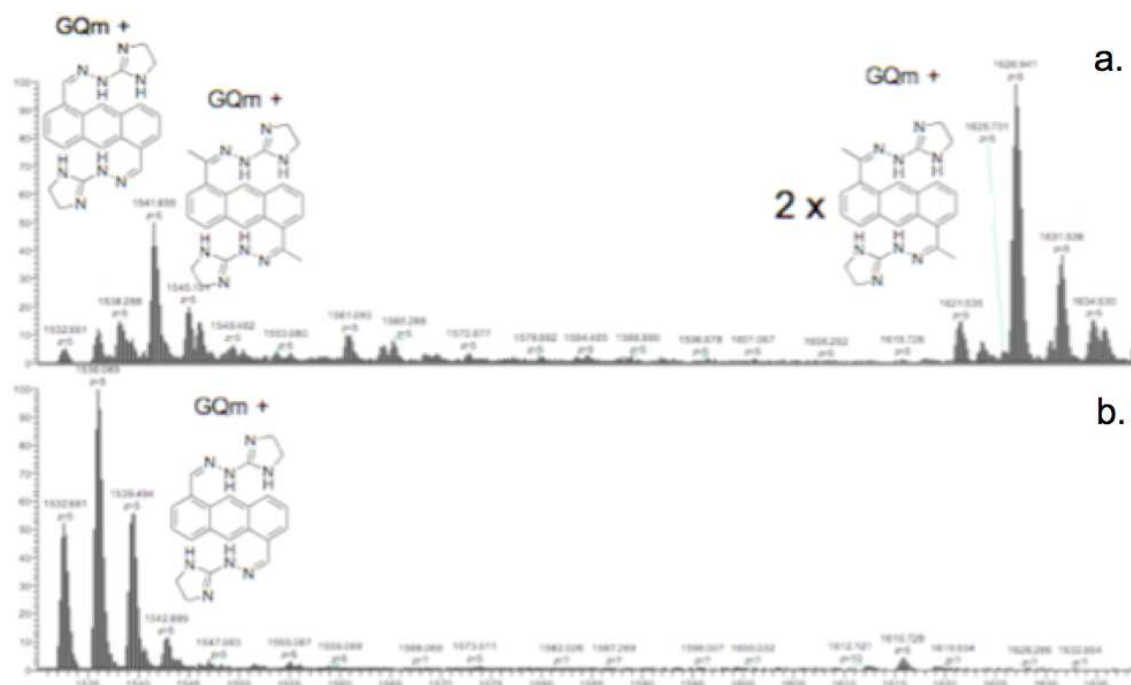


Figure S9. Comparison of binding experiments

S10**IN SILICO CONFORMATIONAL SEARCH**

An *in silico* conformational search was carried out on the three compounds Ant1,5, Ant1,5-Ri and Ant1,5-Ro using Avogadro^{1,2}. The most stable conformations detected are reported in the picture below.

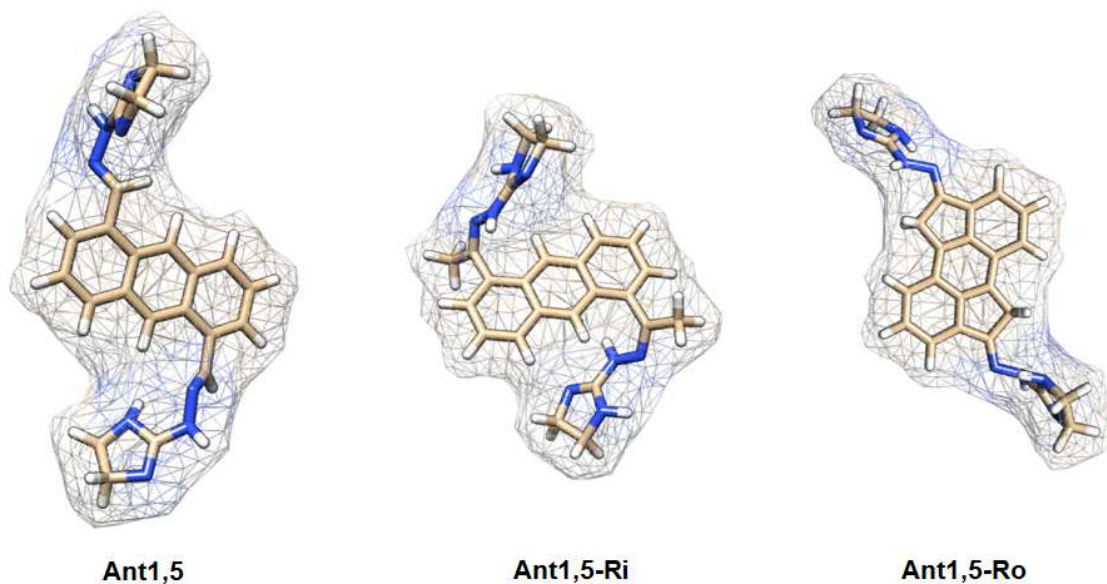


Figure S10. Conformational search

S11

1. Avogadro: an open-source molecular builder and visualization tool. Version 1.0.3. <http://avogadro.openmolecules.net/> (accessed January 2015).

2. M. D. Hanwell, D. E. Curtis, D. C. Lonie, T. Vandermeersch, E. Zurek, G. R. Hutchison, *J Cheminformatics*, 2012, **4**, 1-17.