

Supporting Information

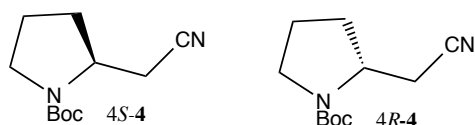
Synthesis and stereochemical assignment of conioidine A. DNA and HSA binding studies of the four diastereomers

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Characterization data for compounds:	pp. S3-S9
¹ H NMR and ¹³ C NMR spectra for compounds in Scheme 1:	pp. S10-S33
DNA/HSA binding studies on the four diastereomers of 1 :	pp. S34-S61
Molecular Docking experiments	pp. S62-S67
Preliminary MTT assay results form 4 <i>R</i> , 6 <i>R</i> - 1 and 4 <i>R</i> , 6 <i>S</i> - 1	p. S68

General Information

Distilled water was used in all of the experiments. Organic extracts were dried over Na_2SO_4 , filtered, and concentrated using a rotary evaporator at aspirator pressure (20-30 mmHg). Chromatography refers to flash chromatography and was carried out on SiO_2 (silica gel 60, 230-400 mesh). All glassware used in the reactions described below were flame-dried under vacuum and then flushed with argon gas at room temperature prior to the addition of reagents and solvents. ^1H and ^{13}C NMR spectra were measured in CDCl_3 at 400 MHz and 100 MHz, respectively, using Me_4Si as internal standard. Chemical shifts are reported in ppm downfield (δ) from Me_4Si . UV spectra were recorded on a diode array spectrophotometer over the 190-500 nm range with subtraction of a solvent blank. Fluorescence spectra were recorded over the 520-670 nm range for ethidium bromide displacement experiments (the maximum emission wavelength was 590 nm when the excitation wavelength was 520 nm; Ex Slit (nm) = 10.0; Em Slit (nm) = 10.0; Scan Speed (nm/min) = 200) and over the 320-410 nm range for HSA binding experiments (the maximum emission wavelength was 338 nm when the excitation wavelength was 280 nm; Ex Slit (nm) = 10.0; Em Slit (nm) = 10.0; Scan Speed (nm/min) = 200). ITC thermograms were recorded for titrations performed at 25°C under the following conditions: DP = 6, 307 rpm, 5 μL injections, 10s duration with 130s, 2s filter, initial delay of 60s, total of 45 injections.



Boc prolinol **4S-3** and **4R-3**, derived from *L*-proline and *D*-proline, respectively, by the procedure of Pericas,¹ (1.5 g, 7.44 mmol) was dissolved in CH₂Cl₂ (7 mL) and cooled to 0 °C, and Et₃N (1.24 mL, 8.93 mmol) and TsCl (1.56 g, 8.2 mmol) was added, along with a catalytic quantity of DMAP (146 mg, 1.19 mmol). The reaction mixture was stirred for one hour at room temperature and was then diluted with saturated NaHCO₃ solution (10 mL) and ether (20 mL). The phases were separated and the aqueous layer was further extracted with ether (2 x 25 mL). The combined organic extracts were once washed with 20 ml sat. aq. NaCl, dried over anhydrous Na₂SO₄, and concentrated under reduced pressure. The crude tosylate was then dissolved in DMF (8 mL) and NaCN (474 mg, 9.69 mmol) was added. The solution was heated at 70°C for 12 hours, and was then diluted with saturated NaHCO₃ solution (10 mL) and ether (20 mL). The phases were separated and the aqueous layer was further extracted with ether (2 x 25 mL). The combined organic extracts were once washed with 20 ml sat. aq. NaCl, dried over anhydrous Na₂SO₄, and concentrated under reduced pressure. Purification of the residue by flash chromatography (SiO₂, 10:1→4:1 hexanes:ethyl acetate) afforded nitrile **4S-4** (935 mg, 4.4 mmol, 60%) and **4R-4** (1.17 g, 5.6 mmol, 75%).

4S-4: (as a mixture of carbamate rotamers).

¹H NMR (400 MHz, CDCl₃) δ 3.99 (s, 1H); 3.39 (m, 2H); 2.79-2.65 (m, 2H); 2.15 (m, 1H); 2.03-1.85 (m, 3H); 1.48 (s, 9H).

¹³C NMR (100 MHz, CDCl₃) δ 154.5, 80.4, 80.0, 53.7, 47.1, 31.2, 30.4, 29.3, 28.4, 23.6, 23.0, 22.8, 22.3.

$\alpha_D^{25} = -54.1$ (c=0.024, CH₂Cl₂). literature $\alpha_D^{25} = -87.7$ (c=0.018, CHCl₃).

These data are in full accord with those previously reported for **4S-4**: Mahato, C., Mukherjee, S., Kundu, M., Pramanik, A. *J. Org. Chem.* **2019**, *84*, 1053.

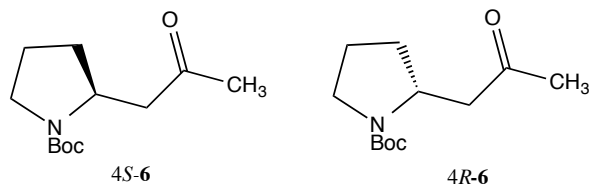
4R-4: (as a mixture of carbamate rotamers).

¹H NMR (400 MHz, CDCl₃) δ 3.96 (m, 1H); 3.60 (m, 2H); 3.44 (m, 1H); 3.31 (m, 1H); 2.00 (m, *J*=7.5 Hz, 1H); 1.85 (m, *J*=6.7 Hz, 1H); 1.57 (m, 1H); 1.47 (s, 9H).

¹³C NMR (100 MHz, CDCl₃) δ 157.1, 80.2, 67.6, 60.2, 47.5, 28.6, 28.4, 24.0.

$\alpha_D^{25} = +38.8$ (c=0.046, CH₂Cl₂). literature $\alpha_D^{25} = +95.0$ (c=0.4, CHCl₃).

These data are in full accord with those previously reported for **4R-4**: Alza, E.; Cambeiro, X. C.; Jimeno, C.; Pericas, M.A. *Org. Lett.* **2007**, *9*, 3717.



Nitrile **4S-4**²⁴ or **4R-4**⁵ (1.0 g, 4.7 mmol) was dissolved in methanol (4.7 mL) and 3N NaOH (4.7 mL, 14.1 mmol) and heated to 100 °C for one hour, at which time a homogenous solution was obtained. The solution was cooled to room temperature and concentrated *in vacuo* to remove methanol. Then ether (10 mL) was added and the solution was acidified with 3N HCl (4.5 mL). The phases were separated and the aqueous layer was further extracted with ether (2 x 25 mL). The combined organic extracts were once washed with 20 ml sat. aq. NaCl, dried over anhydrous Na₂SO₄, and concentrated under reduced pressure. The crude acid (4.7 mmol) was then dissolved in CH₂Cl₂ (11 mL) and cooled to 0 °C. Carbonyl diimidazole (856 mg, 5.28 mmol) was added and the solution was allowed to stir at room temperature for 30 minutes. The solution was then cooled to 0°C and Et₃N (1.68 mL, 12 mmol) and *N*-methyl-*N*-methoxylamine hydrochloride (582 mg, 6 mmol) were added and the solution was stirred at room temperature overnight. The mixture was diluted with CH₂Cl₂ (10 mL) and saturated NaHCO₃ solution (20 mL) and the phases were separated. The aqueous layer was further extracted with ether (2 x 25 mL). The combined organic extract was once washed with 20 ml sat. aq. NaCl, dried over anhydrous Na₂SO₄, and concentrated under reduced pressure. The crude Weinreb amide (~4 mmol) was dissolved in THF (10 mL) and cooled to 0°C. A solution of methylmagnesium bromide (1.5 mL, 4.5 mmol, 3M in ether) was added dropwise and the solution was allowed to stir at room temperature for 4 hours. The reaction mixture was then quenched with saturated NH₄Cl solution (10 mL) and ether (10 mL) was added. The phases were separated and the aqueous layer was further extracted with ether (2 x 25 mL). The combined organic extract was once washed with 20 ml sat. aq. NaCl, dried over anhydrous Na₂SO₄, and concentrated under reduced pressure. Purification of the residue by flash chromatography (SiO₂, 20:1→4:1 hexanes:ethyl acetate) afforded ketone **4S-6** (586.8 mg, 2.6 mmol, 55%) and ketone **4R-6** (363 mg, 1.6 mmol, 34%).

4S-6 (as a mixture of carbamate rotamers): ¹H NMR (400 MHz, CDCl₃) δ 4.16 (m, 1H); 3.32 (m, 2H); 3.15, 2.95 (dd, *J*=15.3, 15.6 Hz, 1H); 2.44 (m, 1H); 2.17 (s, 3H); 2.08 (m, 1H); 1.84 (m, 2H); 1.67 (m, 1H); 1.47 (s, 9H).

¹³C NMR (100 MHz, CDCl₃) δ 207.5, 154.3, 79.5, 53.4, 47.8, 46.4, 31.5, 30.5, 28.4, 23.5.

HRMS (ESI) calculated for C₁₂H₂₁NNaO₃ 250.1419 found 250.1376 (M+Na)⁺.

$\alpha_D^{25} = -36.5^\circ$ (c=0.019, CH₂Cl₂). Literature $\alpha_D^{25} = -36.9$ (c=1.39, CHCl₃).

These data are in full accord with those previously reported for **4S-6** : Seki, H., Georg, G. I. *Eur. J. Org. Chem.* **2014**, 18, 3777-3783.

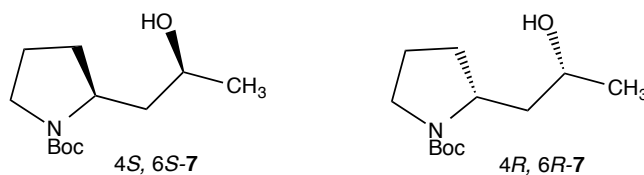
4*R*-**6** (as a mixture of carbamate rotamers): ^1H NMR (400 MHz, CDCl_3) δ 4.09 (m, 1H); 3.29 (t, $J=5.9$ Hz, 2H); 2.96 (d, $J=16.0$ Hz, 1H); 2.37 (dd, $J=9.6, 16.0$ Hz, 1H); 2.11 (s, 3H); 2.01 (m, 1H); 1.79 (m, 2H); 1.60 (m, 1H); 1.42 (s, 9H).

^{13}C NMR (100 MHz, CDCl_3) δ 207.3, 154.2, 79.3, 53.4, 48.2, 46.3, 31.1, 30.4, 28.5, 23.2.

HRMS (ESI) calculated for $\text{C}_{12}\text{H}_{21}\text{NNaO}_3$ 250.1419 found 250.1400 ($\text{M}+\text{Na}$) $^+$.

$\alpha_{\text{D}}^{25}=+18.6$ ($c=0.047$, CHCl_3). Literature $\alpha_{\text{D}}^{25}=+31.6$ ($c=2.5$, CHCl_3).

These data are in full accord with those previously reported for 4*R*-**6**: Davies, S. G., Fletcher, A. M., Roberts, P. M.; Smith, A. D. *Tetrahedron* **2009**, *65*, 10192-10213.



Ketone **4S-6** or **4R-6** (120 mg, 0.53 mmol) was dissolved in THF (1 mL) and the mixture was cooled to 0 °C. Then $\text{Zn}(\text{BH}_4)_2$ (1 mL of a 4M solution, 4 mmol) was added and the mixture was stirred at 0 °C for 6 hours. The mixture was quenched by addition of a saturated solution of Rochelle's salt (5 mL) and stirred at room temperature for 2 hours. After dilution of the mixture with EtOAc (10 mL), the phases were separated and the aqueous layer was further extracted with ether (2 x 25 mL). The combined organic extract was once washed with 20 ml sat. aq. NaCl, dried over anhydrous Na_2SO_4 , and concentrated under reduced pressure. Purification of the residue by flash chromatography (SiO_2 , 10:1 \rightarrow 3:1 hexanes:ethyl acetate) afforded alcohols **4S, 6S-7** (76.4 mg, 0.33 mmol, 63%) or **4R, 6R-7** (70.3 mg, 0.31 mmol, 58%).

4S,6S-7 (as a mixture of carbamate rotamers): ^1H NMR (400 MHz, CDCl_3) δ 5.13 (br s, 1H); 4.15 (m, 1H); 3.71 (m, 1H); 3.29 (t, $J=5.8$ Hz, 2H); 1.96-1.82 (m, 3H), 1.58 (m, 1H); 1.44 (s, 9H); 1.17 (d, $J=6.2$ Hz, 3H).

^{13}C NMR (100 MHz, CDCl_3) δ 156.5, 79.8, 63.8, 53.7, 46.5, 45.5, 31.1, 28.4, 23.5, 22.5.

HRMS (ESI) calculated for $\text{C}_{12}\text{H}_{23}\text{NNaO}_3$ 252.1576 found 252.1555 ($\text{M}+\text{Na}$) $^+$.

$\alpha_{\text{D}}^{25} = -5.8^\circ$ ($c=0.005$, CHCl_3). literature: $\alpha_{\text{D}}^{25} = -11.2^\circ$ ($c=0.2$, CHCl_3).

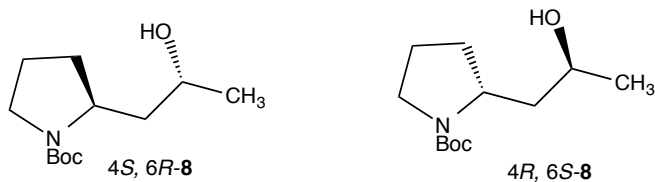
4R,6R-7 (as a mixture of carbamate rotamers): ^1H NMR (400 MHz, CDCl_3) 4.16 (m, 1H); 3.72 (m, 1H); 3.30 (m, 2H); 1.99-1.85 (m, 3H); 1.60 (m, 1H); 1.47 (s, 9H); 1.40 (m, 2H); 1.18 (d, $J=6.0$ Hz, 3H).

^{13}C NMR (100 MHz, CDCl_3) δ 156.5, 79.8, 63.7, 53.8, 46.4, 45.6, 31.1, 28.4, 23.4, 22.6.

HRMS (ESI) calculated for $\text{C}_{12}\text{H}_{23}\text{NNaO}_3$ 252.1576 found 252.1478 ($\text{M}+\text{Na}$) $^+$.

$\alpha_{\text{D}}^{25} = +7.5^\circ$ ($c=0.017$, CHCl_3). literature: $\alpha_{\text{D}}^{25} = +10.9^\circ$ ($c=0.7$, CHCl_3).

These data are in full accord with those previously reported for **4S,6S-7** and **4R,6R-7**: Davies, S. G., Fletcher, A. M., Roberts, P. M.; Smith, A. D. *Tetrahedron* **2009**, *65*, 10192-10213.



Ketone **4S-6** or **4R-6** (320 mg, 1.41 mmol) was dissolved in THF (5 mL) and the mixture was cooled to -78 °C. Then $\text{LiAlH}(\text{O}i\text{Bu})_3$ (1.07 g, 4.24 mmol, 3 equiv) was added and the mixture was warmed to 0 °C and stirred at this temperature for 6 hours. The mixture was quenched with a few drops of water and diluted with EtOAc (10 mL). The solution was cooled to 0 °C, filtered through celite, and concentrated *in vacuo*. Purification of the residue by flash chromatography (SiO_2 , 10:1 \rightarrow 3:1 hexanes:ethyl acetate) afforded alcohol **4S, 6R-8** (293 mg, 1.28 mmol, 91%) or **4R, 6S-8** (267 mg, 1.17 mmol, 83%).

4S,6R-8 (as a mixture of carbamate rotamers): ^1H NMR (400 MHz, CDCl_3) 3.95 (br s, 1H); 3.80 (m, 1H); 3.30 (m, 2H); 1.97 (m, 1H); 1.81 (m, 2H); 1.62 (m, 1H); 1.42 (s, 9H); 1.16 (d, $J=5.0$ Hz, 3H).

^{13}C NMR (100 MHz, CDCl_3) 155.4, 79.5, 66.3, 55.5, 46.3, 45.3, 32.1, 28.4, 23.9, 23.7.

HRMS (ESI) calculated for $\text{C}_{12}\text{H}_{23}\text{NNaO}_3$ 252.1576 found 252.1597 ($\text{M}+\text{Na}$) $^+$.

$\alpha_{\text{D}}^{25} = -24.8^\circ$ ($c=0.076$, CHCl_3). Literature: $\alpha_{\text{D}}^{25} = -80.2^\circ$ ($c=0.1$, CHCl_3)

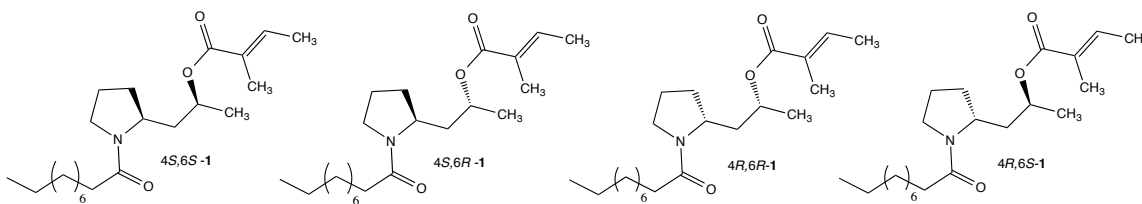
4R,6S-8 (as a mixture of carbamate rotamers): ^1H NMR (400 MHz, CDCl_3) 3.89 (br s, 1H); 3.65 (m, $J=2.9$ Hz, 1H); 3.62 (br s, 1H); 3.24 (m, 2H); 1.91-1.86 (m, 1H); 1.74 (m, 3H); 1.59 (m, 1H); 1.37 (s, 9H); 1.12 (d, $J=6.2$ Hz, 3H).

^{13}C NMR (100 MHz, CDCl_3) δ 155.2, 79.4, 66.0, 55.2, 46.2, 44.9, 31.7, 28.4, 23.9, 23.7.

HRMS (ESI) calculated for $\text{C}_{12}\text{H}_{23}\text{NNaO}_3$ 252.1576 found 252.1579 ($\text{M}+\text{Na}$) $^+$.

$\alpha_{\text{D}}^{25} = +33.3^\circ$ ($c=0.019$, CHCl_3). Literature: $\alpha_{\text{D}}^{25} = +78.5^\circ$ ($c=1.0$, CHCl_3)

These data are in full accord with those previously reported for **4S,6R-8** and **4R,6S-8**: Davies, S. G., Fletcher, A. M., Roberts, P. M.; Smith, A. D. *Tetrahedron* **2009**, *65*, 10192-10213.



Alcohol 4S, 6S-7, 4R, 6R-7, 4S, 6R-8, or 4R, 6S-8 (150 mg, 0.66 mmol) was dissolved in 1:1 TFA:CH₂Cl₂ (1.5 mL) at room temperature and the mixture was stirred for 30 minutes, at which time it was concentrated *in vacuo*. The crude residue was azeotroped with benzene (5 mL) twice, and then CH₂Cl₂ (1.5 mL) was added. Triethylamine (0.25 mL, 1.6 mmol) was added and the mixture was cooled to 0 °C. Then decanoyl chloride (0.15 mL, 0.72 mmol) was added dropwise and the mixture was warmed to room temperature and stirred overnight. Then a solution of saturated NaHCO₃ (5 mL) was added and the layers were separated. The aqueous layer was further extracted with ethyl acetate (2 x 25 mL). The combined organic extract was once washed with 20 mL sat. aq. NaCl, dried over anhydrous Na₂SO₄, and concentrated under reduced pressure. The crude intermediate alcohol (~189 mg, 0.67 mmol) was dissolved in CH₂Cl₂ (2 mL) and cooled to 0 °C. Then pyridine (0.216 mL, 2.68 mmol), DMAP (163 mg, 1.34 mmol), and 2,3-dimethylacryloyl chloride (158 mg, 1.34 mmol) were added successively and the mixture was allowed to warm to room temperature and stir overnight. Dichloromethane (10 mL) and a saturated solution of NaHCO₃ were added (10 mL) and the layers were separated. The aqueous layer was further extracted with ethyl acetate (2 x 25 mL). The combined organic extract was once washed with 20 mL sat. aq. NaCl, dried over anhydrous Na₂SO₄, and concentrated under reduced pressure. Purification of the residue by flash chromatography (SiO₂, 5:1 hexanes:ethyl acetate → 2:1 hexanes: ethyl acetate) afforded the four diastereomers 4S, 6S-1, 4R, 6R-1, 4S, 6R-1, and 4R, 6S-1.

4S,6R-1 (101 mg, 0.344 mmol, 51%, as a mixture of amide rotamers): ¹H NMR (400 MHz, CDCl₃) δ 6.89 (m, 1H); 4.99 (m, 1H); 4.20, 3.85 (m, 1H); 3.47-3.40 (m, 2H); 2.31 (t, *J*=7.6 Hz, 1H); 2.21 (t, *J*=8.1 Hz, 1H); 1.89-1.75 (m, 5H); 1.77 (s, 3H); 1.74 (d, *J*=7.0 Hz, 3H); 1.64 (m, 2H); 1.44 (m, 1H); 1.27 (d, *J*=6.2 Hz, 3H); 1.27-1.24 (m, 12H); 0.86 (t, *J*=6.7 Hz, 3H).

¹³C NMR (100 MHz, CDCl₃):

δ 171.8, 167.8, 137.4, 137.1, 128.8, 128.6, 68.5, 68.3, 54.4, 53.8, 46.7, 45.3, 41.8, 39.2, 35.1, 34.3, 31.8, 30.1, 29.6, 29.5, 29.4, 29.3, 29.2, 25.5, 24.8, 23.9, 22.6, 21.8, 20.9, 20.5, 14.4, 14.0, 12.1, 12.0.

HRMS (ESI) calculated for C₂₂H₄₀NO₃ 366.2990 found 366.2958 (M+H)⁺.

α_D²⁵ = -9.9° (c=0.013, CH₃OH)

4S,6S-1 (84 mg, 0.28 mmol, 43%, as a mixture of amide rotamers). ¹H NMR (400 MHz, CDCl₃) δ 6.82 (m, 1H); 4.96 (q, *J*=6.4 Hz, 1H); 4.08, 3.83 (m, 1H); 3.38 (m, 2H); 2.21 (t, *J*=8.3 Hz, 2H); 2.08 (m, 1H); 1.91-1.78 (m, 9H); 1.66-1.58 (m, 3H); 1.31-1.25 (m, 16H); 0.88 (t, *J*=6.7 Hz, 3H).

^{13}C NMR (100 MHz, CDCl_3): δ 171.6, 167.5, 136.7, 129.0, 69.6, 54.9, 46.6, 39.3, 35.0, 31.8, 29.5, 29.4, 29.2, 24.7, 24.1, 22.6, 20.0, 14.2, 14.0, 11.9.

HRMS (ESI) calculated for $\text{C}_{22}\text{H}_{39}\text{NNaO}_3$ 388.2828 found 388.2838 ($\text{M}+\text{Na}$) $^+$.

$\alpha_{\text{D}}^{25} = -38.5^\circ$ ($c=0.013$, CH_3OH)

4*R*,6*S*-1: (110 mg, 0.37 mmol, 56%, as a mixture of amide rotamers). ^1H NMR (400 MHz, CDCl_3): δ 6.90 (m, 1H); 5.00 (m, 1H); 4.20, 3.85 (m, 1H); 3.47-3.38 (m, 2H); 2.28 (t, $J=7.8$ Hz, 1H); 2.25 (t, $J=7.1$ Hz, 2H); 1.91-1.76 (m, 9H); 1.64 (m, 2H); 1.44 (m, 1H); 1.41-1.27, (m, 16H); 0.89 (t, $J=6.6$ Hz, 3H).

^{13}C NMR (100 MHz, CDCl_3): δ 171.8, 167.8, 137.3, 128.8, 68.5, 54.4, 53.8, 46.7, 45.3, 41.8, 39.2, 35.1, 34.3, 31.8, 30.1, 29.6, 29.5, 29.4, 29.3, 25.5, 24.8, 23.9, 22.6, 21.8, 20.9, 20.5, 14.4, 14.0, 12.1.

HRMS (ESI) calculated for $\text{C}_{22}\text{H}_{40}\text{NO}_3$ 366.2990 found 366.3042 ($\text{M}+\text{H}$) $^+$.

$\alpha_{\text{D}}^{25} = +8.0^\circ$ ($c=0.013$, CH_3OH)

4*R*,6*R*-1: (70 mg, 0.19 mmol, 29%, as a mixture of amide rotamers). ^1H NMR (400 MHz, CDCl_3): δ 6.82 (q, $J=4.3$ Hz, 1H); 4.96 (q, $J=4.0$ Hz, 1H); 4.08 (m, 1H); 3.41-3.35 (m, 2H); 2.21 (t, $J=8.1$ Hz, 2H); 2.08 (m, 1H); 1.91-1.80 (m, 6H); 1.78 (d, $J=8.0$ Hz, 3H); 1.64 (m, 3H); 1.32-1.30 (m, 13H); 1.31 (d, $J=8.0$ Hz, 3H); 0.89 (t, $J=7.0$ Hz, 3H).

Literature ^1H NMR data for Conioidine A 1 : δ 6.80 (dq, $J=7.5$, 1.1 Hz, 1H); 4.90 (m, 1H); 4.08 (m, 1H); 3.35 (m, 2H); 2.15 (t, $J=6.0$ Hz, 2H); 2.03, 1.62 (m, 2H); 1.90 (m, 2H); 1.85 (m, 2H); 1.80 (d, $J=1.1$ Hz, 3H); 1.78 (d, $J=7.5$ Hz, 3H); 1.31 (d, $J=7.2$ Hz, 3H); 1.20-1.61 (m, 14H); 0.86 (t, $J=6.5$ Hz, 3H).

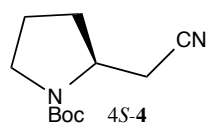
^{13}C NMR (100 MHz, CDCl_3): δ 171.6, 167.5, 136.7, 129.0, 69.6, 54.9, 46.6, 39.3, 35.1, 31.8, 29.5, 29.2, 24.7, 24.1, 22.6, 20.0, 14.3, 14.0, 12.0.

Literature ^1H NMR data for Conioidine A 1 : δ 171.8, 167.5, 136.8, 129.0, 69.5, 55.1, 46.7, 39.2, 34.9, 31.8, 29.5, 29.4, 29.2, 29.1, 24.8, 24.1, 22.6, 14.3, 14.1, 12.0.

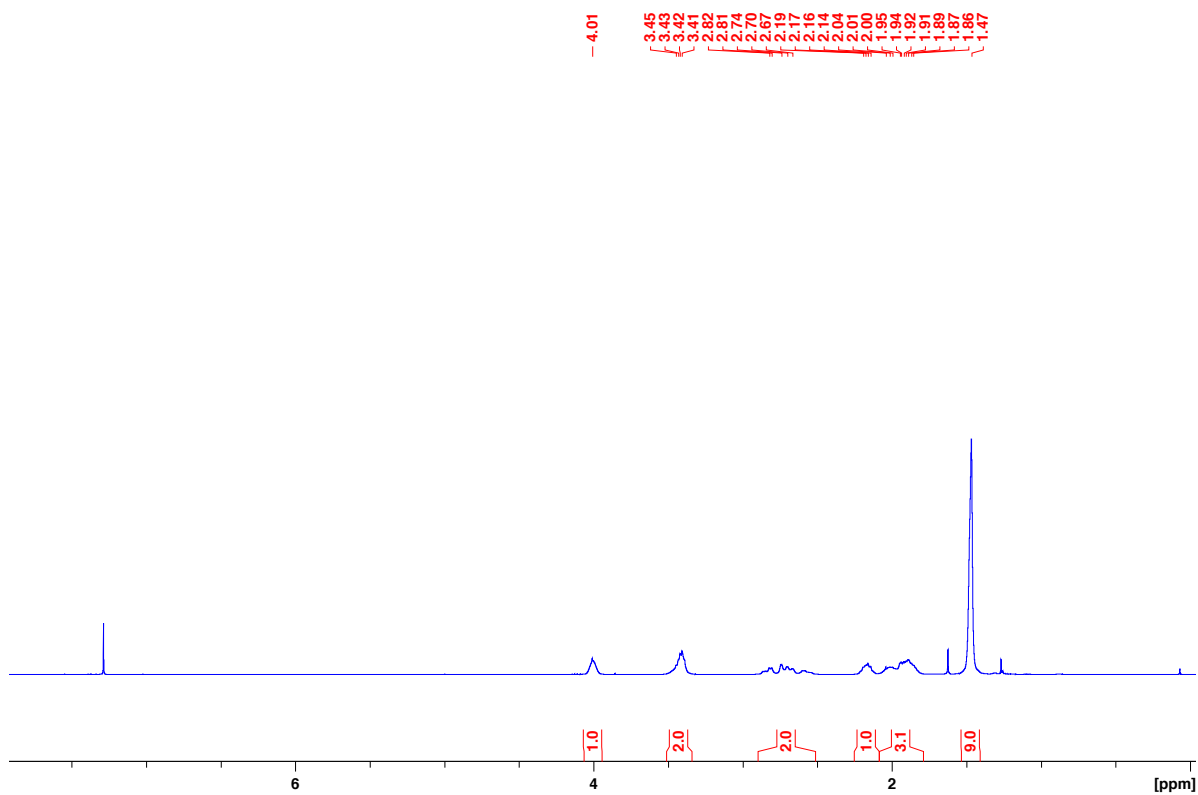
HRMS (ESI) calculated for $\text{C}_{22}\text{H}_{39}\text{NaO}_3$ 388.2828 found 388.2832 ($\text{M}+\text{Na}$) $^+$.

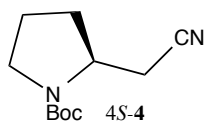
$\alpha_{\text{D}}^{25} = +25.7^\circ$ ($c=0.034$, CH_3OH); literature 1 : $\alpha_{\text{D}}^{25} = +48.2^\circ$ ($c=0.2$, CH_3OH).

1 G. W. Chan, D. Berry, C. W. DeBrosse, M. E. Hemling, L. MacKenzie-LoCasto, P. H. Offen, and J. W. Westley *J. Nat. Prod.* 1993, **56**, 708.

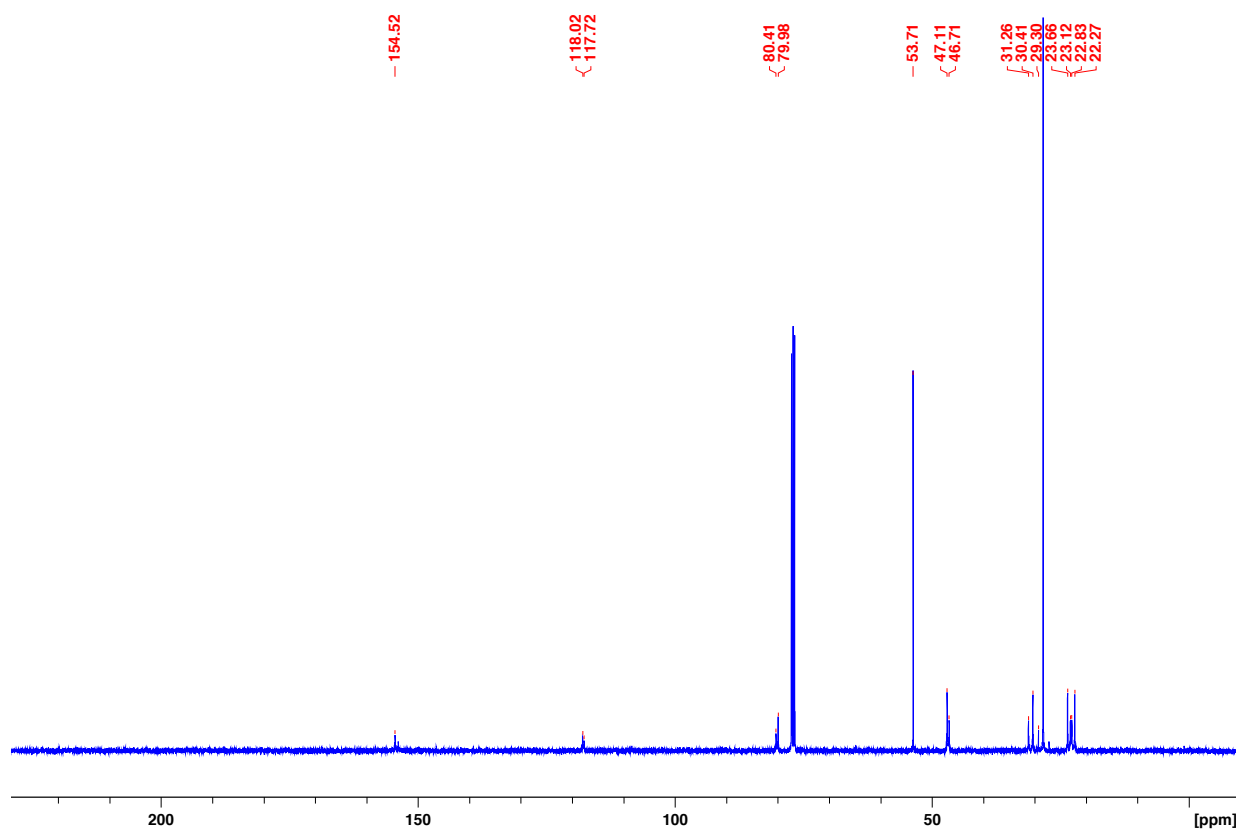


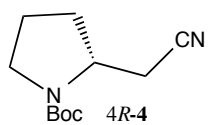
^1H NMR (400 MHz) (as a mixture of carbamate rotamers)



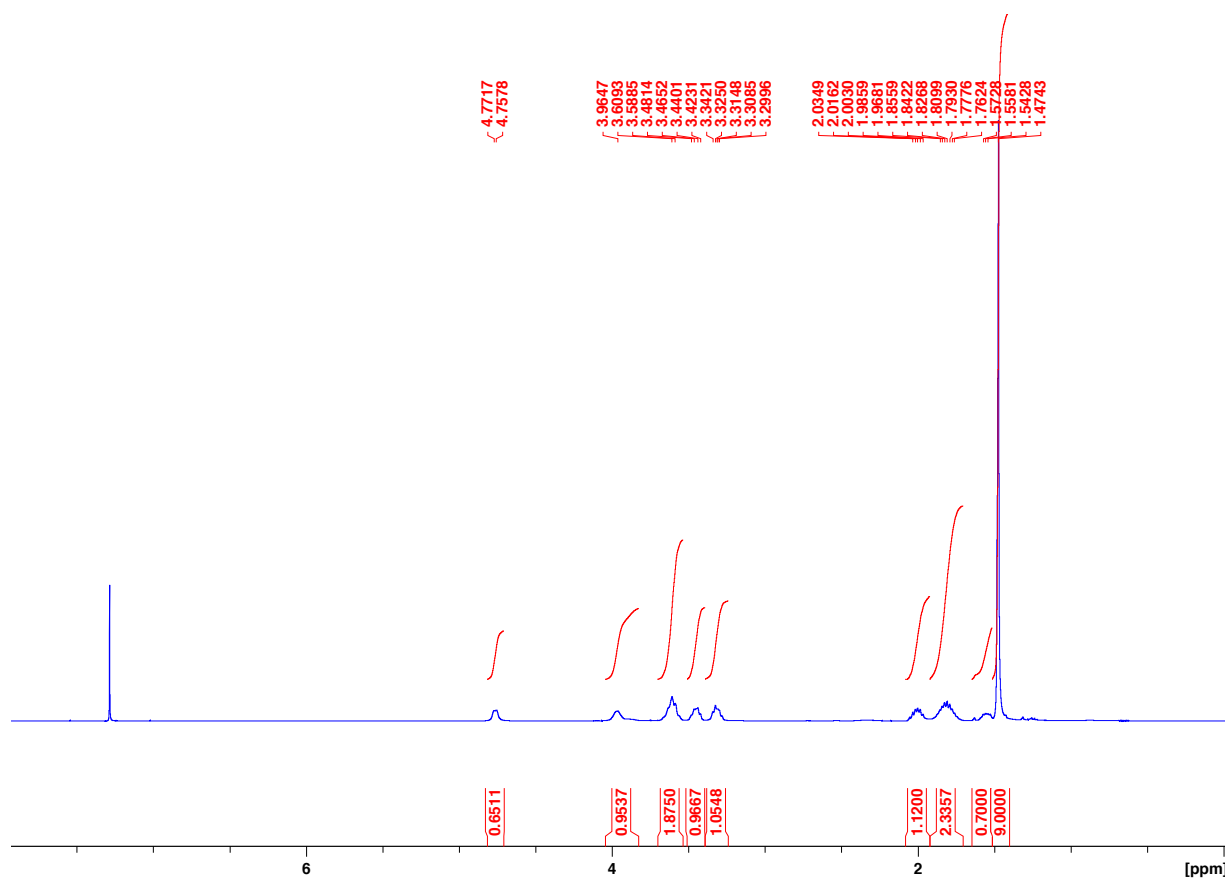


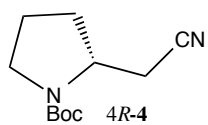
^{13}C NMR (100 MHz) (as a mixture of carbamate rotamers)



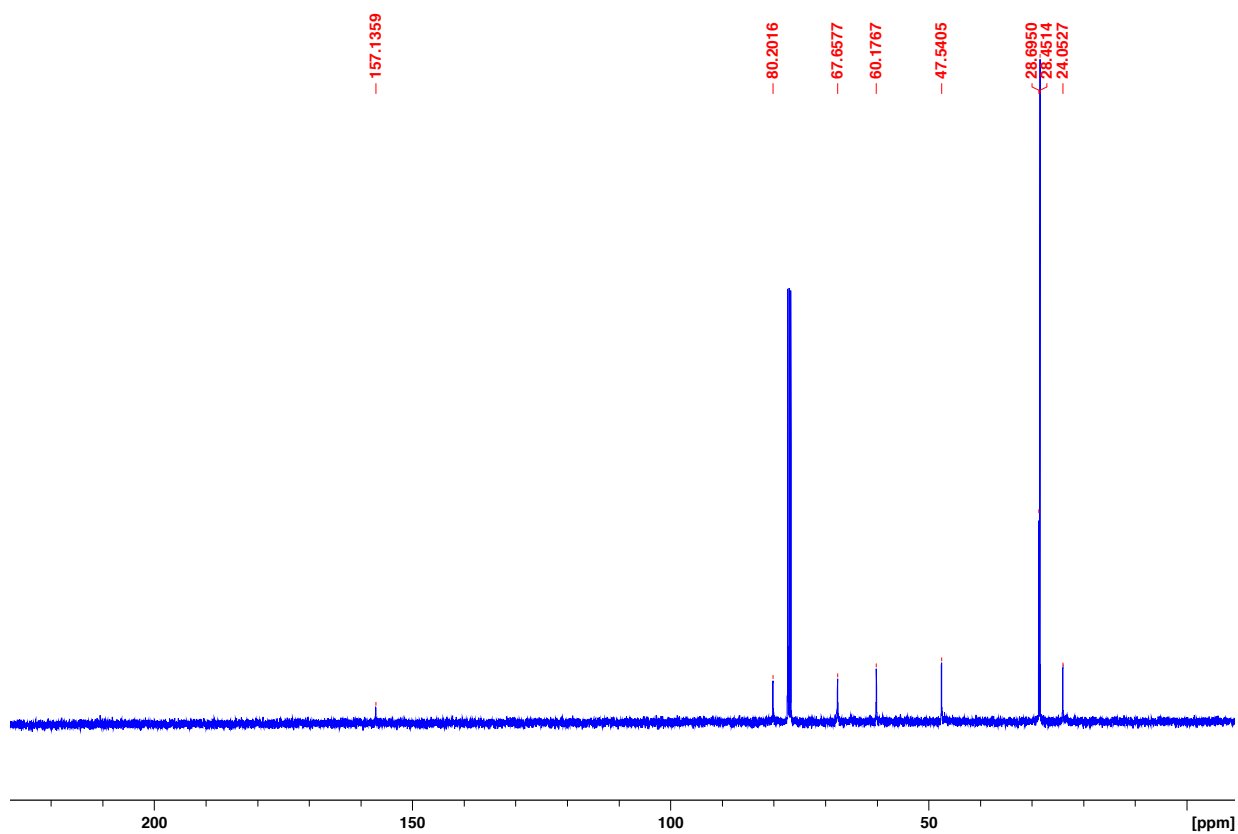


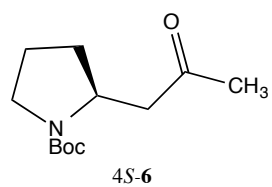
^1H NMR (400 MHz) (as a mixture of carbamate rotamers)



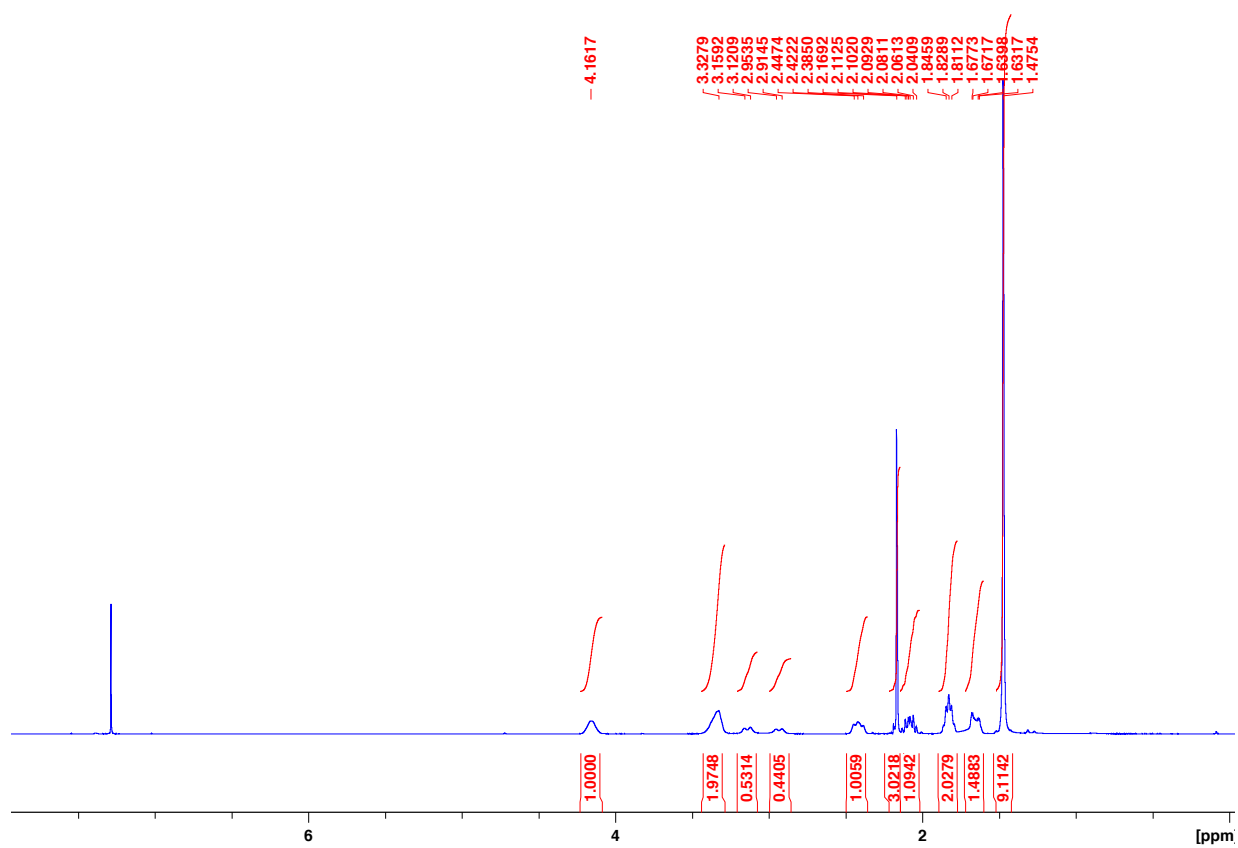


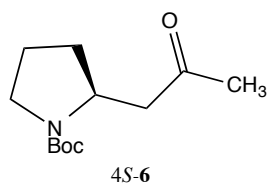
^{13}C NMR (100 MHz) (as a mixture of carbamate rotamers)



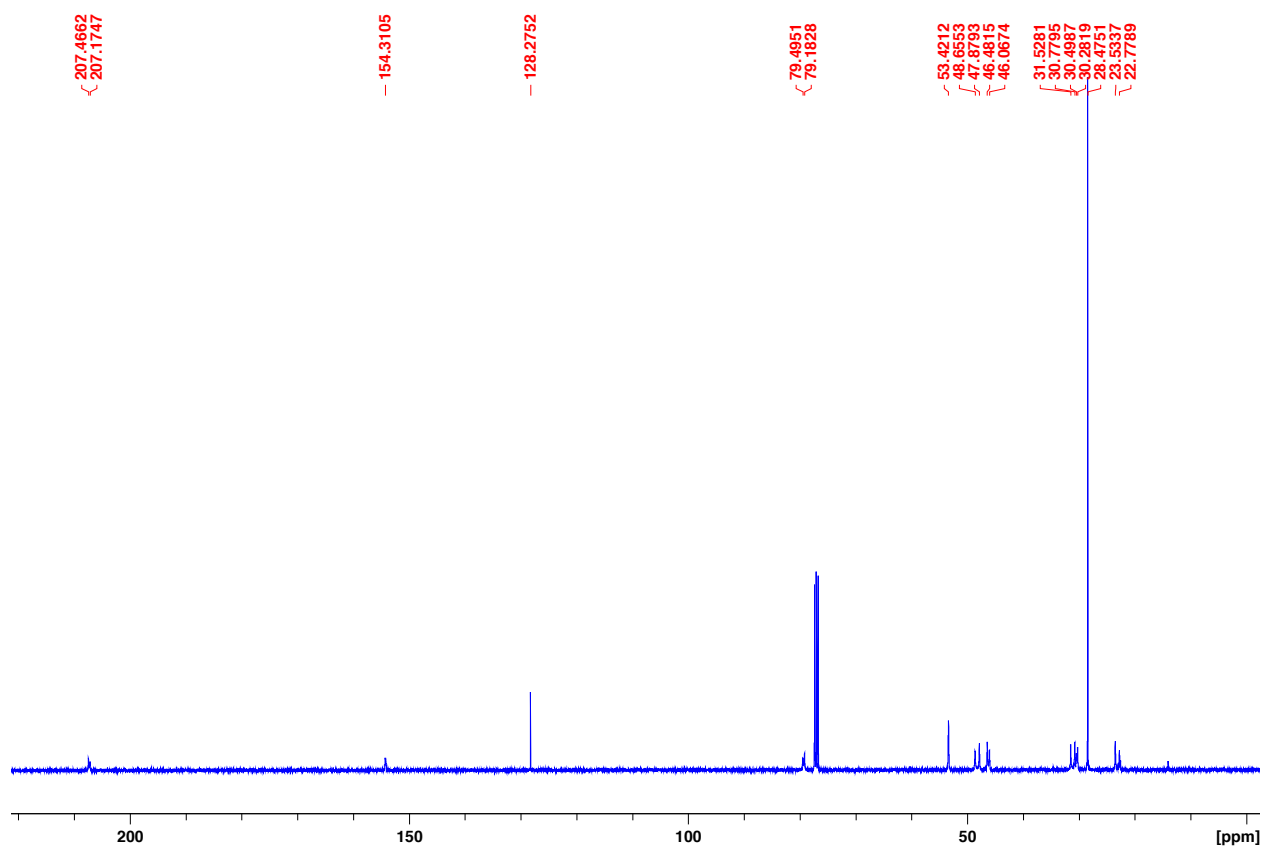


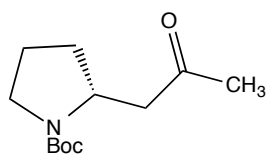
^1H NMR (400 MHz) (as a mixture of carbamate rotamers)





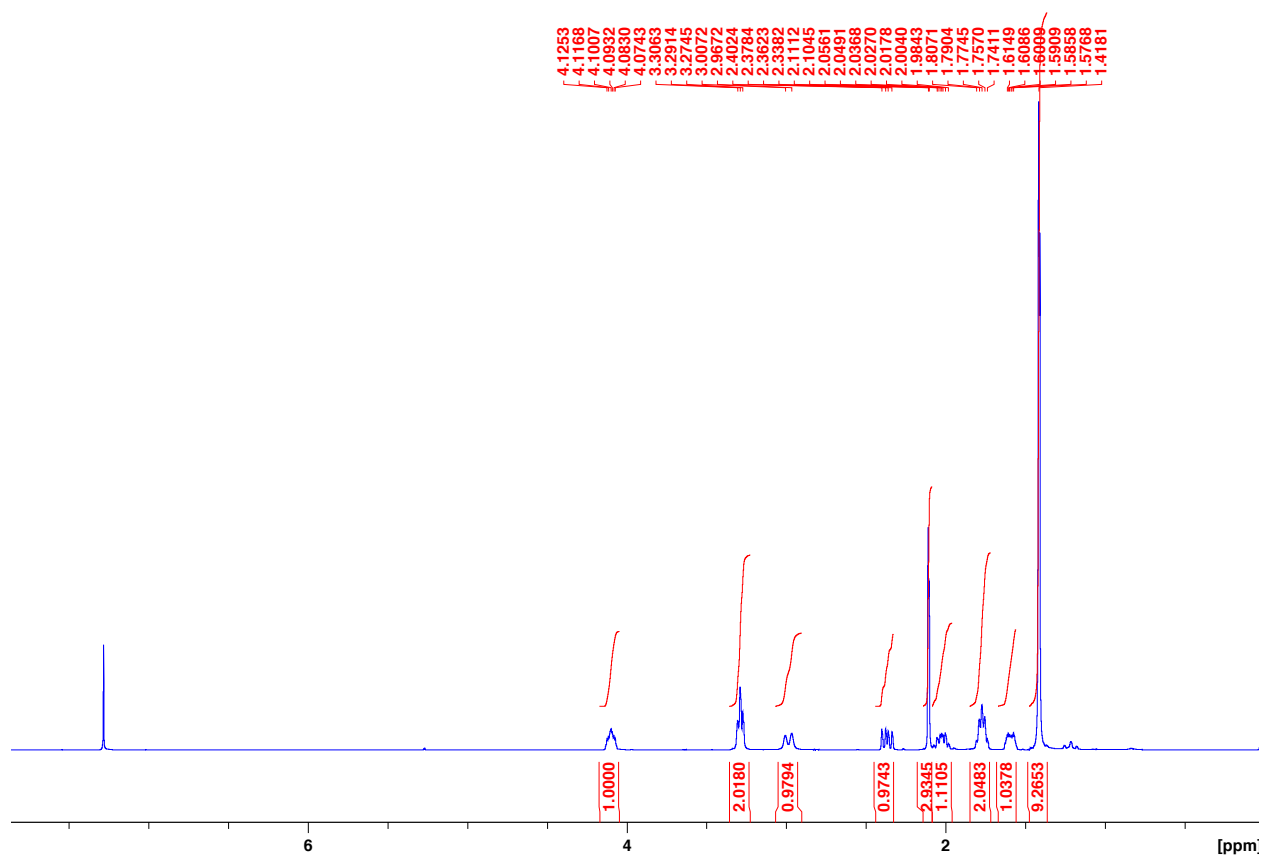
^{13}C NMR (100 MHz) (as a mixture of carbamate rotamers)

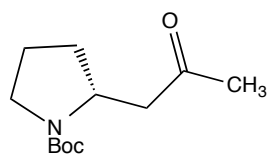




4R-6

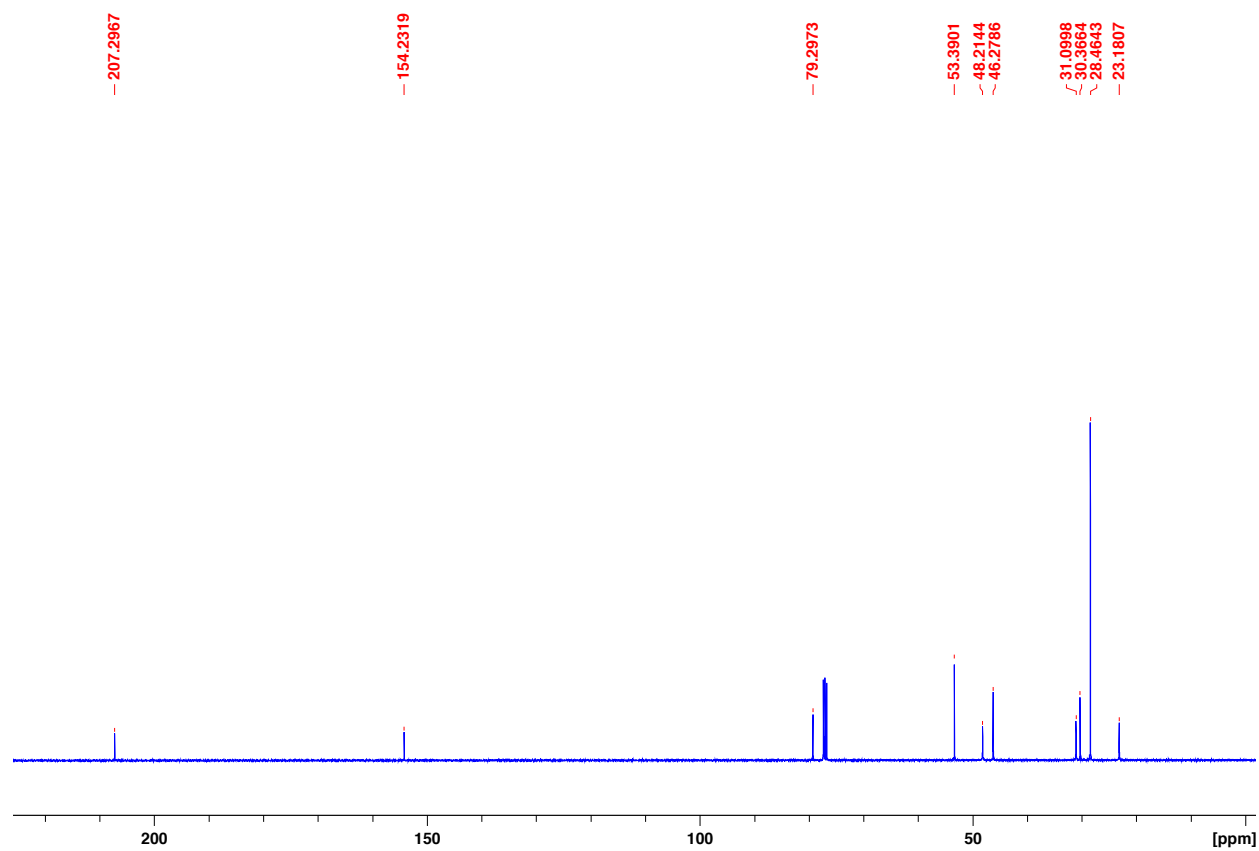
^1H NMR (400 MHz) (as a mixture of carbamate rotamers)

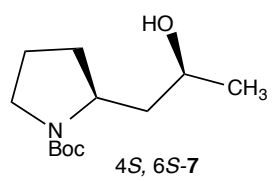




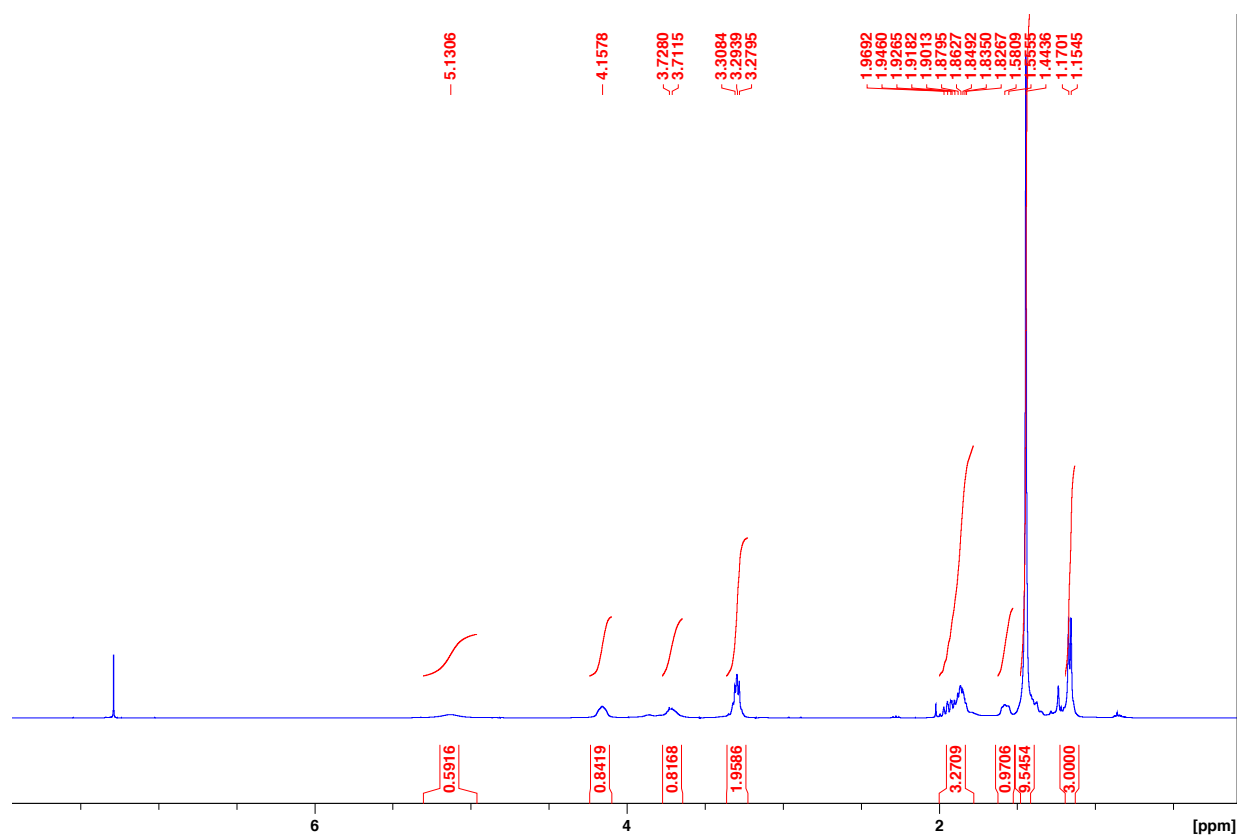
4R-6

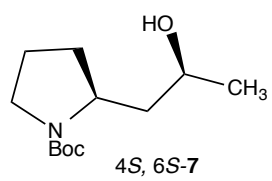
^{13}C NMR (100 MHz) (as a mixture of carbamate rotamers)



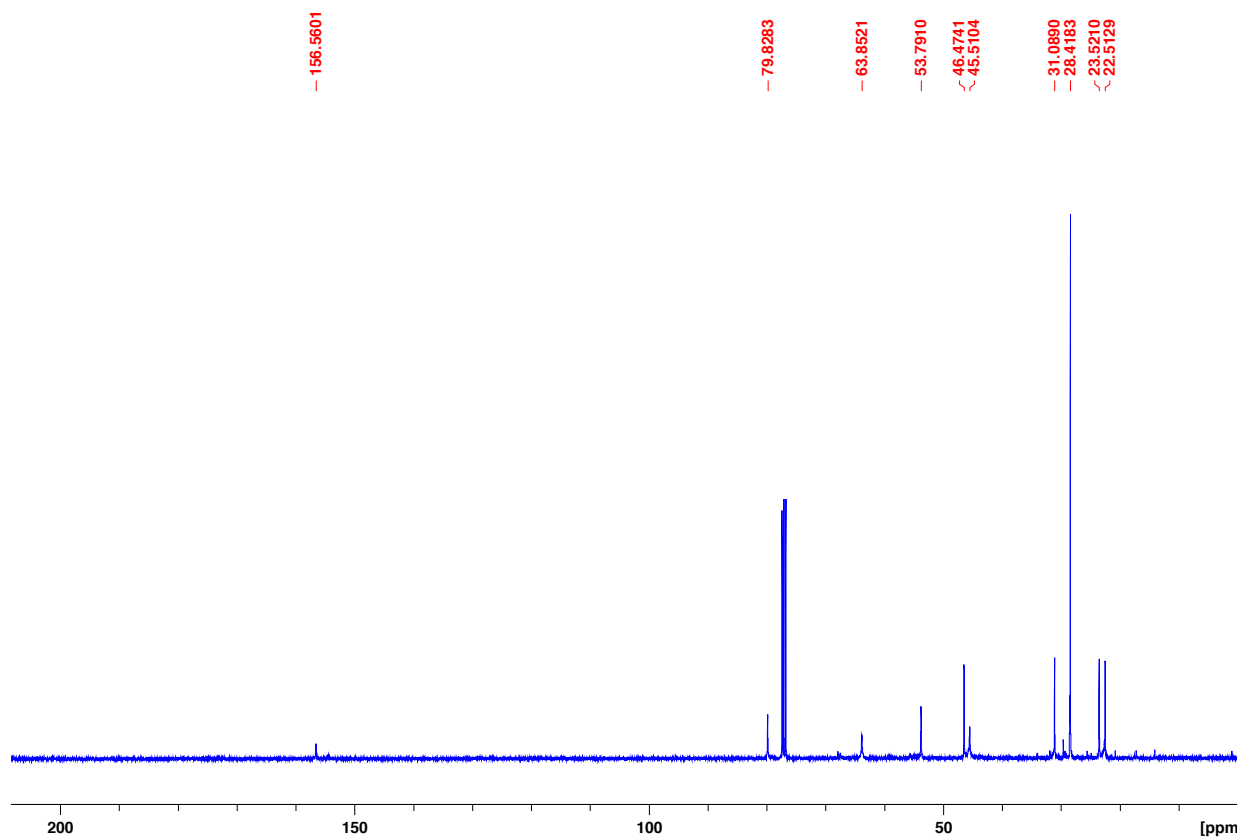


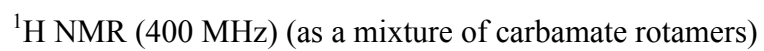
^1H NMR (400 MHz) (as a mixture of carbamate rotamers)

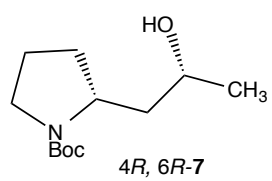




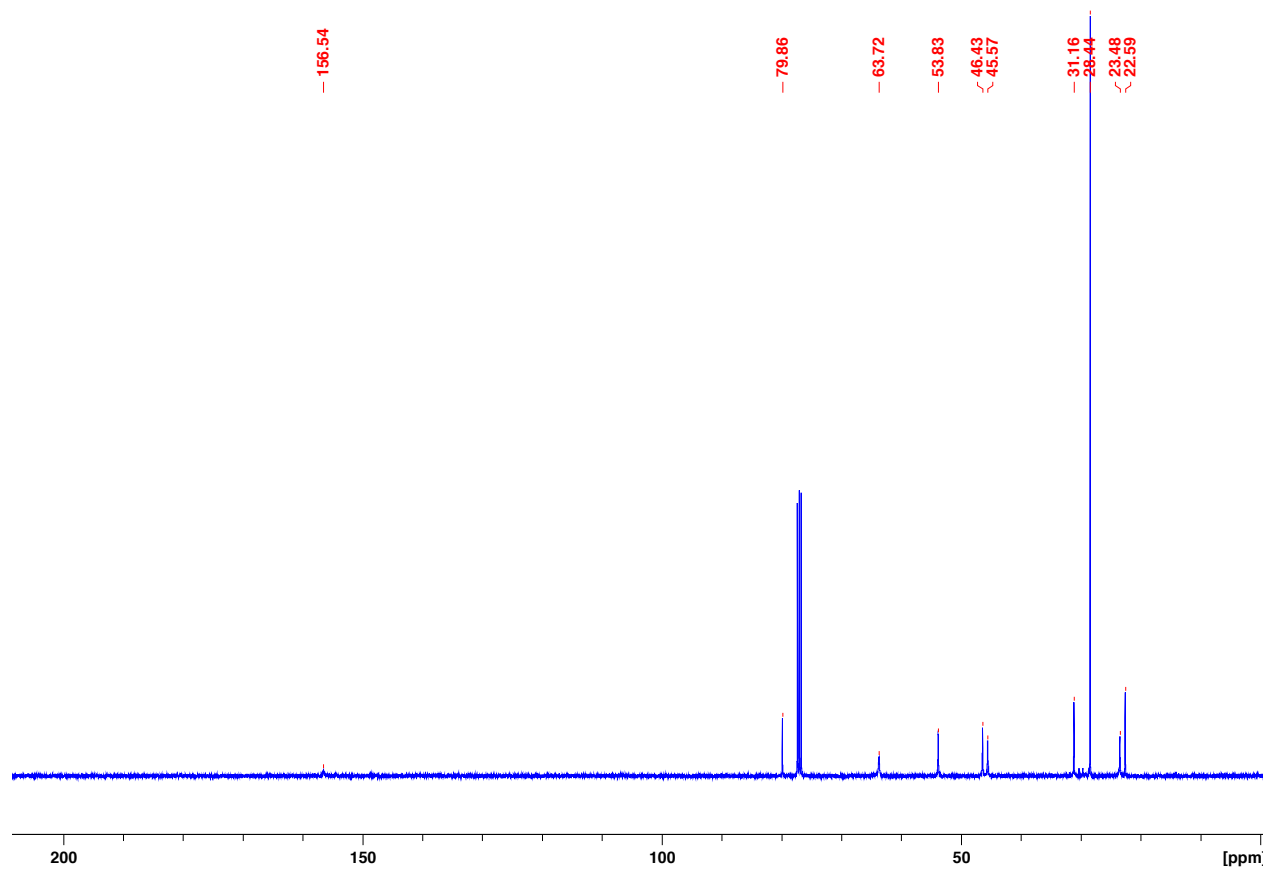
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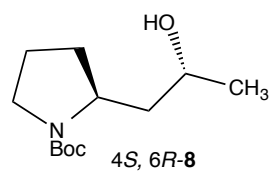




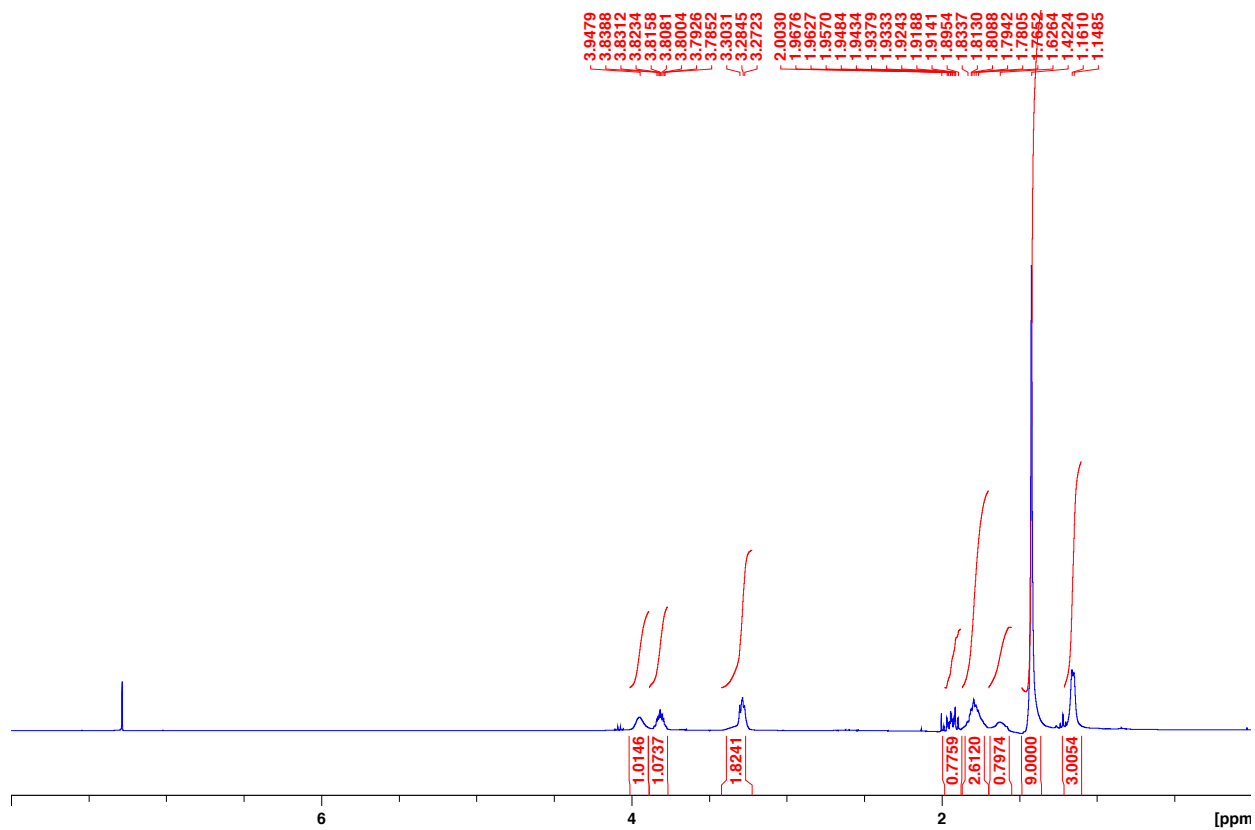


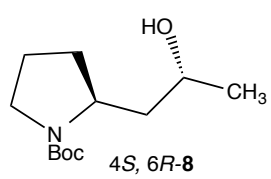
^{13}C NMR (100 MHz) (as a mixture of carbamate rotamers)



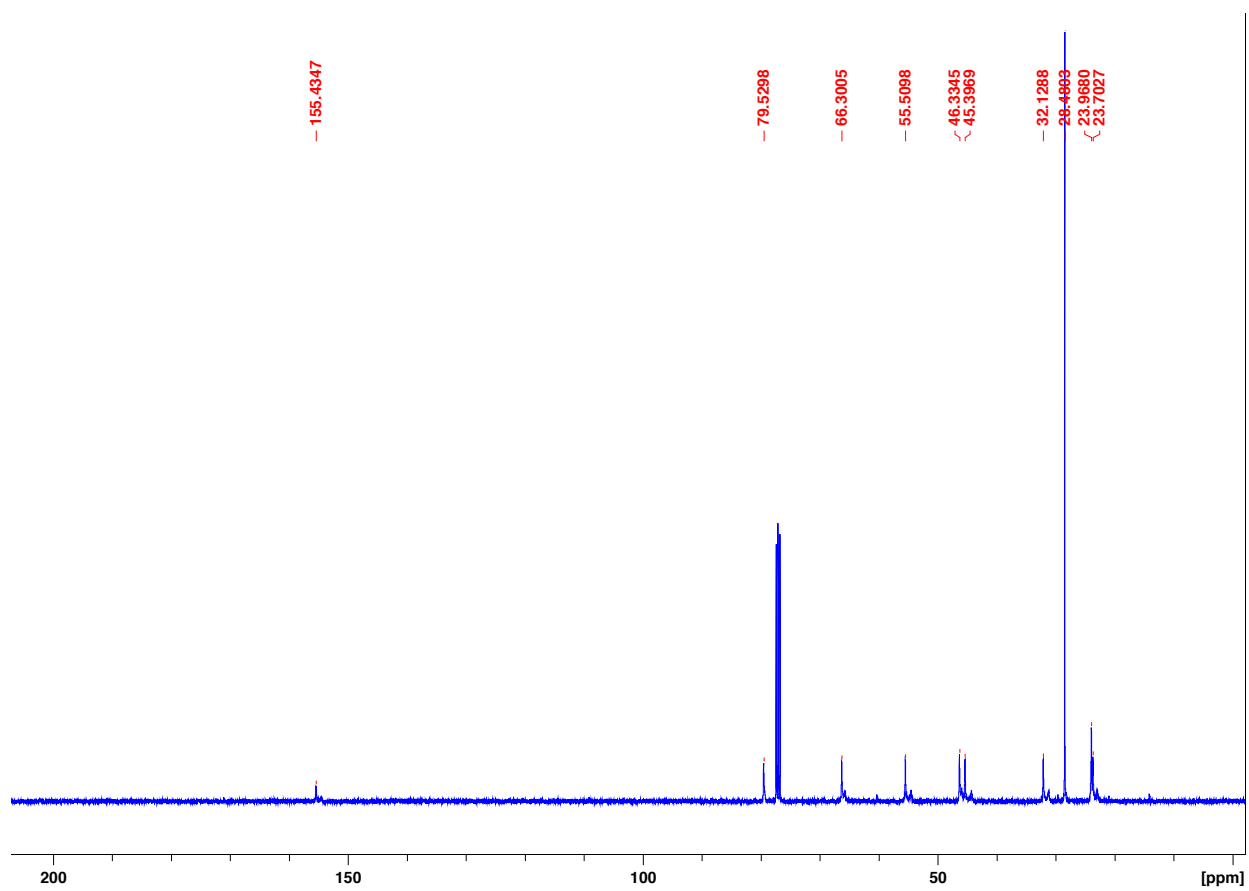


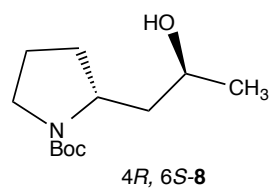
^1H NMR (400 MHz) (as a mixture of carbamate rotamers)



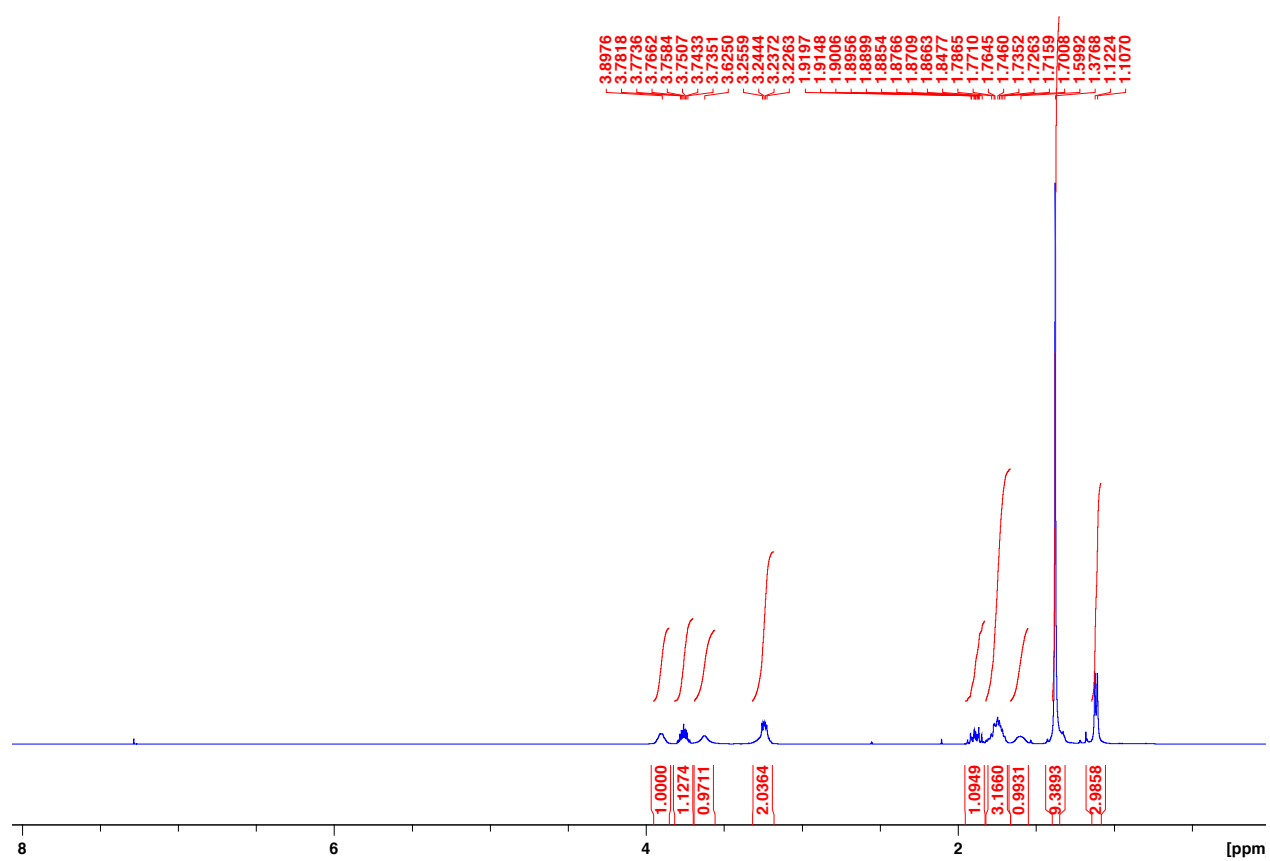


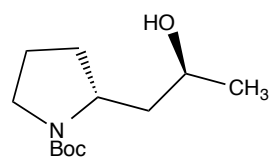
^{13}C NMR (100 MHz) (as a mixture of carbamate rotamers)





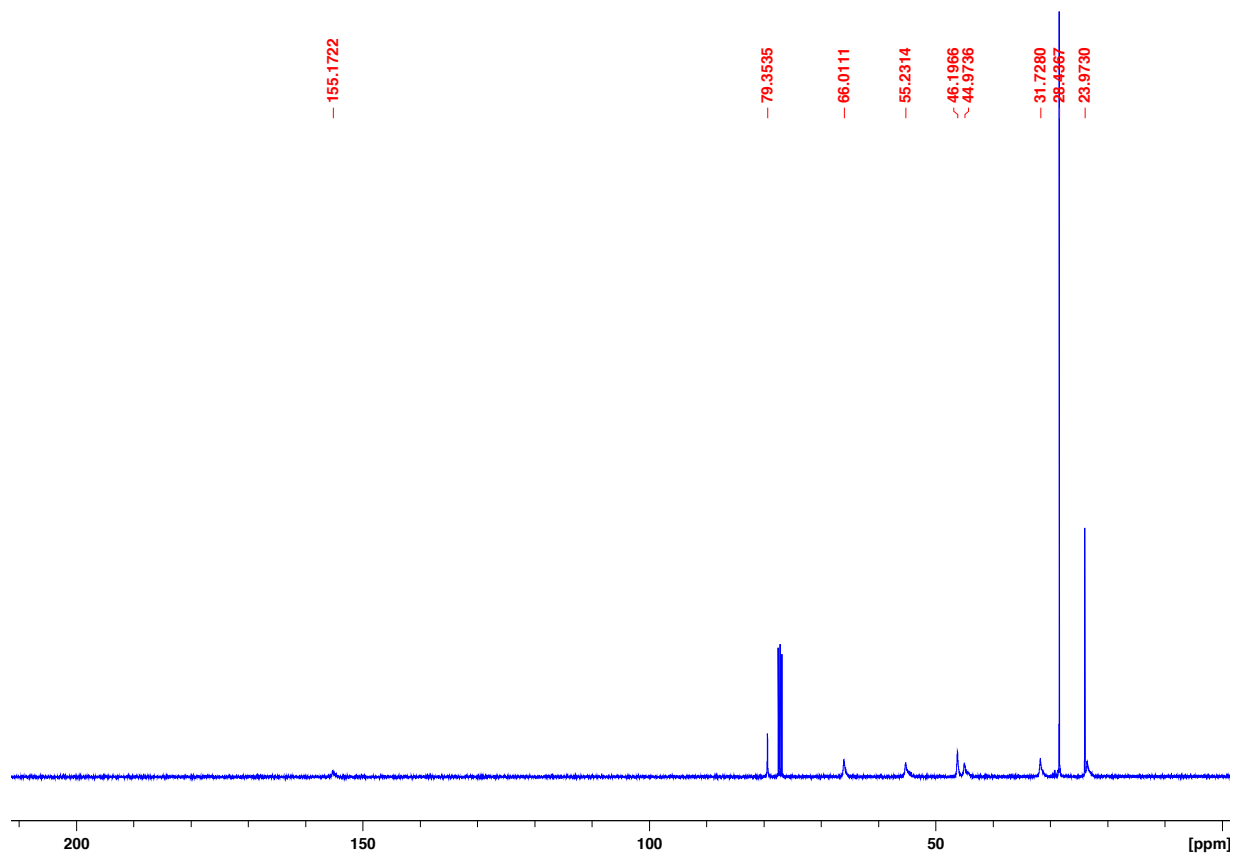
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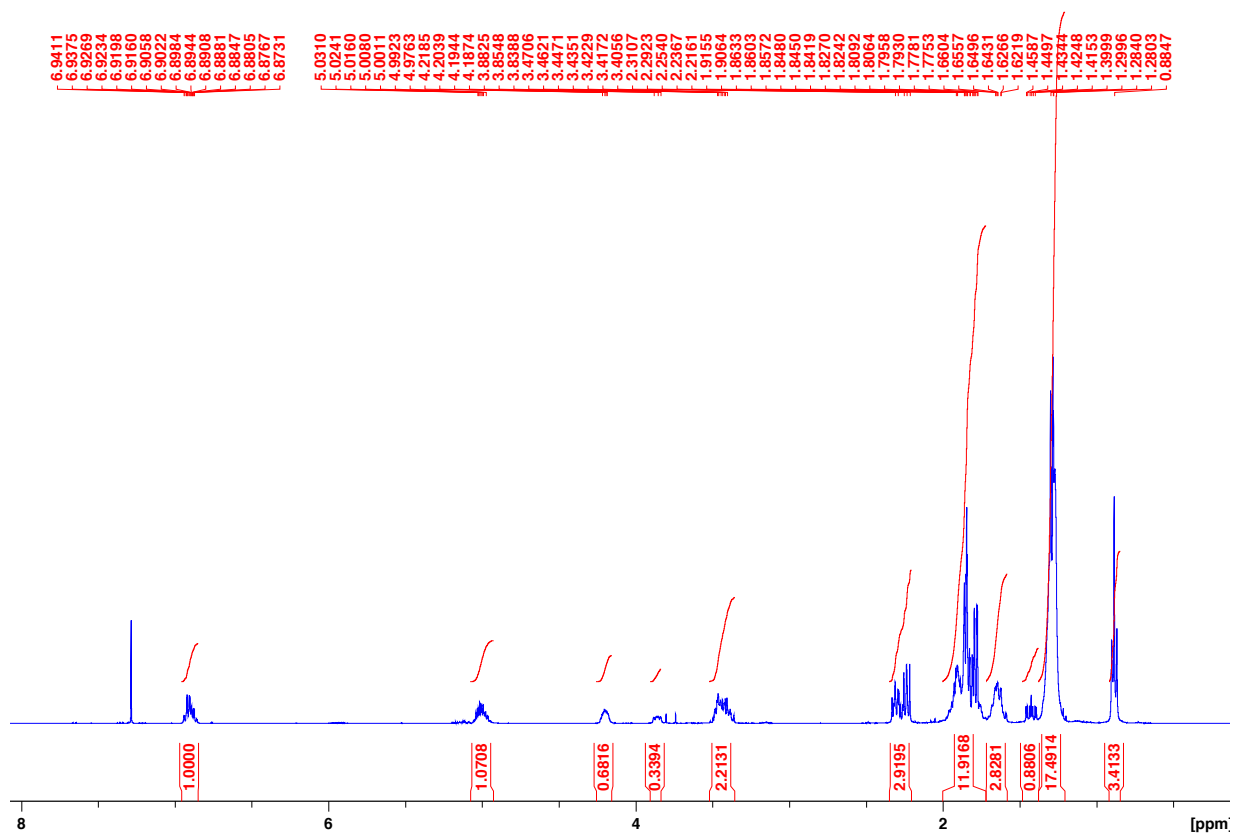
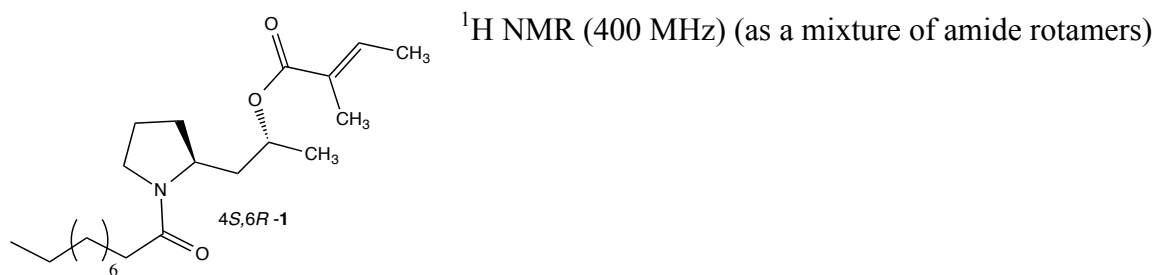


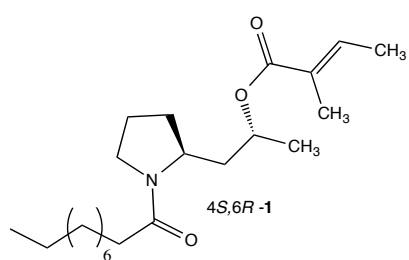


4R, 6S-8

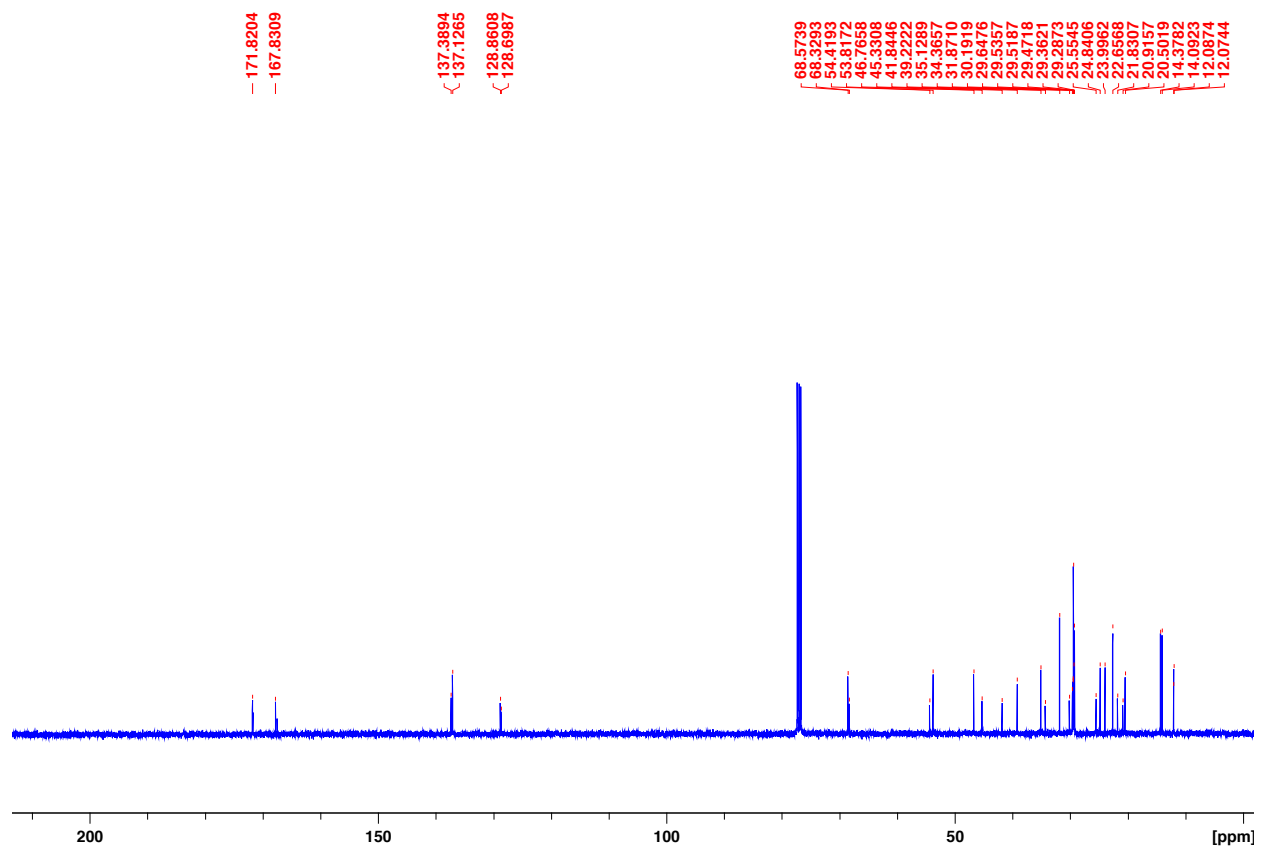
¹³C NMR (100 MHz) (as a mixture of carbamate rotamers)

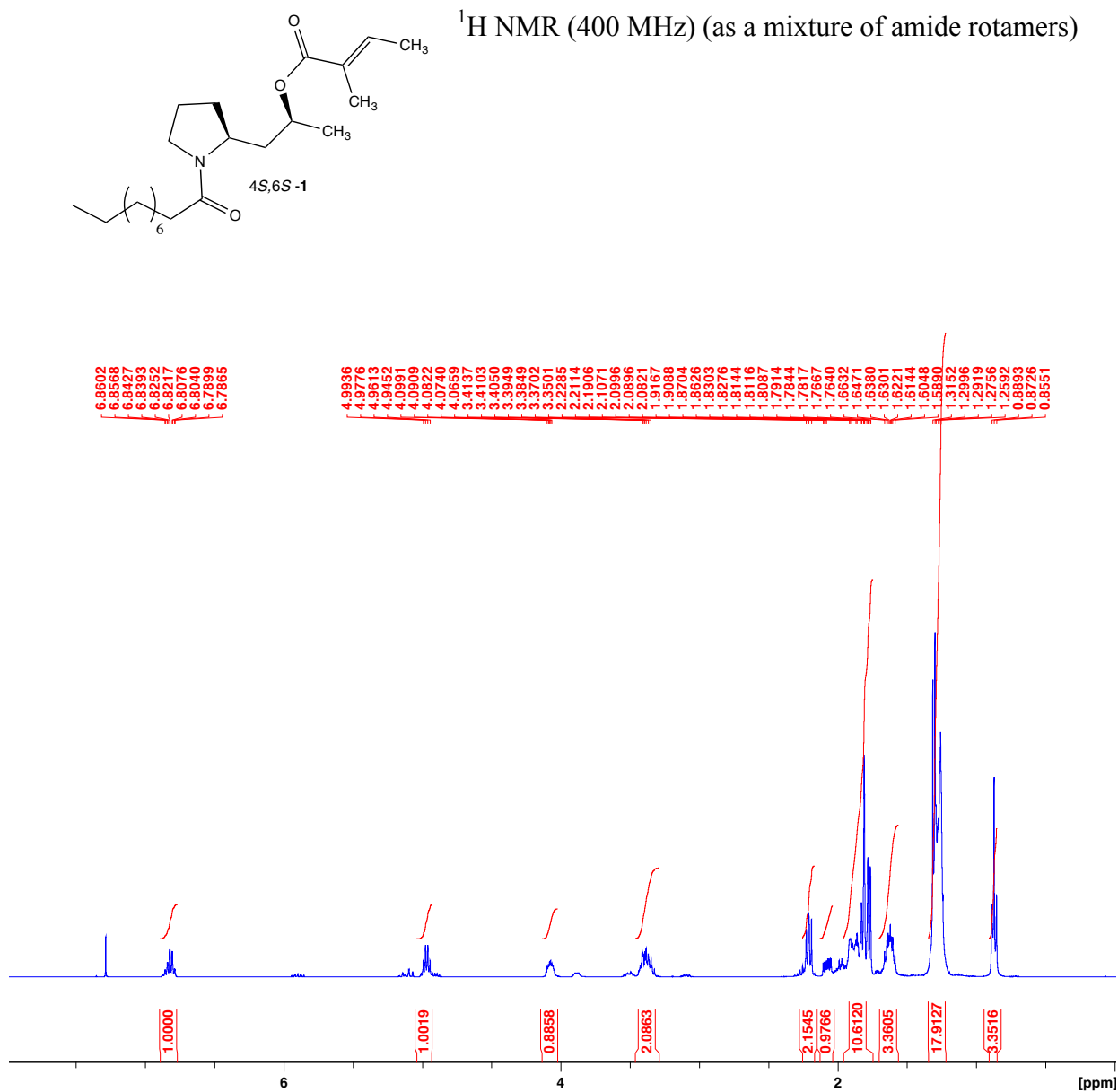


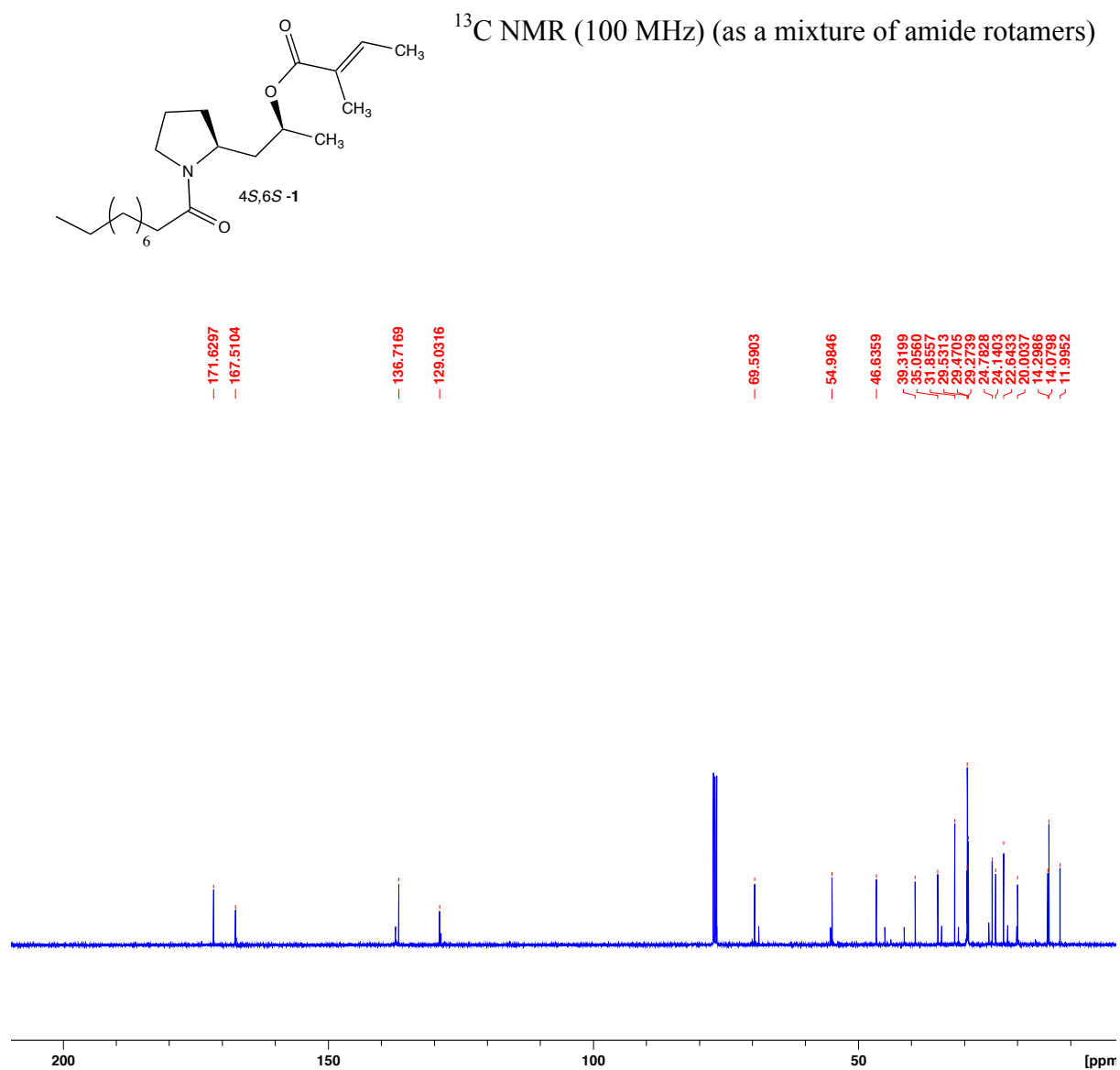


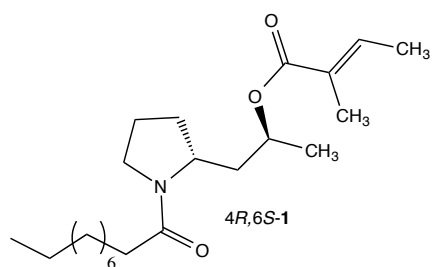


^{13}C NMR (100 MHz) (as a mixture of amide rotamers)

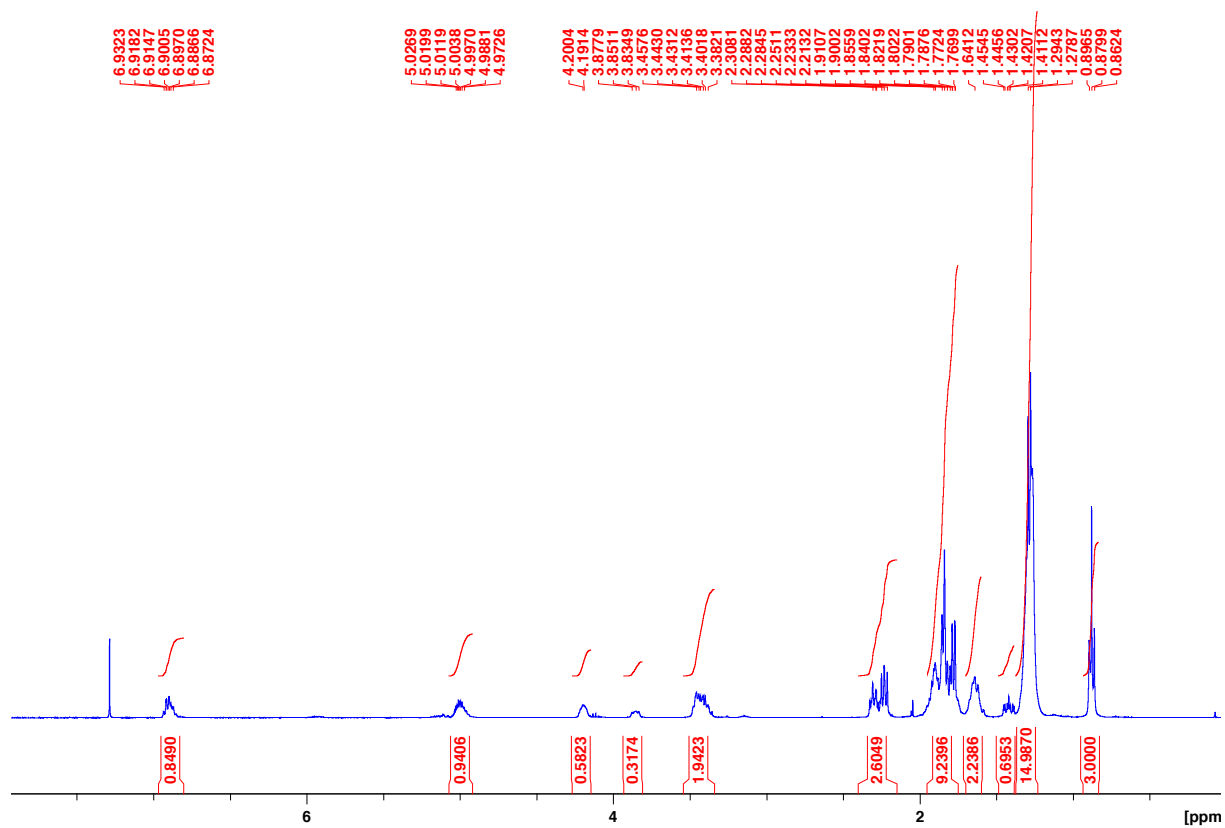


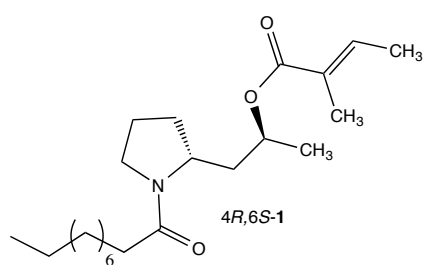




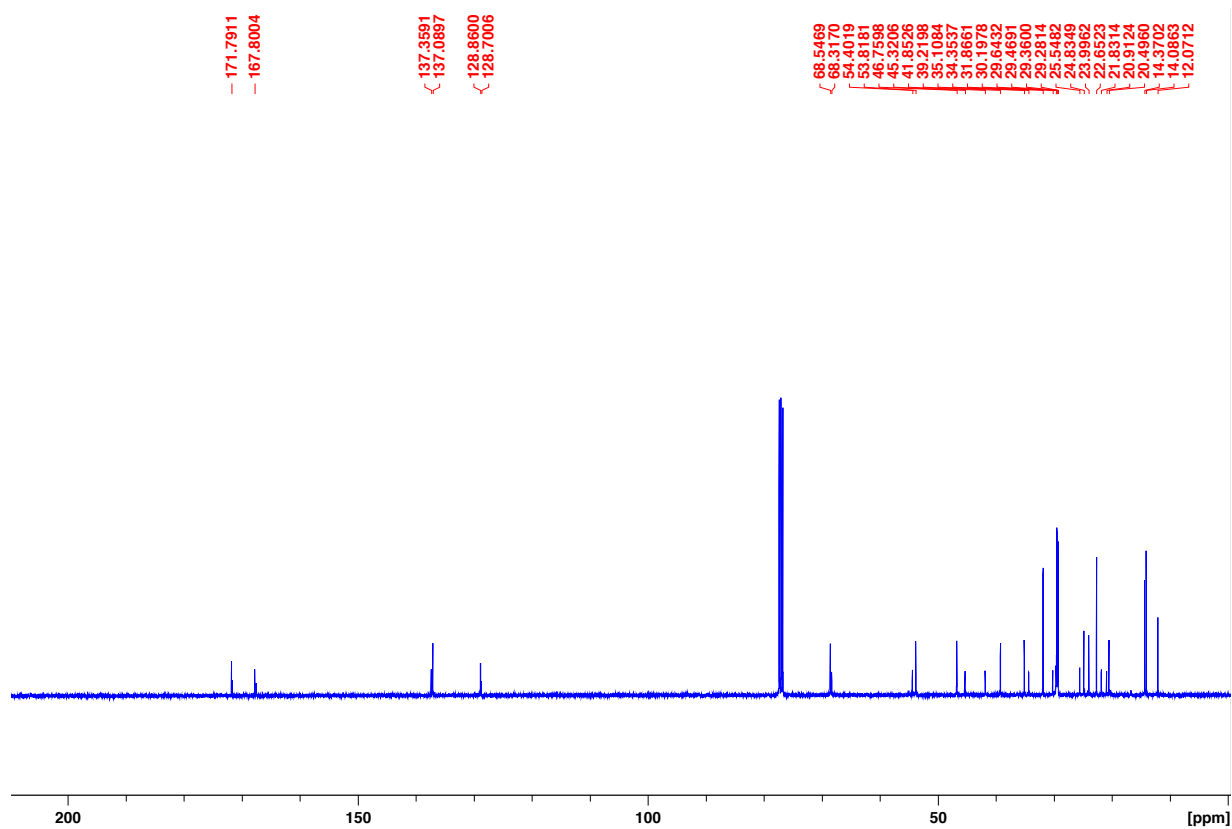


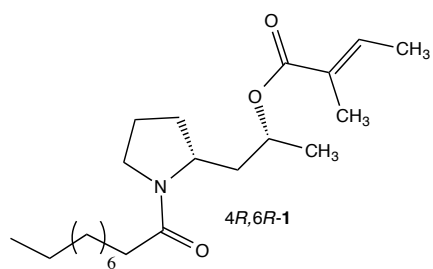
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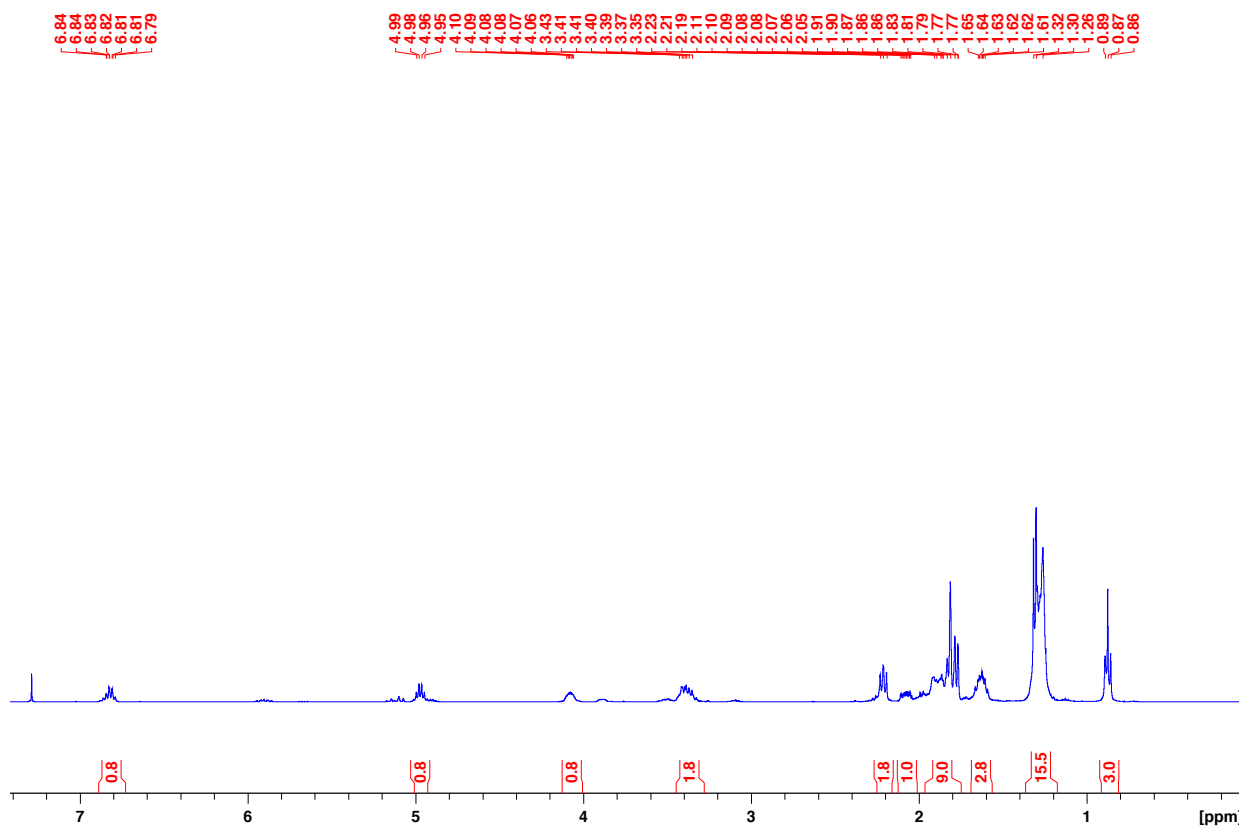


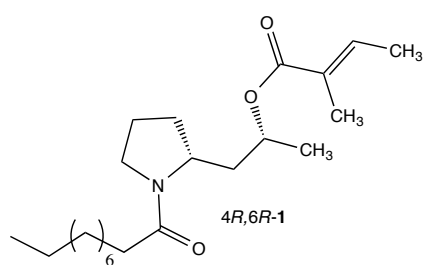
^{13}C NMR (100 MHz) (as a mixture of amide rotamers)



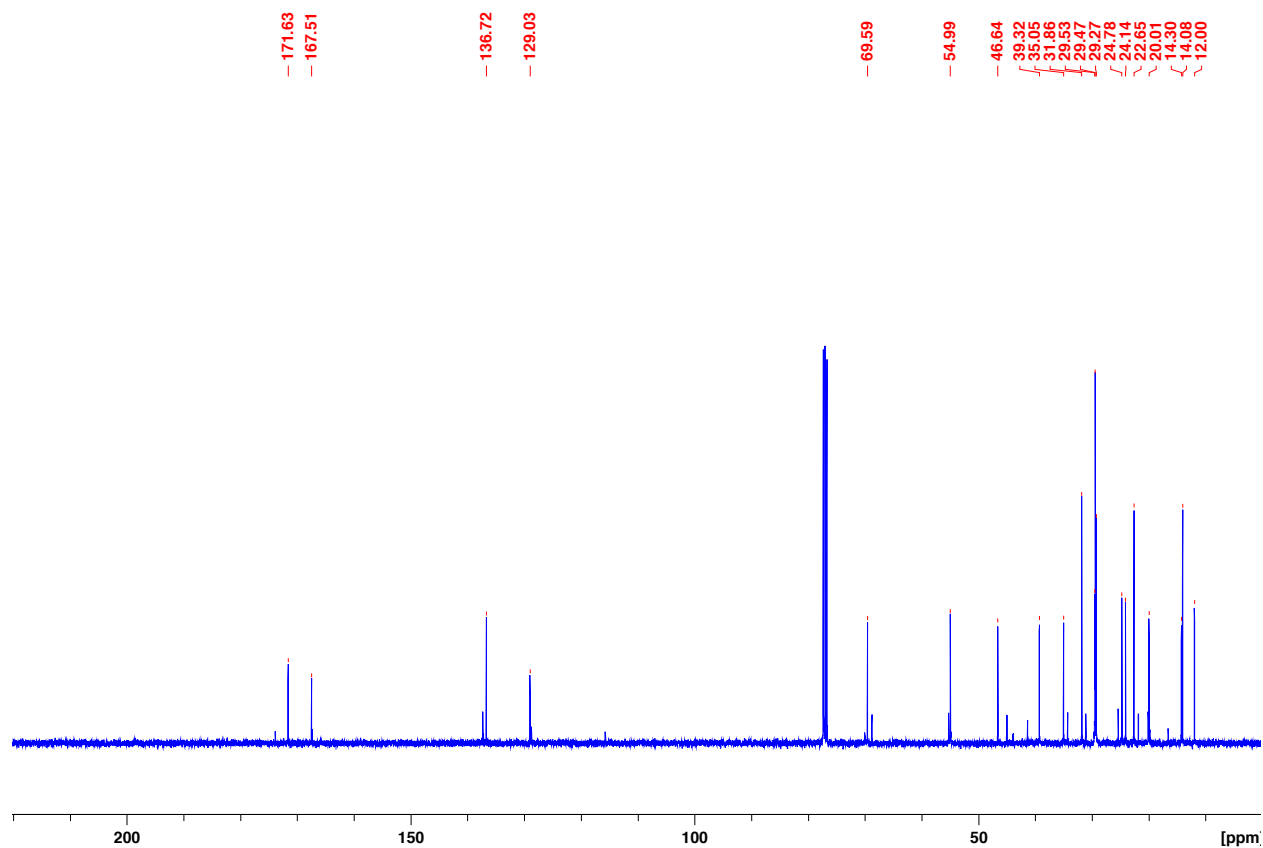


^1H NMR (400 MHz) (as a mixture of amide rotamers)





^{13}C NMR (100 MHz) (as a mixture of amide rotamers)



Binding studies

1. DNA Binding Studies

Materials: CT-DNA was purchased from CALBIOCHEM. Solutions of CT-DNA were prepared in 10mM Tris-EDTA buffer at pH 5.48 (as described in Jenkins: Jenkins, T.C. Optical Absorbance and Fluorescence Techniques for Measuring DNA-Drug Interactions. In *Methods in Molecular Biology, Drug-DNA Interaction Protocols*; Fox, K.R., Ed. Humana: Totowa, 1997; Vol 90, pp.195-217) and gave a 1.83:1 absorbance ratio at 260 nm and 280 nm. DNA and ligand concentrations were determined using 8452A HP Diode Array Spectrophotometer: CT-DNA, $\epsilon_{260} = 6,600 \text{ M}^{-1} \text{ cm}^{-1} \text{ bp}^{-1}$ (1.82:1 absorbance ratio at 260 nm and 280 nm); Ethidium Bromide, $\epsilon_{480} = 5,450 \text{ M}^{-1} \text{ cm}^{-1}$; HSA, $\epsilon_{280} = 35,700 \text{ M}^{-1} \text{ cm}^{-1}$

EB competitive experiments

Constant concentrations of CT-DNA (10 μM) and EtBr (10 μM) were titrated with increasing concentrations of the ligands (from 1 mM and 100 μM stock solutions). The maximum emission wavelength was 590 nm when the excitation wavelength was 520 nm. Fluorescence titrations were recorded from 520 nm to 692 nm after an equilibration period of 3 min. Ex Slit (nm) = 10.0; Em Slit (nm) = 10.0; Scan Speed (nm/min) = 200. $K_{app} = 9.5 \times 10^6 \text{ M}^{-1} \times 10/C_{50}$

Circular Dichroism Studies.

Small aliquots (0.6-5.0 μL) of a concentrated **1** solution (1 mM) were added to a solution of HSA (15 μM in pH=6.81 in Tris-HCl buffer) inverted twice, and incubated for 5 min at 20 °C. The CD spectra were then recorded as an average of three scans from 220 to 310 nm and data recorded in 0.1 nm increments

Thermal denaturation studies

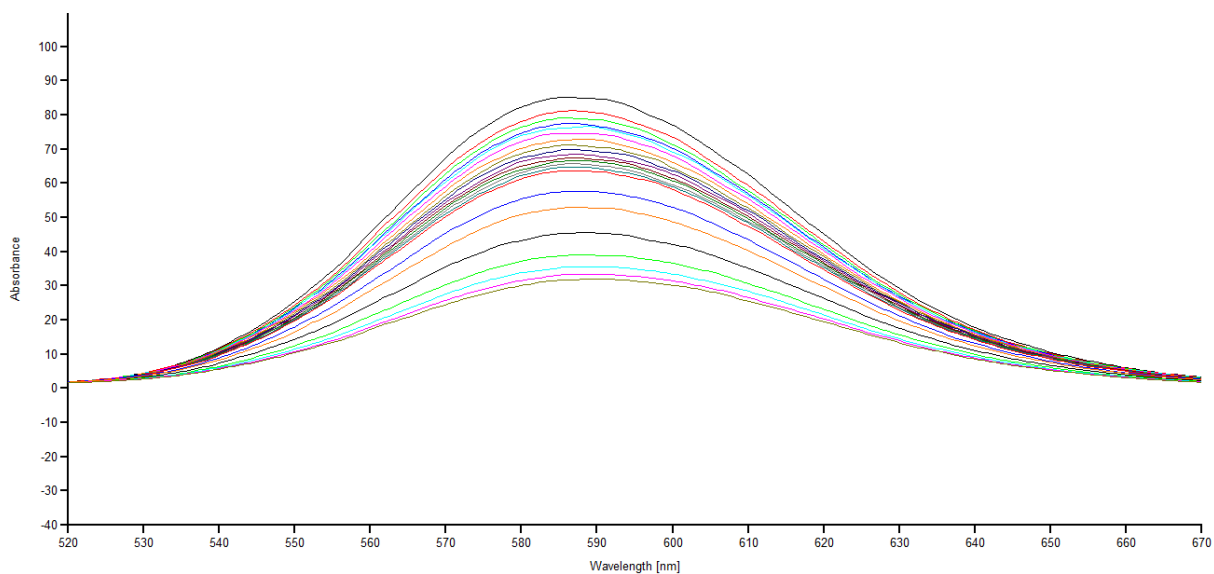
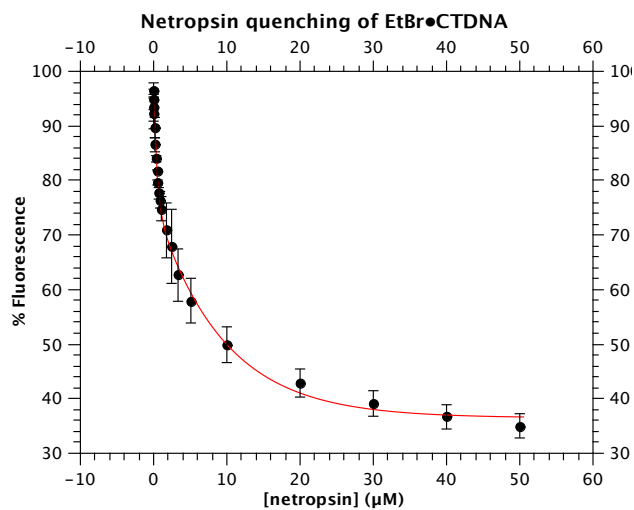
UV thermal denaturation samples (2 mL) were prepared by mixing CT-DNA in Tris-HCl buffer (pH 6.81) in 1cm path length quartz cuvettes. The DNA to ligand ratio was 40:1. Thermal denaturation spectra were obtained from HP Diode Array Spectrophotometer connected to 89090A Peltier Temperature Controller. Absorbance readings were taken for temperature ranging from 25 °C to 90 °C. Temperature was increased gradually with a speed of 1°C/min with an absorbance reading every 2°C. First derivative plots were used to determine the T_M value.

References for extinction coefficients:

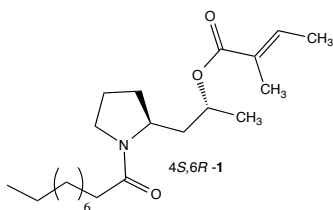
EtBr: Saucier, J. M.; Festy, B.; LePecq, J.-B. *Biochimie* **1971**, 53, 973.

HSA: Bordbar, A.-K.; Taheri-Kafrani, A. *Colloids and Surfaces B: Biointerfaces* **2007**, 55, 84.

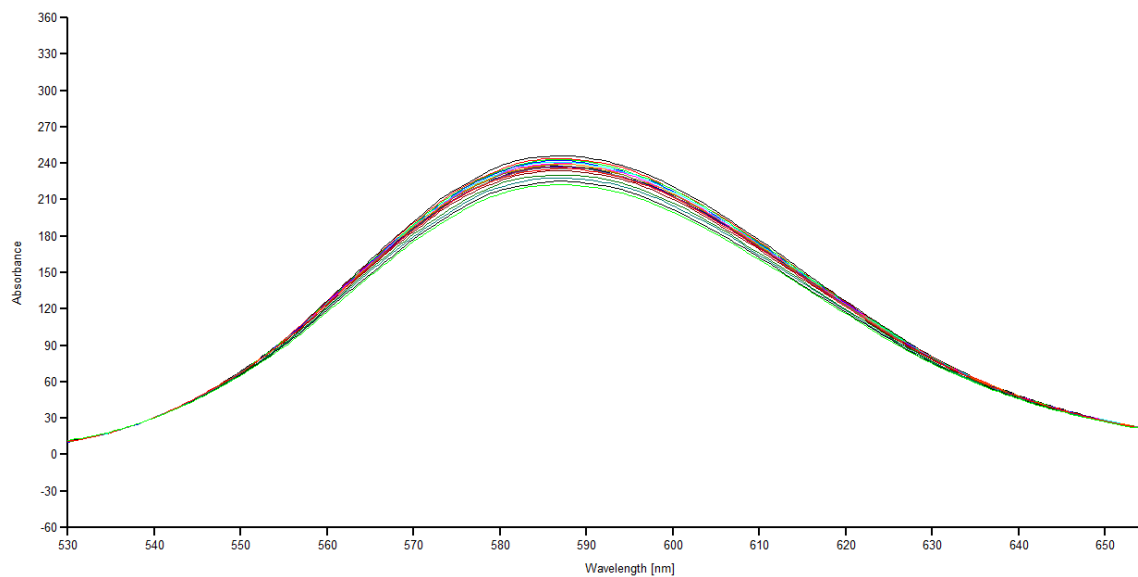
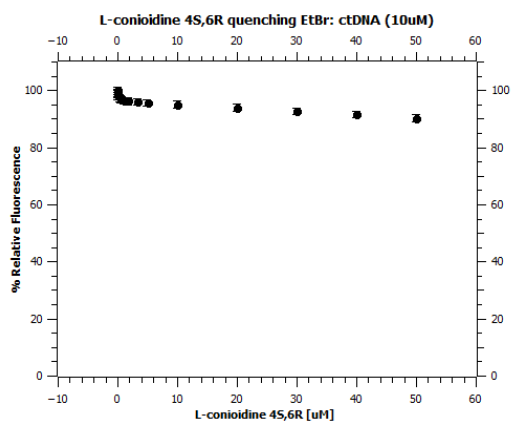
Positive Control for ethidium displacement studies: CT DNA (10 μM) and ethidium bromide (5 μM) were titrated with increasing amounts of netropsin over the 0.01 μM to 50 μM range (pH=7.21, $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$ buffer): 0.01, 0.02, 0.04, 0.05, 0.10, 0.20, 0.33, 0.45, 0.56, 0.63, 0.71, 0.83, 1.0, 1.25, 1.67, 2.5, 3.3, 5.0, 10.0, 20.0, 30.0, 40.0, 50.0 μM . Measured $C_{50}=10 \pm 2.5 \mu\text{M}$; $K_{app}=5.1 \pm 0.3 \times 10^6 \text{ M}^{-1}$.



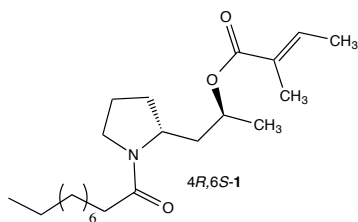
CT DNA binding data for 4*S*,6*R*-1



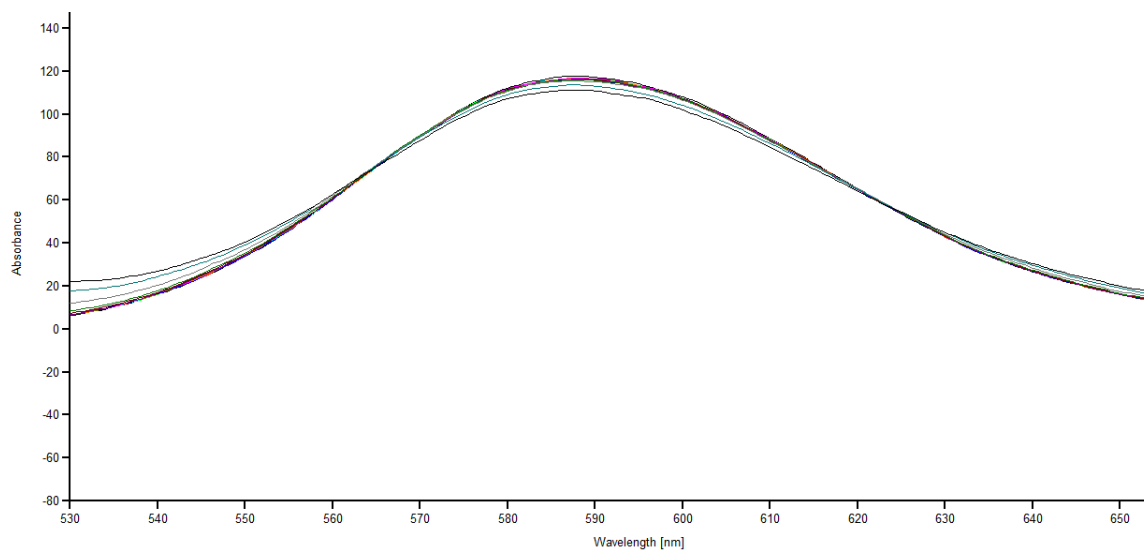
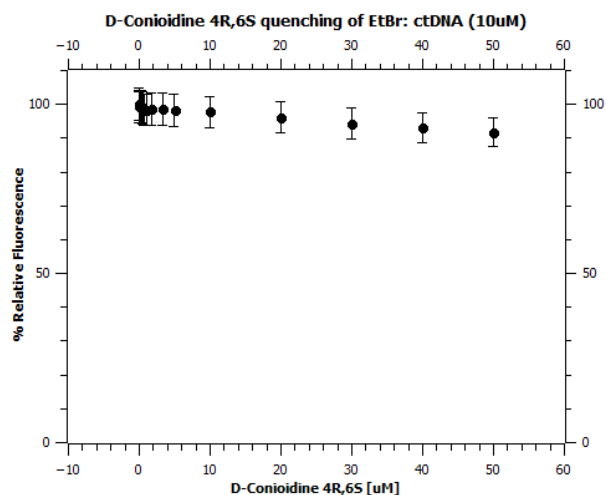
Ethidium displacement studies: CT DNA (10 μM) and ethidium bromide (10 μM) were titrated with increasing amounts of 4*S*,6*R*-1 over the 0.01 μM to 50 μM range (pH=6.81, Tris-HCl buffer): 0.01, 0.02, 0.05, 0.1, 0.38, 0.56, 0.71, 1.0, 1.67, 3.3, 5.0, 10.0, 20.0, 30.0, 40.0, 50.0 μM . Approximate C_{50} = 380 μM ; K_{app} = $\sim 2.3 \times 10^5 \text{ M}^{-1} \cdot \text{bp}^{-1}$.



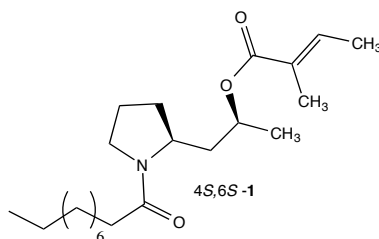
CT DNA binding data for 4*R*,6*S*-1



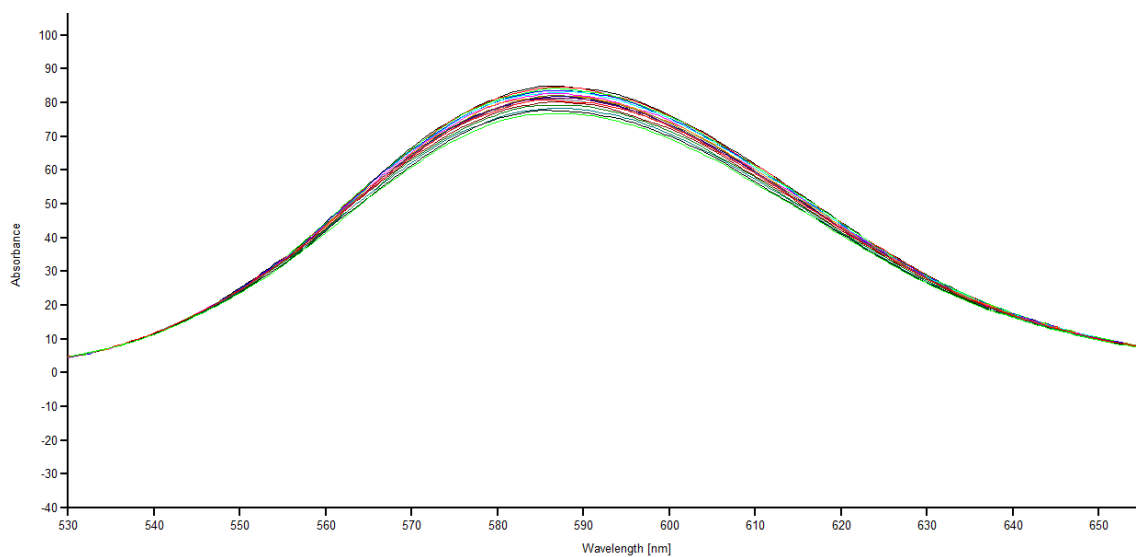
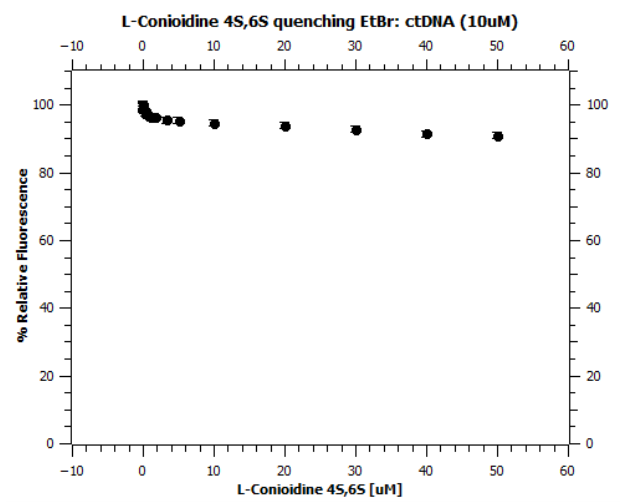
Ethidium displacement studies: CT DNA (10 μM) and ethidium bromide (10 μM) were titrated with increasing amounts of 4*R*,6*S*-1 over the 0.01 μM to 50 μM range (pH=6.81, Tris-HCl buffer): 0.01, 0.02, 0.05, 0.1, 0.38, 0.56, 0.71, 1.0, 1.67, 3.3, 5.0, 10.0, 20.0, 30.0, 40.0, 50.0 μM . Approximate C_{50} = 340 μM ; K_{app} = $\sim 2.6 \times 10^5 \text{ M}^{-1} \cdot \text{bp}^{-1}$.



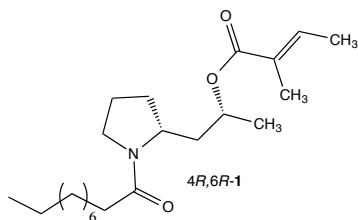
CT DNA binding data for 4*S*,6*S*-1



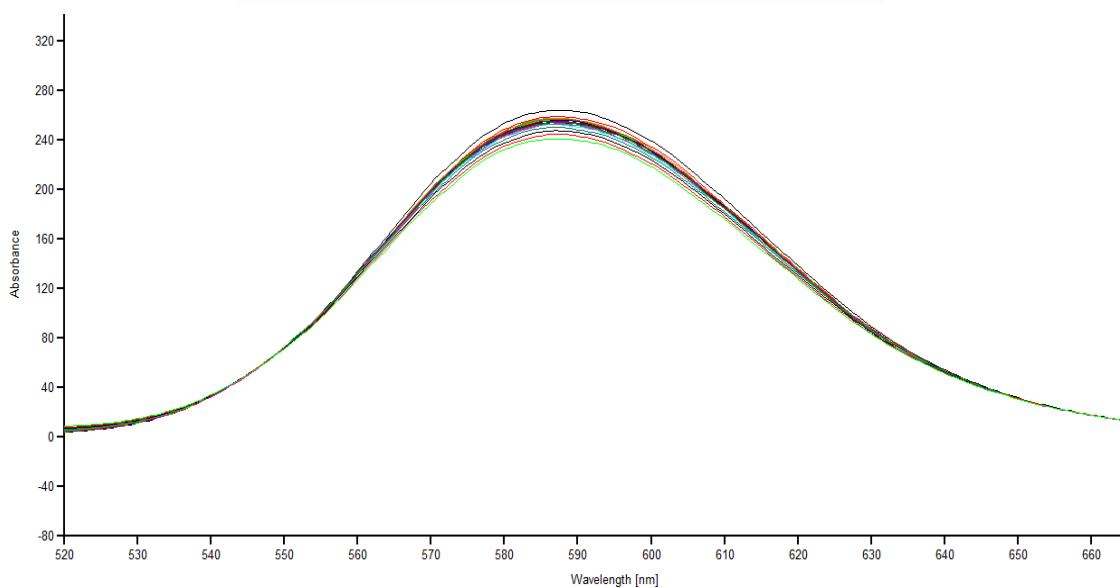
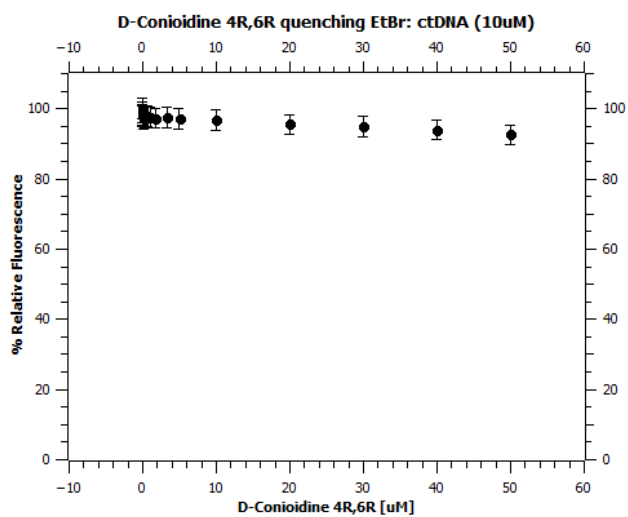
Ethidium displacement studies: CT DNA (10 μM) and ethidium bromide (10 μM) were titrated with increasing amounts of 4*S*,6*S*-1 over the 0.01 μM to 50 μM range (pH=6.81, Tris-HCl buffer): 0.01, 0.02, 0.05, 0.1, 0.38, 0.56, 0.71, 1.0, 1.67, 3.3, 5.0, 10.0, 20.0, 30.0, 40.0, 50.0 μM . Extrapolated C_{50} = 470 μM ; K_{app} = $\sim 1.9 \times 10^5 \text{ M}^{-1} \cdot \text{bp}^{-1}$.



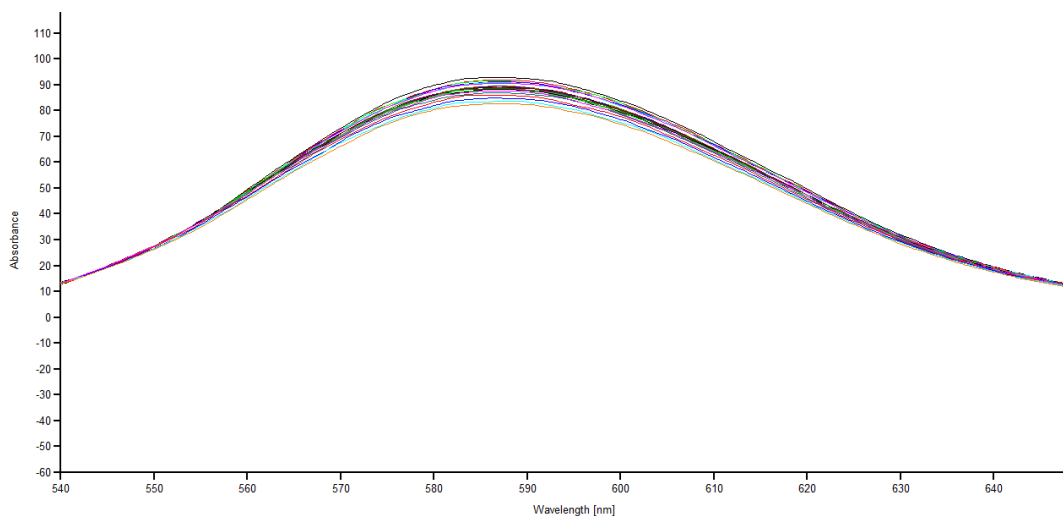
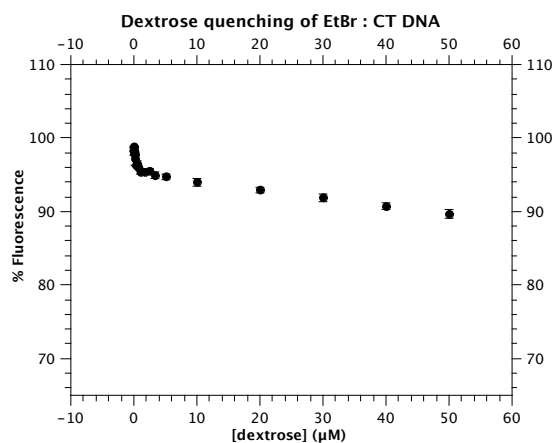
CT DNA binding data for 4*R*,6*R*-1



Ethidium displacement studies: CT DNA (10 μM) and ethidium bromide (10 μM) were titrated with increasing amounts of 4*R*,6*R*-1 over the 0.01 μM to 50 μM range (pH=6.81, Tris-HCl buffer): 0.01, 0.02, 0.05, 0.1, 0.38, 0.56, 0.71, 1.0, 1.67, 3.3, 5.0, 10.0, 20.0, 30.0, 40.0, 50.0 μM . Extrapolated C_{50} = 480 μM ; K_{app} = $\sim 1.8 \times 10^5 \text{ M}^{-1} \cdot \text{bp}^{-1}$.

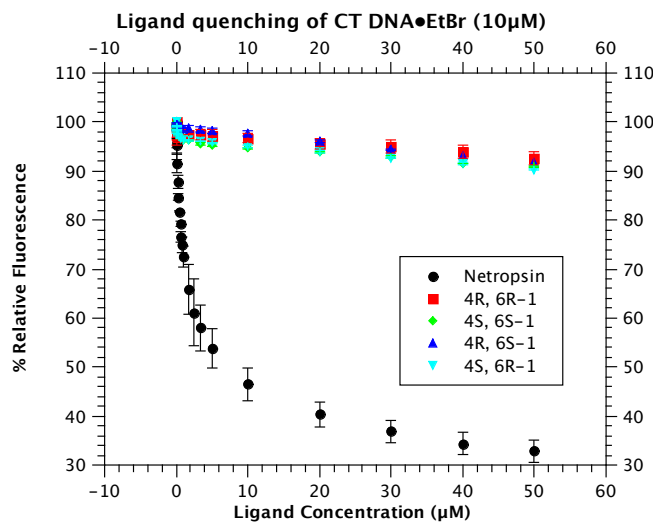


Negative Control for ethidium displacement studies: CT DNA (10 μM) and ethidium bromide (10 μM) were titrated with increasing amounts of dextrose over the 0.005 μM to 50 μM range (pH=7.21, $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$ buffer): 0.005, 0.01, 0.02, 0.04, 0.05, 0.10, 0.20, 0.33, 0.45, 0.56, 0.63, 0.71, 0.83, 1.0, 1.25, 1.67, 2.5, 3.3, 5.0, 10.0, 20.0, 30.0, 40.0, 50.0 μM . Extrapolated $C_{50}=390 \mu\text{M}$; $K_{app}\sim 2.7 \times 10^5 \text{ M}^{-1}$.

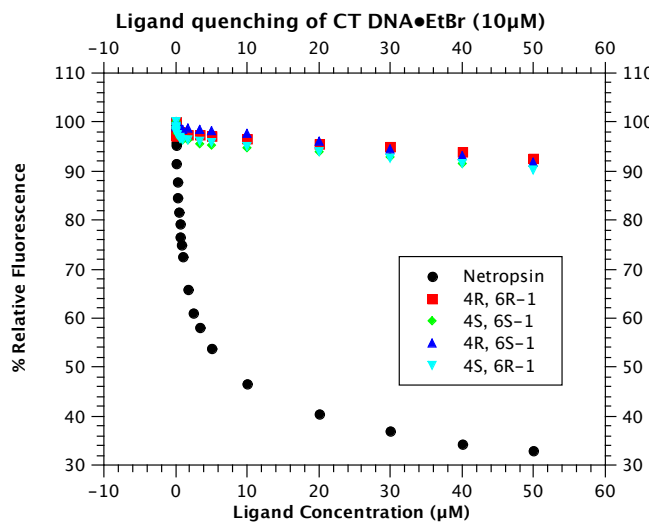


Summary of data: Plot of % relative fluorescence vs. ligand concentration for 4*R*, 6*R*-1, 4*S*, 6*S*-1, 4*R*, 6*S*-1, and 4*S*, 6*R*-1, and control compound netropsin binding to CT DNA with displacement of ethidium bromide.

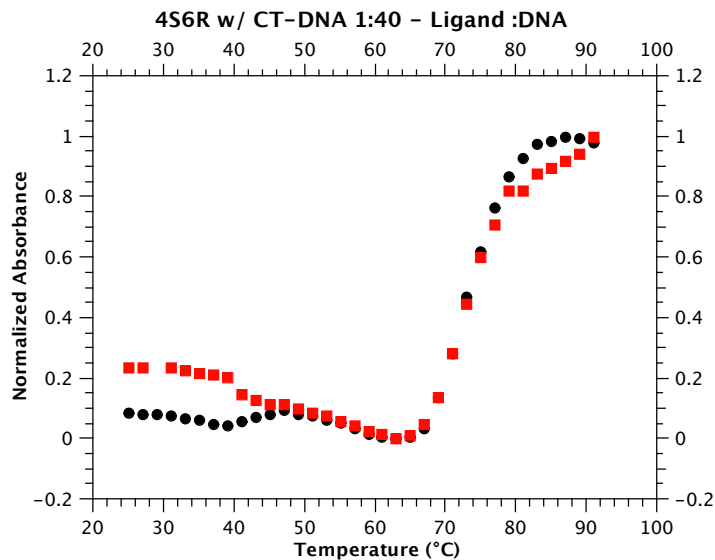
With error bars



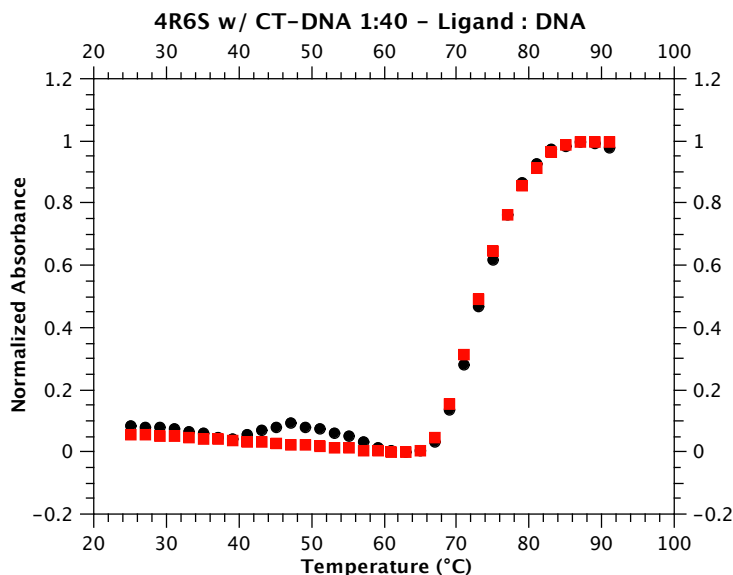
Without error bars



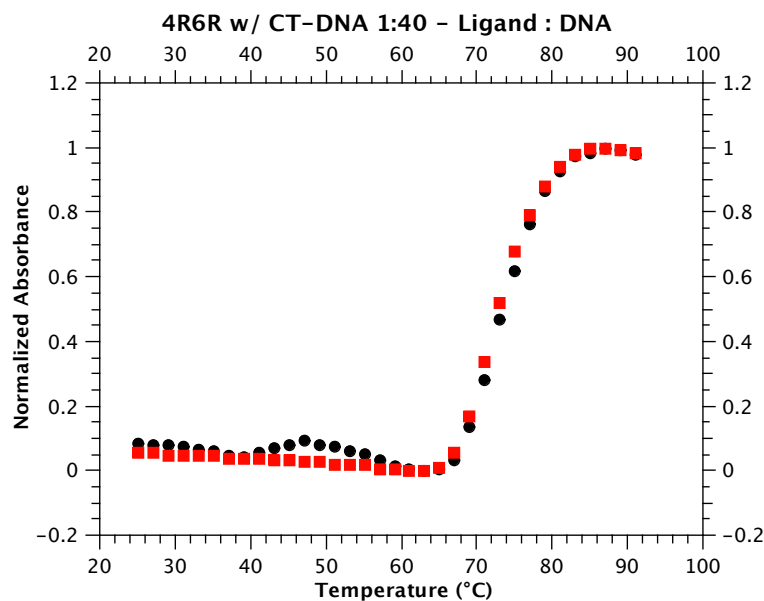
Monitoring the absorbance of CT DNA (100 $\mu\text{M/bp}$) at 260 nm in the presence and absence of 4*S*, 6*R*-1 (2.5 μM) in Tris-HCl buffer, pH=6.81, at temperatures from 25 $^{\circ}\text{C}$ –90 $^{\circ}\text{C}$ in 2 $^{\circ}\text{C}$ intervals. $T_M(\text{CT DNA})= 73.9$ $^{\circ}\text{C}$; $T_M(\text{CT DNA}+4\text{S}, 6\text{R-1}) =74.3$ $^{\circ}\text{C}$; $\Delta T_m = +0.4$ $^{\circ}\text{C}$. CT DNA alone, black; CT DNA + 4*S*, 6*R*-1, red.



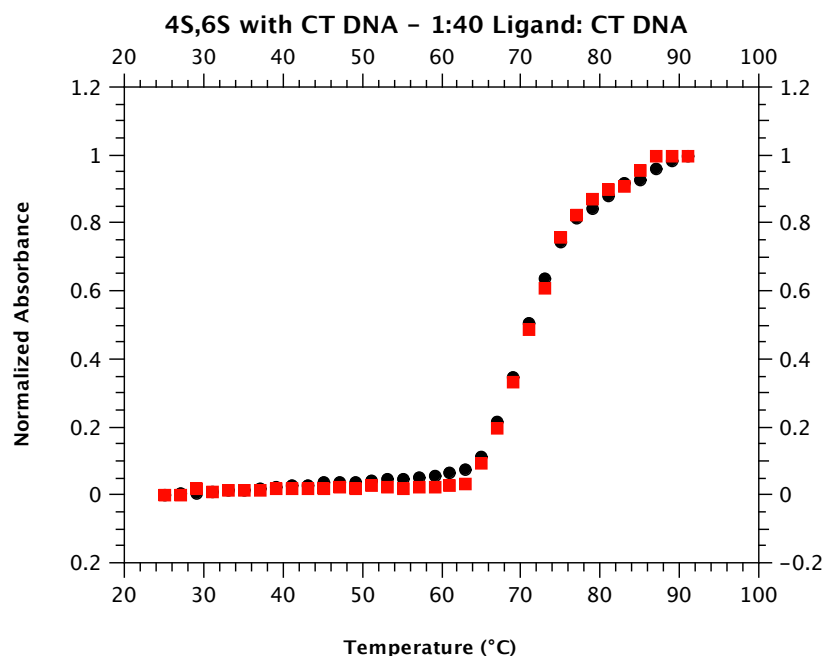
Monitoring the absorbance of CT DNA (100 $\mu\text{M bp}$) at 260 nm in the presence and absence of 4*R*, 6*S*-1 (2.5 μM) in Tris-HCl buffer, pH=6.81, at temperatures from 25 $^{\circ}\text{C}$ –90 $^{\circ}\text{C}$ in 2 $^{\circ}\text{C}$ intervals. $T_M(\text{CT DNA})= 73.9$ $^{\circ}\text{C}$; $T_M(\text{CT DNA}+4\text{R}, 6\text{S-1}) =73.5$ $^{\circ}\text{C}$; $\Delta T_m = -0.4$ $^{\circ}\text{C}$. CT DNA alone, black; CT DNA + 4*R*, 6*S*-1, red



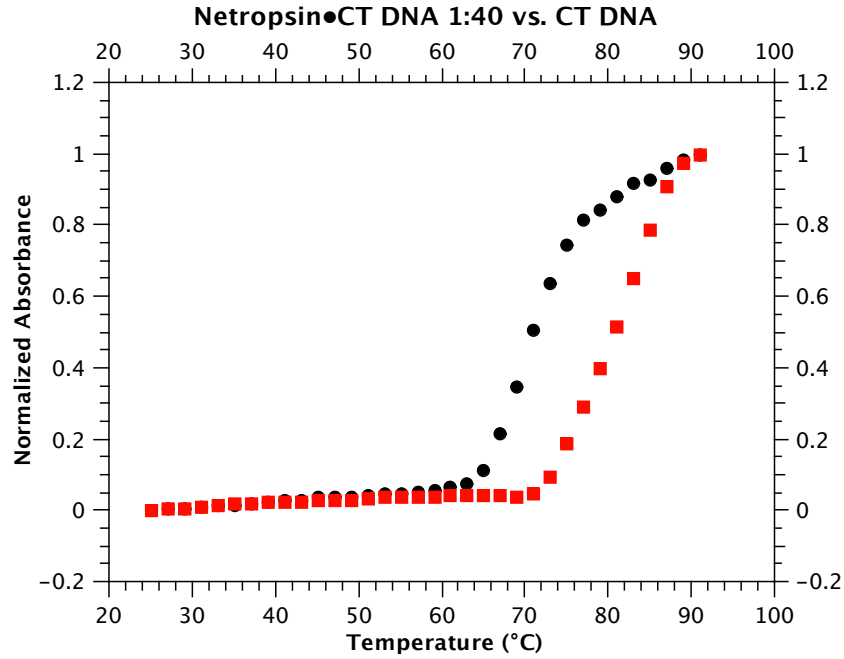
Monitoring the absorbance of CT DNA (100 μ M bp) at 260 nm in the presence and absence of 4R, 6R-1 (2.5 μ M) in Tris-HCl buffer, pH=6.81, at temperatures from 25 $^{\circ}$ C –90 $^{\circ}$ C in 2 $^{\circ}$ C intervals. T_M (CT DNA)= 73.9 $^{\circ}$ C; T_M (CT DNA+4R, 6R-1) =73.2 $^{\circ}$ C; ΔT_m = -0.7 $^{\circ}$ C. CT DNA alone, black; CT DNA + 4R, 6R 1, red.



Monitoring the absorbance of CT DNA (100 μ M bp) at 260 nm in the presence and absence of 4S, 6S-1 (2.5 μ M) in Tris-HCl buffer, pH=6.81, at temperatures from 25 $^{\circ}$ C –90 $^{\circ}$ C in 2 $^{\circ}$ C intervals. T_M (CT DNA)= 71.3 $^{\circ}$ C; T_M (CT DNA+4S, 6S-1) =71.1 $^{\circ}$ C; ΔT_m = -0.2 $^{\circ}$ C. CT DNA alone, black; CT DNA + 4R, 6R 1, red.



Control experiment: Monitoring the absorbance of CT DNA (100 μ M bp) at 260 nm in the presence and absence of netropsin (2.5 μ M) in Tris-HCl buffer, pH=6.81, at temperatures from 25 $^{\circ}$ C –90 $^{\circ}$ C in 2 $^{\circ}$ C intervals. T_M (CT DNA)= 71.1 $^{\circ}$ C; T_M (CT DNA+netropsin) =81.3 $^{\circ}$ C; ΔT_m =+10.2 $^{\circ}$ C. CT DNA alone, black; CT DNA + netropsin, red.



Fluorescence quenching experiments:

Technique:

Quenching of HSA fluorescence monitored at 338 nm was observed upon titration with ligands over the 0.5-10 μM range. $[\text{HSA}] = 10 \mu\text{M}$ (pH=6.81, Tris-HCl buffer); excitation wavelength 280 nm. Each addition of drug was 10 μL from a 1 mM stock of drug. There was a two-minute waiting period between each successive addition of drug.

Analysis:

$$F_0/F = 1 + K_{SV}[Q];$$

K_{SV} – Stern-Volmer quenching constant

$[Q]$ – concentration of the ligand

F_0 = initial fluorescence in absence of ligand

F = measured fluorescence after the addition of ligand

$$F_0/F_0 - F = 1/f_a K_a \cdot 1/[Q] + 1/f_a$$

f_a = fraction of accessible fluorophore

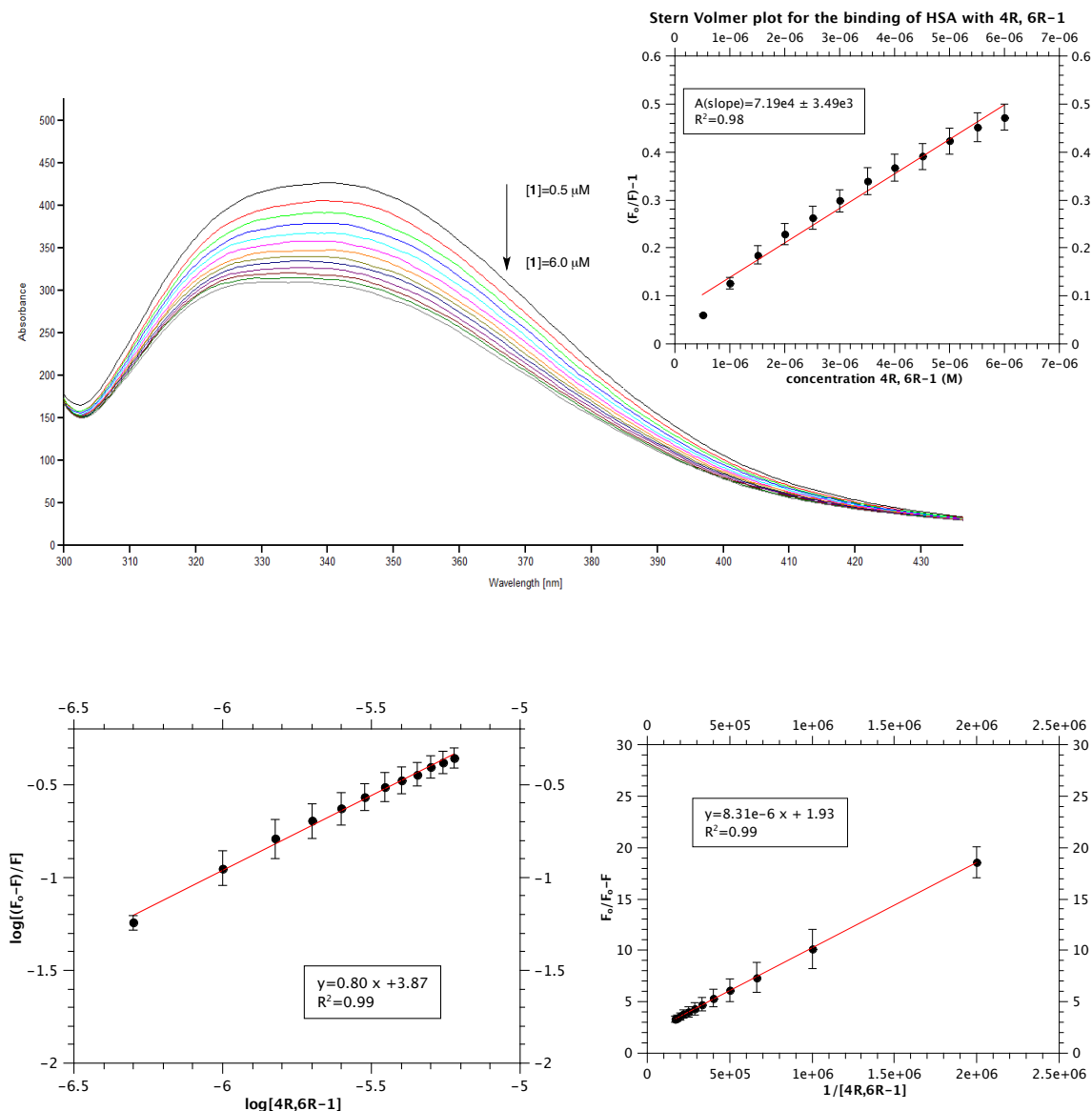
K_a = effective quenching constant for the accessible fluorophores

$$\log [(F_0 - F)/F] = \log K_b + n \log [Q]$$

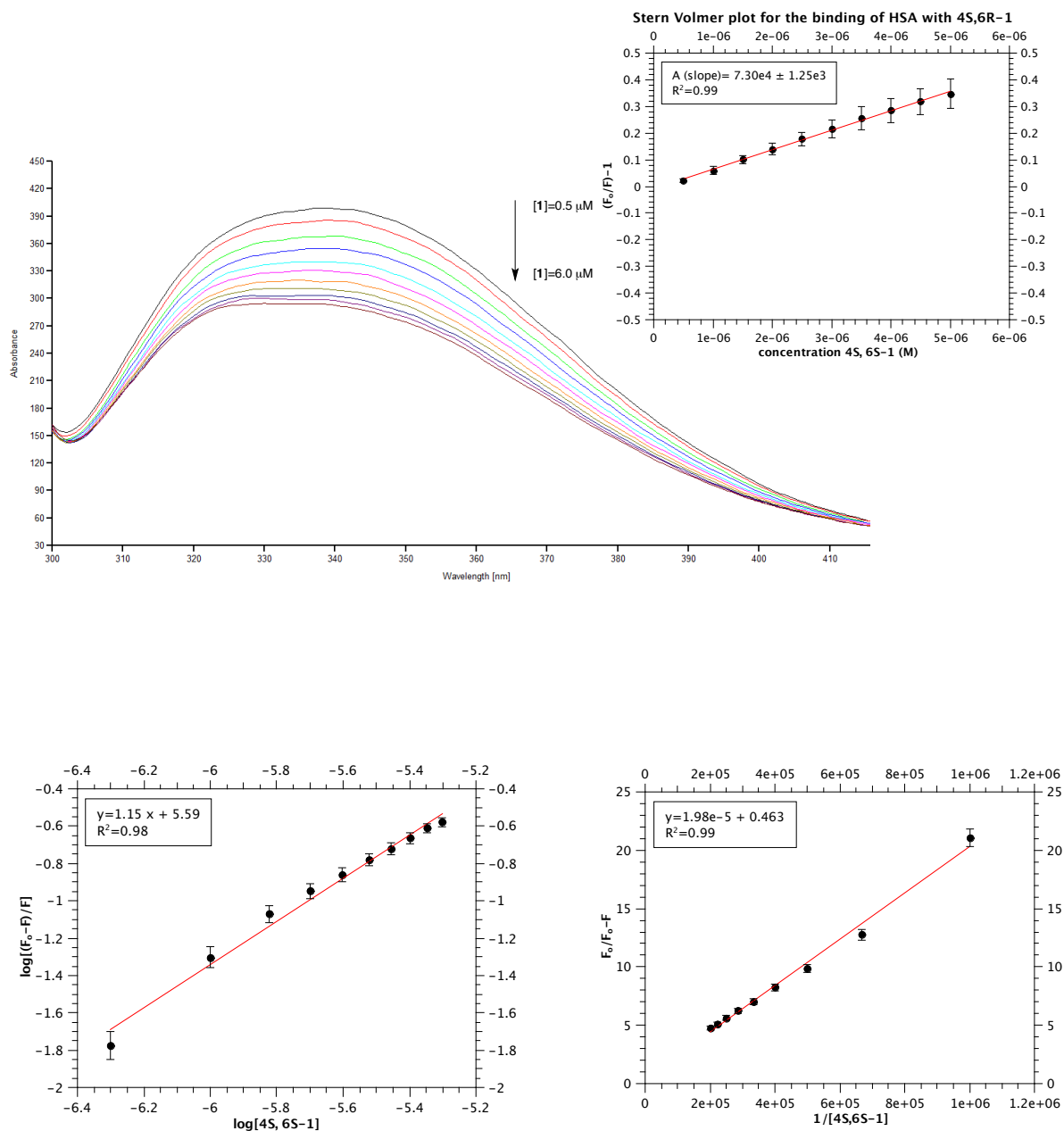
K_b = binding constant

n = number of binding sites

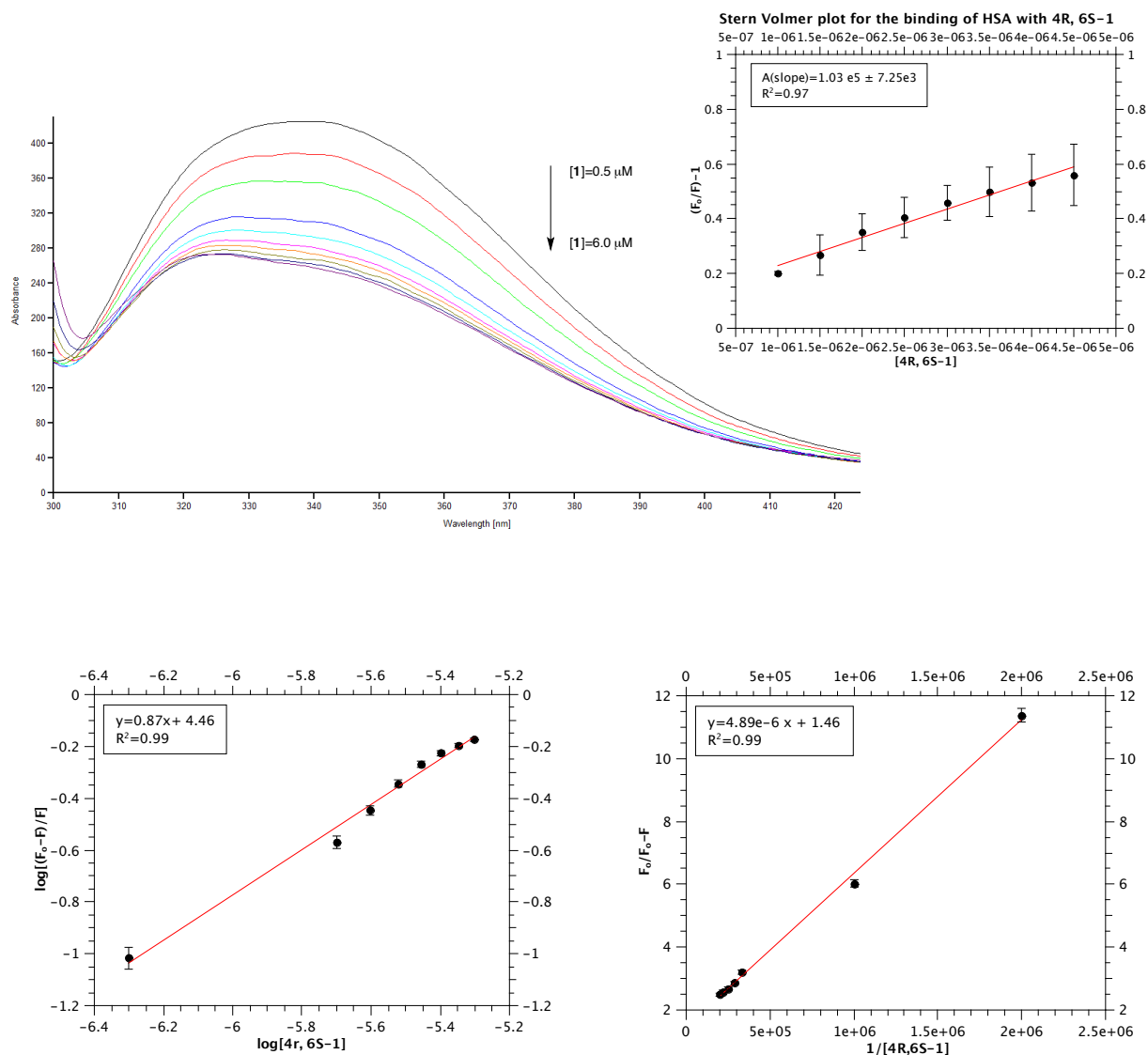
Quenching of HSA fluorescence monitored at 338 nm upon titration with 4*R*,6*R*-1 over the 0.5-6 μM range. [HSA]= 10 μM (pH=6.81, Tris-HCl buffer); excitation wavelength 280 nm. Each addition of drug was 10 μL from a 1 mM stock, to give final drug concentrations of: 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, and 6.0 μM . Addition of higher concentrations of 4*R*,6*R*-1 resulted in precipitation of the HSA•4*R*,6*R*-1 complex. $K_{SV}=7.2\pm0.4 \times 10^4 \text{ M}^{-1}$; $K_b=7.5\pm0.6 \times 10^3 \text{ M}^{-1}$; $n=0.8$; $K_a=2.3\pm0.3 \times 10^5 \text{ M}^{-1}$; $f_a=0.55$.



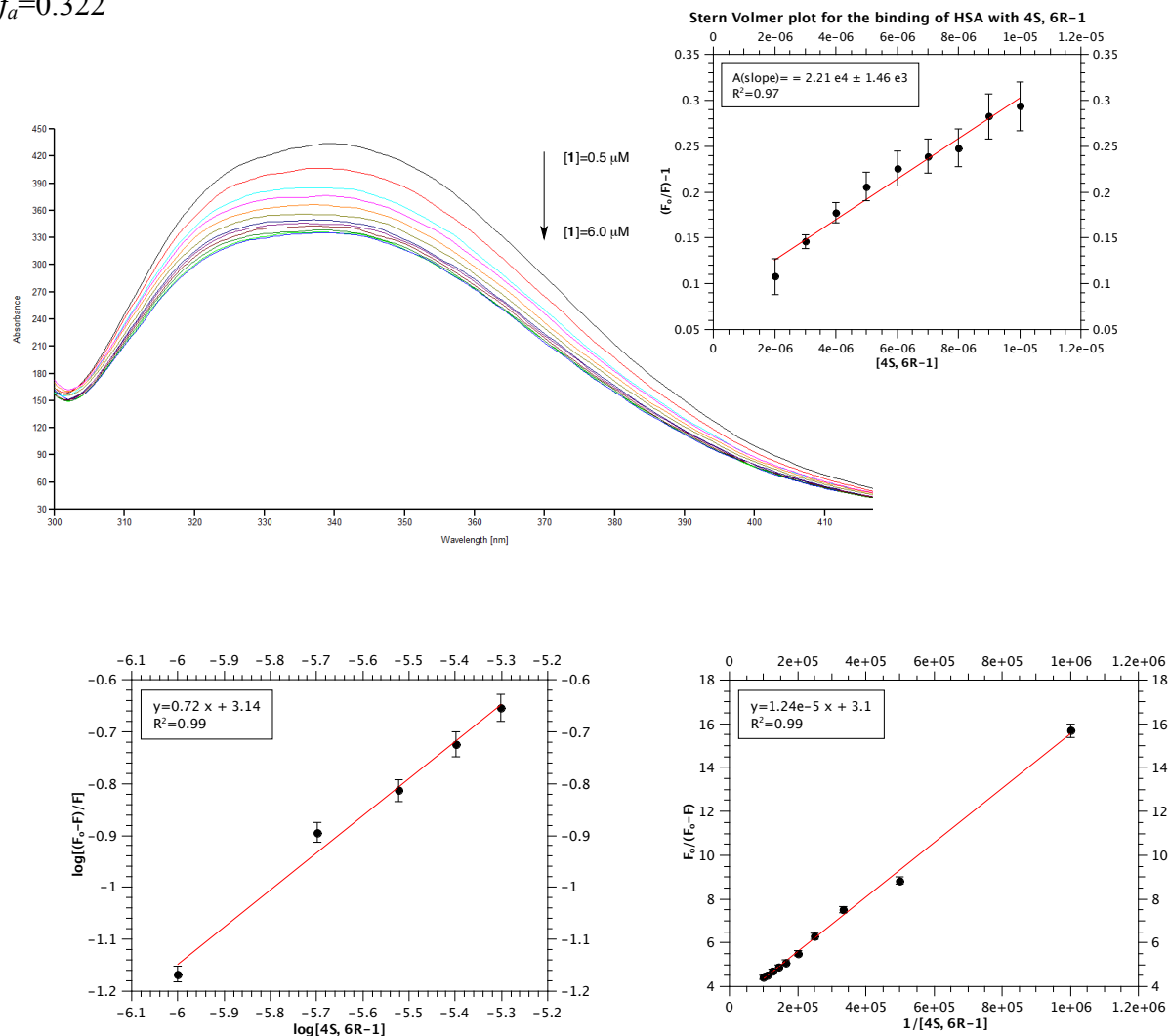
Quenching of HSA fluorescence monitored at 338 nm upon titration with 4S,6S-1 over the 0.5-6 μM range. [HSA]= 10 μM (pH=6.81, Tris-HCl buffer); excitation wavelength 280 nm. Each addition of drug was 10 μL from a 1 mM stock, to give final drug concentrations of: 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, and 6.0 μM . Addition of higher concentrations of 4S,6S-1 resulted in precipitation of the HSA•4S,6S-1 complex. $K_{SV}=7.3\pm1.2 \times 10^4 \text{ M}^{-1}$; $K_b=3.9\pm 0.2 \times 10^5 \text{ M}^{-1}$; $n=1.2$; $K_a=2.3\pm0.1 \times 10^4 \text{ M}^{-1}$; $fa=2.15$.



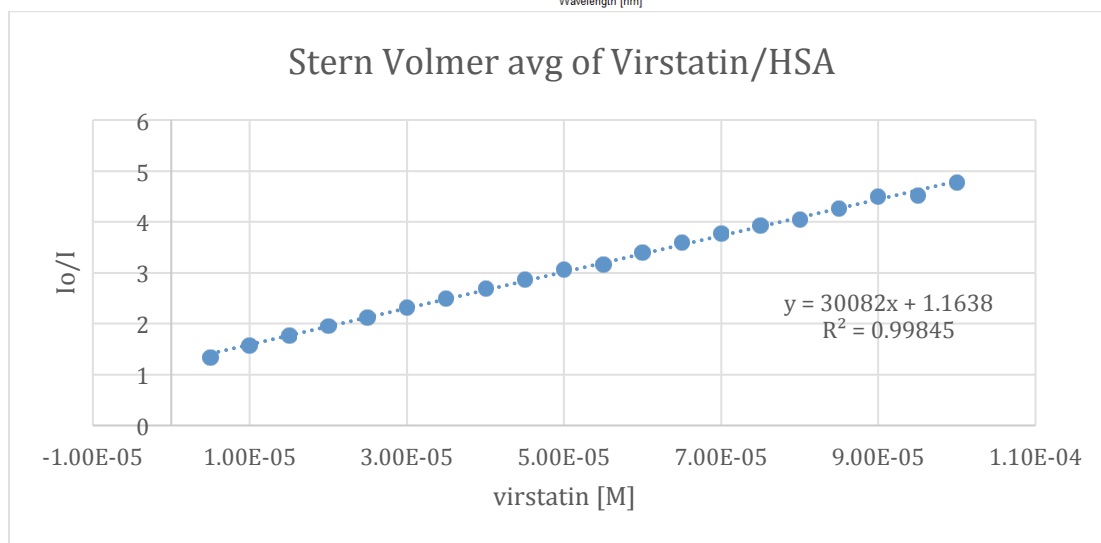
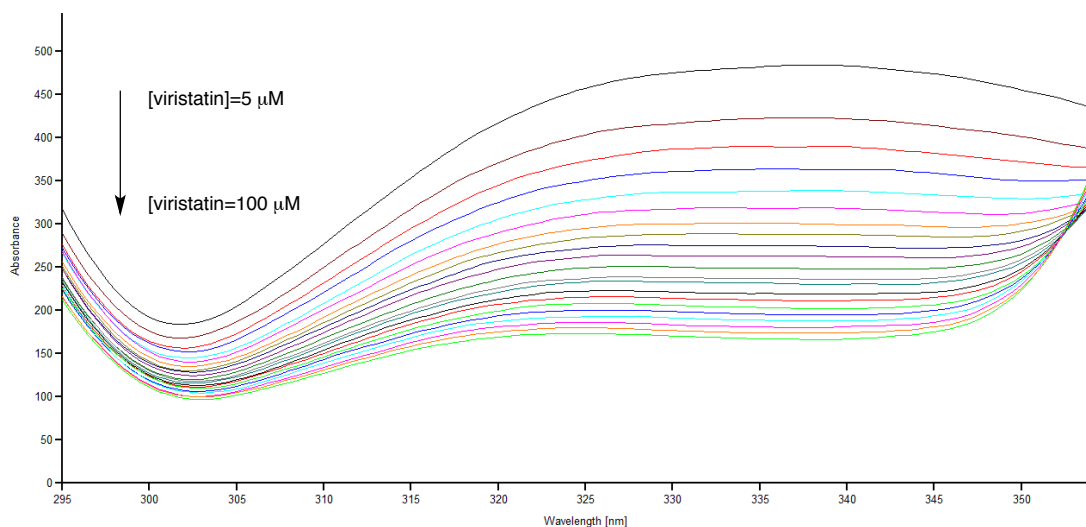
Quenching of HSA fluorescence monitored at 338 nm upon titration with 4R,6S-1 over the 0.5-6 μM range. [HSA]= 10 μM (in pH=6.81, Tris-HCl buffer); excitation wavelength 280 nm. Each addition of drug was 10 μL from a 1 mM stock, to give final drug concentrations of: 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, and 6.0 μM . Addition of higher concentrations of 4R,6S-1 resulted in precipitation of the HSA•4R,6S-1 complex. $K_{SV}=1.3\pm0.1 \times 10^5 \text{ M}^{-1}$; $K_b=2.9\pm 0.1 \times 10^4 \text{ M}^{-1}$; $n=0.87$; $K_a=2.9\pm0.2 \times 10^5 \text{ M}^{-1}$; $f_a=0.68$



Quenching of HSA fluorescence monitored at 338 nm upon titration with 4*S*,6*R*-**1** over the 0.5-6 μM range. [HSA]= 10 μM (in pH=6.81, Tris-HCl buffer); excitation wavelength 280 nm. Each addition of drug was 10 μL from a 1 mM stock, to give final drug concentrations of: 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, and 6.0 μM . Addition of higher concentrations of 4*S*,6*R*-**1** resulted in precipitation of the HSA•4*S*,6*R*-**1** complex. $K_{SV}=2.1\pm0.2 \times 10^4 \text{ M}^{-1}$; $K_b=1.4\pm 0.1 \times 10^3 \text{ M}^{-1}$; $n=0.72$; $K_a=7.8\pm0.2 \times 10^4 \text{ M}^{-1}$; $f_a=0.322$

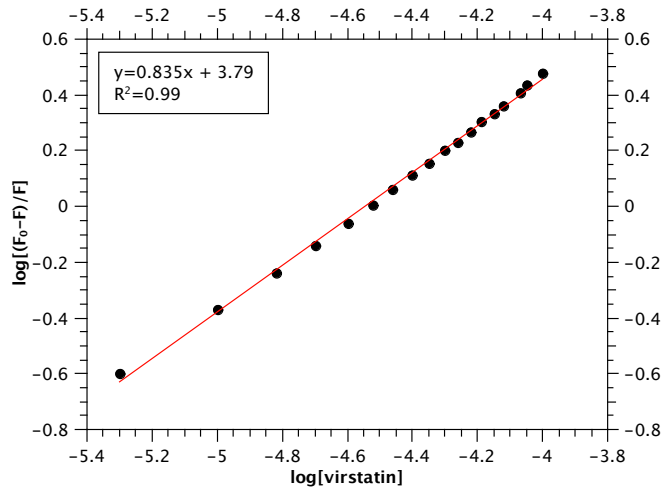
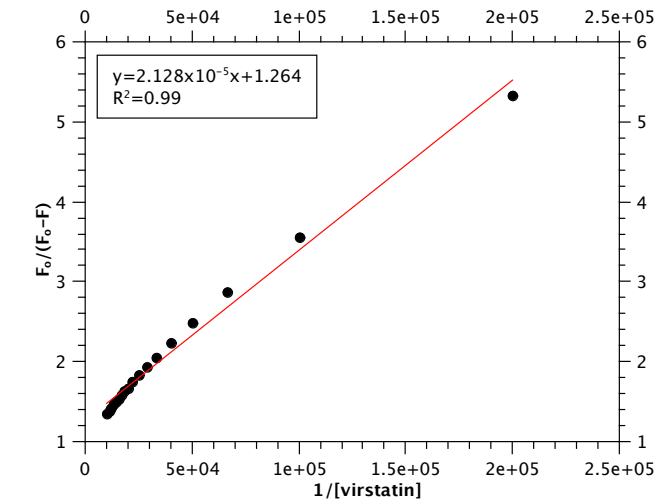


Quenching of HSA fluorescence monitored at 338 nm upon titration with viristatin over the 5-100 μM range. [HSA]= 1 μM (pH=7.21, $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$ buffer); excitation wavelength 280 nm. Each addition of drug was 10 μL from a 1mM stock, to give final drug concentrations of: 5.0, 10.0, 15.0, 20.0, 25.0, 30.0, 35.0, 40.0, 45.0, 50.0, 55.0, 60.0, 65.0, 70.0, 75.0, 80.0, 85.0, 90.0, 95.0, and 100.0 μM .

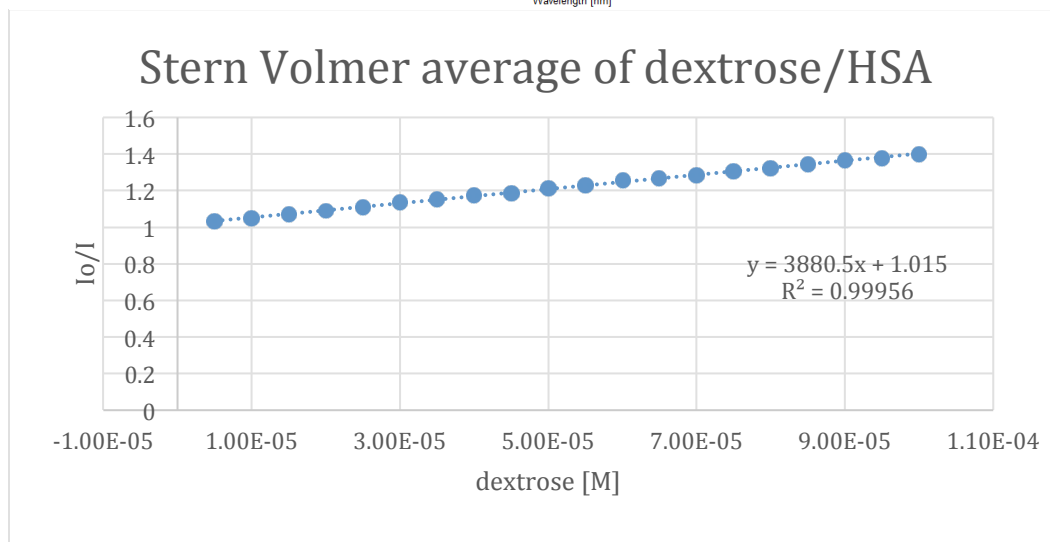
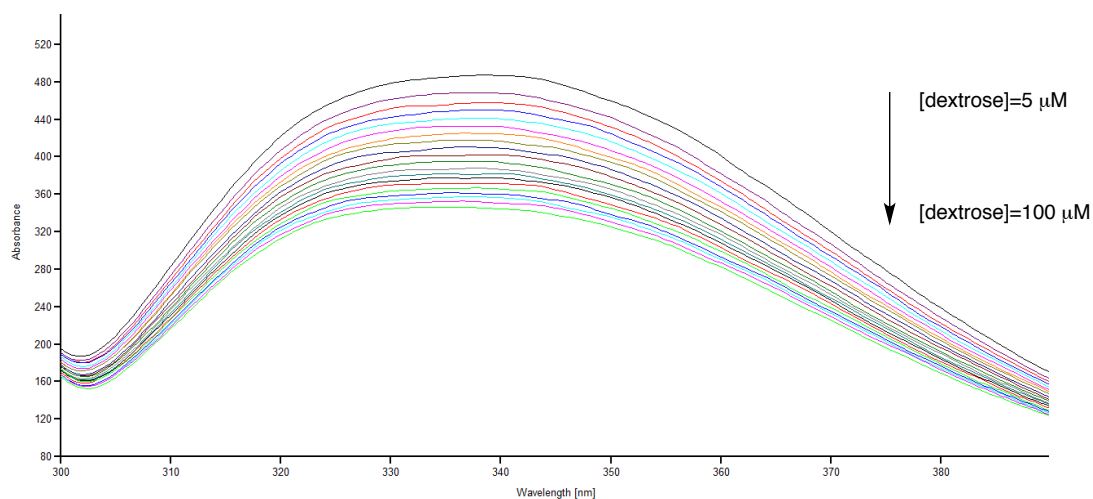


Positive Control Virstatin (average of three trials):

$K_{SV}=3.0\pm0.8 \times 10^4 \text{ M}^{-1}$; $f_a=0.80\pm0.04$; $K_a=6.8\pm1.9 \times 10^4 \text{ M}^{-1}$; $K_b=6.59\pm1.42 \times 10^3 \text{ M}^{-1}$;
 $n=0.84\pm0.02$

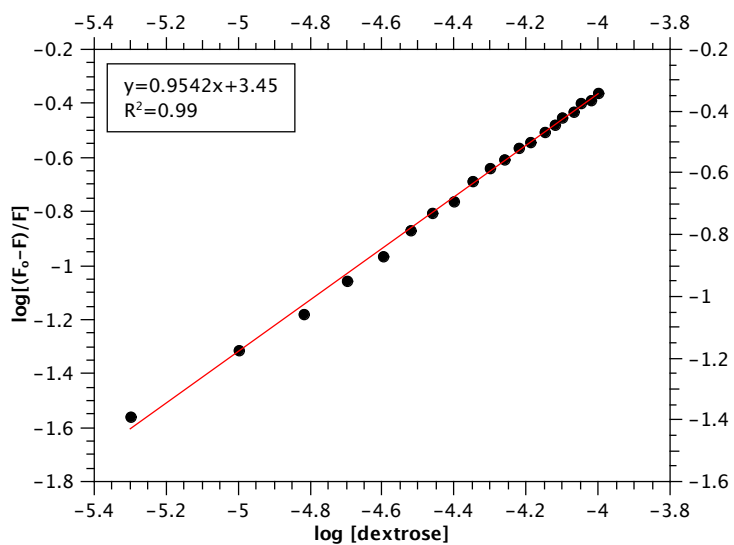
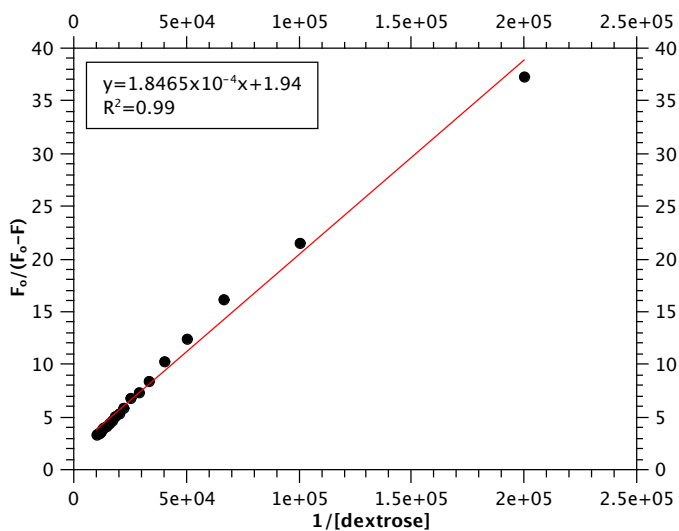


Quenching of HSA fluorescence monitored at 338 nm upon titration with dextrose over the 5-100 μM range. [HSA]= 1 μM (pH=7.21, $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$ buffer); excitation wavelength 280 nm. Each addition of drug was 10 μL from a 1mM stock, to give final drug concentrations of: 5.0, 10.0, 15.0, 20.0, 25.0, 30.0, 35.0, 40.0, 45.0, 50.0, 55.0, 60.0, 65.0, 70.0, 75.0, 80.0, 85.0, 90.0, 95.0, 100.0 μM



Negative Control Dextrose (average of two trials):

$K_{SV}=3.8\pm0.5 \times 10^3 \text{ M}^{-1}$; $f_a=0.41\pm0.10$; $K_a=1.7\pm0.6 \times 10^4 \text{ M}^{-1}$; $K_b=1.70\pm1.11 \times 10^3 \text{ M}^{-1}$;
 $n=0.90\pm0.09$



Summary:

Table 1. HSA binding parameters for 4R, 6R-I, 4S, 6S-I, 4R, 6S-I, 4S,6R-I, virstatin (positive control) and dextrose (negative control) obtained by fluorescence spectroscopy.^{20,21}

	K_{SV}^2	K_a^2	f_a	K_b^2	n
4R, 6R-1	7.2±0.4	23.0±3.0	0.55	0.75±0.03	0.8
4S, 6S-1	7.3±1.2	2.3±0.1	2.15	39.0±2.0	1.2
4R, 6S-1	13.0±1.0	29.0±2.0	0.68	2.9±0.1	0.87
4S, 6R-1	2.1±0.2	7.8±0.2	0.322	0.14±0.01	0.72
Virstatin	3.0±0.8	6.8±1.9	0.80	0.66±0.14	0.84
Dextrose	0.38±0.05	1.70±0.6	0.41	0.17±0.11	0.90

¹ K_{SV} is the Stern-Volmer quenching constant; K_a is the effective quenching constant for the accessible fluorophores; f_a is the fraction of accessible fluorophores; K_b is the binding constant; n is the number of binding sites. ²Values reported are in units of ($\times 10^4 M^{-1}$).

Table 2. HSA binding parameters for 4R, 6R-I, 4S, 6S-I, 4R, 6S-I, 4S, 6R-I, virstatin (positive control) and dextrose (negative control) obtained by ITC. ¹Units for $K = \times 10^4 M^{-1}$. ²Units for $\Delta G = kcal/mol$. ³Units for $\Delta H = kcal/mol$. ⁴Units for $\Delta S = cal/mol \cdot K$.

	K^1	ΔG^{o2}	ΔH^{o3}	ΔS^4	N
4R, 6R-1	6.4±0.2	-6.5±0.5	-1.9±0.3	15.5±0.7	3.38
4S, 6S-1	7.1±0.1	-6.6±0.8	-2.4±0.4	14.3±1.3	1.57
4R, 6S-1	16.0±3.0	-7.0±0.3	-1.6±0.1	18.2±0.8	1.83
4S, 6R-1	5.9±1.2	-6.5±0.3	-29.3±0.1	-76.5±0.7	4.55
virstatin	98.1±0.1	-9.6±0.6	-83.2±0.1	-247±0.5	1.94

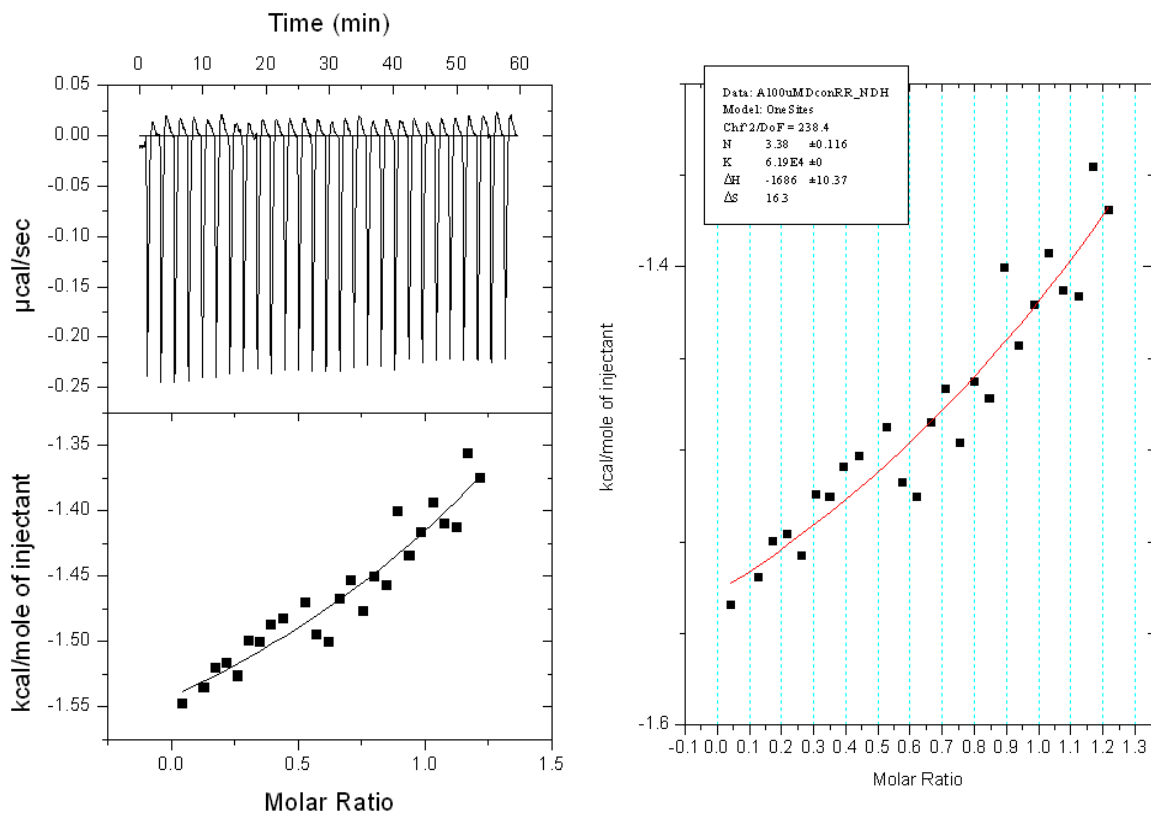
For reference, the binding constant of warfarin for drug site I on HSA is $2.75 \times 10^5 M^{-1}$.

See: Villamor, J. P.; Zaton, A. M. *J. Biochem. Biophys. Method.* **2001**, 48, 33-41.

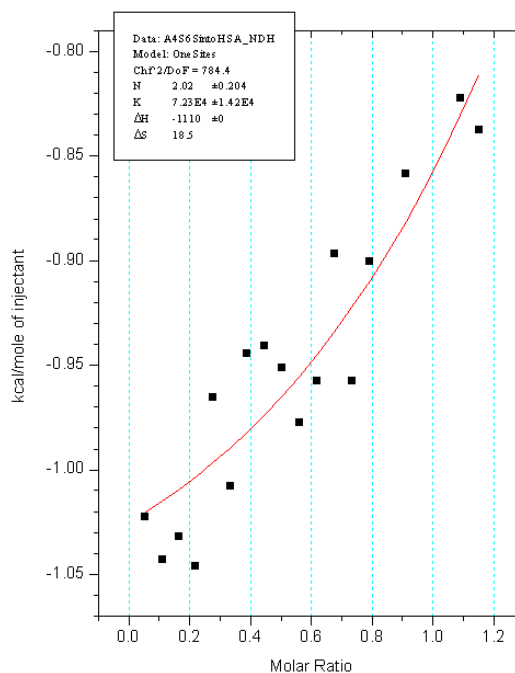
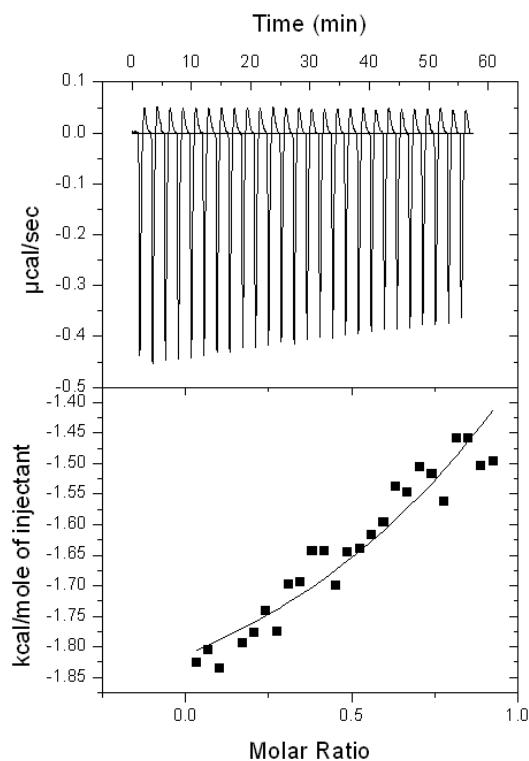
For reference, the binding constant of ibuprofen for drug site II on HSA is $2.97 \times 10^6 M^{-1}$

See: Li, Z.-M.; Wei, C.-W.; Zhang, Y.; Wang, S.-S.; Liu, Y.-N. *J. Chromatogr. B* **2011**, 879, 1934.

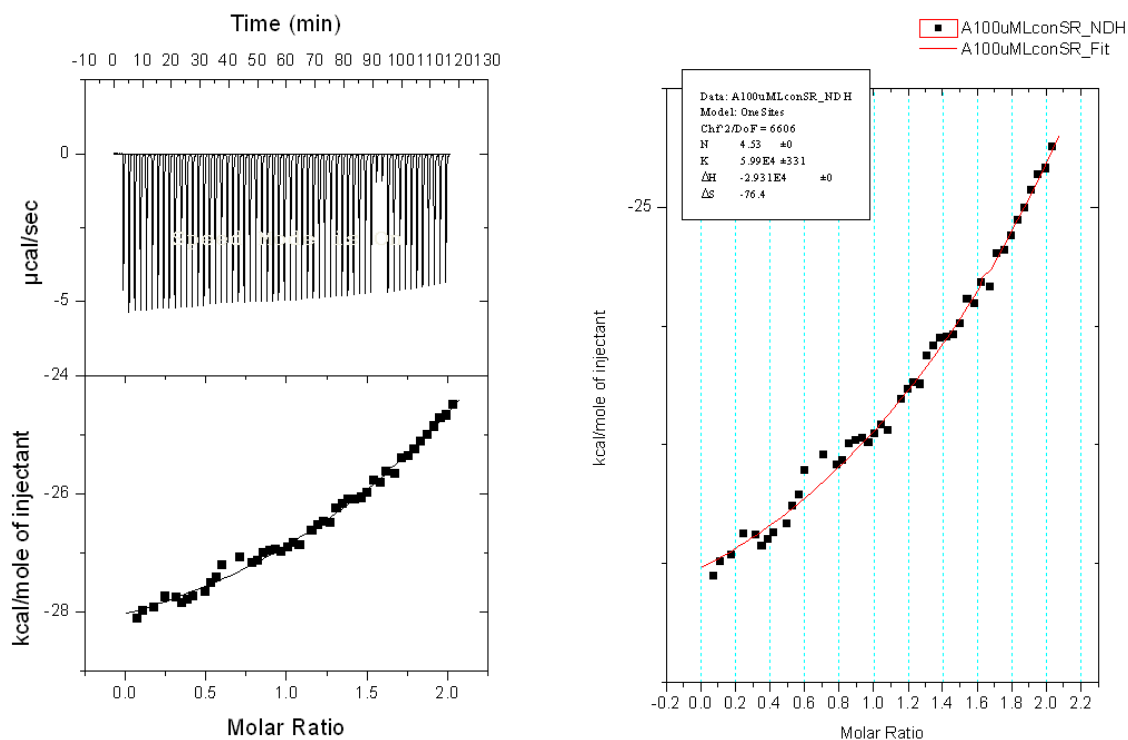
ITC trace for the binding of 4*R*, 6*R*-1 to HSA: 100 μM 4*R*, 6*R*-1 in pH=6.81 Tris-HCl buffer, titrated against 57 μM HSA (in pH=6.81, Tris-HCl buffer) at 25 °C. DP = 6, 307 rpm, 5 μL injections, 10s duration with 130s, 2s filter, initial delay of 60s, total of 45 injections. $K_b=6.4\pm0.2 \times 10^4 \text{ M}^{-1}$; $\Delta H=-1.9\pm0.3 \text{ kcal/mol}$; $\Delta S=15.5 \pm 0.7 \text{ cal/mol}\cdot\text{K}$.



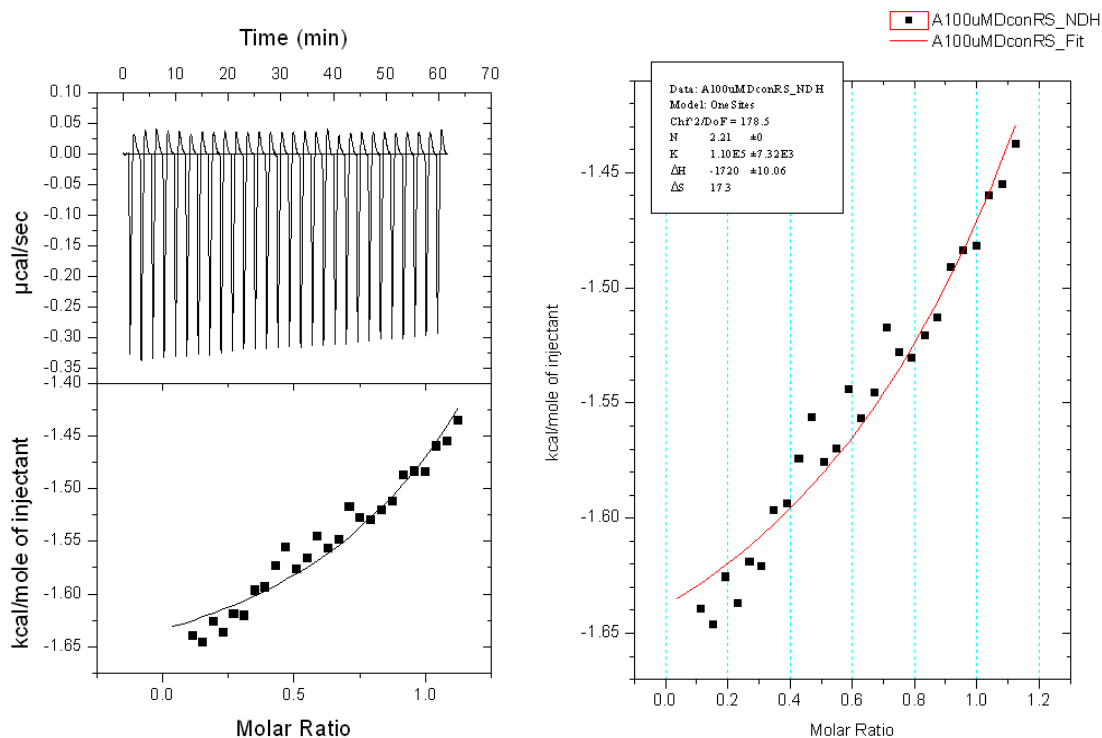
ITC trace for the binding of 4S, 6S-1 to HSA: 100 μM 4S, 6S-1 in pH=6.81 Tris-HCl buffer, titrated against 57 μM HSA (in pH=6.81, Tris-HCl buffer) at 25 $^{\circ}\text{C}$. DP = 6, 307 rpm, 5 μL injections, 10s duration with 130s, 2s filter, initial delay of 60s, total of 45 injections. $K_b=7.16\pm1.42 \times 10^4 \text{ M}^{-1}$; $\Delta H=-1.8\pm0.4 \text{ kcal/mol}$; $\Delta S=16.4 \pm 1.3 \text{ cal/mol}\cdot\text{mol}$.



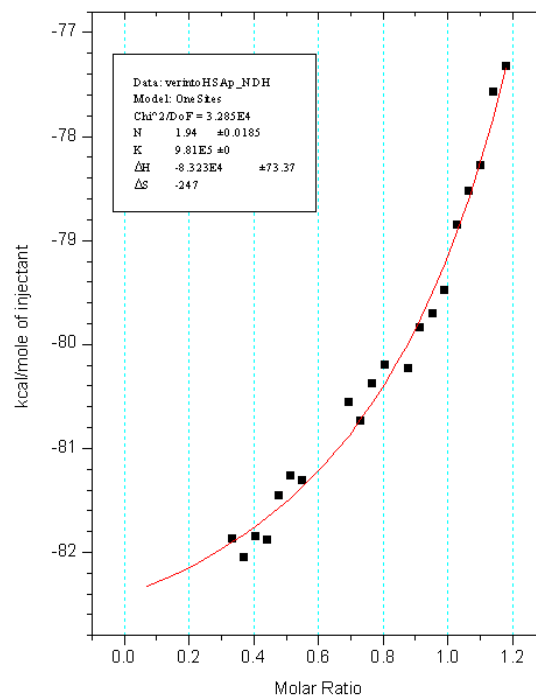
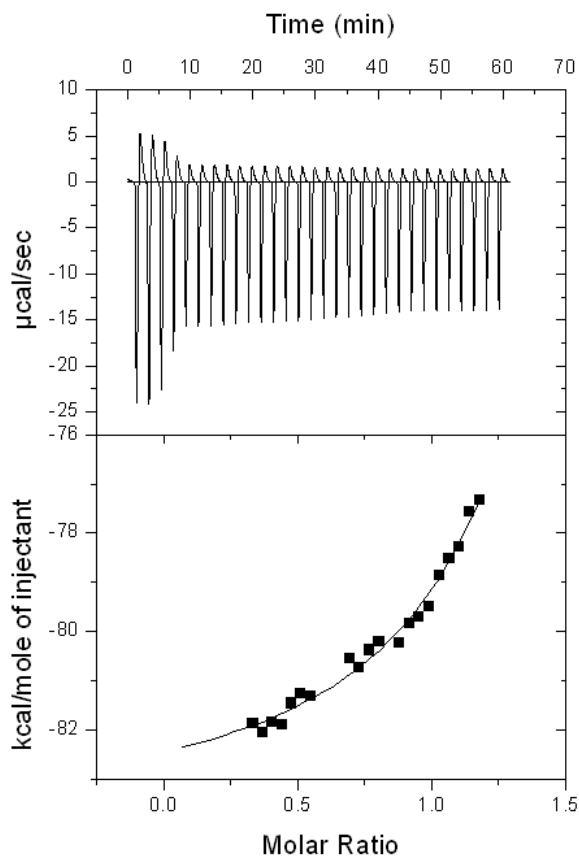
ITC trace for the binding of 4*S*, 6*R*-1 to HSA: 100 μ M 4*R*, 6*R*-1 in pH=6.81 Tris-HCl buffer, titrated against 57 μ M HSA (in pH=6.81, Tris-HCl buffer) at 25 °C. DP = 6, 307 rpm, 5 μ L injections, 10s duration with 130s, 2s filter, initial delay of 60s, total of 45 injections. $K=5.9\pm1.2 \times 10^4 \text{ M}^{-1}$; $\Delta H=-29.3\pm0.1 \text{ kcal/mol}$; $\Delta S=-76.4 \pm 0.7 \text{ cal/mol}\cdot\text{K}$.



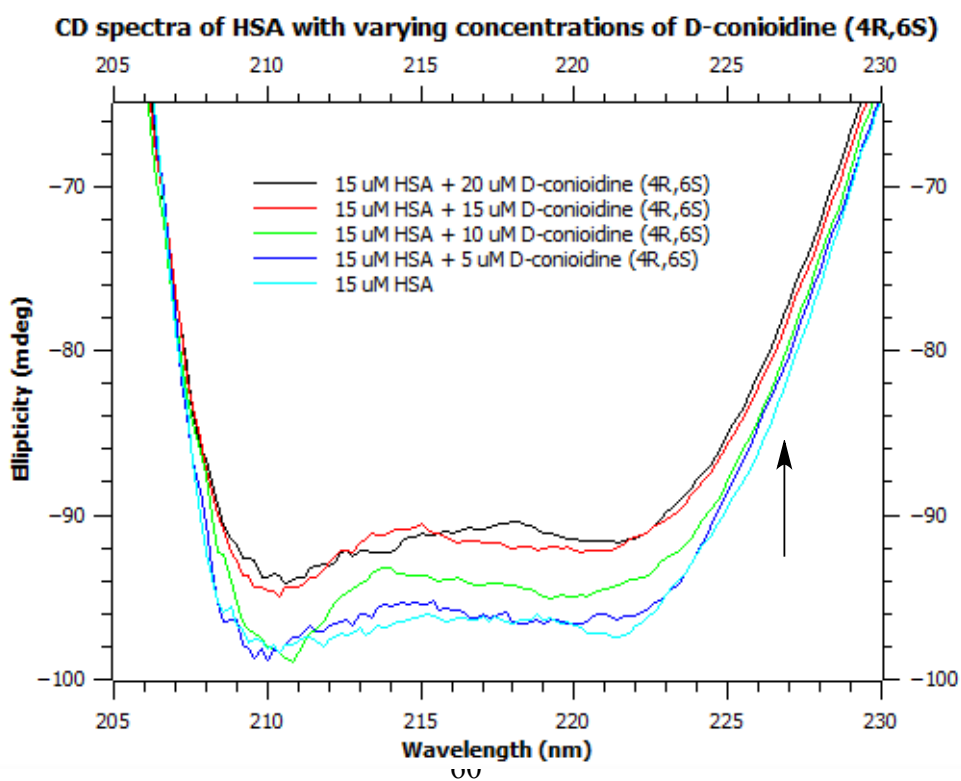
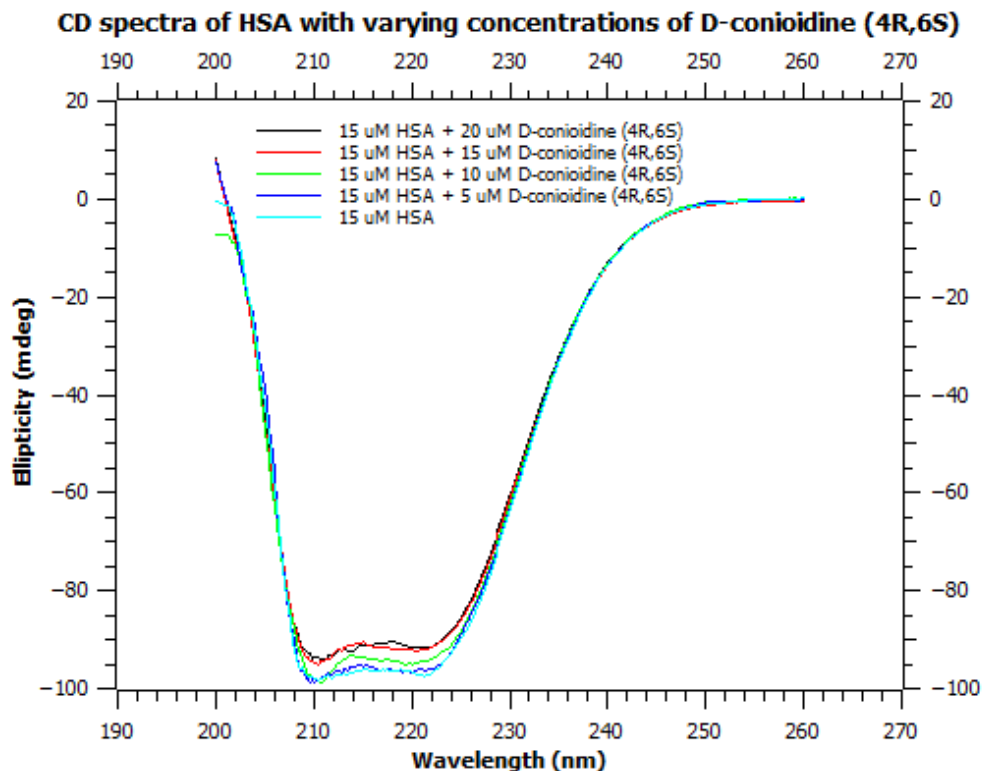
ITC trace for the binding of 4*R*, 6*S*-1 to HSA: 100 μ M 4*R*, 6*R*-1 in pH=6.81 Tris-HCl buffer, titrated against 57 μ M HSA (in pH=6.81, Tris-HCl buffer) at 25 $^{\circ}$ C. DP = 6, 307 rpm, 5 μ L injections, 10s duration with 130s, 2s filter, initial delay of 60s, total of 45 injections. $K=1.6\pm0.3 \times 10^5 \text{ M}^{-1}$; $\Delta H=-1.6\pm0.1 \text{ kcal/mol}$; $\Delta S=18.2 \pm 0.8 \text{ cal/mol}\cdot\text{K}$.



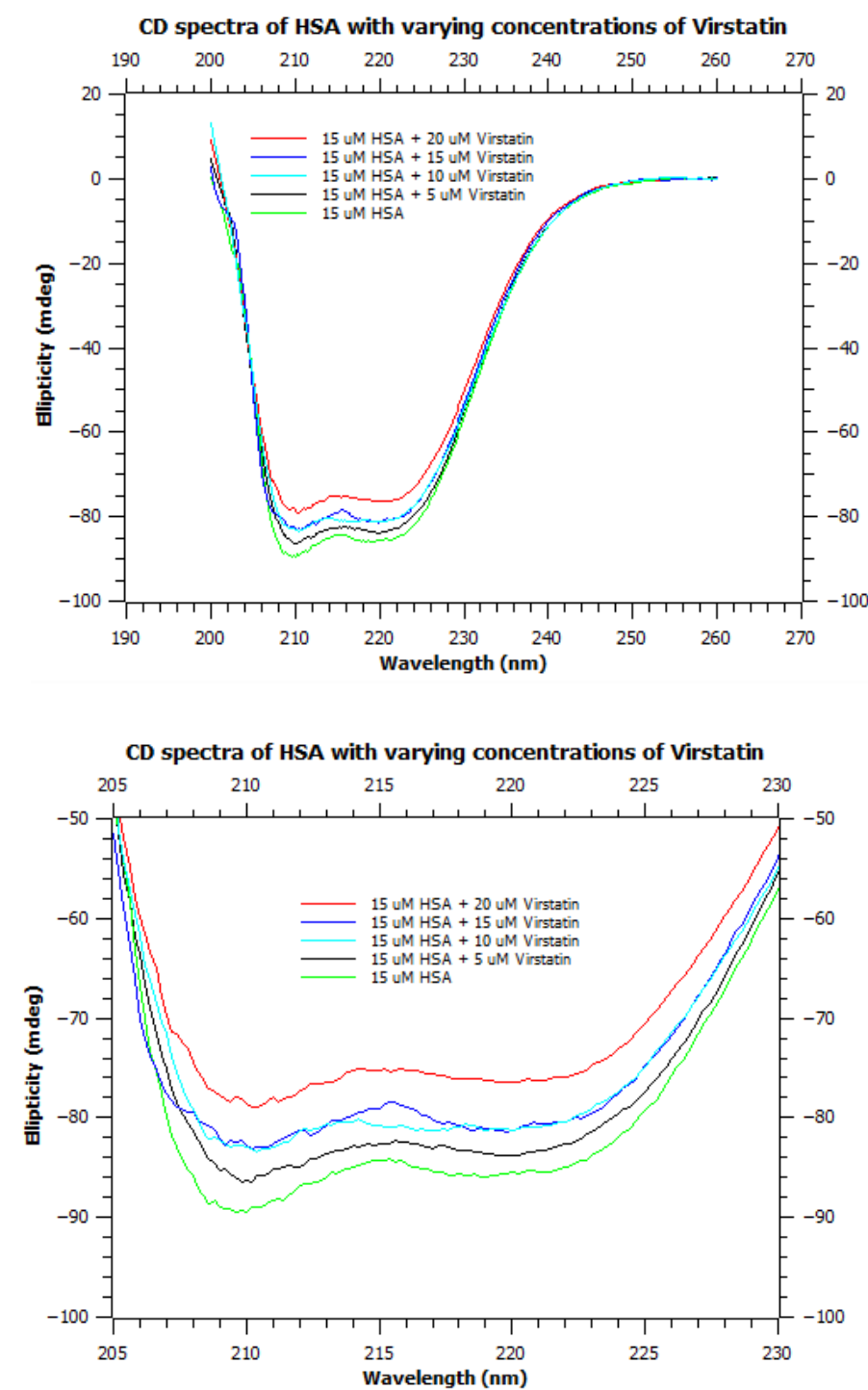
Control: ITC trace for the binding of virstatin to HSA: 500 μM virstatin in pH=7.21 $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$ buffer, titrated against 60 μM HSA (in pH=7.21 $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$ buffer) at 25 $^\circ\text{C}$. DP = 6, 307 rpm, 5 μL injections, 10s duration with 130s, 2s filter, initial delay of 60s, total of 45 injections. $K=9.81 \times 10^5 \text{ M}^{-1}$ (literature $K=6.09 \times 10^5 \text{ M}^{-1}$); $N = 1.94$ (literature $N=0.29$). $\Delta H=-83.2 \pm 0.1 \text{ kcal/mol}$; $\Delta S=-247 \pm 0.7 \text{ cal/mol}\cdot\text{K}$.



The 200-260 nm region of the CD spectrum of solutions of HSA (15 μ M in pH=6.81 in Tris-HCl buffer) in the absence (light blue line) and presence of various concentrations of 4*R*, 6*S*-1: blue line 5.0 μ M 4*R*, 6*S*-1; green line 10 μ M 4*R*, 6*S*-1; red line 15 μ M 4*R*, 6*S*-1; black line 20 μ M 4*R*, 6*S*-1.



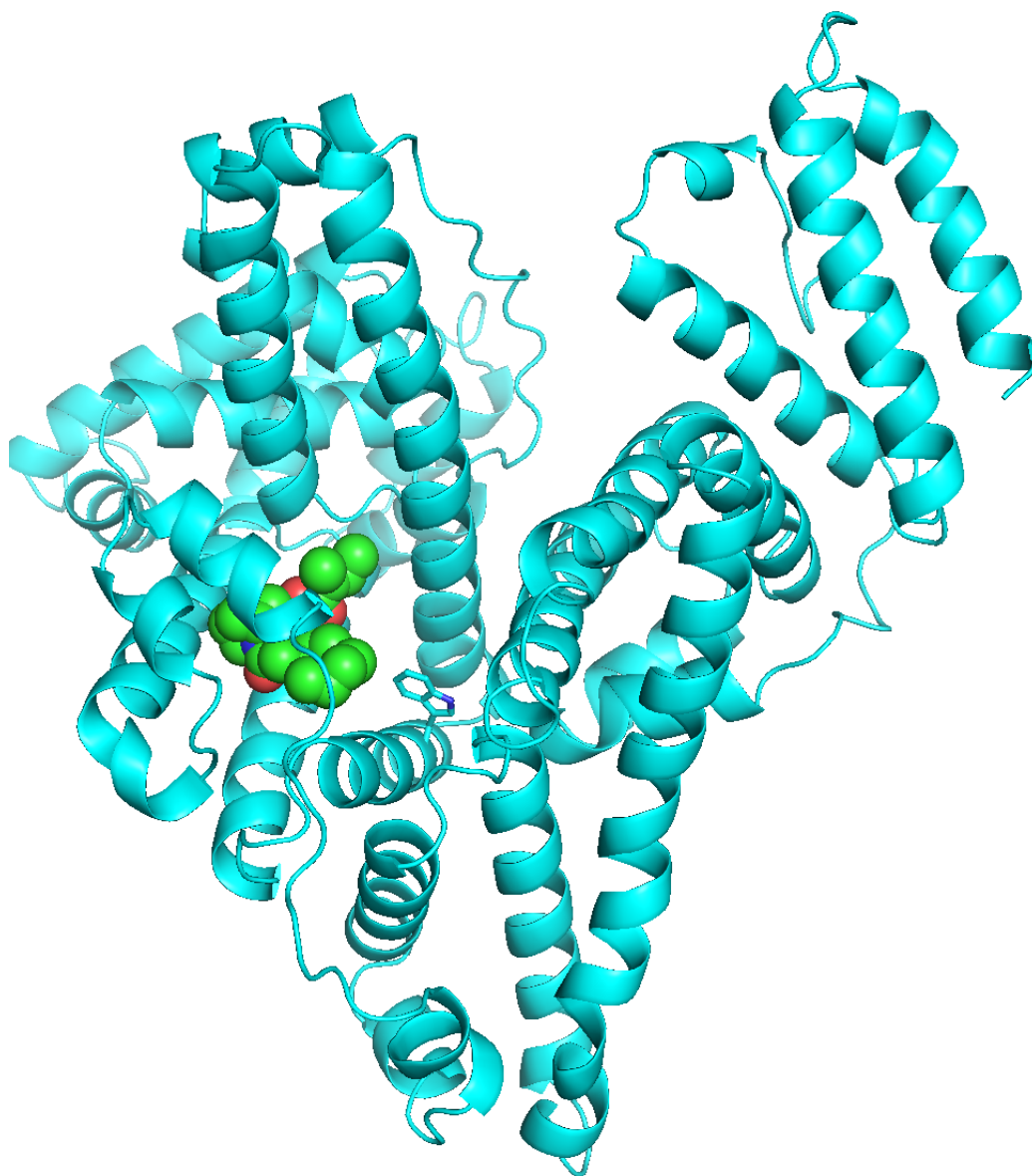
Control experiment: The 200-260 nm region of the CD spectrum of solutions of HSA (15 μM in pH=6.81 in Tris-HCl buffer) in the absence (green line) and presence of various concentrations of virstatin: blue line 5.0 μM virstatin; light blue line 10 μM virstatin; dark blue line 15 μM virstatin; red line 20 μM virstatin.



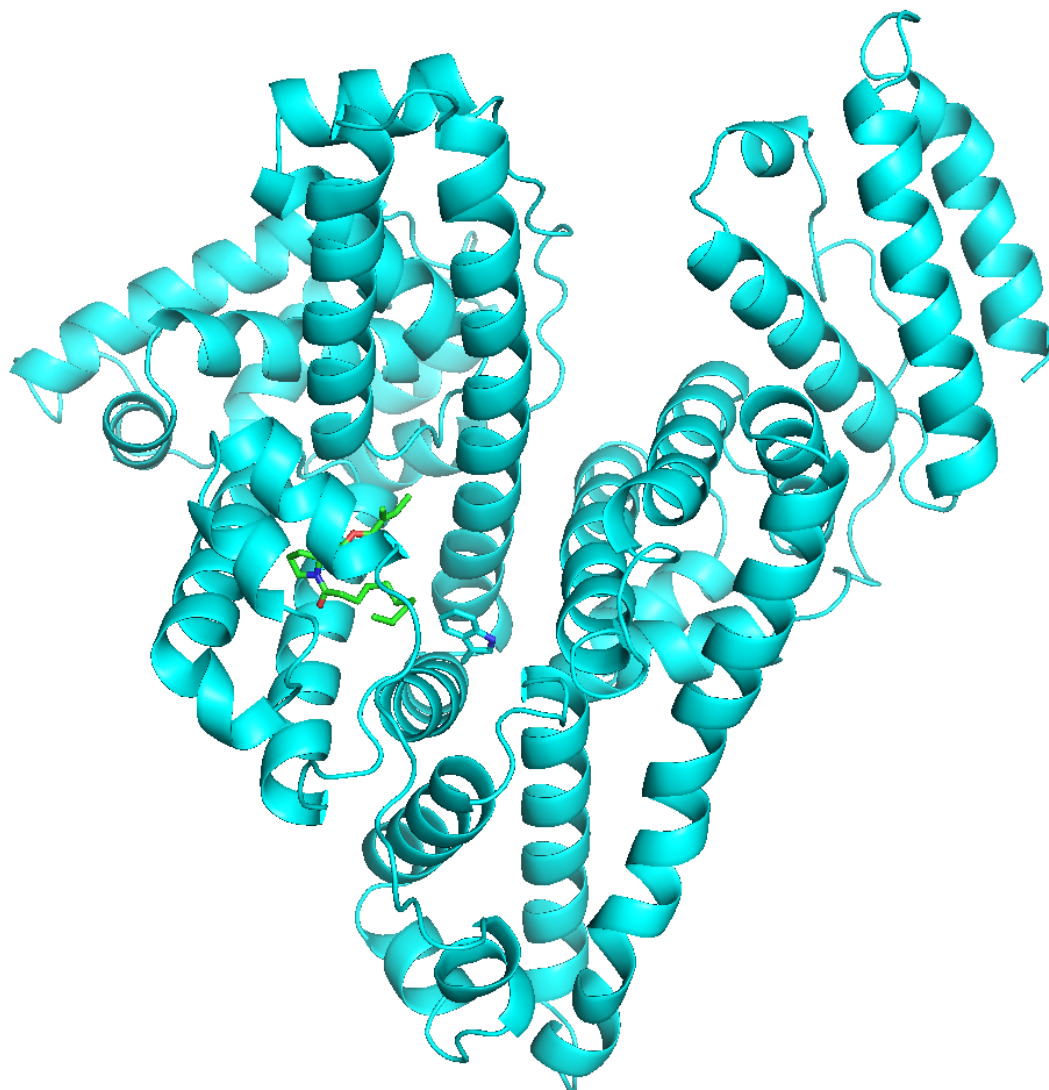
Model for the binding of 4*R*, 6*R*-1 (green, red, blue) to HSA site I (warfarin site) in proximity to the Trp214 residue (shown), generated by Autodock Vina¹ using HSA (light blue, PDB 1AO6)² and 4*R*, 6*R*-1 minimized by Spartan 14 for Macintosh (Wavefunction, Inc., Irvine CA).

¹ Trott, O.; Olson, A. J. *J. Comput. Chem.* 2010, **31**, 455.

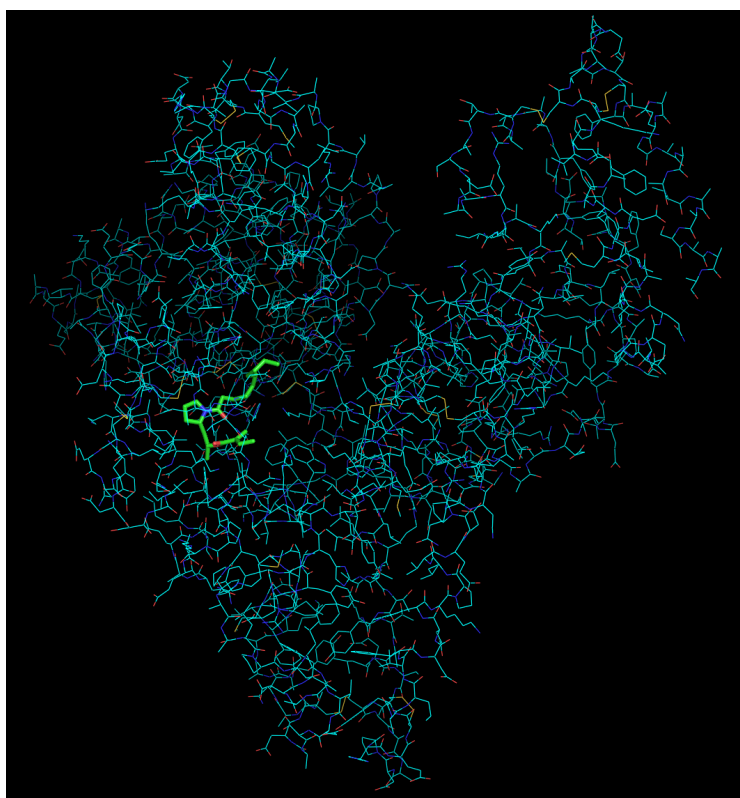
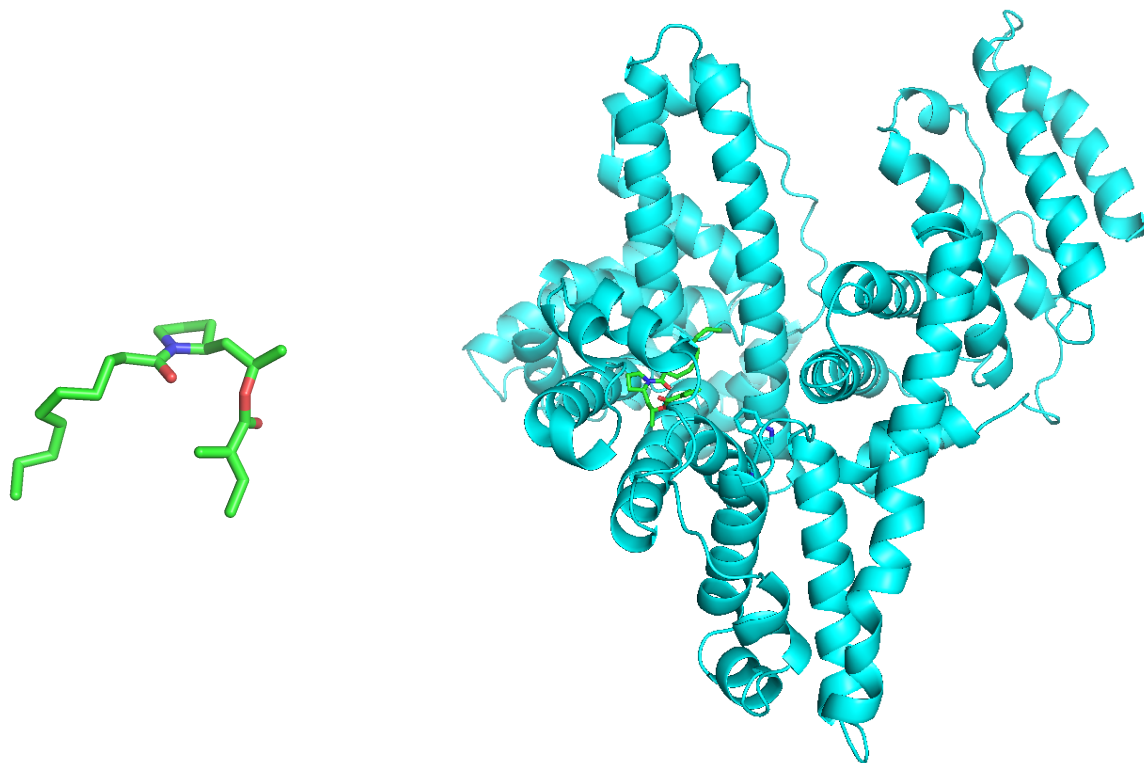
² Sugio, S.; Kashima, A.; Mochizuki, S.; Noda, M. Kobayashi, K. *Protein Eng.* **1999**, *12*, 439.



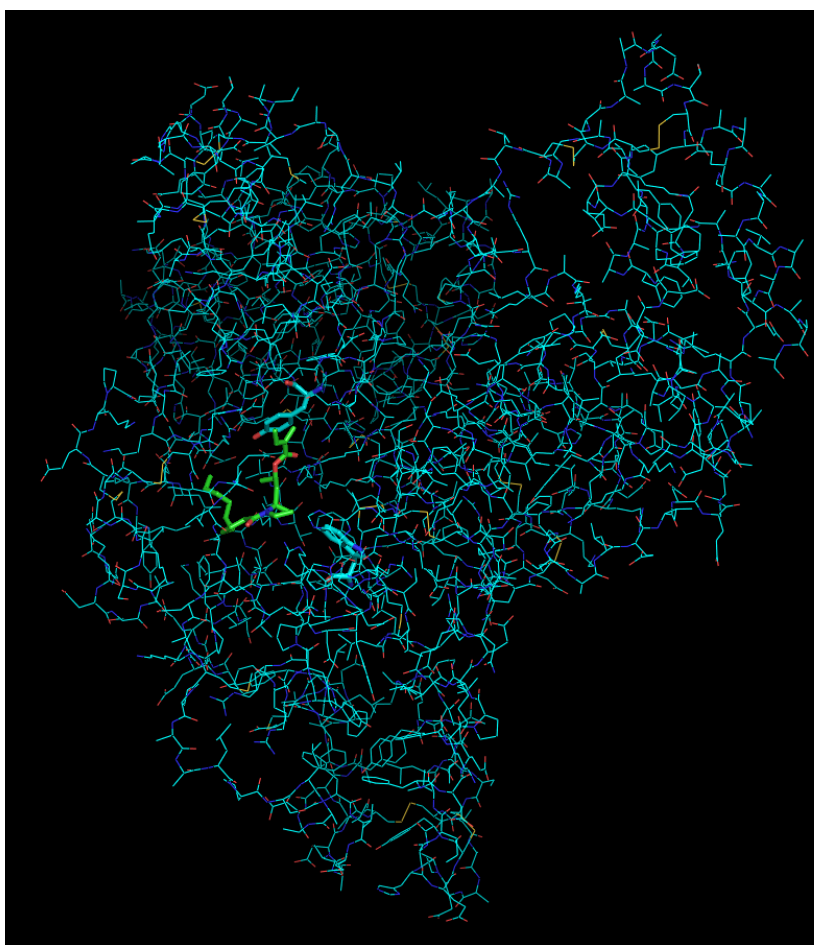
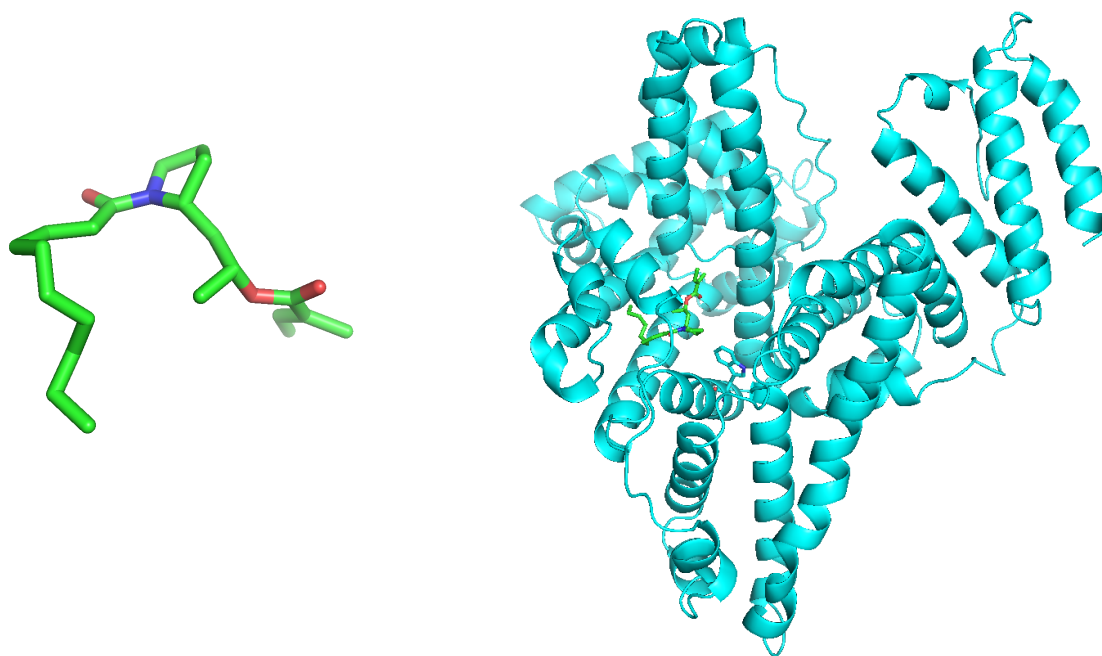
Alternative view of the binding of 4*R*, 6*R*-1 to HSA.



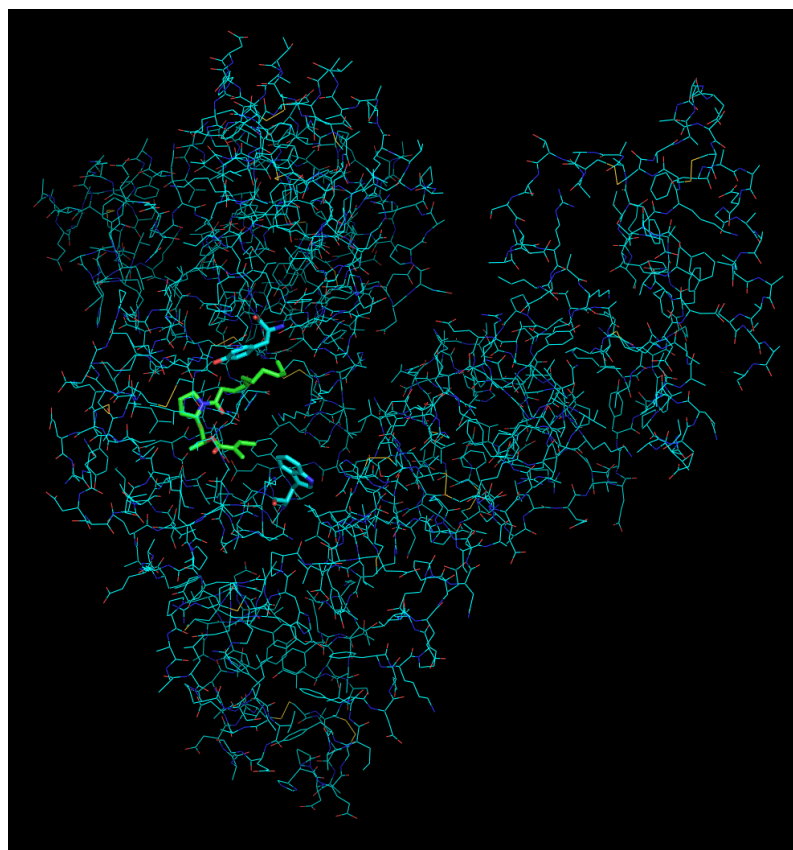
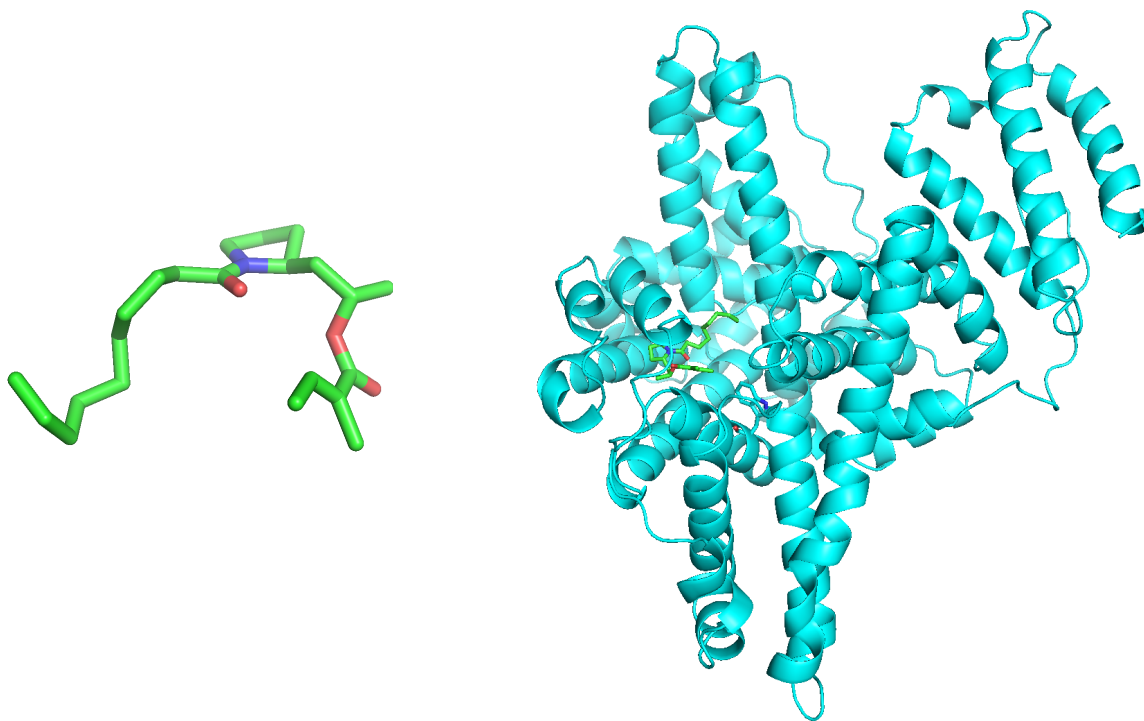
Model for the binding of 4*S*, 6*R*-**1** (green, red, blue) to HSA site I (-7.8 kcal/mol)



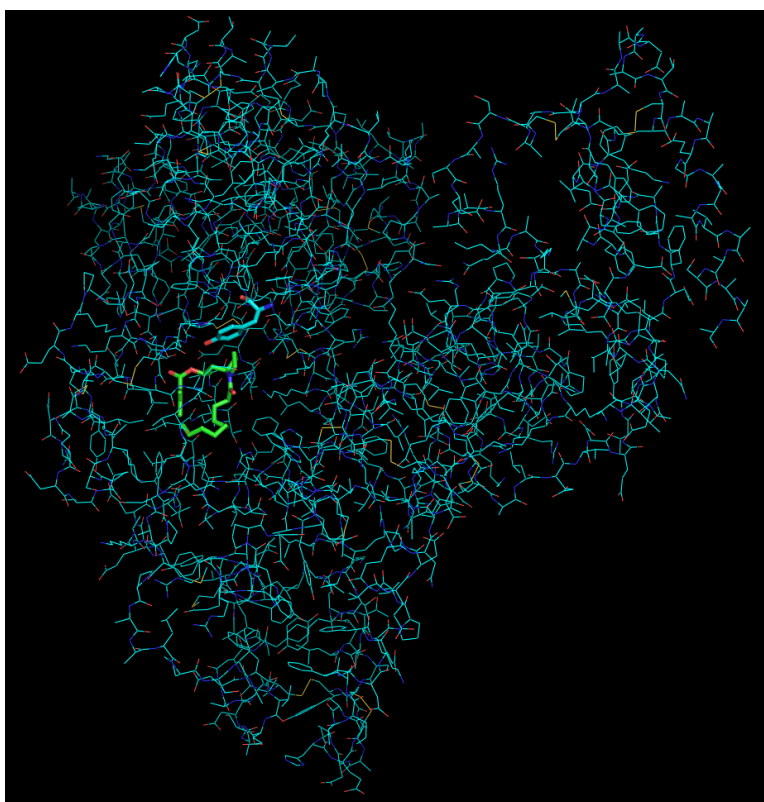
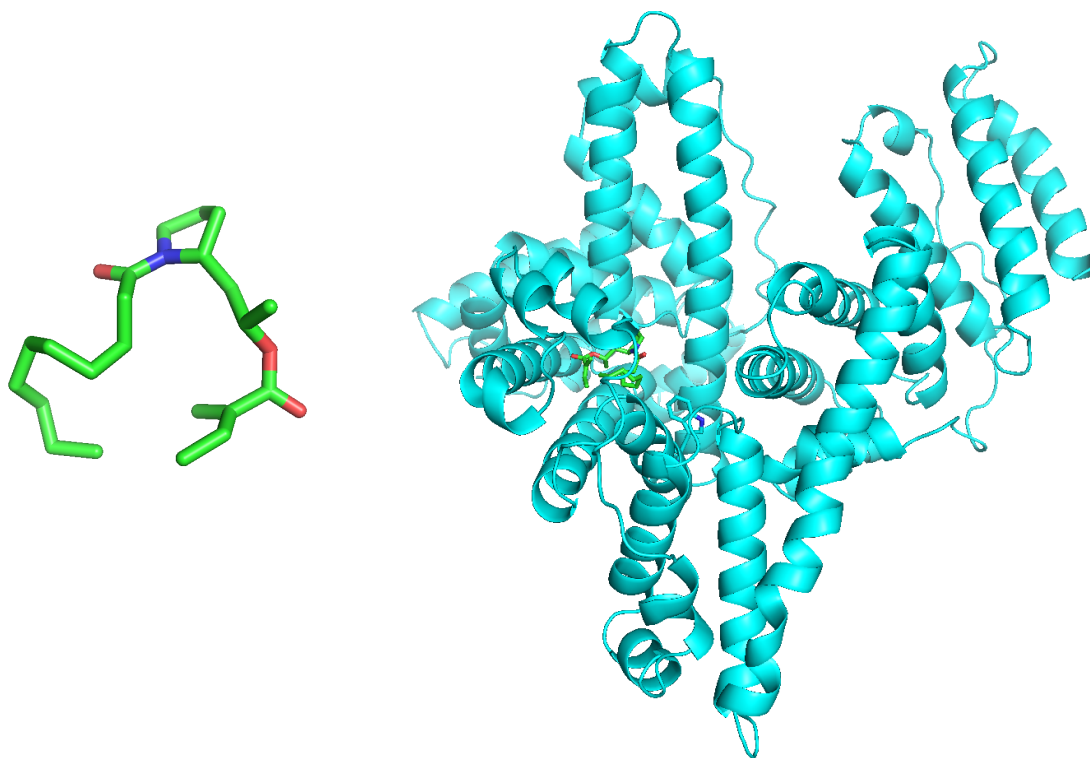
Model for the binding of *4R*, *6R*-**1** (green, red, blue) to HSA site I (-7.6 kcal/mol)



Model for the binding of 4*S*, 6*S*-1 (green, red, blue) to HSA site I (-7.5 kcal/mol)

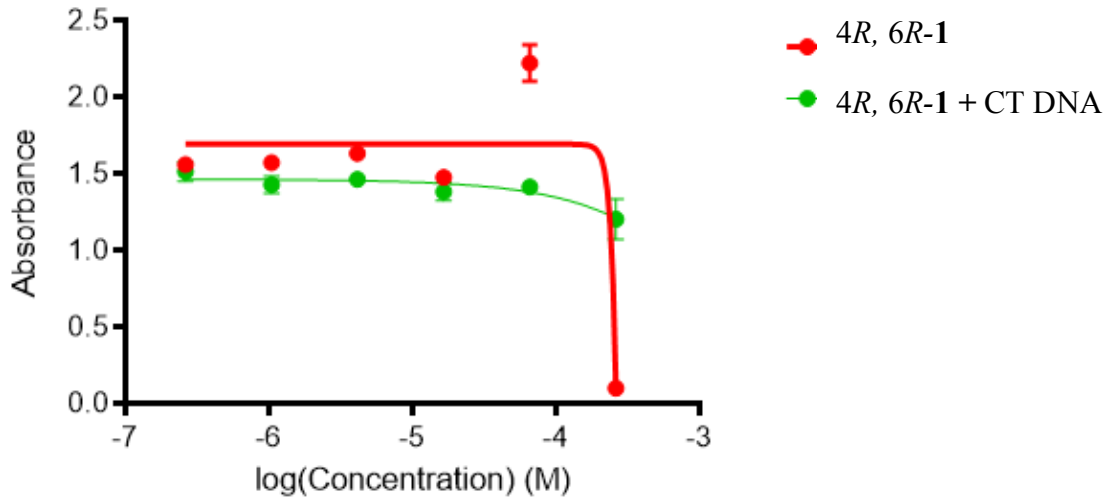


Model for the binding of 4*R*, 6*S*-**1** (green, red, blue) to HSA site I (-7.2 kcal/mol)



Preliminary MTT assay results for dosing of MCF-7 cells with varying concentrations of 4*R*, 6*R*-1 in the presence and absence of CT DNA

IC_{50} (4*R*, 6*R*-1) = 0.447 mM; IC_{50} (4*R*, 6*R*-1 + CT DNA)=321 mM



Preliminary MTT assay results for dosing of MCF-7 cells with varying concentrations of 4*S*, 6*S*-1 in the presence and absence of CT DNA

IC_{50} (4*S*, 6*S*-1) = 0.150 mM; IC_{50} (4*S*, 6*S*-1 + CT DNA)=5.11 mM

