Electronic Supplementary Material (ESI) for Analyst.

Naphthalene-Functionalized Resorcinarenes as Potent and Highly- Selective, Fluorescent Self-Quenching, Sensors for Kynurenic Acid

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SUPPORTING INFORMATION

CONTENTS

A. GENERAL INFORMATION

Kynurenic acid (KynA), tryptophan (Trp) and solvents used for syntheses were purchased from Sigma Aldrich or Oakwood Chemicals (Estill SC, USA). The receptors (**1-3**) were synthesized according to reported procedures.[1,2] All ^{1}H NMR, ITC, fluorescence titration and UV-visible spectroscopy experiments were carried out in 70% DI water/buffer, 30% DMSO at 298 K on a Bruker Avance 400, Microcal VP-ITC 2000, Horiba Jobin-Yvon Fluorolog 3, and Agilent Cary-100 spectrophotometers respectively.

B. SYNTHESIS

The sodium sulfonated resorcinarene (**1**),[1] and N-alkyl ammonium resorcinarene salts (**2**,**3**)[2] were synthesized according to reported procedures. *N*-Napthyl ammonium resorcinarene chloride (**4**) was synthesized according to the following procedure:

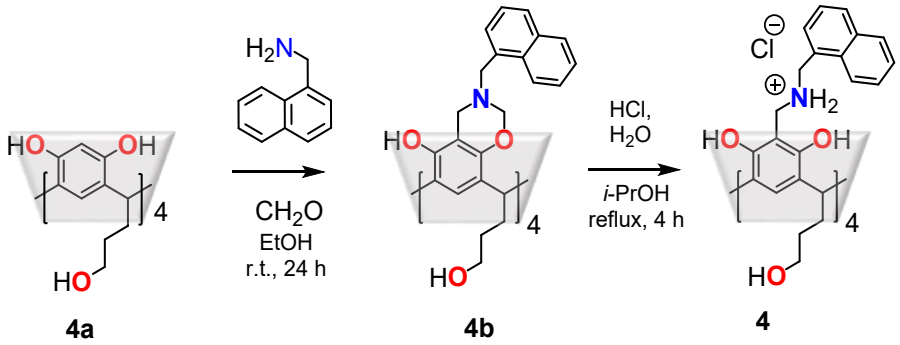

Figure S1. Synthesis of receptor **4**.

The resorcinarene **4a** was synthesized by reported procedure.[3] To a solution of **4a** (5.0 g, 6.9 mmol), excess formaldehyde solution 36% (28 mL) and ethanol (60 ml), 1-napthylmethylamine (4.3 mL, 29 mmol) in ethanol (15 mL) is slowly added and stirred at room temperature for 24 hours. The precipitate (**4b**) that separated is filtered, dried and used in the next step without further purification. To a solution of **4b** (0.5 g, 0.4 mmol), 1.5 mL of concentrated hydrochloric acid and 2 mL of H_2O in 25 mL of isopropanol is added. The mixture is heated to boiling, refluxing for 4 hours. Water and formaldehyde are removed by azeotropic distillation with chloroform. The remaining isopropanol is evaporated, and the crude product triturated with diethyl ether to give receptor **4** without further purification (0.23 g, 42 %).

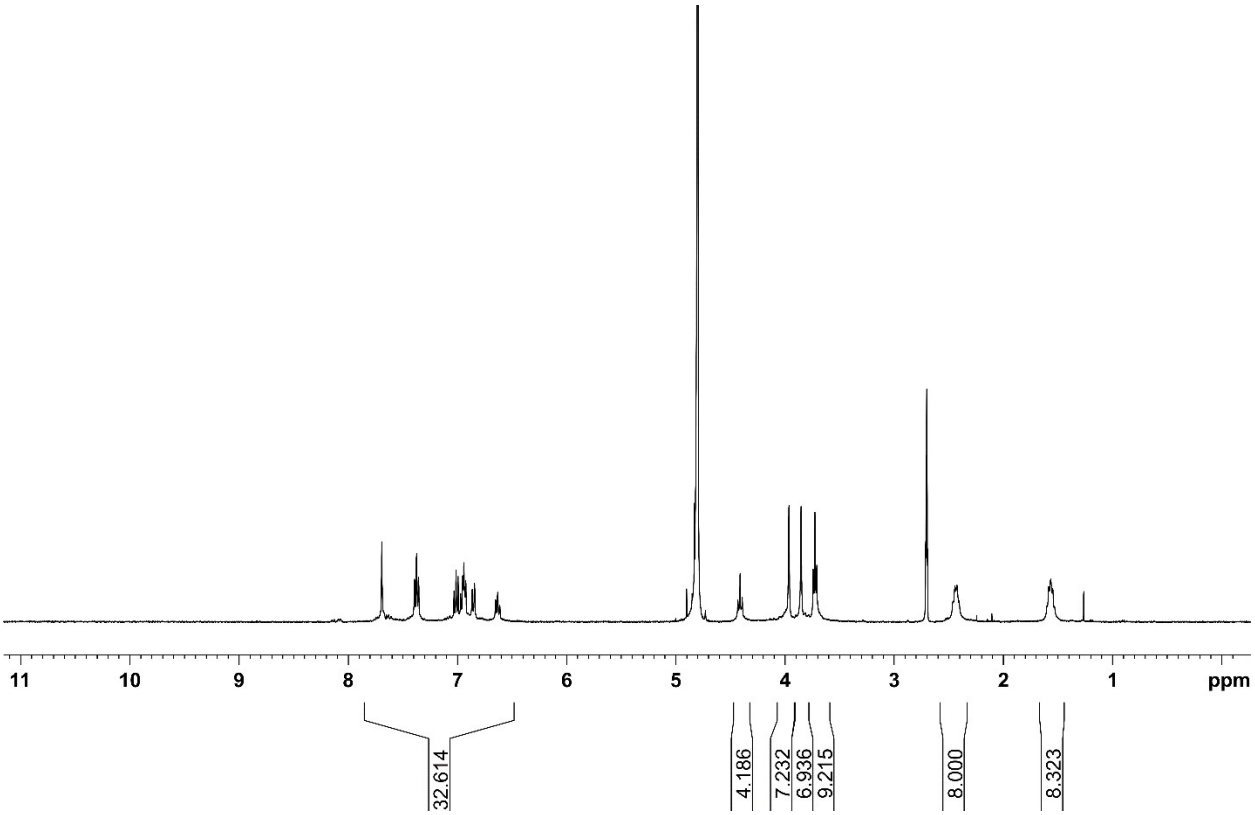

Figure S2. ^{1}H NMR of *N*-naphthyl ammonium resorcinarene chloride **4** in 90%D_2O/10%DMSO at 298 K.

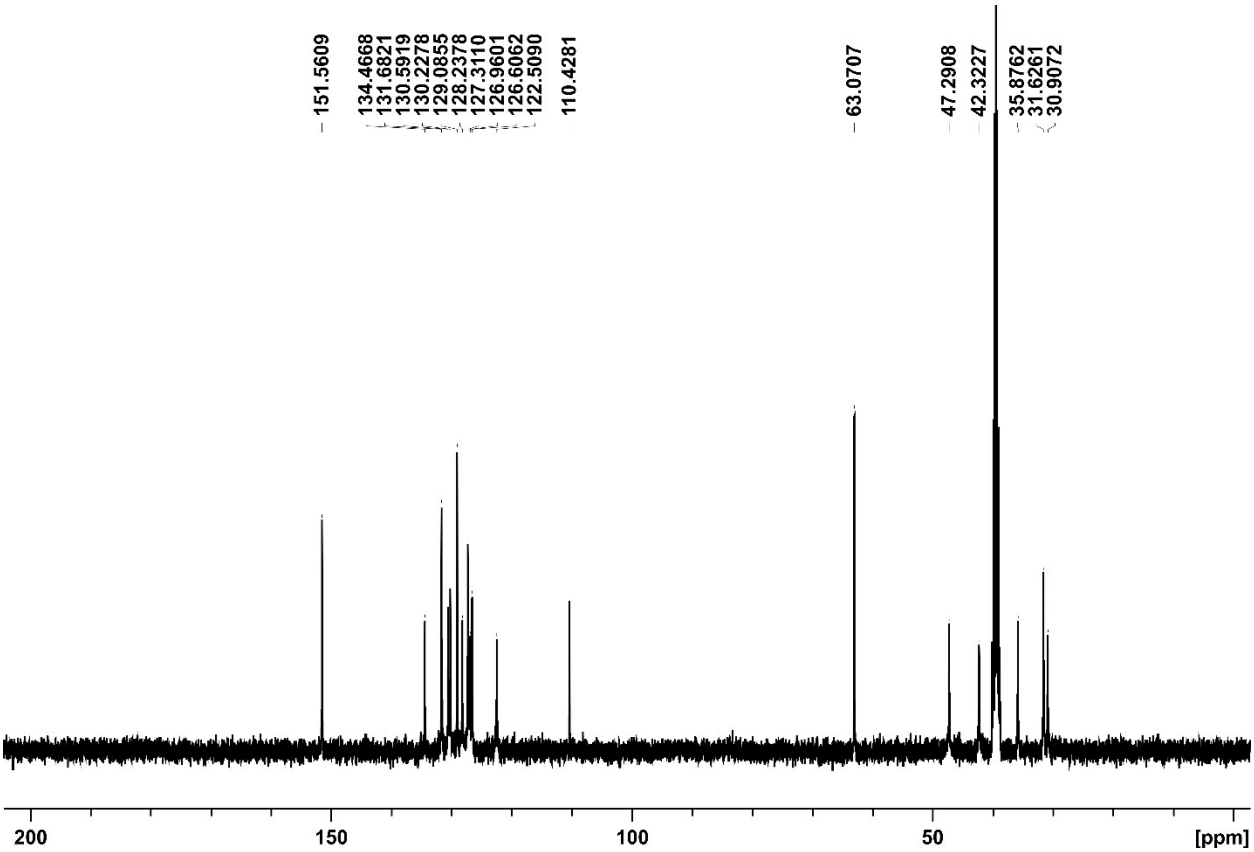

Figure S3. ^{13}C NMR of *N*-naphthyl ammonium resorcinarene chloride **4** in 90%D_2O/10%DMSO at 298 K.

Receptor 4: Melting point > 300°C. ESI-TOF-HRMS (Positive ion mode, sprayed from MeOH): *m/z* Found 699.34303 $[M-4Cl-2H]^+$; calc. 699.3429, m/z found 1433.66197 $[M-3Cl-2H]^+$; calc. 1433.6551. ^1H NMR (400 MHz in D_2O/[D_6]DMSO 9/1) δ_{ppm}: 1.56 (m, 8H,CH_2), 2.43 (q, J=7.66Hz, 8H, CH_2), 3.72 (t, J=6.4Hz, 8HCH_2), 3.85 (s, 8HCH_2), 3.96 (s, 8HCH_2), 4.41 (t, J=8.21Hz, 4H, CH) 6.60-7.7 (m, Ar, 32H): ^{13}C NMR: (100 MHz, 298 K in CD_3OD) δ_{ppm} = 30.9, 31.6, 35.9, 42.3, 47.2, 63.1, 110.4, 122.5, 126.6, 127.0, 127.3, 128.2, 129.1, 130.8, 131.7, 134.5, 151.6.

C. NMR SPECTROSCOPY

i) Proton NMR Experiment

For sample preparation, stock solutions of the receptors **1-4** (2 mM), KynA (2 mM), and Trp (2 mM) were prepared in 70% D_2O and 30% DMSO. For the pure **1-4**, KynA and Trp samples, 250 µL of the stock solution was transferred to an NMR tube and diluted with 250 µL of pure solvent mixture to make a 1 mM sample concentration.

For a 1:1 (**1-4**·KynA/Trp) mixture, 250 µL of **1-4** and 250 µL of KynA/Trp was pipetted into a clean NMR tube making 1:1 equimolar sample with 1 mM concentration of each component in the mixture.

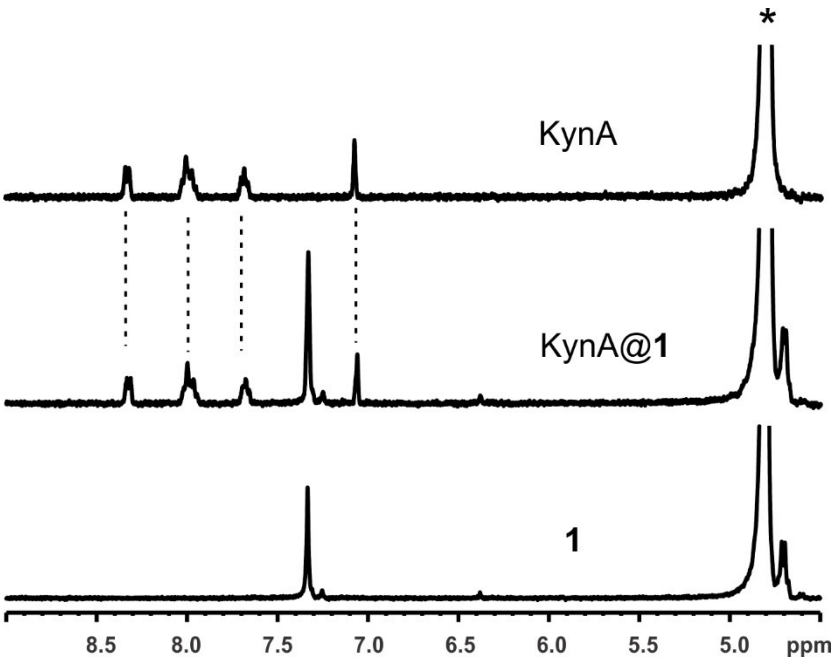

Figure S4. ^{1}H NMR spectra (70% D_2O, 30% D_6-DMSO, 298 K) of sulfonated resorcinarene (**1**), KynA and equimolar mixtures of KynA and **1**.

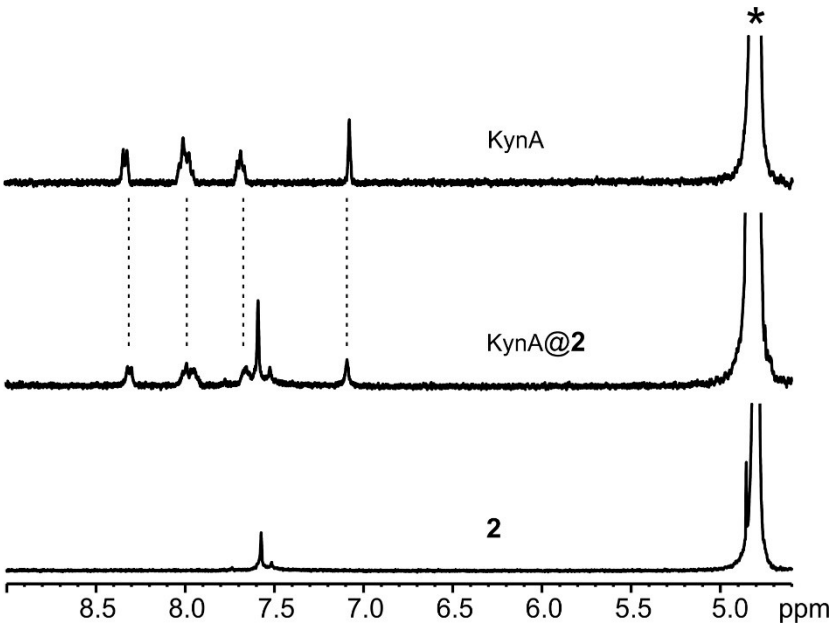

Figure S5. ^{1}H NMR spectra (70% D_2O, 30% D_6-DMSO, 298 K) of C3-hydroxyl resorcinarene salt (**2**), KynA, and equimolar mixtures of KynA and **2**.

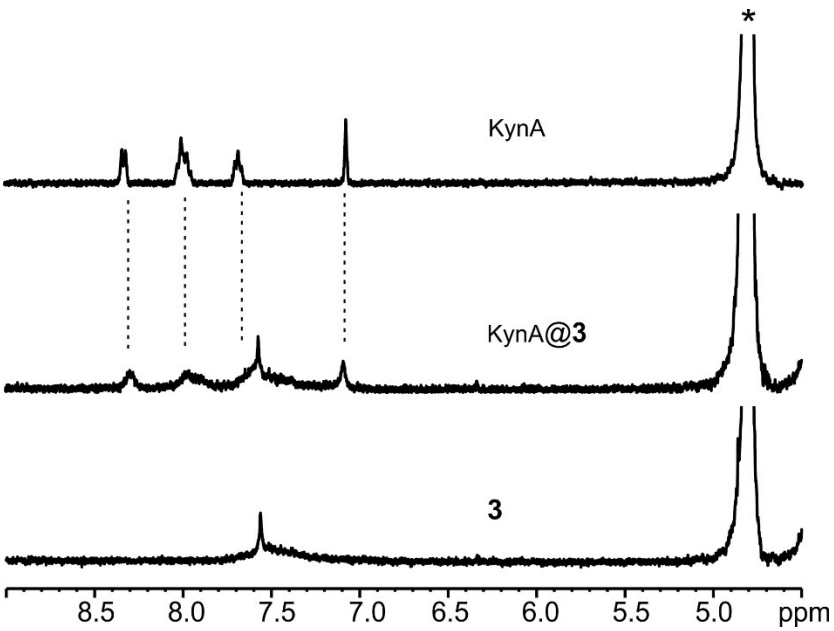

Figure S6. ^{1}H NMR spectra (70% D_2O, 30% D_6-DMSO, 298 K) of cyclohexyl resorcinarene salt (**3**), KynA, and equimolar mixtures of KynA and **3**

iii) 2D NOESY and DOSY NMR Experiment

2D NOESY NMR experiments were performed on a 500 MHz Bruker Avance III spectrometer at a temperature of 298 K. The mixing time was set to 200 µs. The 90° pulse was determined to be 8.7 µs. Spectra were recorded with 4k × 1k complex data points using 16 or 20 scans per t1 increment and 16 dummy scans at 25 and 70 °C. Diffusion ordered NMR spectroscopy (DOSY) measurements were performed using a Bruker Avance 500 MHz spectrometer equipped with a Great 1/10 pulsed gradient unit and a direct probe at 298 K. A LED29 pulse sequence (ledbpgp2s) was used for the diffusion experiments with a sine-shape pulsed gradient duration δ (P30) of 1.0−1.2 ms incremented from 0.68 to 32.4 G cm^{-1} in 16 steps. The pulsed gradient separation Δ (D20) was 50 ms, the spoil gradient (P19) was set to 1100 µs, and the eddy current delay (D21) was 5 ms. The reported diffusion coefficients were obtained using the T1/T2 relaxation module in TopSpin 4.1.4 software.

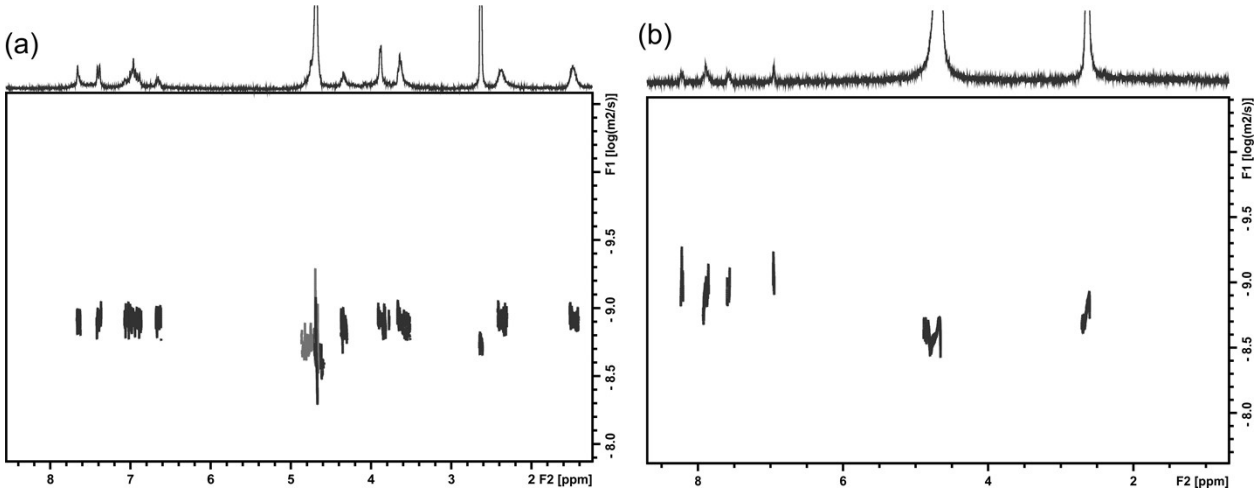

Figure S7. 2D DOSY NMR spectra of (a) receptor **4** and (b) KynA in 70%D_2O, 30% D_6[DMSO] at 298 K.

ii) Proton NMR Competition Experiment

NMR competition titration experiments were carried out in 70% D_2O, 30% D_6-DMSO at 298 K on a Bruker Avance 400 MHz spectrometer. 250 ul of 2mM pure KynA and Trp were charged into the sample tube. Due to the limited solubility of KynA, only a fraction of the KynA dissolved. Careful integration reveals a 1:8 equivalent KynA:Trp mixture which was recalculated to give a final concentration ratio of 0.125:1. 5µl aliquots of 20mM receptor **4** was titrated in the sample and the ^{1}H NMR recorded. Up to 6 equivalents (0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.4, 3.0, 6.0 equivalents) of **5** was added to KynA and Trp.

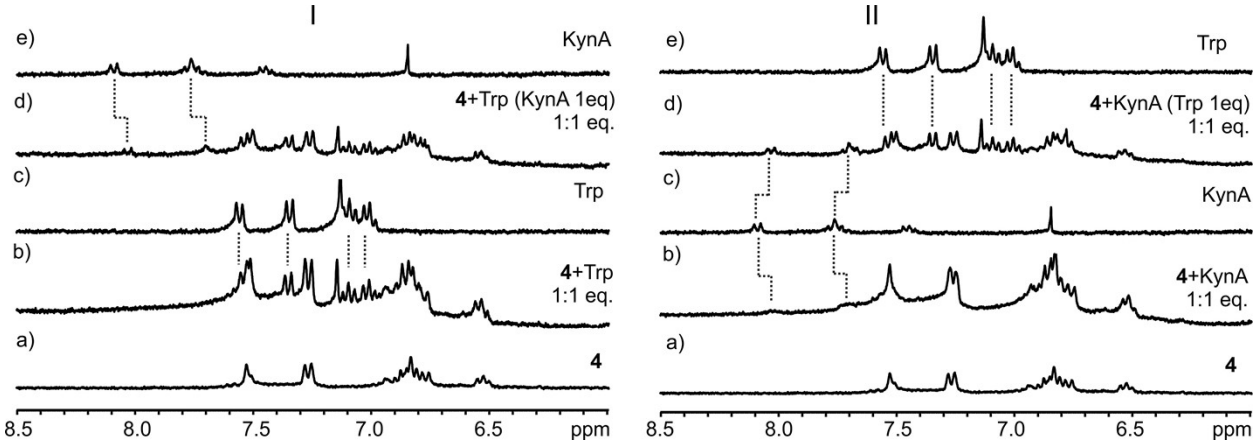

Figure S8. Competitive NMR titration in 70%D_2O, 30% D_6[DMSO] at 298 K. (I) a) receptor **4**, b) 1:1 equivalent of **4** and Trp, c) pure Trp, d) addition of 1 equivalent of KynA into a 1:1 mixture of **4** and Trp, e) pure KynA. (II) a) receptor **4**, b) 1:1 equivalent of **4** and KynA, c) pure KynA, d) addition of 1 equivalent of Trp into a 1:1 mixture of **4** and KynA, e) pure Trp. Dashed lines give an indication of the signal changes in ppm.

D. ISOTHERMAL CALORIMETRY

A VP-ITC instrument by MicroCal was used to determine the molar enthalpy (ΔH) of complexation. Subsequent fitting of the data to a 1:1 binding model using Origin software provides association constant (K), change in enthalpy (ΔH) and entropy (ΔS). The ITC experiment was carried out by filling the sample cell with the guest (0.25 mM), filling the syringe with the receptor (5.0 mM), and titrating via computer-automated injector at 298 K. Blank titrations into plain solvent were also performed and subtracted from the corresponding titration to remove any effect from the heats of dilution from the titrant.

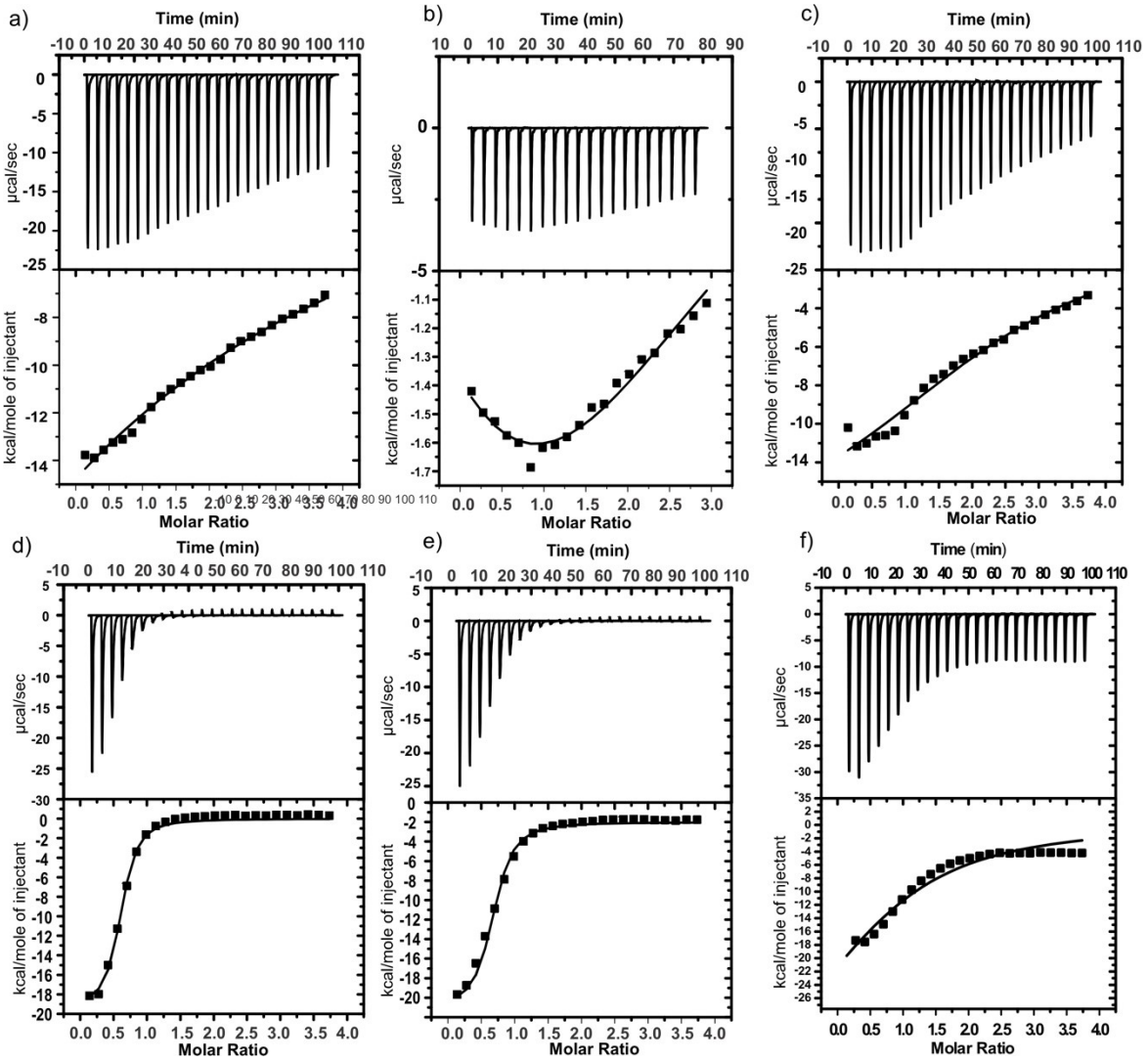

Figure S9: ITC traces of the titration of receptors with KynA (**a-e**) and Trp (**f**) at 298 K. (a) KynA@**1**, (c) KynA@**3**, (d) KynA@**4** in 70% H_2O/30% DMSO were fitted to a one set of site binding model**.** (b) KynA@**2** all in 70% H_2O/30% DMSO was fitted to two set of sites binding model. (e) KynA@**4** and (f) Trp@**4** in 70% Tris buffer pH 7.4/30% DMSO were fitted to one set of site binding model.

E. FLUORESCENCE SPECTROSCOPY

Fluorescence titration experiments were carried out in 70% H_2O, 30% DMSO at 298 K. 2.0 ml of 0.1 mM of KynA was pipetted into the sample cuvette. 5 µl aliquots of 20.0 mM receptor **4** at 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.4, 3.0, 6.0 equivalents were titrated in the sample and emission spectrum recorded.

In the reverse titration, 2.0 ml of 0.1 mM receptor **4** was pipetted into the sample cuvette. 5µl aliquots of 10.0 mM KynA (at constant sonication) at 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.4, 3.0, 6.0 equivalents were titrated in the sample and emission spectrum recorded.

REVERSE TITRATION

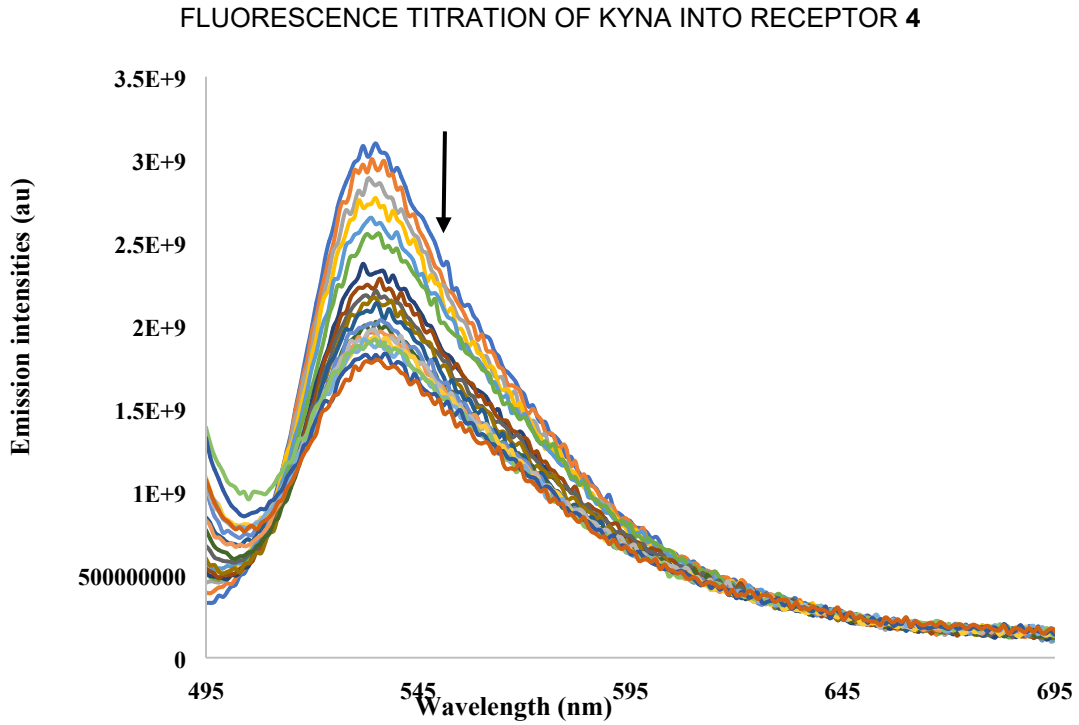

Figure S10. Steady state emission spectra (λ_{exc} = 475nm) of receptor **4** in H_2O/DMSO (70%/30%, 298 K) at different equivalents of KynA: 1) 0.00, 0.30, 0.60, 0.90, 1.19, 1.49, 1.78, 2.08, 2.37, 2.66, 2.96, and 3.25.

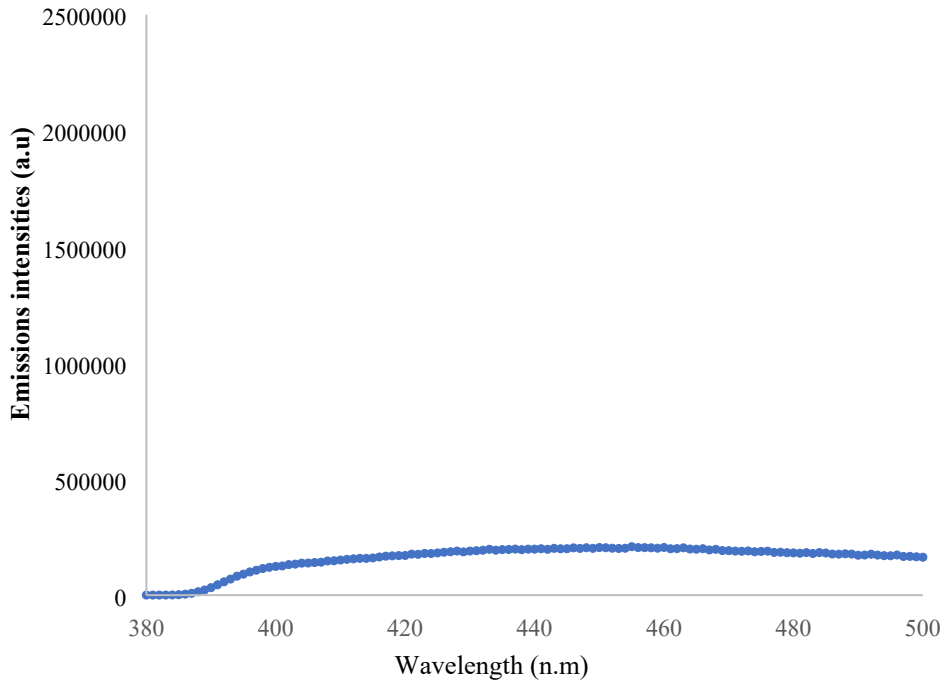

Figure S11. Steady state emission spectra (λ_{exc} = 350nm) of 3mM receptor **4** in H_2O/DMSO (70%/30%, 298 K)

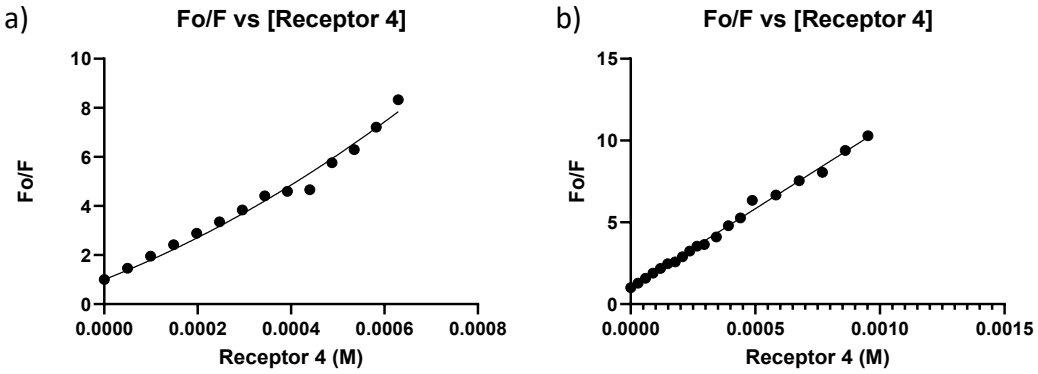

Figure S12. a) Stern-Volmer plot of first 14 titration points to reveal positive curvature, b) full stern-volmer plot of extended titration points.

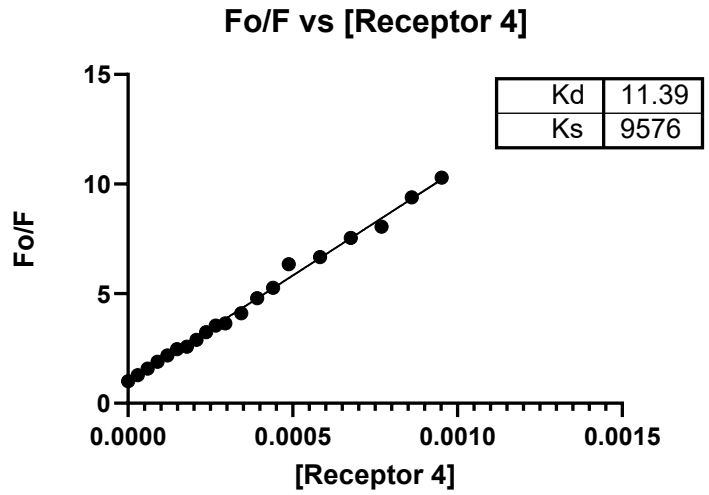

Figure S13. Modified Stern-Volmer regression analysis plot of Fo/F against increasing concentrations (logarithmic) of receptor 4.

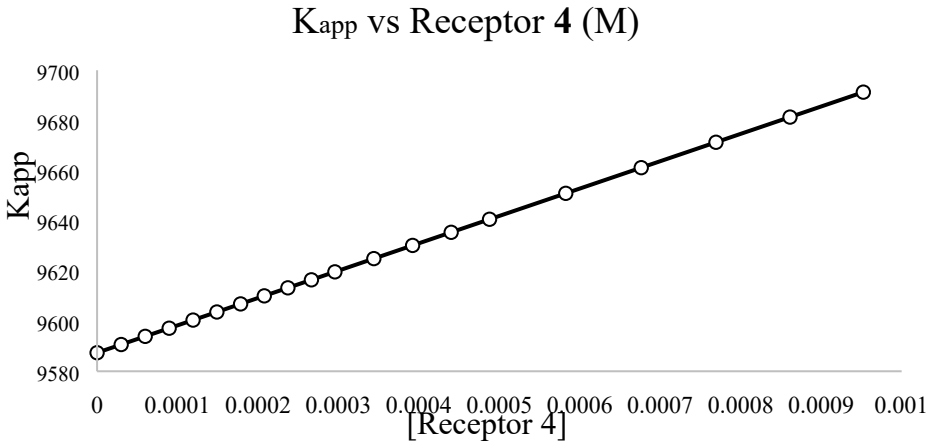

Figure S14. Plot of Kapp vs [receptor **4**] showing a linear plot with a specific slope.

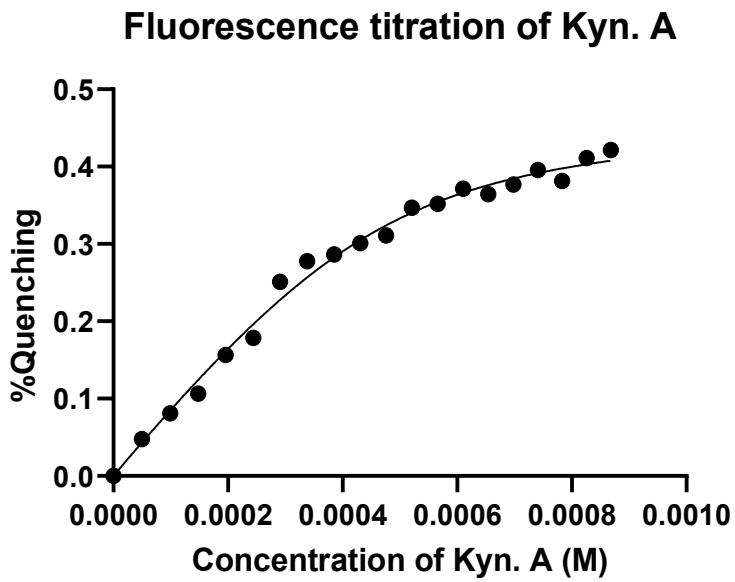

Figure S15. Fitting of percentage quenching of receptor **4** with concentration of KynA.

F. UV VISIBLE SPECTROSCOPY

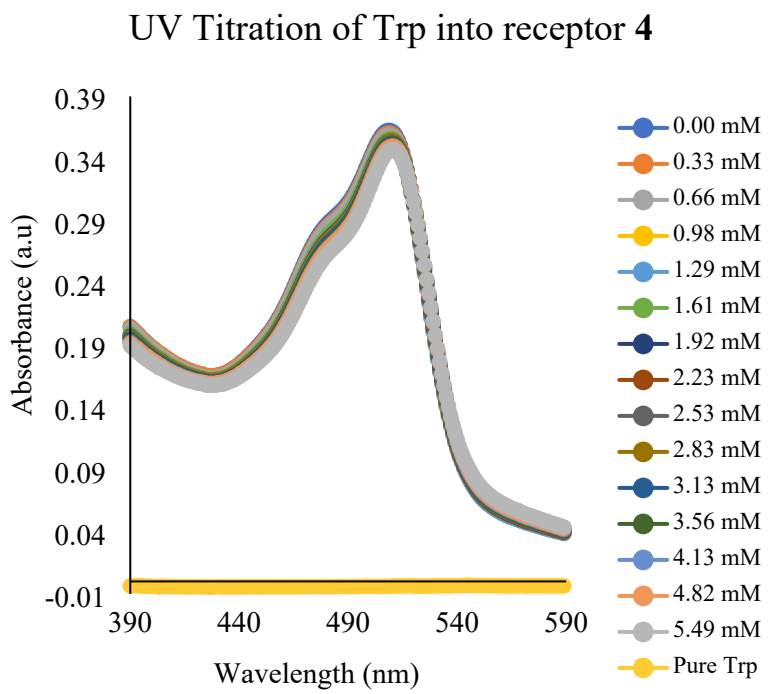

Figure S16. UV-Vis titration of increasing concentrations of Tryptophan (Trp, mM) into a solution of receptor **4** in 50:50 H_2O/DMSO system, monitoring absorbance of receptor **4**.

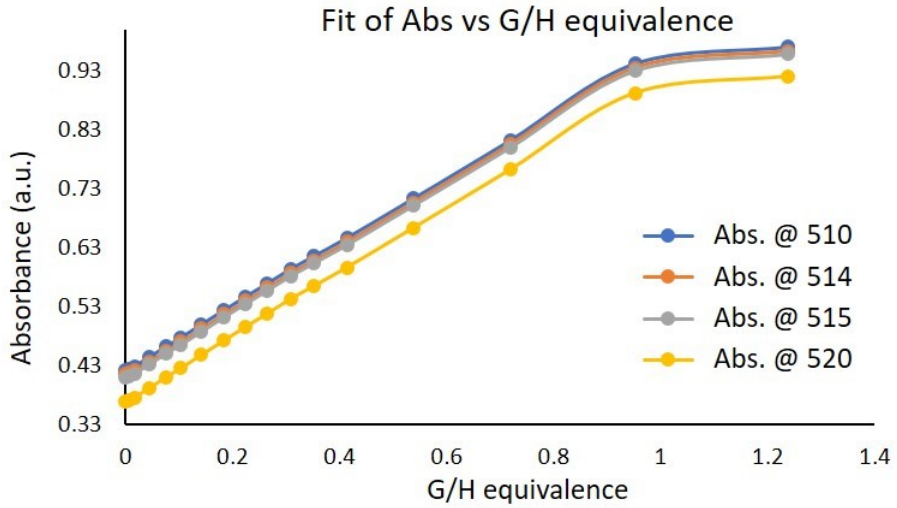

Figure S17. Output bindfits from absorbance of receptor **4** at 510nm, 514nm, 515nm and 520nm.

Table S1. Output fit quality

	510nm	**514nm**	**515nm**	**520nm**	**Total**
Root Mean Square (RMS)	0.020	0.020	0.020	0.020	0.020
Covariance	0.014	0.0134	0.014	0.014	0.014

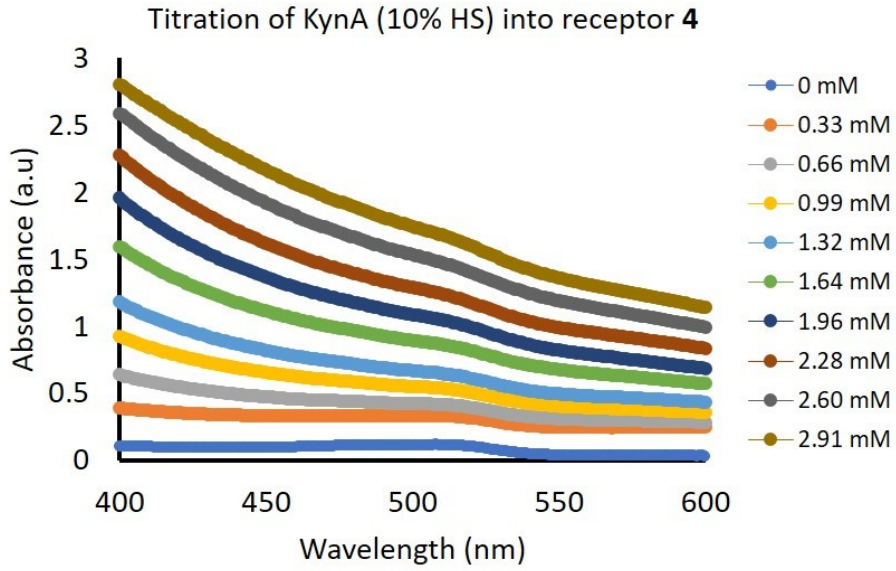

Figure S18. UV-Vis titration of increasing concentration of 60mM KynA in 50:40:10 DMSO/H_2O/Human Serum, into a solution of 3mM receptor **4** in 90:10 H_2O/DMSO system.

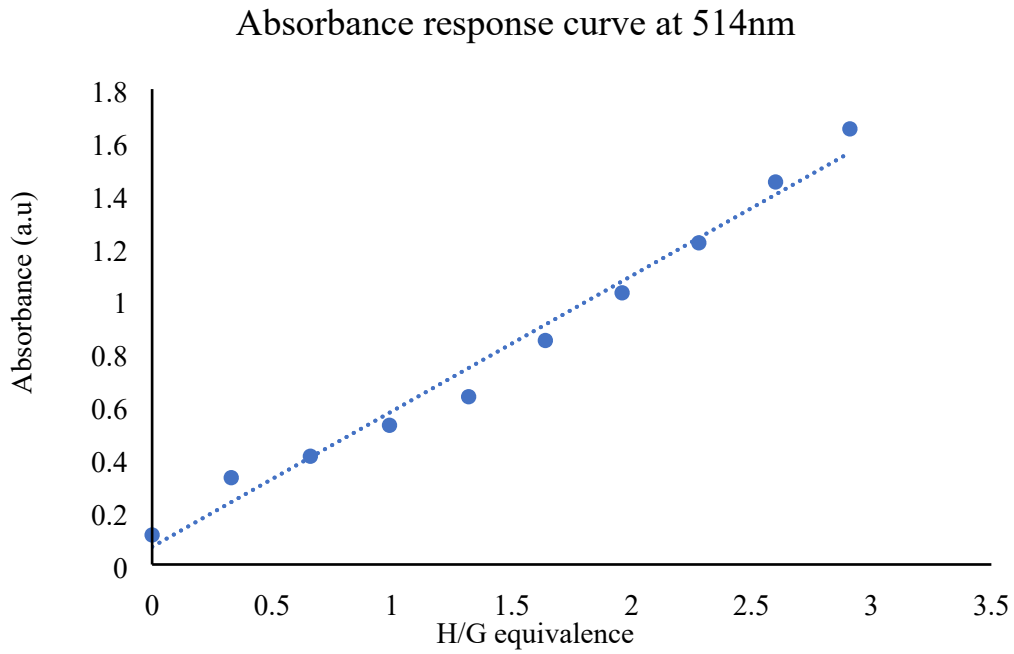

Figure S19. Linear response plot of absorbance at 514nm vs equivalence of host/guest in 10% HSA samples.

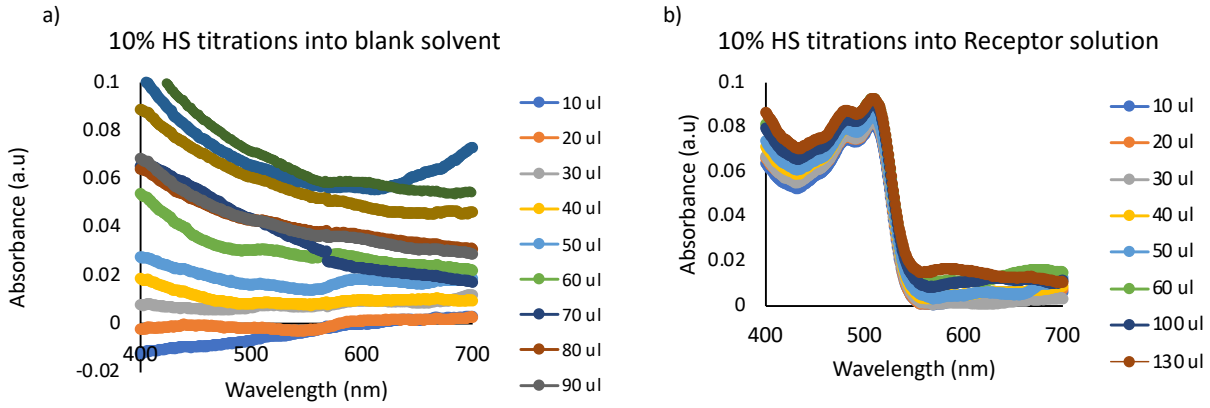

Figure S20. a) UV-Vis titration of increasing concentration of 50:40:10 DMSO/H_2O/Human Serum (HS), into a blank solution of 90:10 H_2O/DMSO system. b) UV-Vis titration of increasing concentration of 50:40:10 DMSO/H_2O/Human Serum, into a solution of 3mM receptor **4** in 90:10 H_2O/DMSO system in the absence of KynA.

G. ADDITIONAL COMPUTATIONAL DETAIL

The computational part of this study was performed with the Gaussian 16 Rev. C suite of programs.[4] The Symmetry Adapted Perturbation Theory (SAPT) analysis were performed using the Psi4 package.[5] The structural optimizations confirmed by frequency calculations were performed at the B3LYP-D3(BJ) level with Grimme's dispersion correction, [6] and the Becke-Johnson damping functions employed. The def2-SVP basis set was used throughout. The solvent optimization using SCRF=(PCM,Solvent=Water) solvation model leads to very similar geometry of the gas phase optimized geometry of the receptor **4** with O---N and O---O distances slightly longer by ca. 0.01-0.02Å, respectively. Cl^- counterions are also slightly more distant from the respective protons by 0.1Å from H-N and 0.03Å from H-O compared to gas-phase data. It is not unexpected since the dielectric continuum favors more charge separation (the dipole moment of solvent-optimized structure increases to 14.1D). The xyz coordinate files of both structures are shown below along with the host-guest, KynA@**4**, gas-phase optimized structure. The gas-phase thermodynamic results lead to the following values

Thermodynamic calculations at 298°K					
	KynA@**4**	Receptor **4**	KynA	ΔG°, kcal/mol	TΔS, kcal/mol
G° , a.u.	-6419.605084	-5754.391504	-665.178367	-22.10	
S, cal/mol K	386.818	343.449	27.129		4.84

The SAPT approach defines the interaction energy as a following sum of the electrostatic (elst) exchange (exch), induction (ind), exchange-induction (ex-ind), dispersion (disp), and exchange-dispersion counterpart (ex-disp).

$$E_{SAPT} = E_{elst} + E_{exch} + E_{ind,r} + E_{ex\text{-}ind,r} + E_{disp} + E_{ex\text{-}disp} + \delta_{HF} \quad \text{(S1)}$$

where "r" in the subscripts refers to the response-inclusive treatment of induction and the last term stands for higher order induction and residual effects. In this work we employ a SAPT variant based on the zeroth-order wavefunction obtained within the Hartree-Fock approximation. Evaluation of energy terms utilizes the density fitting and Laplace transformation techniques.[7] The induction term (Tables 2 and 3 in the main manuscript) has been combined with its exchange and residual contributions, while the dispersion has been combined with its exchange counterpart.

Another way of confirming the stability of the optimized host-guest complex, is by evaluating the interaction energy of host-guest complex as a difference of the total energies of the supermolecule (KynA@**4**) and its constituents,

$$E_{int} = E(\text{KynA@}\mathbf{4}) - E(\text{KynA; DCBS}) - E(\mathbf{4}\text{; DCBS}) \quad \text{(S2)}$$

where DCBS stands for Dimer-Centered Basis Set to make sure that the interaction energy is free from the effects of the basis set superposition error (BSSE).[8] E_{int} obtained from B3LYP-D3 total energies amounts to -35.8 kcal/mol. This result is not inconsistent with the perturbation theory estimate given that SAPT was based on the Hartree-Fock description of the monomer wavefunctions. We can also offer a gas-phase estimate of the free energy as $\Delta G^{o}(298°K)$ = -22.1 kcal/mol out of which, 13.5 kcal/mol of BSSE should be subtracted.

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176
Gas-phase Receptor 4
C    0.00000000  0.00000000  0.00000000
C   -0.23789400  2.52278700 -1.23116600
C    0.70789800  0.28533300 -1.18132200
C   -0.80912400  1.00887200  0.53198400
C   -0.91907700  2.29026200 -0.02365300
C    0.51862500  1.50604600 -1.84202800
H   -1.36560100  0.79494700  1.44465400
C   -1.79551500  3.37540200  0.60193100
H   -1.37015600  4.34214000  0.29619200
C   -3.19598700  3.30989700 -0.00079000
C   -5.71896500  3.07140900 -1.23143500
C   -3.48135000  4.01688200 -1.18264000
C   -4.20485600  2.50121100  0.53188200
C   -5.48628200  2.39085300 -0.02359700
C   -4.70227400  3.82747600 -1.84293300
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H   -7.53812000  1.94008000  0.29700200
C   -6.50619700  0.11410700 -0.00030700
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H   -2.21010500  2.42591300  2.55024200
H   -2.38443500  4.18933300  2.52820600
C    0.04164200 -1.40716500  2.13468300
H    0.87825100 -0.81170000  2.52941700
H   -0.88523300 -0.98532800  2.55069800
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O   -8.05371100  0.76706700 -1.70982200
O   -3.96274900 -4.74336500 -1.71029700
O   -6.28352800 -3.60329500 -1.88313200
O    1.54862200 -0.65272900 -1.70862200
O    0.40721100 -2.97228400 -1.88298500
H    2.44197500 -0.23553900 -1.96372500
H    0.93028700 -2.13305600 -1.77869300
H   -1.06093100  4.24075800 -1.77941700
H   -2.95967900  5.75015000 -1.96641500
H   -7.43744400  2.24855700 -1.77847300
H   -8.94702500  0.35020100 -1.96559700
H   -3.54555400 -5.63666400 -1.96551900
H   -5.44409800 -4.12618800 -1.77945000
C   -7.50562500 -1.62316900 -3.24209900
H   -7.94255800 -0.72119800 -3.68376400
H   -6.67576600 -1.97967800 -3.86539200
C   -4.93381500  4.30895600 -3.24245500
H   -5.29033500  3.47907400 -3.86571500
H   -4.03196300  4.74601200 -3.68425900
C    1.00046700  1.73712300 -3.24149800
H    0.17083300  2.09380600 -3.86498800
H    1.43729500  0.83504700 -3.68304400
C   -1.57262200 -4.19461500 -3.24226800
H   -1.21571300 -3.36477600 -3.86536600
H   -2.47467000 -4.63116300 -3.68416900
N   -8.56512300 -2.67865400 -3.27545500
N   -5.98948000  5.36830200 -3.27561300
N    2.06022400  2.79234500 -3.27467700
N   -0.51755000 -5.25453400 -3.27563400
H   -6.75724900  5.03326700 -2.67953100
H   -8.23024700 -3.44651000 -2.67939700
H    0.25038600 -4.92006300 -2.67945000
H    1.72542600  3.56030300 -2.67870100
H    0.13234600 -2.43570100  2.51474500
H   -5.74634700 -3.32976100  2.51385800
H   -0.76014500  3.44404600  2.51370900
H   -6.63907400  2.54997500  2.51426200
H   -5.50456400  6.22807300 -2.83839000
C   -6.49089100  5.71384100 -4.63595200
H   -5.65219700  6.18250500 -5.16599500
H   -9.42484800 -2.19359900 -2.83833300
C   -8.91053200 -3.18004900 -4.63587200
H   -9.37932800 -2.34141400 -5.16589600
H   -9.67594800 -3.95907800 -4.49828900
```

```
H   -1.00304300 -6.11412800 -2.83864500
C   -0.01628900 -5.60008100 -4.63601800
H   -0.85521500 -6.06823600 -5.16614300
H    0.76218200 -6.36609500 -4.49853200
H    2.91974400  2.30704300 -2.83744500
C    2.40597700  3.29357400 -4.63506600
H    3.17129400  4.07269100 -4.49742300
H   -7.26973600  6.47944800 -4.49832600
H    2.87497600  2.45488700 -5.16481900
C   -7.00843000  4.51023900 -5.38835400
H   -8.45236000  3.94638200 -3.88893000
C   -8.00419600  3.71080300 -4.85697800
C   -6.86645900  3.04927700 -7.33004800
C   -8.44695800  2.53766200 -5.52174300
C   -6.43318500  4.16070600 -6.64013100
C   -7.87865300  2.20603100 -6.79768800
C   -9.42164600  1.67170700 -4.95429300
H   -5.62986800  4.77744000 -7.04751200
H   -7.92124400  0.80244700 -8.45397800
H   -6.42129700  2.79532100 -8.29536800
C   -9.84361900  0.54702500 -5.63009100
H   -9.83323300  1.88595900 -3.96608400
H   -10.5767890 -0.10253100 -5.14793600
C   -9.31096900  0.24140400 -6.90857100
H   -9.67389100 -0.63709100 -7.44872800
C   -8.34290400  1.04489900 -7.47504300
C   -7.70670400 -3.69729100 -5.38808700
H   -7.14465800 -5.14313600 -3.88985000
C   -6.90807300 -4.69396000 -4.85719000
C   -6.24406900 -3.55355400 -7.32837000
C   -5.73455000 -5.13638800 -5.52150100
C   -7.35592400 -3.12068400 -6.63888600
C   -5.40165700 -4.56670100 -6.79650600
C   -4.86941200 -6.11204900 -4.95448800
H   -7.97199400 -2.31663200 -7.04581300
H   -3.99672500 -4.60785300 -8.45168900
H   -5.98912200 -3.10733900 -8.29294300
C   -3.74433600 -6.53364900 -5.62986300
H   -5.08460500 -6.52465700 -3.96691000
H   -3.09538600 -7.26755300 -5.14801800
C   -3.43748300 -5.99962300 -6.90746800
H   -2.55869800 -6.36226500 -7.44734500
C   -4.24014500 -5.03058600 -7.47345900
C    0.50183500 -4.39660200 -5.38821400
H    1.94614100 -3.83376600 -3.88877200
C    1.49802500 -3.59776200 -4.85673600
C    0.36040300 -2.93512200 -7.32956100
C    1.94128000 -2.42467300 -5.52126700
C   -0.07331600 -4.04652100 -6.63987900
C    1.37300100 -2.09244800 -6.79706700
C    2.91642300 -1.55931800 -4.95369200
H   -0.87695200 -4.66278000 -7.04734100
H    1.41600200 -0.68839800 -8.45295600
H   -0.08473400 -2.68072100 -8.29477700
```

```
C    3.33876600 -0.43459500 -5.62919000
H    3.32805600 -1.77404900 -3.96560800
H    4.07228100  0.21448400 -5.14691500
C    2.80608800 -0.12835600 -6.90751100
H    3.16930600  0.75015700 -7.44744300
C    1.83766200 -0.93131100 -7.47413500
C    1.20228600  3.81053400 -5.38770200
H    0.63968600  5.25661300 -3.88989700
C    0.40337500  4.80718200 -4.85718800
C   -0.25995600  3.66609300 -7.32822700
C   -0.77013200  5.24921600 -5.52179300
C    0.85185200  3.23358600 -6.63844400
C   -1.10268100  4.67917600 -6.79673000
C   -1.63560700  6.22477300 -4.95511400
H    1.46813100  2.42953200 -7.04505600
H   -2.50735400  4.71964100 -8.45214800
H   -0.51464900  3.21960100 -8.29274000
C   -2.76069000  6.64594400 -5.63075000
H   -1.42067600  6.63763500 -3.96758200
H   -3.40992800  7.37975600 -5.14915100
C   -3.06720700  6.11157200 -6.90829300
H   -3.94599700  6.47387800 -7.44838800
C   -2.26419800  5.14263500 -7.47396400
Cl  -2.52812000 -7.23309400 -2.42151400
Cl  -10.5438360 -0.66836000 -2.42124100
Cl  -3.97853100  7.34639500 -2.42347200
Cl   4.03854600  0.78131300 -2.42108200

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Water solvent optimized Receptor 4
C    0.00000000  0.00000000  0.00000000
C    2.52747000  0.24353000 -1.22276600
C    0.29925000 -0.72094800 -1.17005600
C    0.99975200  0.82283800  0.53003700
C    2.28156400  0.93734900 -0.02373100
C    1.52587400 -0.54236500 -1.82373200
H    0.77771700  1.38618500  1.43654300
C    3.35864900  1.82318300  0.60254500
H    4.32849900  1.40281600  0.30057500
C    3.28663400  3.22367500  0.00038900
C    3.04306100  5.75087900 -1.22295100
C    4.00847100  3.52311500 -1.16907500
C    2.46315400  4.22324700  0.52975800
C    2.34871000  5.50497700 -0.02422200
C    3.82990200  4.74952900 -1.82309000
H    1.89926300  4.00115900  1.43592500
C    1.46280300  6.58212100  0.60182200
H    1.88295400  7.55193000  0.29941800
C    0.06207300  6.50991500  0.00026100
C   -2.46608100  6.26691100 -1.22118900
C   -0.23789100  7.23153200 -1.16921400
C   -0.93734100  5.68676800  0.53044000
C   -2.21946000  5.57250200 -0.02265600
C   -1.46493100  7.05334300 -1.82221500
```

H	-0.71477000	5.12295700	1.43652700
C	-3.29622700	4.68649900	0.60389300
H	-4.26622200	5.10685300	0.30236700
C	-1.40044300	-0.07242200	0.60222000
H	-1.82065500	-1.04224300	0.29993200
C	-3.22441600	3.28608800	0.00154300
C	-2.98170400	0.75878900	-1.22171900
C	-3.94667200	2.98675900	-1.16769600
C	-2.40079400	2.28646100	0.53059300
C	-2.28669100	1.00470800	-0.02337600
C	-3.76861300	1.76020500	-1.82164200
H	-1.83646400	2.50854700	1.43648700
C	-3.26752200	4.69205800	2.13617300
H	-2.33653900	4.27739400	2.54907200
H	-4.09936800	4.09086400	2.53262700
C	1.46912900	6.55453800	2.13412900
H	1.05482900	5.62378800	2.54791900
H	0.86800700	7.38658300	2.53027800
C	3.33055800	1.81742600	2.13483000
H	2.39981700	2.23221200	2.54815100
H	4.16266900	2.41842500	2.53102100
C	-1.40607600	-0.04493600	2.13452600
H	-0.80464900	-0.87691200	2.53035700
H	-0.99173200	0.88585500	2.54818000
O	3.72076700	0.24341000	-1.87146100
O	4.85713200	2.58705300	-1.69270800
O	3.04266700	6.94394000	-1.87208100
O	0.69835200	8.07934900	-1.69382400
O	-4.79518700	3.92307200	-1.69109900
O	-3.65972900	6.26700700	-1.86926000
O	-0.63720600	-1.56865900	-1.69446800
O	-2.98179800	-0.43430000	-1.87076400
H	-0.21965500	-2.46405600	-1.90990300
H	-2.14369600	-0.95283300	-1.76048500
H	4.24019900	1.08077500	-1.75989600
H	5.75250100	3.00527600	-1.90691500
H	2.20479200	7.46265000	-1.76101400
H	0.28080400	8.97482400	-1.90895800
H	-5.69066700	3.50515800	-1.90547500
H	-4.17858800	5.42924400	-1.75799800
C	-1.71349200	7.57441400	-3.20612300
H	-0.82267000	8.03934600	-3.64074800
H	-2.04821600	6.75385700	-3.85268300
C	4.35095300	4.99692500	-3.20721600
H	3.53024500	5.33050300	-3.85418900
H	4.81641500	4.10590100	-3.64087100
C	1.77346600	-1.06230100	-3.20824700
H	2.10786200	-0.24120000	-3.85429800
H	0.88230100	-1.52673000	-3.64269700
C	-4.29029000	1.51249600	-3.20548000
H	-3.47000900	1.17818700	-3.85260800
H	-4.75543300	2.40357700	-3.63932100
N	-2.79811900	8.60644600	-3.21897600
N	5.38239800	6.08203000	-3.22116300

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N    2.85796600 -2.09441700 -3.22285100
N   -5.32236300  0.42792400 -3.21857500
H    5.03022900  6.84086800 -2.62284800
H   -3.55669500  8.25466500 -2.62013400
H   -4.97035300 -0.33088200 -2.62015400
H    3.61679700 -1.74337100 -2.62390300
H   -2.43385000 -0.14507500  2.51436700
H   -3.36704800  5.71984100  2.51615600
H    3.43005800  0.78957800  2.51464400
H    2.49709100  6.65450200  2.51351100
H    6.24120400  5.65314900 -2.77165500
C    5.71330200  6.62060300 -4.57810600
H    6.19729500  5.80896700 -5.13396200
H   -2.36849800  9.46504000 -2.76989400
C   -3.33751100  8.93820000 -4.57538000
H   -2.52615600  9.42245300 -5.13141700
H   -4.13446800  9.67870500 -4.41902500
H   -6.18074900  0.85750200 -2.76894400
C   -5.65452800 -0.11096600 -4.57511200
H   -6.13962400  0.70035600 -5.13047500
H   -6.39442800 -0.90849500 -4.41879000
H    2.42854100 -2.95351900 -2.77453100
C    3.39683500 -2.42435700 -4.57993200
H    4.19332900 -3.16565000 -4.42494400
H    6.45379500  7.41777300 -4.42274300
H    2.58504900 -2.90718600 -5.13661700
C    4.48432600  7.12052400 -5.30011600
H    3.97776000  8.59212400 -3.80853900
C    3.69943000  8.11980700 -4.75329100
C    2.94300800  6.92588500 -7.17556500
C    2.49072000  8.53226500 -5.37273900
C    4.08981300  6.52035700 -6.52709800
C    2.10849900  7.93412800 -6.62074000
C    1.63411500  9.50345200 -4.78325800
H    4.70047300  5.72152100 -6.95101800
H    0.62427400  7.91711500 -8.20723700
H    2.65391500  6.46115300 -8.12126500
C    0.46891400  9.88880500 -5.41103800
H    1.89751800  9.93388400 -3.81496800
H   -0.18205700  10.6118160 -4.91644300
C    0.11171100  9.32634800 -6.66378600
H   -0.80109300  9.65890900 -7.16387700
C    0.90710400  8.36389200 -7.25085500
C   -3.83801300  7.70987000 -5.29804400
H   -5.30792300  7.20125200 -3.80544900
C   -4.83655400  6.92415300 -4.75103700
C   -3.64514800  6.17086900 -7.17556800
C   -5.24943600  5.71610900 -5.37148900
C   -3.23920100  7.31701800 -6.52621400
C   -4.65255000  5.33546200 -6.62056900
C   -6.21988500  4.85866200 -4.78201000
H   -2.44112100  7.92853100 -6.95036500
H   -4.63678400  3.85299300 -8.20871500
H   -3.18142000  5.88299500 -8.12213100
```

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C   -6.60570300  3.69413200 -5.41074900
H   -6.64930000  5.12089900 -3.81294900
H   -7.32812300  3.04242500 -4.91625400
C   -6.04437000  3.33842900 -6.66443000
H   -6.37711400  2.42605300 -7.16515700
C   -5.08268600  4.13468100 -7.25158600
C   -4.42627600 -0.61012900 -5.29884400
H   -3.91589300 -2.08104500 -3.80785700
C   -3.63953500 -1.60856200 -4.75310200
C   -2.88812100 -0.41415400 -7.17674100
C   -2.43129100 -2.01974700 -5.37432900
C   -4.03447100 -0.00993400 -6.52666500
C   -2.05148800 -1.42116900 -6.62286600
C   -1.57271000 -2.98991500 -4.78602900
H   -4.64695400  0.78792400 -6.94983400
H   -0.56920400 -1.40201500 -8.21114200
H   -2.60100200  0.05072000 -8.12297200
C   -0.40771300 -3.37360100 -5.41521300
H   -1.83435400 -3.42083500 -3.81747800
H    0.24485700 -4.09570700 -4.92140600
C   -0.05271800 -2.81049900 -6.66830100
H    0.86008600 -3.14161500 -7.16938000
C   -0.85024800 -1.84923900 -7.25444100
C    3.89807700 -1.19517100 -5.30064900
H    5.36952400 -0.69095700 -3.80807000
C    4.89781000 -0.41154100 -4.75281400
C    3.70588000  0.34744300 -7.17528200
C    5.31172400  0.79715700 -5.37132600
C    3.29887400 -0.79931800 -6.52766100
C    4.71472000  1.18060500 -6.61950100
C    6.28329100  1.65258900 -4.78075400
H    2.49962800 -1.40899000 -6.95228300
H    4.70041700  2.66547000 -8.20543900
H    3.24191800  0.63753700 -8.12105400
C    6.67028200  2.81766000 -5.40776400
H    6.71262000  1.38829600 -3.81220600
H    7.39308300  3.46814500 -4.91225400
C    6.10918900  3.17585600 -6.66082700
H    6.44321400  4.08848900 -7.16028100
C    5.14623400  2.38177300 -7.24886300
Cl  -7.33321600  2.43638200 -2.15325700
Cl  -0.78813100  10.6175050 -2.15765400
Cl   7.39408400  4.07494200 -2.15536500
Cl   0.84893100 -4.10638600 -2.16146700

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KynA@4 from gas-phase optimization
C    0.00000000  0.00000000  0.00000000
C    1.00094600 -2.36758700 -1.14665300
C   -0.60989200 -0.55535600 -1.13807000
C    1.08641700 -0.68455100  0.55048900
C    1.57517400 -1.89666500  0.04426600
C   -0.04473300 -1.66648000 -1.76989200
H    1.56934500 -0.26247700  1.43262200
```

```
C    2.72126200 -2.65606100  0.70857800
H    2.62460400 -3.70533300  0.39416600
C    4.06779000 -2.17688000  0.17595300
C    6.55499900 -1.31049900 -0.82275700
C    4.60478900 -2.74590700 -0.99318500
C    4.79211200 -1.14882000  0.78853800
C    6.04794100 -0.71174900  0.34543400
C    5.79925400 -2.25535300 -1.53702000
H    4.37377900 -0.68847100  1.68336400
C    6.78136800  0.45210100  1.01794300
H    7.85889500  0.28697200  0.87531700
C    6.42325400  1.72329600  0.25074000
C    5.56654800  3.80682200 -1.44040400
C    7.17785100  2.08156900 -0.88188500
C    5.27458400  2.47383800  0.52723500
C    4.85611600  3.56350600 -0.25285600
C    6.68345500  3.02656700 -1.78517300
H    4.69040600  2.21837000  1.41215300
C    3.65833600  4.42891700  0.13088600
H    3.81643300  5.40550900 -0.34900300
C   -0.43744400  1.37763600  0.49184000
H   -1.48939300  1.50100700  0.19613800
C    2.36594600  3.87121700 -0.45600000
C   -0.01658800  2.85395900 -1.54876200
C    1.95417000  4.26145400 -1.74281400
C    1.57532600  2.94272300  0.22882000
C    0.37048200  2.43250100 -0.26569500
C    0.80314500  3.70256800 -2.31315500
H    1.90332600  2.61779600  1.21624400
C    3.57364800  4.68829300  1.63971000
H    3.41421900  3.76943000  2.22307500
H    2.73717300  5.36729100  1.86246200
C    6.53969900  0.55150000  2.52596800
H    5.48872900  0.75581200  2.77882700
H    7.14218400  1.36747900  2.95168900
C    2.63553900 -2.64352700  2.23900300
H    2.71014700 -1.63105900  2.66280300
H    3.44945700 -3.24332500  2.67308600
C   -0.38078300  1.53391300  2.01377900
H   -1.02696400  0.78517300  2.49549400
H    0.63270200  1.39930000  2.41995200
O    1.37217000 -3.51974100 -1.77128800
O    3.94558700 -3.76589300 -1.61571500
O    7.78521800 -1.05422700 -1.34342900
O    8.35580100  1.44130000 -1.13137500
O    2.70687700  5.15959200 -2.44311200
O    5.26400300  4.79462700 -2.32366200
O   -1.72061300  0.04112200 -1.66684000
O   -1.18959500  2.50819000 -2.14694300
H   -2.41220300 -0.64845500 -1.94819600
H   -1.50347700  1.59389900 -1.91158700
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