Supporting Information

Complexation of 5-aminovaleric acid zwitterion in aqueous/methanol solution by heterotopic tri-cationic receptor

Wojciech Walczak, Maciej Zakrzewski, Grzegorz Cichowicz and Piotr Piątek*

Department of Chemistry, University of Warsaw, Pasteura 1, 02-093 Warsaw, Poland

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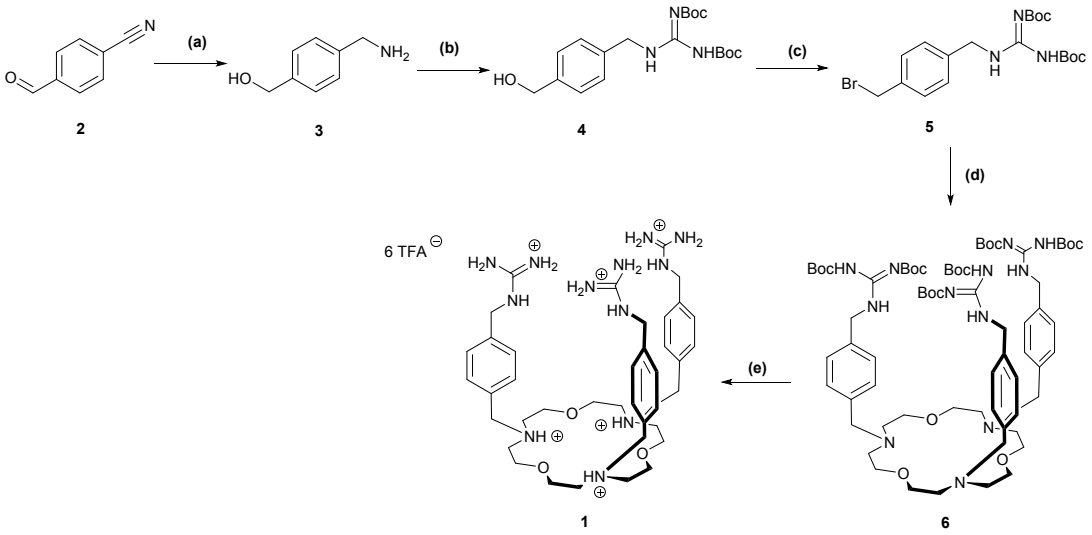
1. Synthetic route to receptor 1 with experimental procedures and NMR spectra



*Scheme 1. Synthetic route to **1***

(4-(aminomethyl)phenyl)methanol (3)

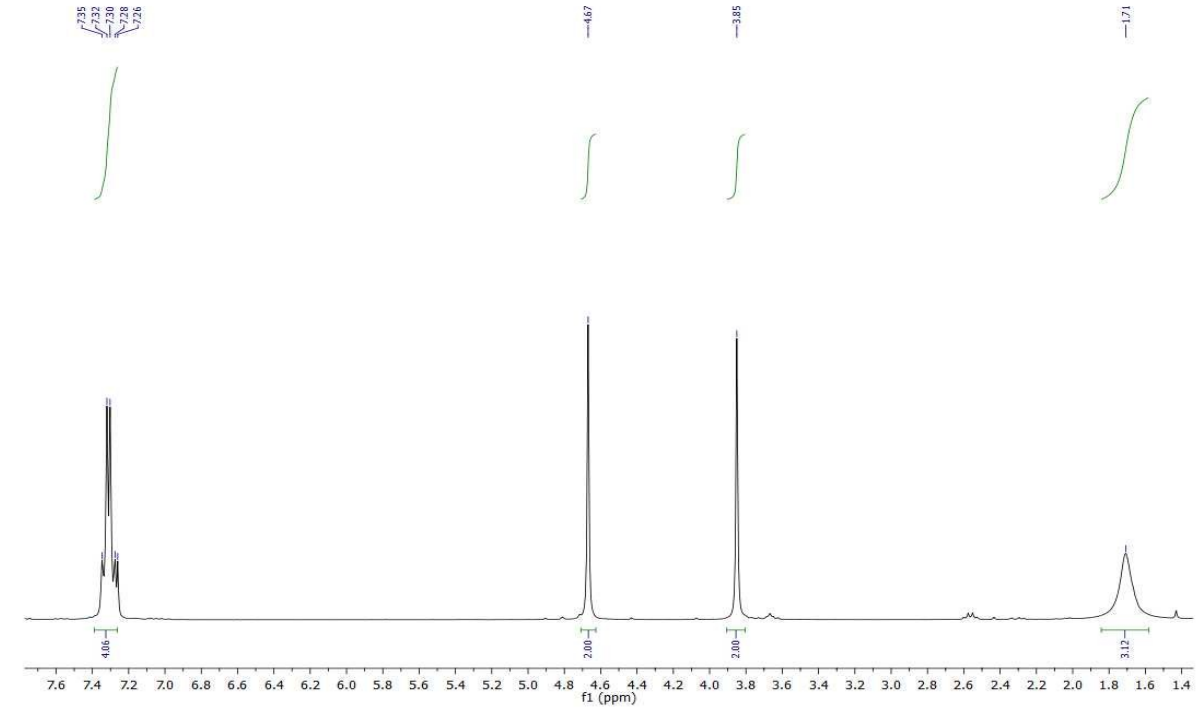


Figure 1. ^{1}H NMR of compound 3.

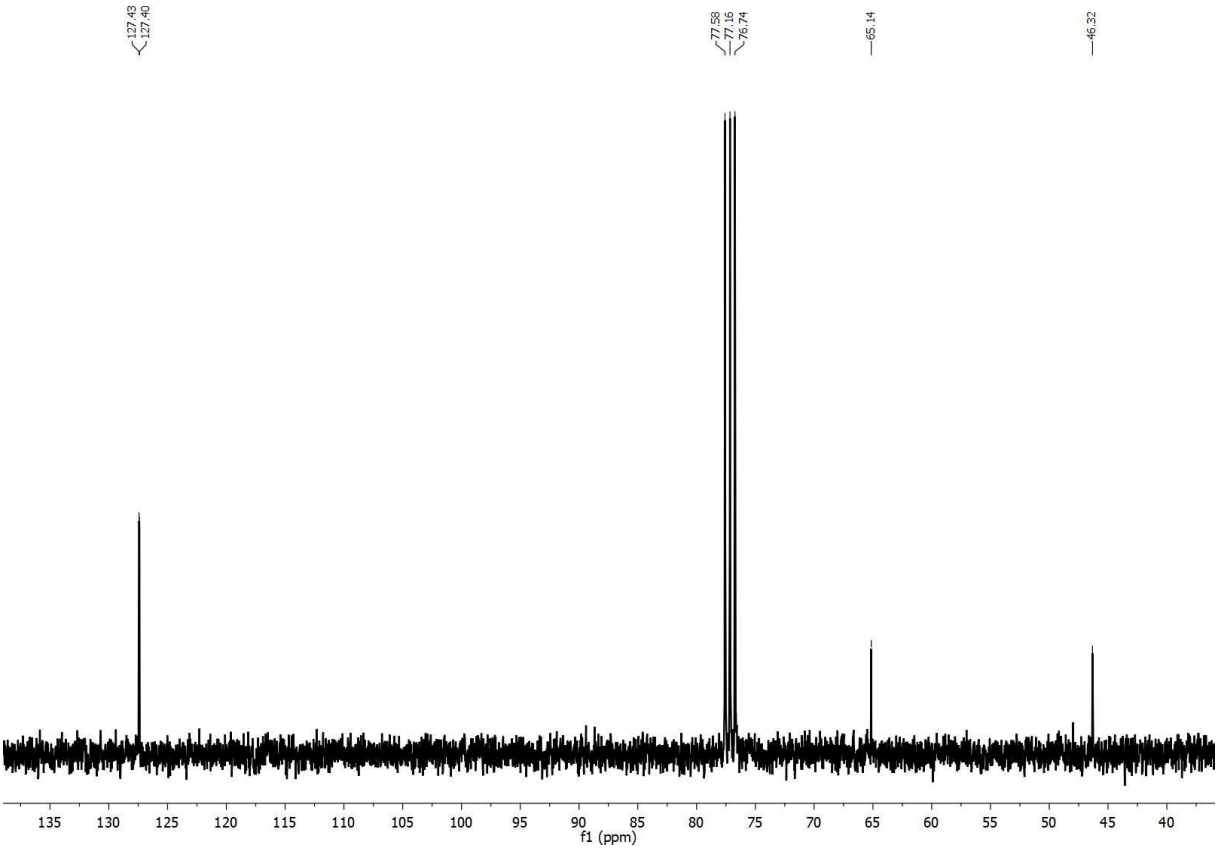


Figure 2.^{13}C NMR of compound 3.

N,N'-diBoc-N''-(4-hydroxymethyl)-benzylguanidine (4)

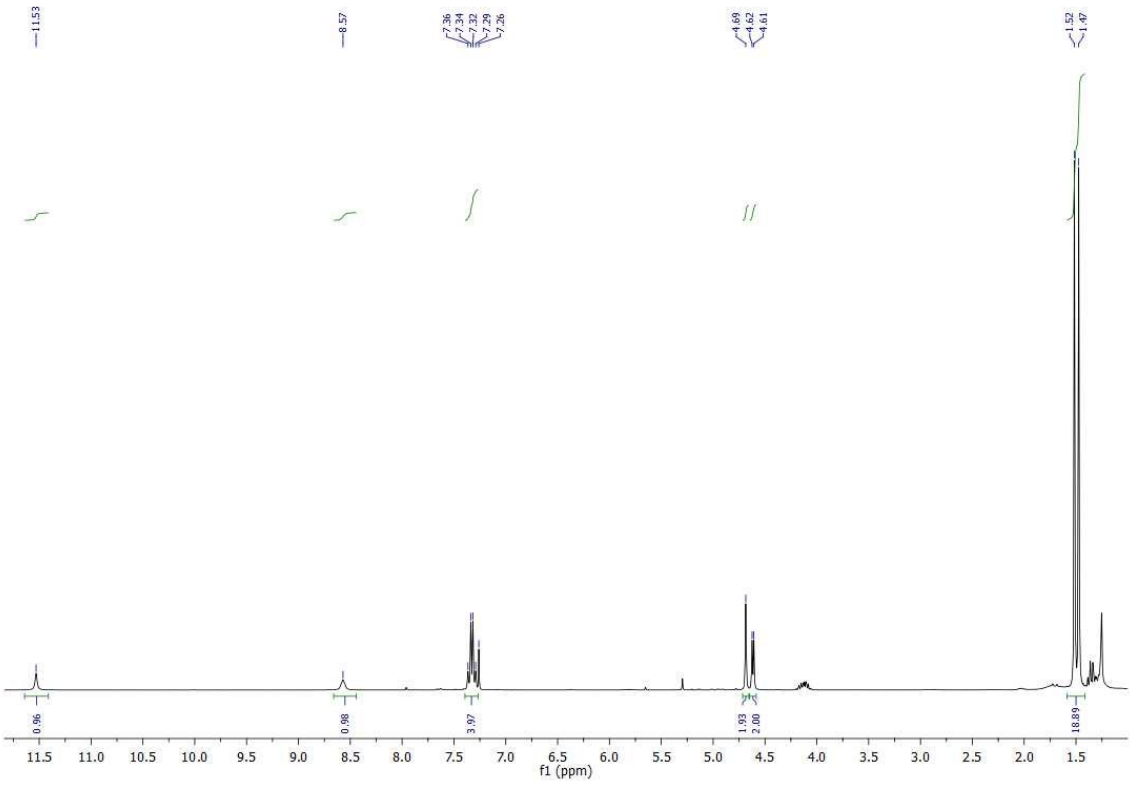


Figure 3. ^{1}H NMR of compound 4.

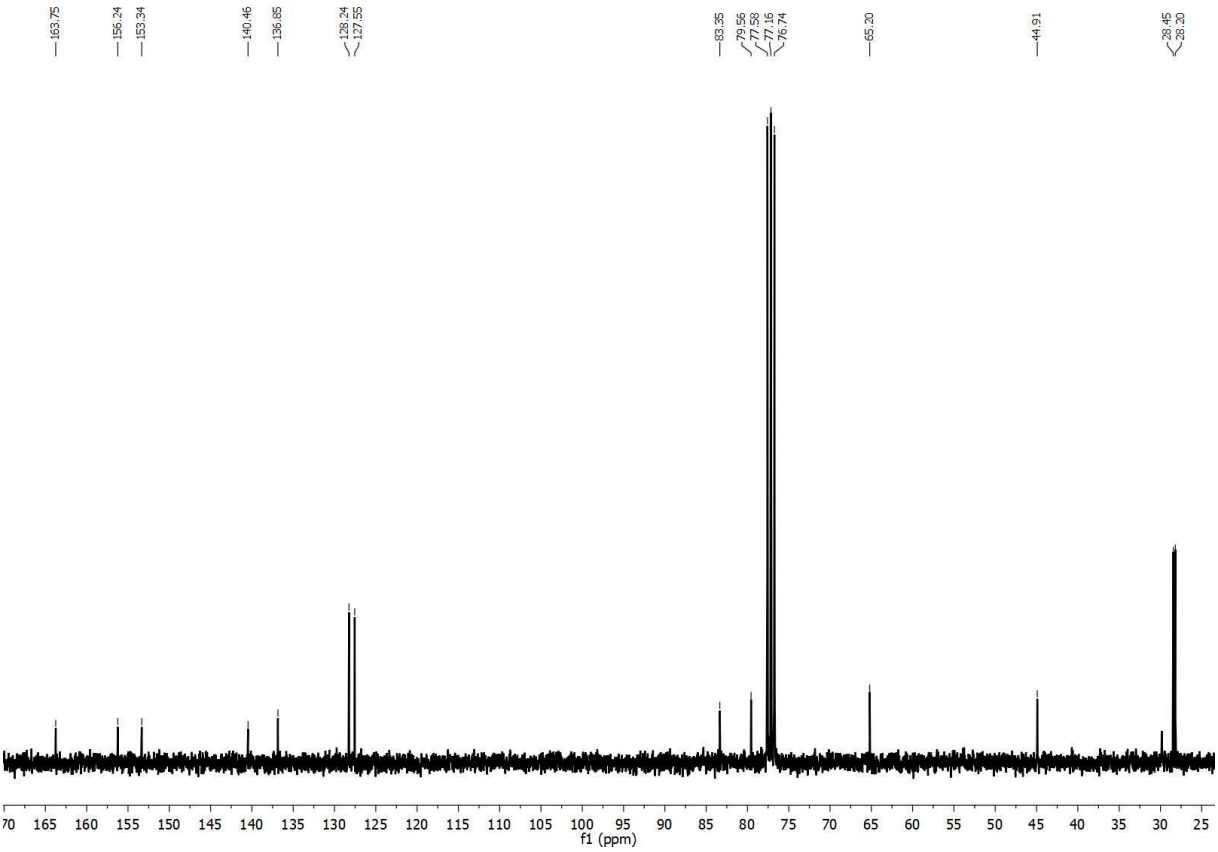


Figure 4^{13}C NMR of compound 4.

N,N'-diBoc-N''-(4-bromomethyl)-benzylguanidine (5)

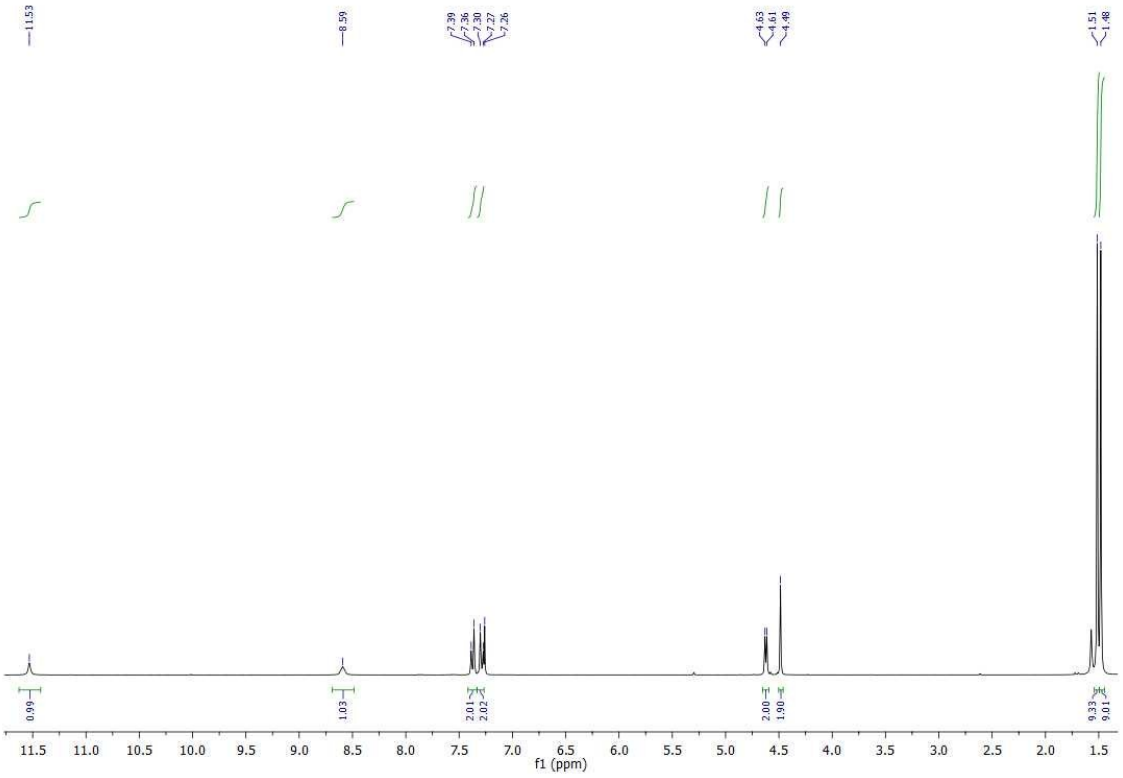


Figure 5. ^{1}H NMR of compound 5.

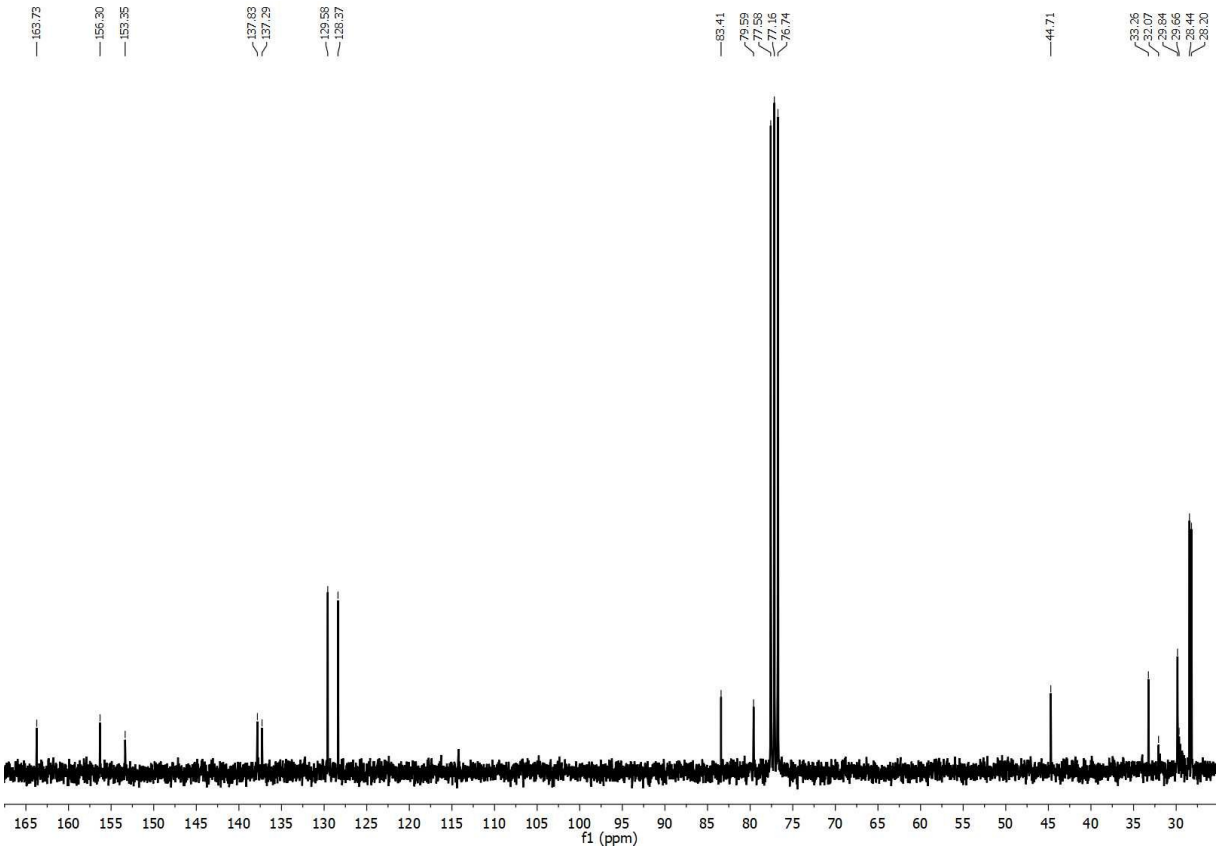


Figure 6. ^{13}C NMR of compound 5.

Tri-[(4-((N,N'-diBoc-guanidine)methy)-benzyl]-triaza-18-crown-6 (6)

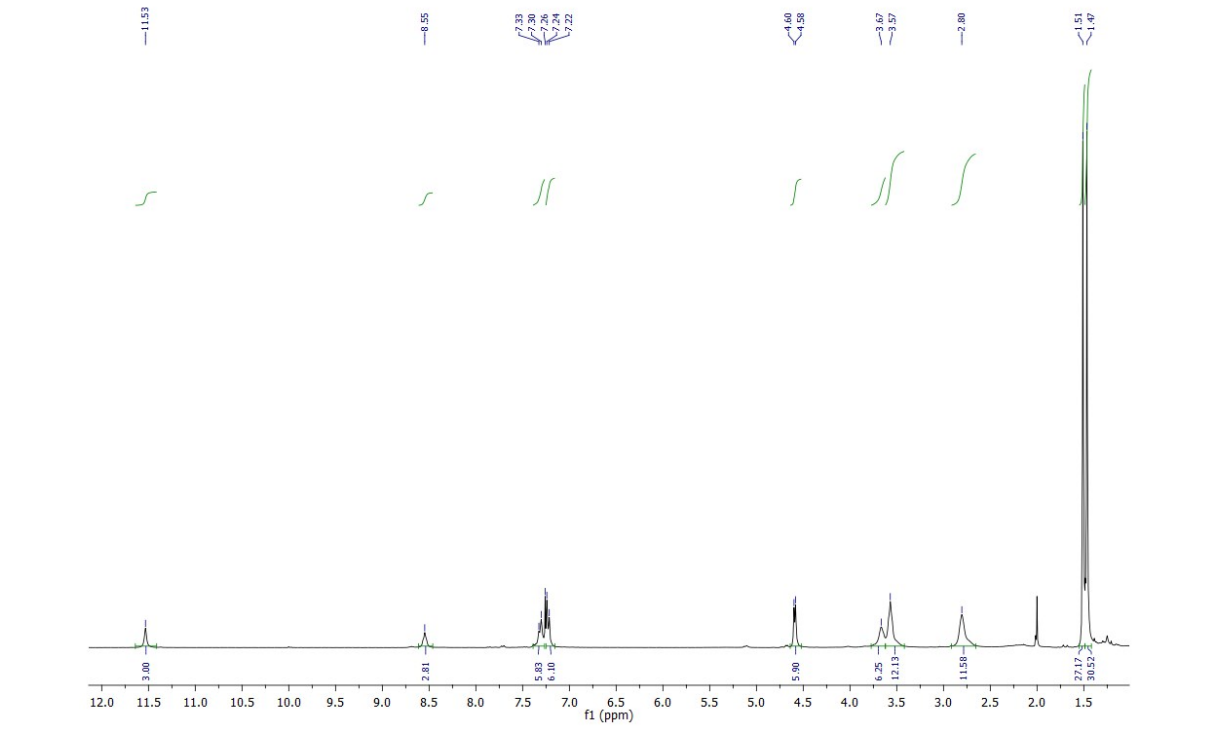


Figure 7. ^{1}H NMR of compound 6.

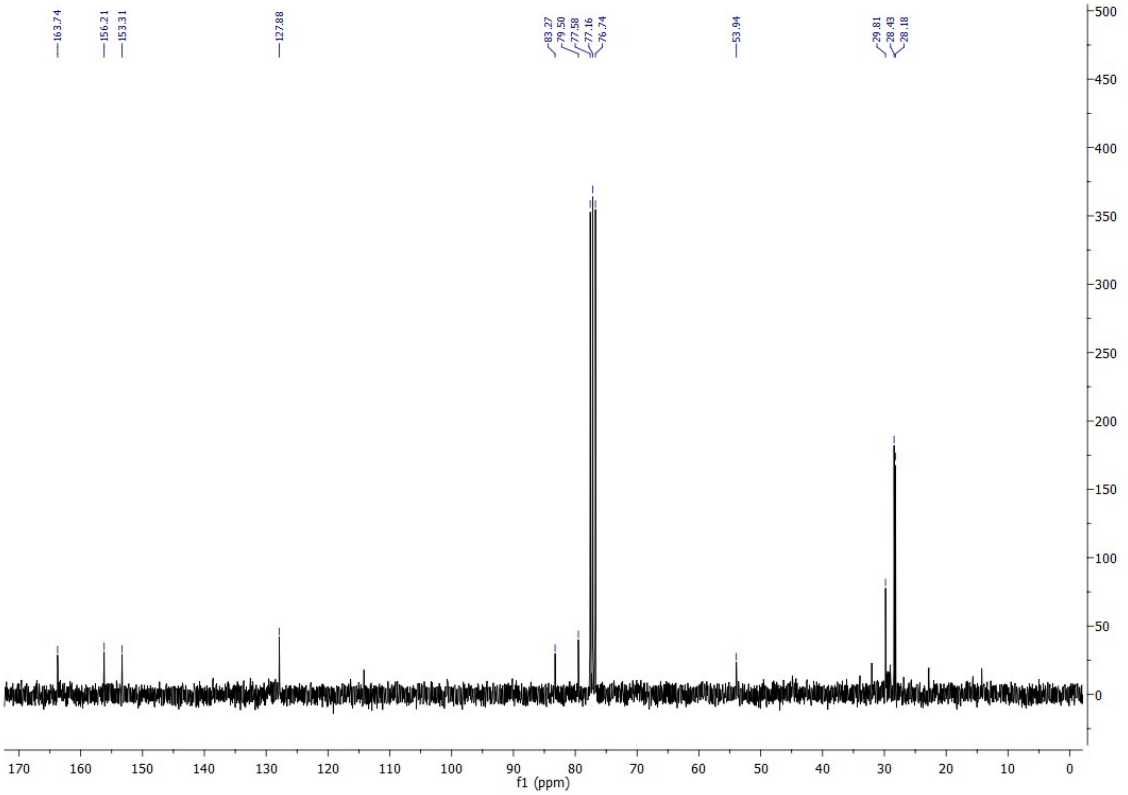


Figure 8. ^{13}C NMR of compound 6.

Trifluoroacetic salt of Tri-[(4-guanidinemethyl)-benzyl]-triaza-18-crown-6 (1)

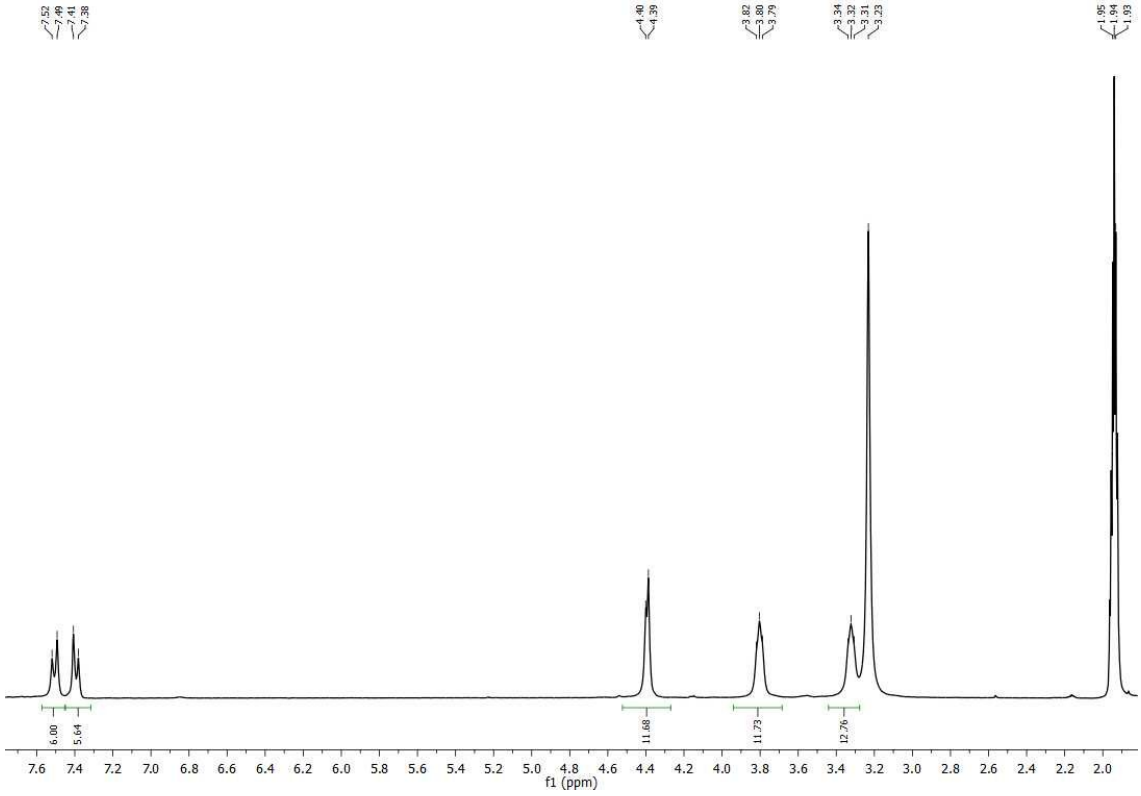


Figure 9. ^{1}H NMR of compound 1.

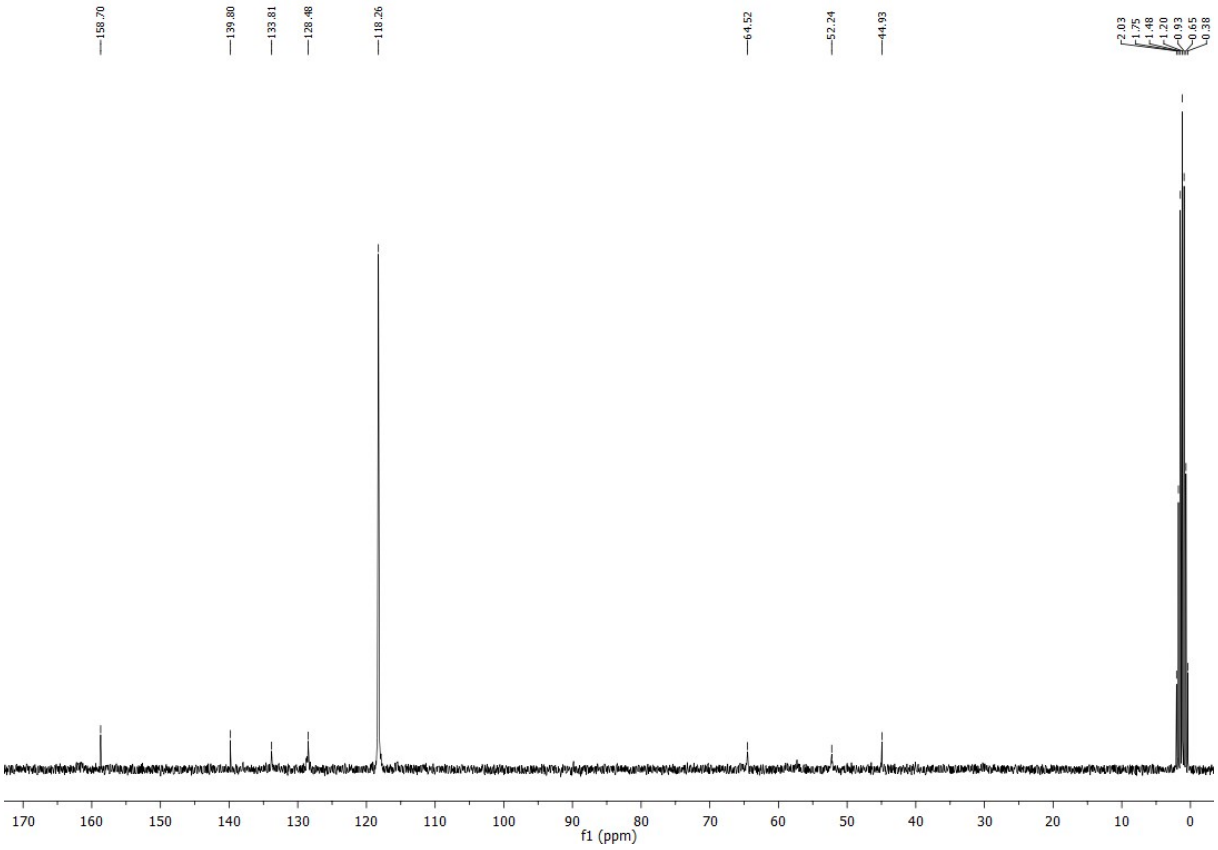


Figure 10. ^{13}C NMR of compound 1.

2. The calculated structure of 1•5-AVA complex

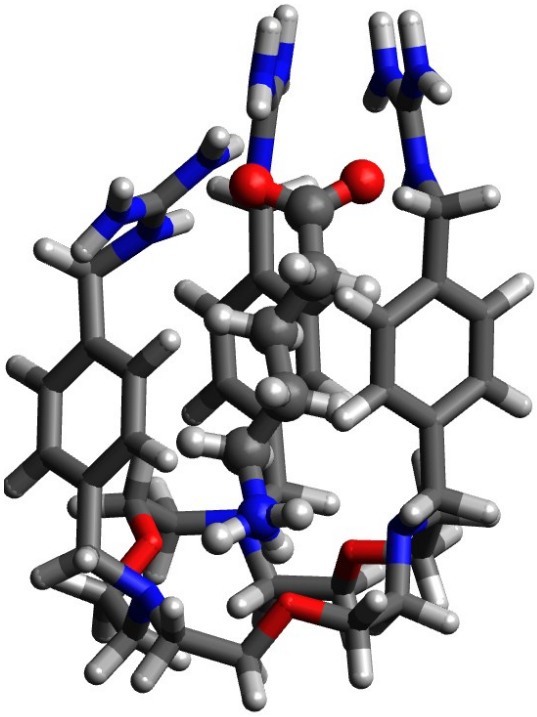
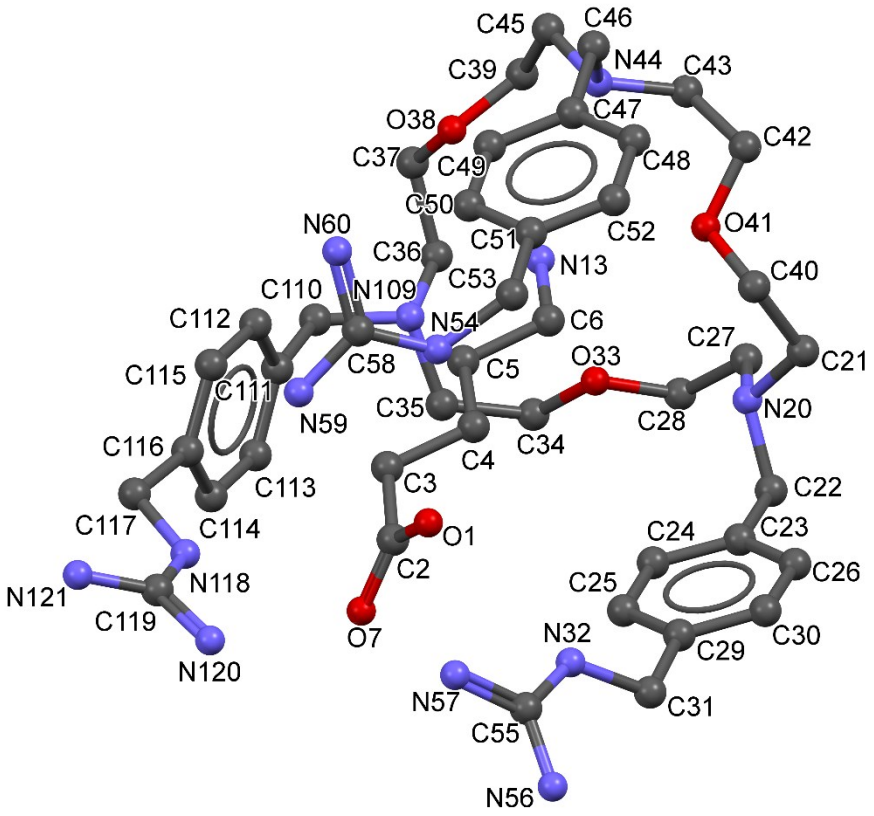


Figure 11. Molecular modelling structure of 1•5-AVA

Atom	Distance [Å]
O(crown)···N(5-AVA)	2.78
O(crown)···N(5-AVA)	2.91
O(crown)···N(5-AVA)	2.79
N(gua)···O(5-AVA)	3.09
N(gua)···O(5-AVA)	3.63
N(gua)···O(5-AVA)	3.33
N(gua)···O(5-AVA)	2.78
N(gua)···O(5-AVA)	2.80
N(gua)···O(5-AVA)	3.07
N(gua)···O(5-AVA)	2.77
N(gua)···O(5-AVA)	3.85
N(gua)···O(5-AVA)	2.91

Table 1. Distances between atoms.

Theoretical calculations for **1**●5-AVA were performed in Gaussian 09 (Revision D.01) [1]. The initial (starting point) geometry was obtained by preoptimization of **1**●5-AVA with universal force field and steepest descent algorithm implemented in Avogadro 1.2 [2]. Geometry optimization and frequency calculation were done with aim of the ωB97xD hybrid functional and 6-311G(2df,2p) basis set [3]. Both calculations were conducted in simulated methanol environment using Polarizable Continuum Model [4]. Ultrafine integration grid method was also applied. Stationary point was found with positive values of all calculated frequencies. Evaluated energy for obtained final geometry is -7374755.61435 kJ/mol and atomic coordinates are provided below:



Atomic coordinates for **1**●5-AVA

Atom	X	Y	Z
O1	3.768185	-0.681034	-0.881192
C2	3.496612	-0.001569	0.158675
C3	2.177927	0.737815	0.180159
C4	0.968154	-0.148300	-0.059908
C5	-0.311462	0.642380	0.114080
C6	-1.515436	-0.228761	-0.124237
O7	4.261445	0.111763	1.136083
H8	2.070968	1.239201	1.140789
H9	2.220030	1.517315	-0.586632
H10	0.998266	-0.588472	-1.056563
H11	0.974160	-0.980995	0.643385
H12	-0.343552	1.058133	1.121328
N13	-2.777306	0.517012	0.112523
H14	-1.531717	-0.590040	-1.147169
H15	-1.520490	-1.091534	0.539594
H16	-0.323193	1.493148	-0.571124
H17	-2.608145	1.450782	0.509874
H18	-3.378019	0.590350	-0.733981
H19	-3.298919	0.036385	0.845873
N20	-2.787935	-3.149196	1.403024
C21	-3.103592	-3.878331	0.172674
C22	-2.043911	-3.982765	2.344001
C23	-0.588559	-4.121903	1.968111
C24	0.296670	-3.072519	2.205797
C25	1.644623	-3.197429	1.917655
C26	-0.089793	-5.288887	1.407314
C27	-3.998051	-2.575544	1.982437
C28	-3.788642	-1.600764	3.120695
C29	2.139394	-4.372058	1.355540
C30	1.256755	-5.409116	1.092122
C31	3.614134	-4.570906	1.118720
N32	4.226735	-3.449089	0.423962
O33	-3.052361	-0.467903	2.701506
C34	-2.836031	0.433001	3.767604
C35	-2.006168	1.635200	3.367600
C36	-3.963441	2.743149	2.438073
C37	-4.588806	3.531562	1.299277
O38	-4.476226	2.875303	0.050394
C39	-5.677020	2.274142	-0.393682
C40	-2.903923	-3.054617	-1.085630
O41	-3.647097	-1.853575	-1.081414
C42	-4.862234	-1.895020	-1.782181
C43	-5.553213	-0.553522	-1.665579
N44	-4.742041	0.610682	-2.045208
C45	-5.522816	1.838670	-1.836793
C46	-4.236348	0.523128	-3.430613
C47	-2.767674	0.177499	-3.534651
C48	-2.343552	-1.065211	-3.989396
C49	-1.798458	1.116823	-3.201142
C50	-0.445855	0.824226	-3.314626
C51	-0.029526	-0.421060	-3.765686
C52	-0.997134	-1.364130	-4.095515
C53	1.425981	-0.792621	-3.974138
N54	2.397175	-0.019518	-3.229180
C55	5.541858	-3.308622	0.269661
N56	6.376967	-4.209529	0.781851
N57	6.005922	-2.266581	-0.404104
C58	2.983525	1.090763	-3.652742
N59	4.012339	1.570914	-2.955195
N60	2.563862	1.725760	-4.742703
H61	-4.125648	-4.278934	0.212165
H62	-2.442039	-4.740431	0.081804
H63	-2.089236	-3.521446	3.328321
H64	-2.499206	-4.978922	2.444744
H65	-0.077594	-2.152844	2.640074
H66	-4.674388	-3.360578	2.360086
H67	-4.530371	-2.063224	1.181419
H68	-3.277507	-2.067595	3.968468
H69	-4.776149	-1.287488	3.478416
H70	1.622963	-6.328524	0.653018
H71	3.767764	-5.493087	0.554593
H72	4.105689	-4.688111	2.088574
H73	3.642830	-2.773622	-0.048718
H74	-2.283787	-0.075511	4.565522
H75	-3.797010	0.740113	4.193355
H76	-1.882581	2.209087	4.299302
H77	-4.199882	3.261093	3.380682
H78	-4.138956	4.517524	1.189538
H79	-5.639544	3.695225	1.551922
H80	-6.489963	3.006249	-0.353648
H81	-5.957727	1.443420	0.262822
H82	-3.155797	-3.656460	-1.964794
H83	-1.852398	-2.776705	-1.165388
H84	-5.535955	-2.655346	-1.370160
H85	-4.683349	-2.159551	-2.829342
H86	-5.869321	-0.422234	-0.630880
H87	-6.467669	-0.599328	-2.274522
H88	-6.525360	1.735219	-2.274224
H89	-5.024130	2.655161	-2.356619
H90	-4.387593	1.489668	-3.909639
H91	-2.100451	2.093860	-2.842446
H92	0.279995	1.577576	-3.036694
H93	-0.694229	-2.343706	-4.444946
H94	1.569855	-1.832771	-3.687792
H95	1.667297	-0.743700	-5.039004
H96	2.778413	-0.409201	-2.359695
H97	6.047572	-5.056201	1.204997
H98	7.369021	-4.098316	0.683045
H99	6.993825	-2.121203	-0.498834
H100	4.446115	2.444097	-3.189686
H101	4.403340	1.011524	-2.214119

H102	3.052907	2.528340	-5.094398
H103	2.320728	-2.380613	2.142095
H104	-3.073076	-1.814882	-4.267246
H105	1.716791	1.458893	-5.209816
H106	5.344171	-1.530261	-0.665243
H107	-4.460789	1.774048	2.470336
H108	-1.010765	1.296818	3.078094
N109	-2.521748	2.510396	2.305601
C110	-1.794644	3.787374	2.345493
C111	-0.323295	3.748947	1.998668
C112	0.089169	4.007888	0.695857
C113	0.653158	3.590293	2.977748
C114	1.998731	3.718566	2.670521
C115	1.432924	4.138747	0.385248
C116	2.401770	4.012761	1.372894
C117	3.856841	4.240895	1.053490
N118	4.612538	2.993830	1.024893
C119	5.859870	2.896231	0.577618
N120	6.442936	1.703992	0.525993
N121	6.514957	3.980216	0.166466
H122	4.196276	2.135595	1.369281
H123	7.396990	1.612834	0.230764
H124	5.891106	0.877115	0.746729
H125	7.459775	3.908602	-0.163557
H126	6.153293	4.901863	0.324532
H127	2.738627	3.622366	3.455603
H128	0.359097	3.405343	4.003206
H129	-0.653523	4.138068	-0.081791
H130	1.728650	4.362933	-0.632112
H131	3.934407	4.725012	0.078778
H132	4.288486	4.913642	1.799164
H133	-1.906901	4.239718	3.342675
H134	-2.264765	4.464737	1.639377
H135	-4.825084	-0.194335	-4.009170
H136	-0.756104	-6.121959	1.219863

3. ^{1}H NMR titrations experiments

^{1}H NMR titration experiments were performed on a 300 MHz BrukerAvance spectrometer, at 298K. All solutions were buffered at pH 8.0 with TRIS (10 mM) in 30% water in methanol (v/v). In each case 0.5 mL of 3.1x10^{-3} M solution of receptor **1** was added to 5 mm NMR tube. Then to the receptor solution titrant solution of zwitterion in receptor solution (from 5.9 x10^{-2} to 6.3x10^{-2} M) was added. After each addition of titrant, a spectrum was registered. Titration isotherms for Bg, Ar and in some cases -CH_2- crown ethers protons were fitted to a 1:1 binding model. All measurements were carried out in at least duplicate using independent samples. The resulting titration data were analyzed using BindFit (v0.5) package, available online at http://supramolecular.org.

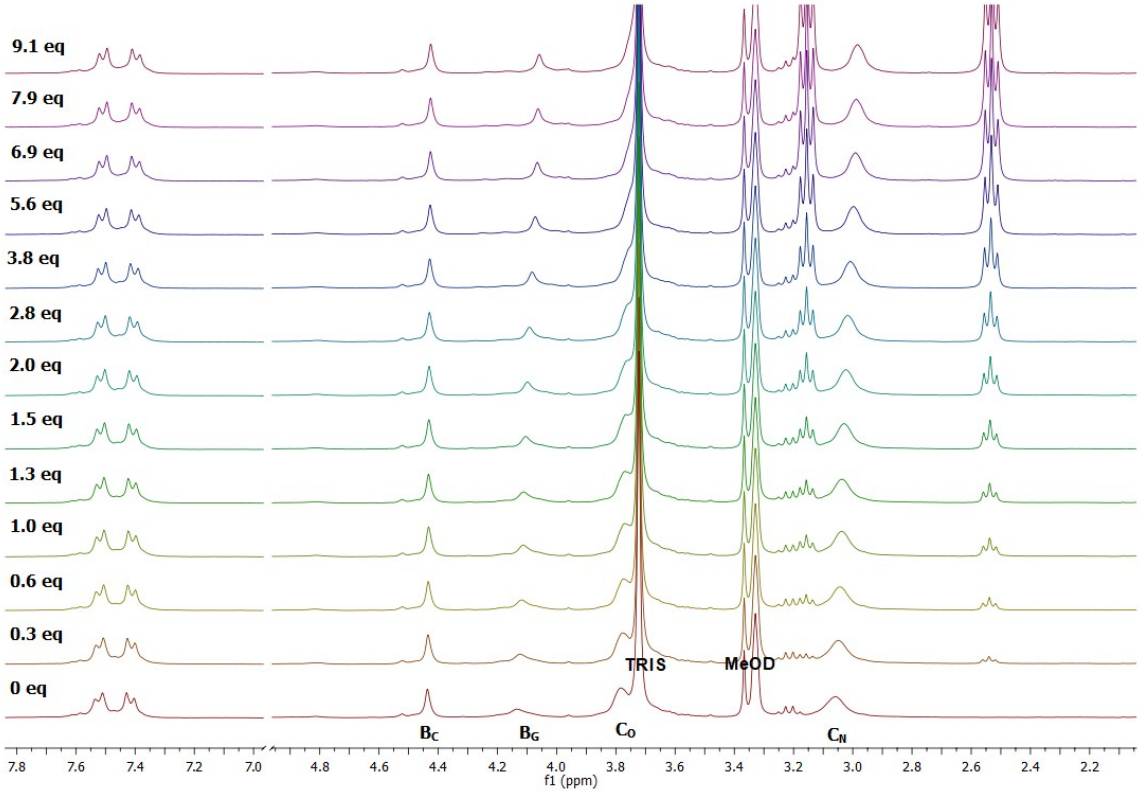


*Figure 12. Partial ^{1}H NMR titration experiment of **1** upon titrant (β-Alanine) addition ($C_{titrant}$ = 6.2 x10^{-2} M, $C_{receptor}$ = 3.1 x10^{-3} M).*

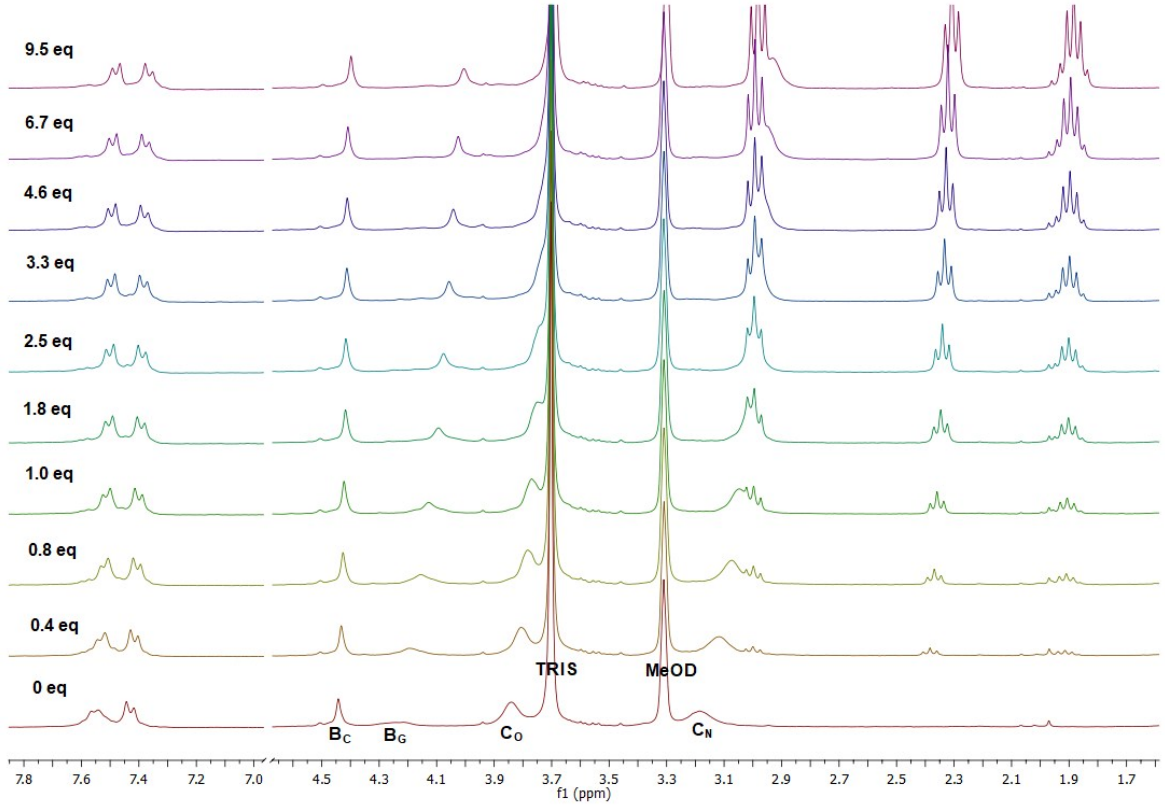


*Figure 13. Partial ^1H NMR titration experiment of **1** upon titrant (GABA) addition ($C_{titrant}$ = 6.3 x10^{-2} M, $C_{receptor}$ = 3.1 x10^{-3} M).*

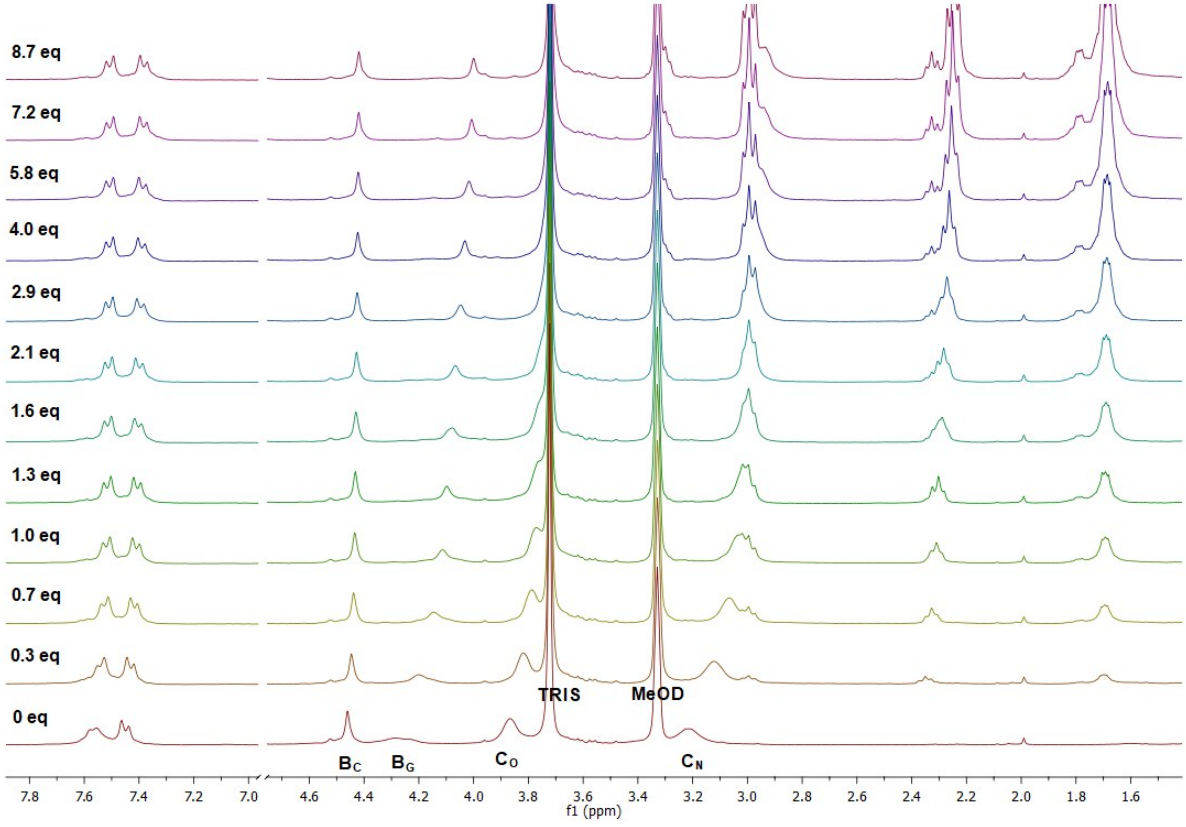


*Figure 14. Partial ^1H NMR titration experiment of **1** upon titrant (5-AVA) addition ($C_{titrant}$ = 5.9 x10^{-2} M, $C_{receptor}$ = 3.1 x10^{-3} M).*

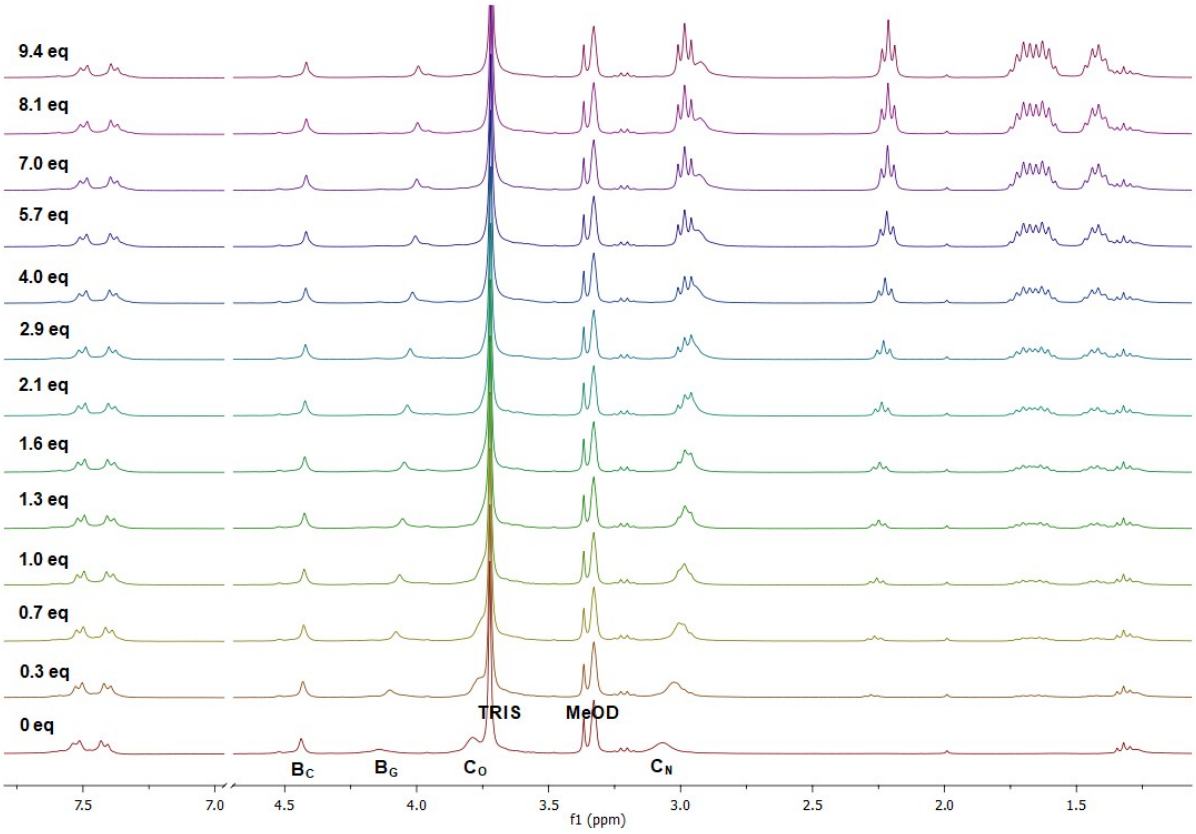


*Figure 15. Partial ^{1}H NMR titration experiment of **1** upon titrant (6-ACA) addition ($C_{titrant}$ = 6.3 x10^{-2} M, $C_{receptor}$ = 3.1 x10^{-3} M).*

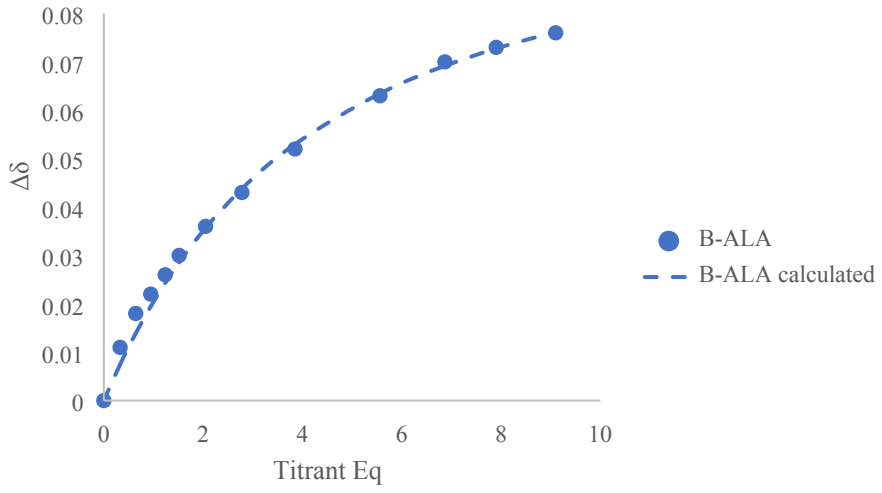


*Figure 16. ^{1}H NMR titration binding isotherm of **1** with β-Alanine.*

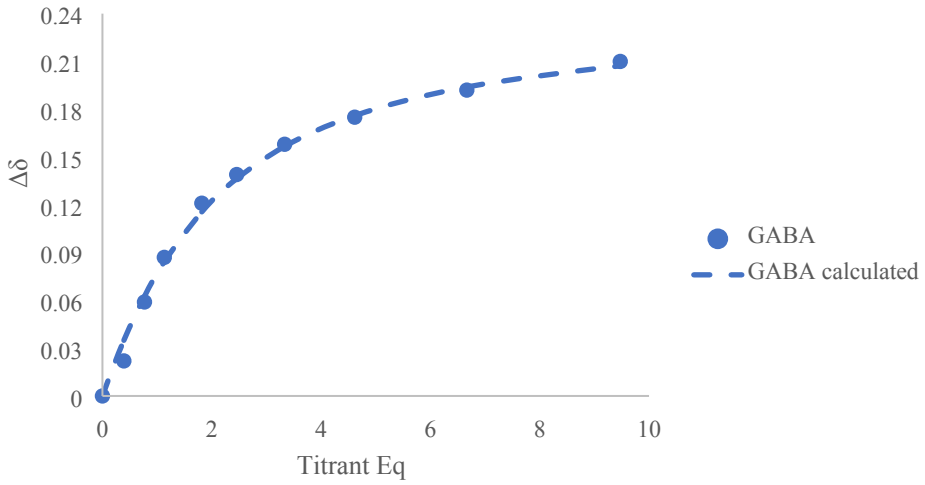


*Figure 17. ^{1}H NMR titration binding isotherm of **1** with GABA.*

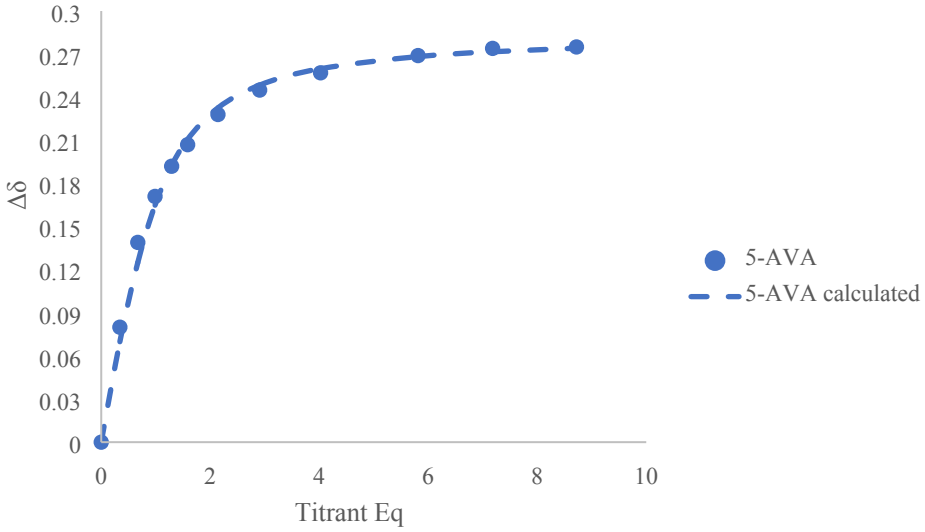


*Figure 18. ^{1}H NMR titration binding isotherm of **1** with 5-AVA.*

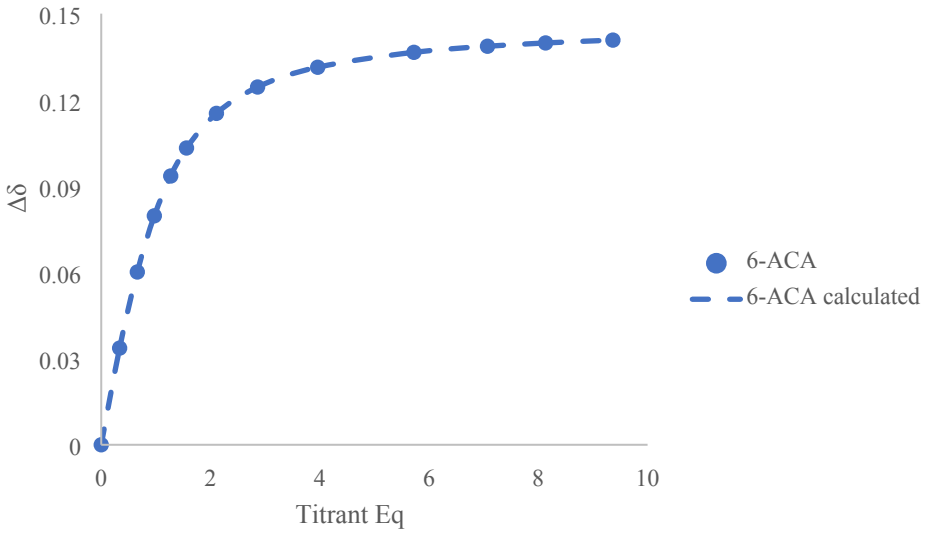


*Figure 19. ^{1}H NMR titration binding isotherm of **1** with 6-ACA.*

Amino Acid	K_a [M^{-1}]
β-Alanine	70
4-Aminobutyric acid	210
5-Aminovaleric acid	1 090
6-Aminocaproic acid	550

*Table 2. ^{1}H NMR spectroscopy K_a values for interactions of **1** with various amino acids (298 K, TRIS buffer pH=8.0, 30% H_2O/MeOH), errors of <10%.*

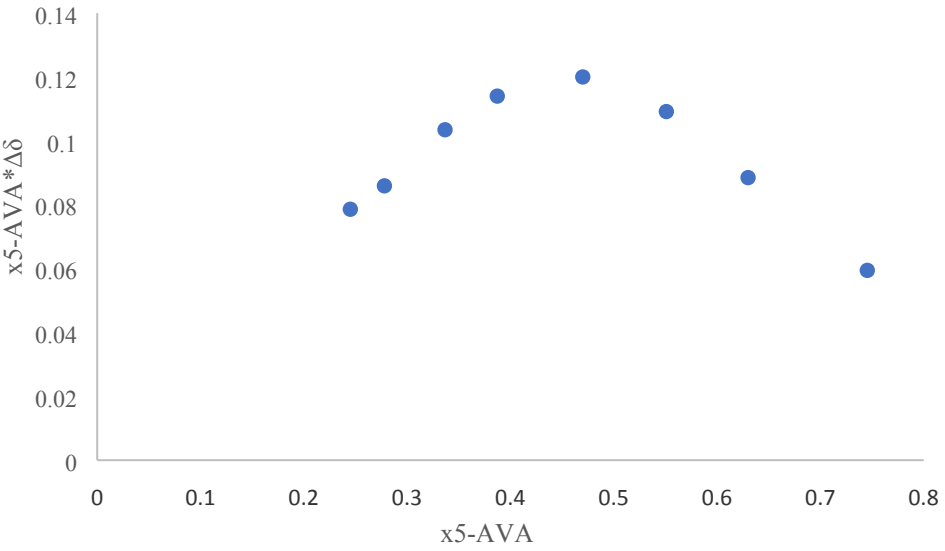


*Figure 20. Job plot for determination of **1**/5-AVA ratio.*

4. UV-Vis titration experiments

UV-Vis Titrations of Indicator and Receptor.

The UV-Vis titration was performed using Thermo Spectronic Unicam UV500 Spectrophotometer at 298K. All solutions were buffered at pH 8.0 with TRIS (10 mM) in 30% water in methanol (v/v). A solution of fluorescein ($3.8x10^{-5}$ M) was prepared in the cuvette, and into this was titrated a stock solution of **1** ($1.9x10^{-3}$ M) and fluorescein, thereby keeping the host concentration constant. All measurements were carried out in at least duplicate using independent samples. The resulting titration data were analyzed using HypSpec package.

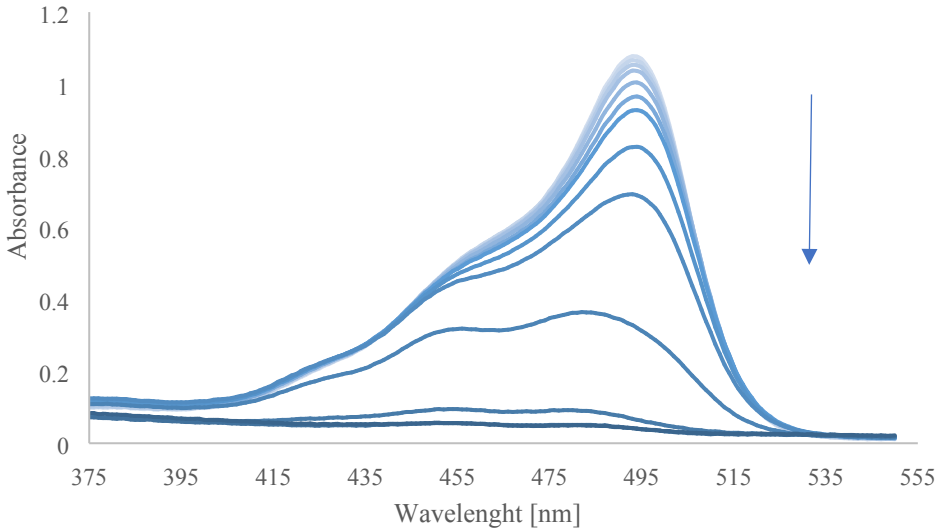


*Figure 21. UV-Vis fluorescein spectrum changes upon titrant (**1**) addition ($C_{receptor}$=1.9 x10^{-3} M, $C_{fluorescein}$=3.8 x10^{-5} M).*

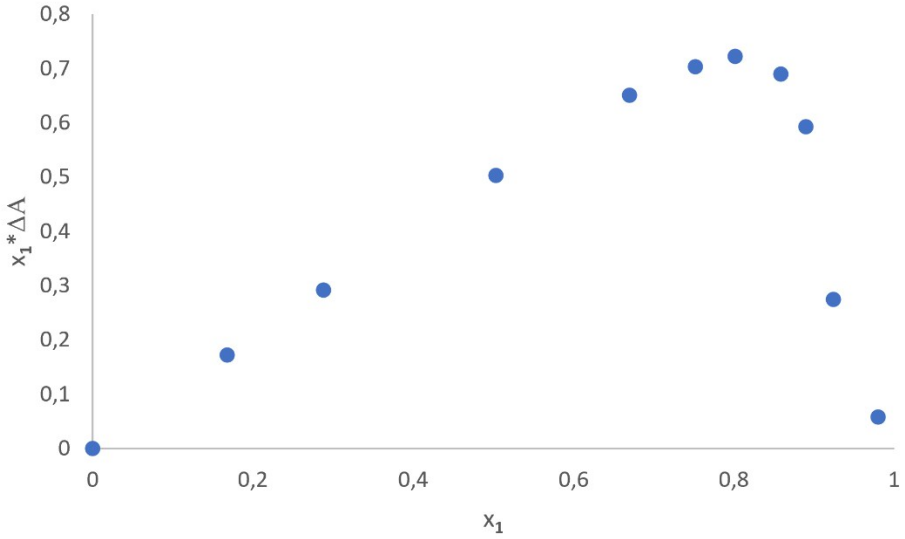


*Figure 22. Job plot for determination of fluorescein/**1** ratio.*

UV-Vis Titration of Receptor/Indicator Ensemble and Guest.

The UV-Vis titration was performed using Thermo Spectronic Unicam UV500 Spectrophotometer at 298K. All solutions were buffered at pH 8.0 with TRIS buffer (10 mM)

in 30% water in methanol (v/v). A solution of fluorescein ($3.8x10^{-5}$ M) and receptor **1** ($3.8x10^{-4}$ M) was prepared in the cuvette, and into this was titrated a stock solution of indicator, host, and guest, keeping the indicator and host concentrations constant. The data were taken at the appropriate wavelength to determine the association constants. The guests concentration in the stock solution was from $6.2x10^{-3}$ to $6.8x10^{-3}$ M. All measurements were carried out in at least duplicate using independent samples. The resulting titration data were analyzed using HypSpec package.

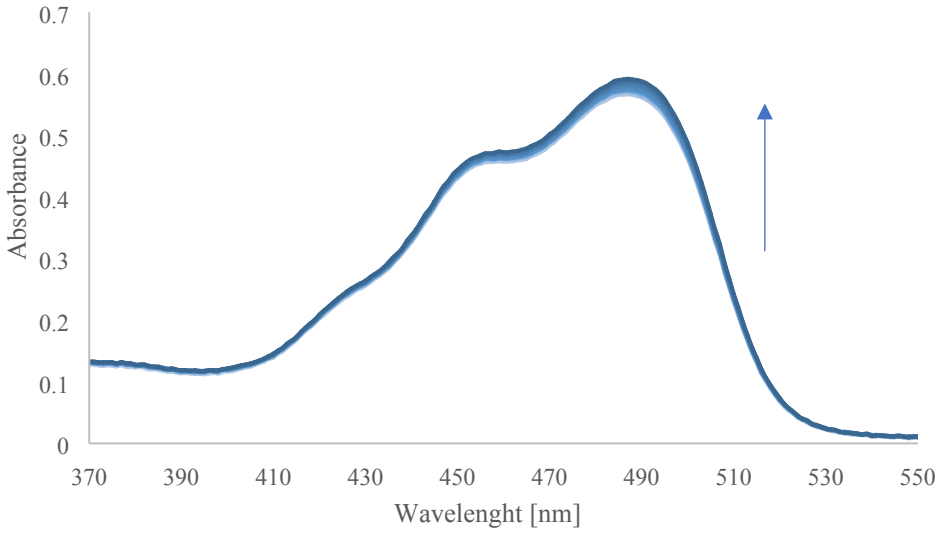


*Figure 23. UV-Vis fluorescein-**1** complex spectrum changes upon titrant (β-Alanine) addition ($C_{titrant}$=6.4 x10^{-3} M, $C_{receptor}$=3.8 x10^{-4} M, $C_{fluorescein}$=3.8 x10^{-5} M).*

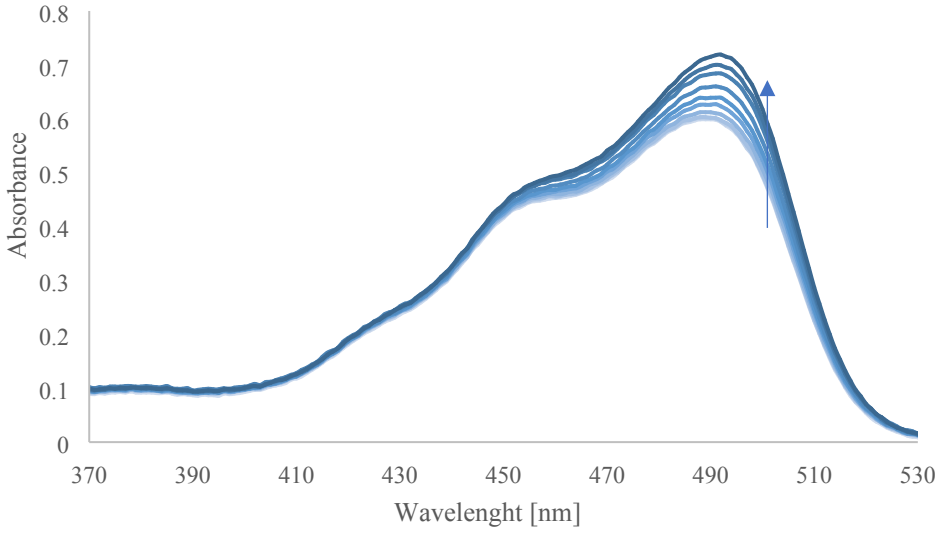


*Figure 24. UV-Vis fluorescein-**1** complex spectrum changes upon titrant (GABA) addition ($C_{titrant}$=6.2 x10^{-3} M, $C_{receptor}$=3.8 x10^{-4} M, $C_{fluorescein}$=3.8 x10^{-5} M).*

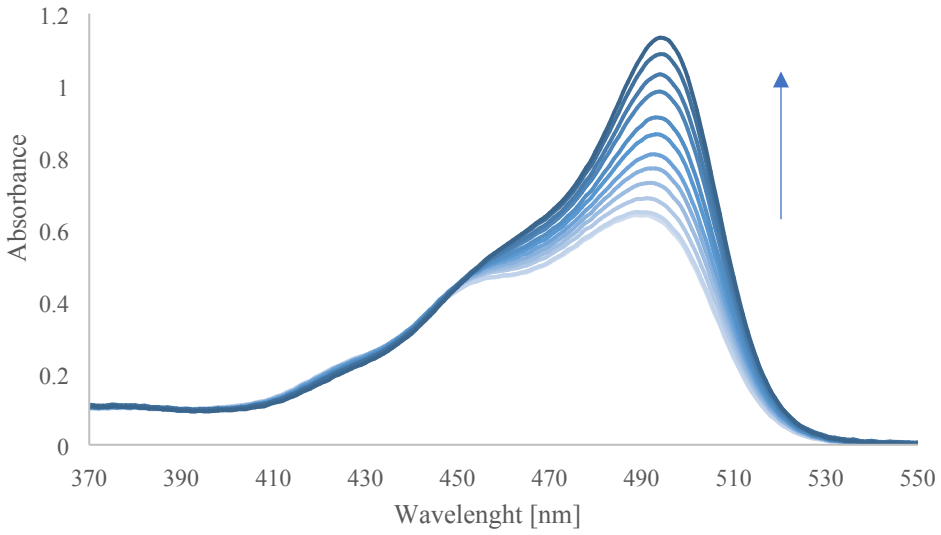


*Figure 25. UV-Vis fluorescein-**1** complex spectrum changes upon titrant (5-AVA) addition ($C_{titrant}$=6.4 x10^{-3} M, $C_{receptor}$=3.8 x10^{-4} M, $C_{fluorescein}$=3.8 x10^{-5} M).*

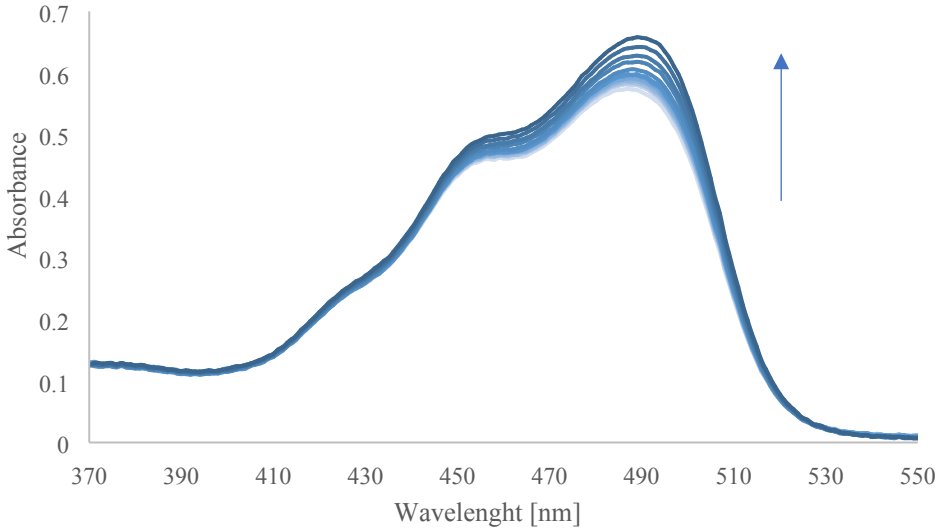


*Figure 26. UV-Vis fluorescein-**1** complex spectrum changes upon titrant (6-ACA) addition ($C_{titrant}$=6.4 x10^{-3} M, $C_{receptor}$=3.8 x10^{-4} M, $C_{fluorescein}$=3.8 x10^{-5} M).*

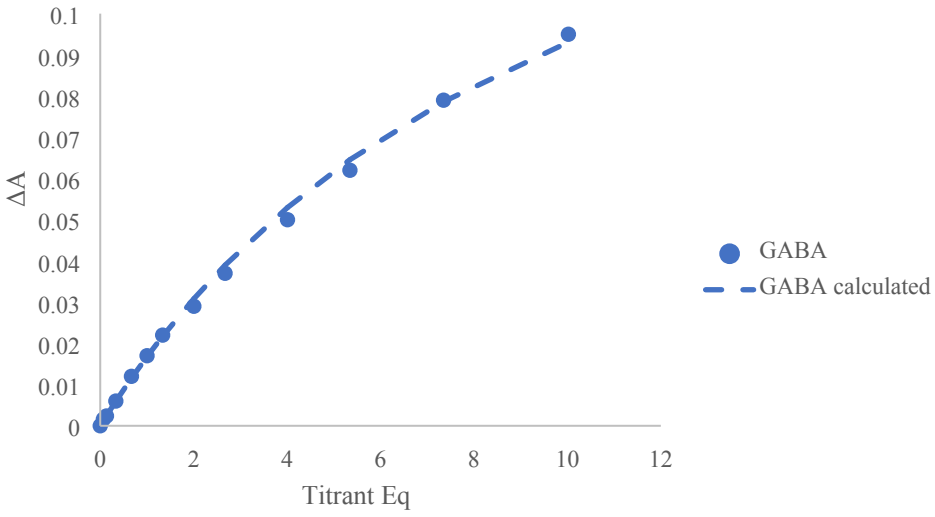


*Figure 27. UV-Vis titration binding isotherm of fluorescein-**1** complex with GABA (λ=493 nm).*

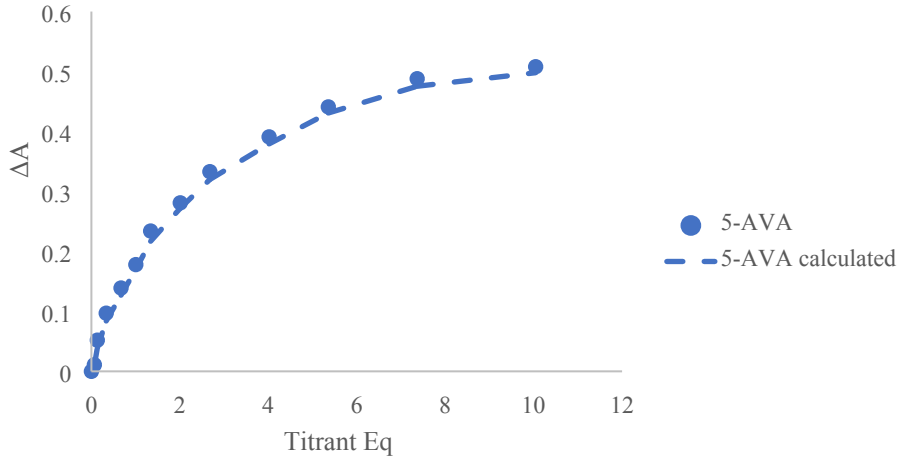


*Figure 28. UV-Vis titration binding isotherm of fluorescein-**1** complex with 5-AVA (λ=493 nm).*

Amino Acid	**K_a [M^{-1}]**
β-Alanine	-[a]
4-Aminobutyric acid	114
5-Aminovaleric acid	690
6-Aminocaproic acid	-[a]

*Table 3. UV-Vis spectroscopy K_a values for interactions of **1** with various amino acids (298 K, TRIS buffer pH=8.0, 30% H_2O/MeOH), errors of <10%. [a]The Absorbance changes were too small to be calculated Ka.*

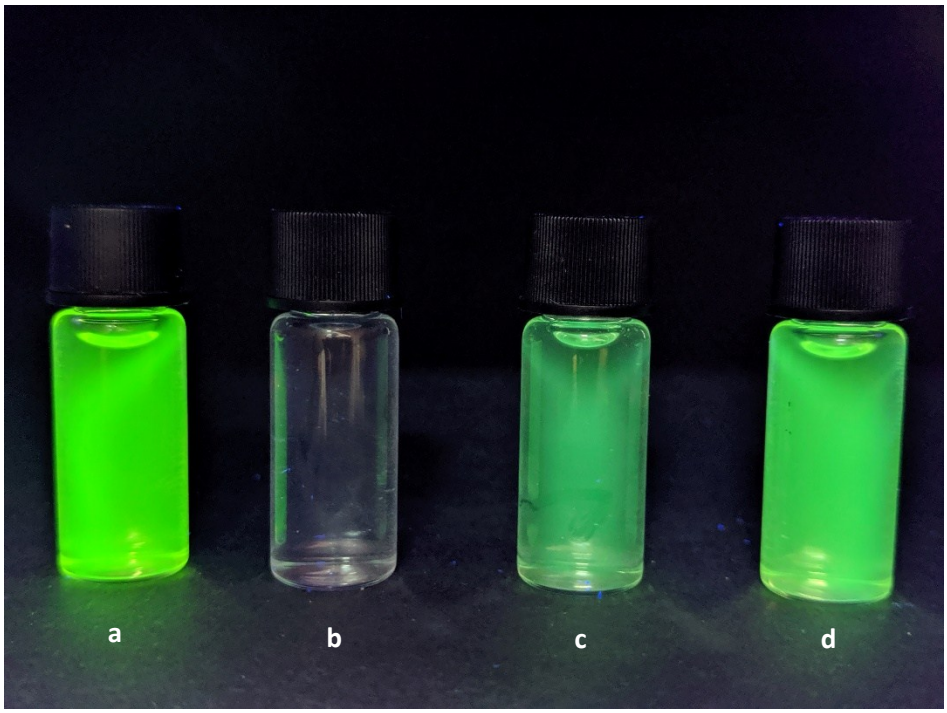


*Figure 29. a) Solution of fluorescein; b) Solution of fluorescein after addition 20 eq of **1**; c) Displacement of fluorescein after addition 20 eq of GABA; d) Displacement of fluorescein after addition 20 eq of 5-AVA. All solutions were prepared in 30% water/ methanol (v/v), 10 mM TRIS, pH=8.0. Radiating on 366 nm wavelength.*

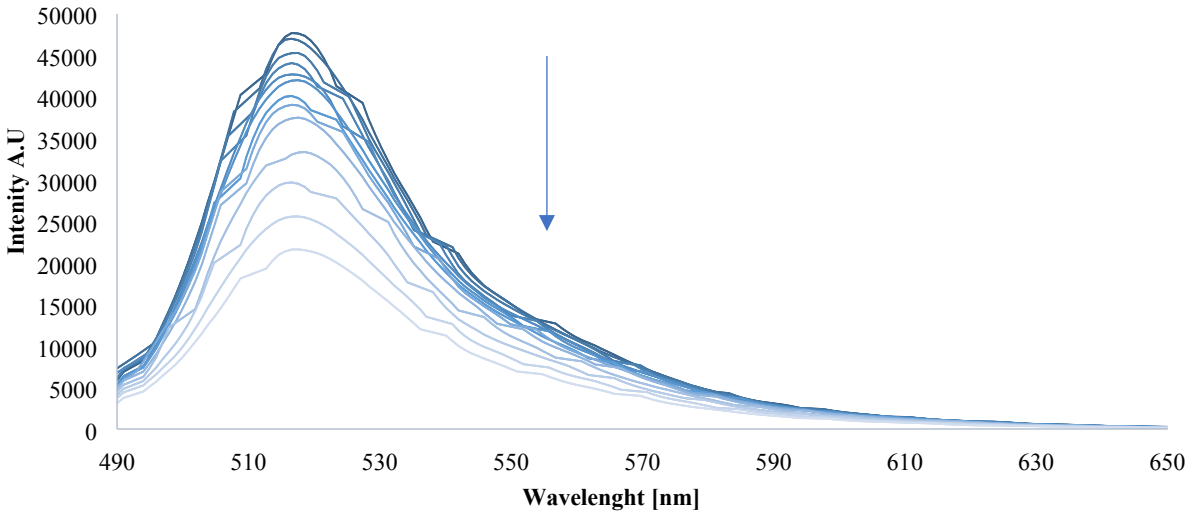


*Figure 30. Emission titration of fluorescein with receptor **1** ($C_{Fluorescin}$=7.7 x10^{-6} M, $C_{receptor}$=4.5 x10^{-4} M), excitation wavelength 494,8 nm, solvent 30% H_2O/MeOH, 10 mM Tris Buffer.*

5. Fluorescein : receptor 1 complex stoichiometry studies

*Figure 31. Job plot for determination of fluorescein/**1** ratio. UV-Vis measurements $C_{receptor}$=1.9 x10^{-3} M, $C_{fluorescein}$=3.8 x10^{-5} M, solvent 30% H_2O/MeOH, 10 mM Tris Buffer*

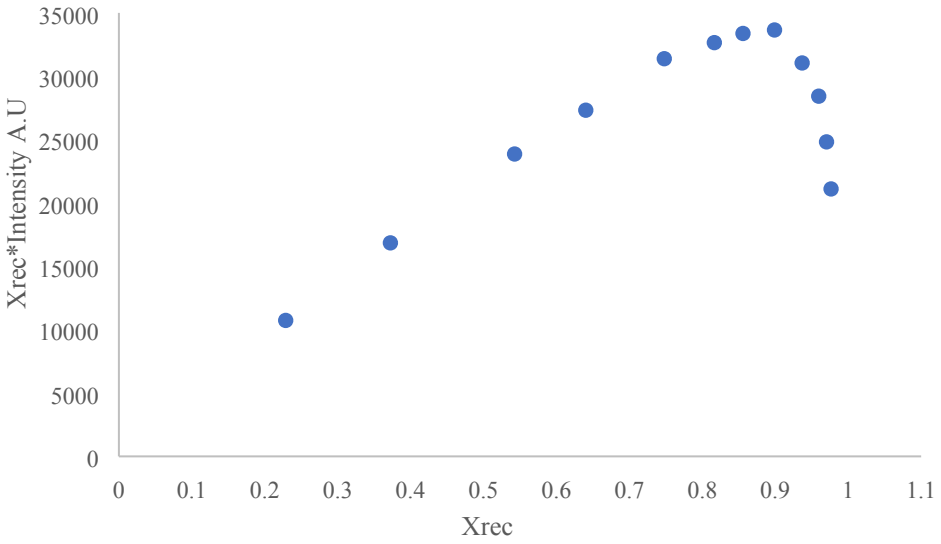


*Figure 32. Job plot for determination of fluorescein/**1** ratio. Fluorescent measurements $C_{Fluorescin}$=7.7 x10^{-6} M, $C_{receptor}$=4.5 x10^{-4} M), , solvent 30% H_2O/MeOH, 10 mM Tris Buffer*

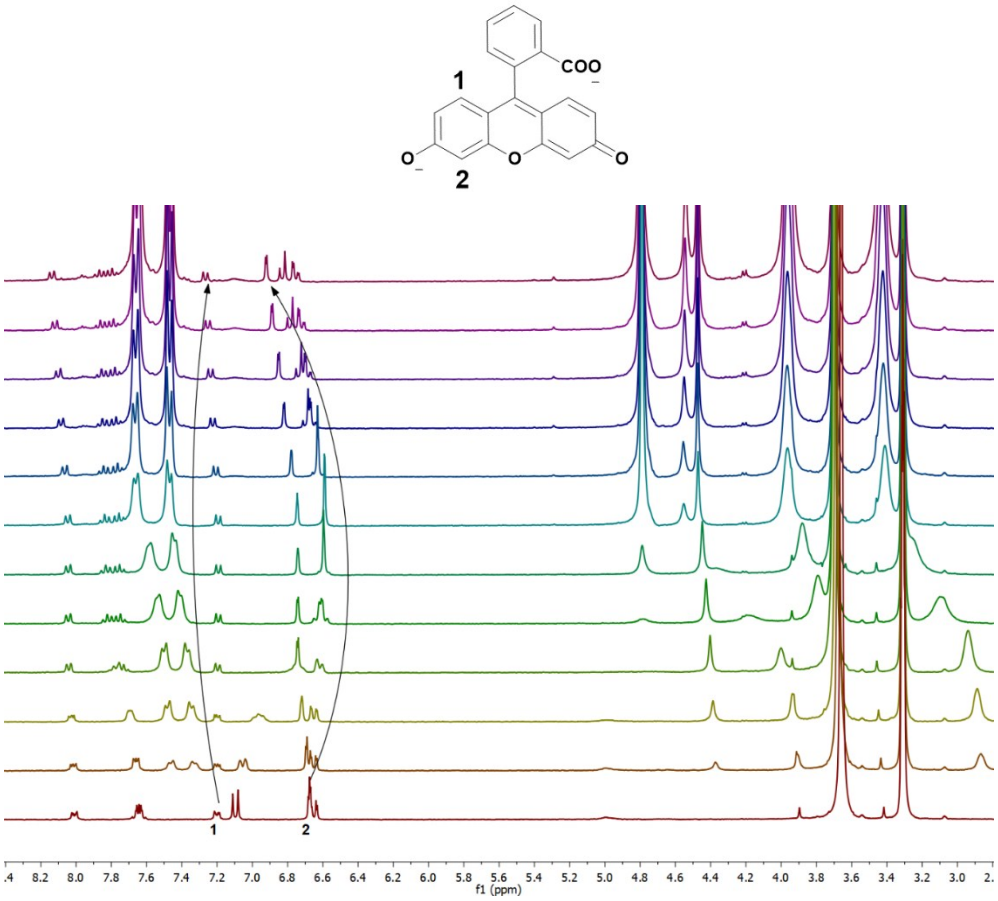


*Figure 33. Partial ^{1}H NMR titration experiment of Fluorescein upon titrant (Receptor **1**) addition ($C_{fluorescein}$ = 5.7x10^{-3} M, $C_{receptor}$ = 1.4 x10^{-2} M).*

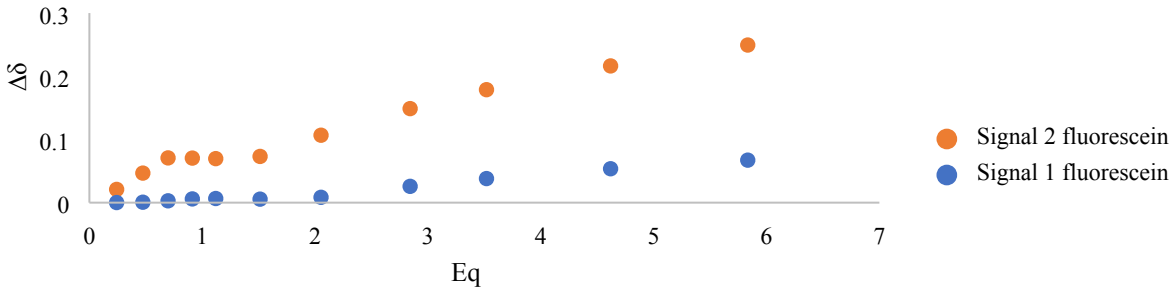


*Figure 34. ^{1}H NMR titration binding isotherm of fluorescein with receptor **1**.*

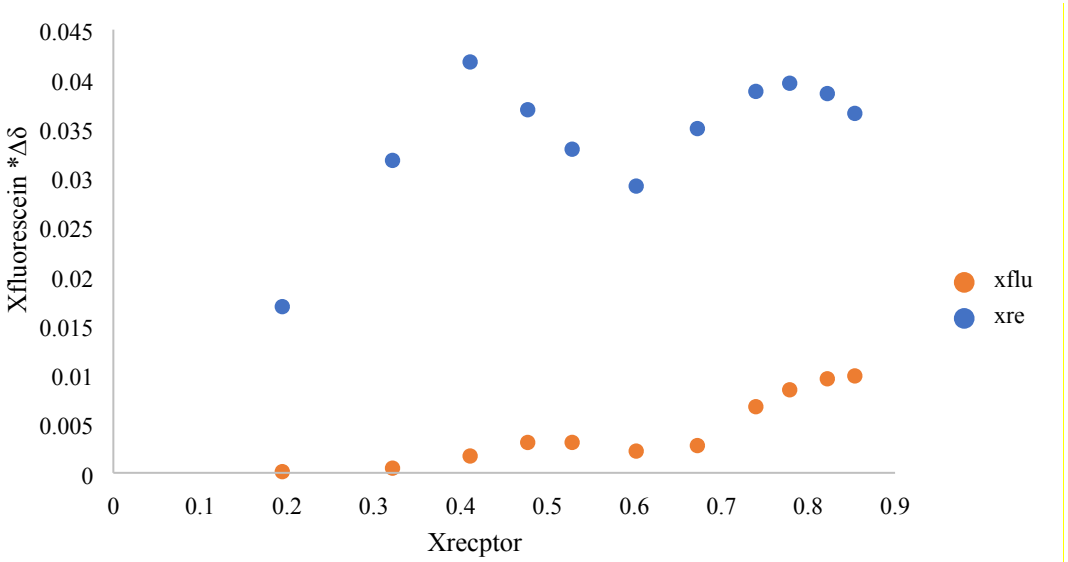


*Figure 35. Job plot fluorescein/receptor **1**. NMR measurements $C_{fluorescein}$ = 5.7x10^{-3} M, $C_{receptor}$ = 1.4 x10^{-2} M, solvent 30% D_2O/CD_3OD, 10 mM Tris Buffer*

Based on Uv/Vis ($C_{receptor}$=1.9 x10^{-3}M, $C_{fluorescein}$=3.8 x10^{-5}M) and Fluorescence ($C_{Fluorescin}$=7.7 x10^{-6} M, $C_{receptor}$=4.5 x10^{-4} M) titration isotherms and Job plots we can conclude that under these experiments conditions complexes of 3:1 Indicator: receptor **1** stoichiometry predominates. However, during NMR experiment performed in much higher concentration ($C_{fluorescein}$ = 5.7x10^{-3}M, $C_{receptor}$ = 1.4 x10^{-2}M, the complex stoichiometry is not well defined and probably the predominant form of the complex is changing during the titration. However, at pH 8 the dianion form of fluorescein is predominant, therefore the formation of the complexes with a different stoichiometry than 1:1 is feasible.

Nevertheless the stoichiometry of fluorescein-receptor **1** complex should not affect the stoichiometry of receptor-5-AVA complex. This kind of multicomponent ensembles are known in the literature.[4] However during the titration fluorescein signal are observed as an average band corresponding to complexes between fluorescein/receptor of different stoichiometry. Based on the displacement isotherms and their very good fit to the 1:1 model, it can be assume that predominant form of complex receptor/zwitterion are 1:1.[5] In other words the response of the multicomponent ensemble to zwitterionic quest can be presented as typical 1:1 complex isotherm.

6. References

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