Supplementary Material (ESI) for Chemical Communications

Supplementary Information

A Fluorescent Molecular Ruler as a Selective Probe for ω-Aminoacids

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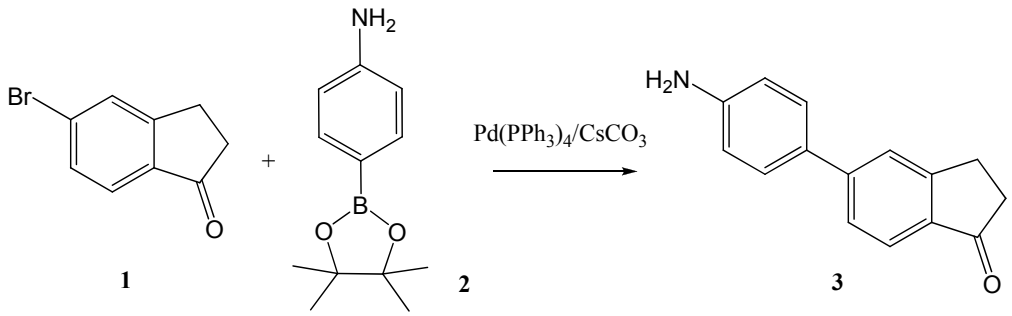
Experimental Part:

Materials and methods: The reactions performed with air sensitive reagents were conducted under dry nitrogen. The solvents were previously distilled under nitrogen over calcium hydride or sodium filaments. Melting points were determined in a Gallenkamp apparatus and are not corrected. Infrared spectra were registered in a Nicolet Impact 410 spectrometer in potassium bromide tablets. NMR spectra were recorded in Varian Mercury-300 and Varian Unity Inova-400 machines, in DMSO-d_6, $CDCl_3$, CD_3CN, CD_3OD. Chemical shifts are reported in ppm with respect to residual solvent protons, coupling constants ($J_{X\text{-}X'}$) are reported in Hz. Elemental analyses of C, H and N were taken in a Leco CHNS-932. Mass spectra were taken in a Micromass AutoSpec machine, by electronic impact at 70 eV. Quantitative UV-visible measures were performed with a Varian, Cary 300 Bio UV spectrophotometer, in 1 cm UV cells at 25ºC. Fluorescence spectra were recorded in a Varian Cary Eclipse spectrofluorometer, in 1 cm quarz cells at 25ºC.

Synthesis of 5-(4-aminophenyl)indan-1-one



$Pd(PPh_3)_4$ (54 mg, 0.047 mmol) was added under nitrogen to a solution of 5-bromo-1-indanone **1** (100 mg 0.470 mmol) in toluene/butanol 4:1 mixture (15 mL) and the mixture was stirred for 30 minutes at room temperature. Then solid 4-aminobenzeneboronic pinacol ester **2** (103 mg, 0.470 mmol) and cesium carbonate (463 mg, 1.42 mmol) dissolved in H_2O (2 mL) were added and the mixture was heated under reflux for 16 h. Then the solvent was evaporated under reduced pressure and the residue was added to water (20 mL) and extracted with CH_2Cl_2 (3 x 30 mL). The combined organic extracts were dried

(Na_2SO_4) and evaporated and the residue was purified by flash chromatography (silica, 3 x 30 cm), CH_2Cl_2, to get 5-(4-aminophenyl)indan-1-one **3** as a light yellow solid (95 mg, 91 %), mp 250-251 ºC.

IR (KBr, cm^{-1}): 3429 and 3335 (NH_2), 1678 and 1592 (C=O), 1297 and 1278, 811.

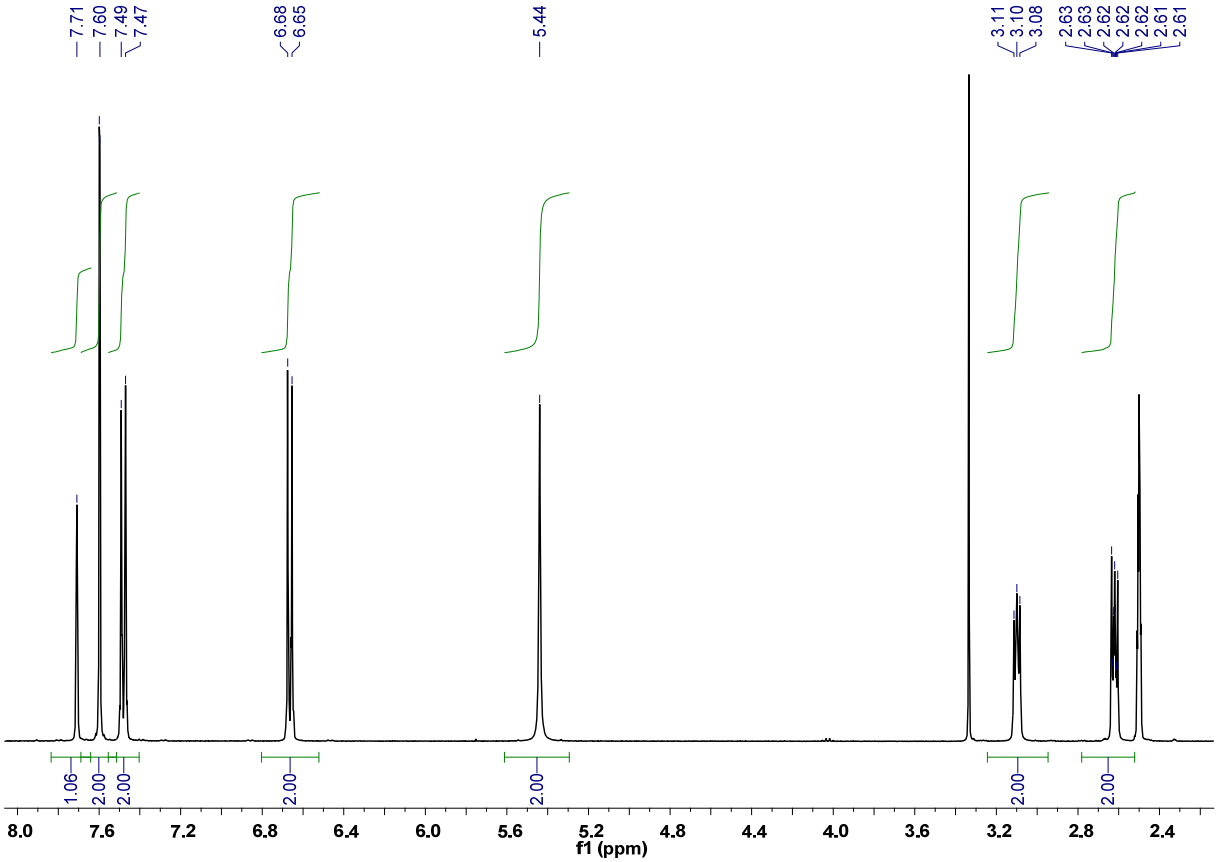
^{1}H NMR (DMSO-d_6, 400MHz) 7.71 (s, 1H, ArH), 7.60 (s, 2H, ArH), 7.48 (d, J = 6 Hz, 2H, ArH), 6.68 (d, J = 6 Hz, 2H, ArH), 5.44 (s, br, exch, 2H, NH_2), 3.10 (m, 2H, CH_2), 2.61 (m, 2H, CH_2).

^{13}C NMR (DMSO-d_6, 100MHz) δ: 205.44 (C=O), 156.14 (C-NH_2), 149.53, 146.91, 134.05, 127.90 (CH), 125.84, 124.60 (CH), 123.25 (CH), 122.79 (CH), 114.13 (CH), 36.10 (CH_2), 25.44 (CH_2).

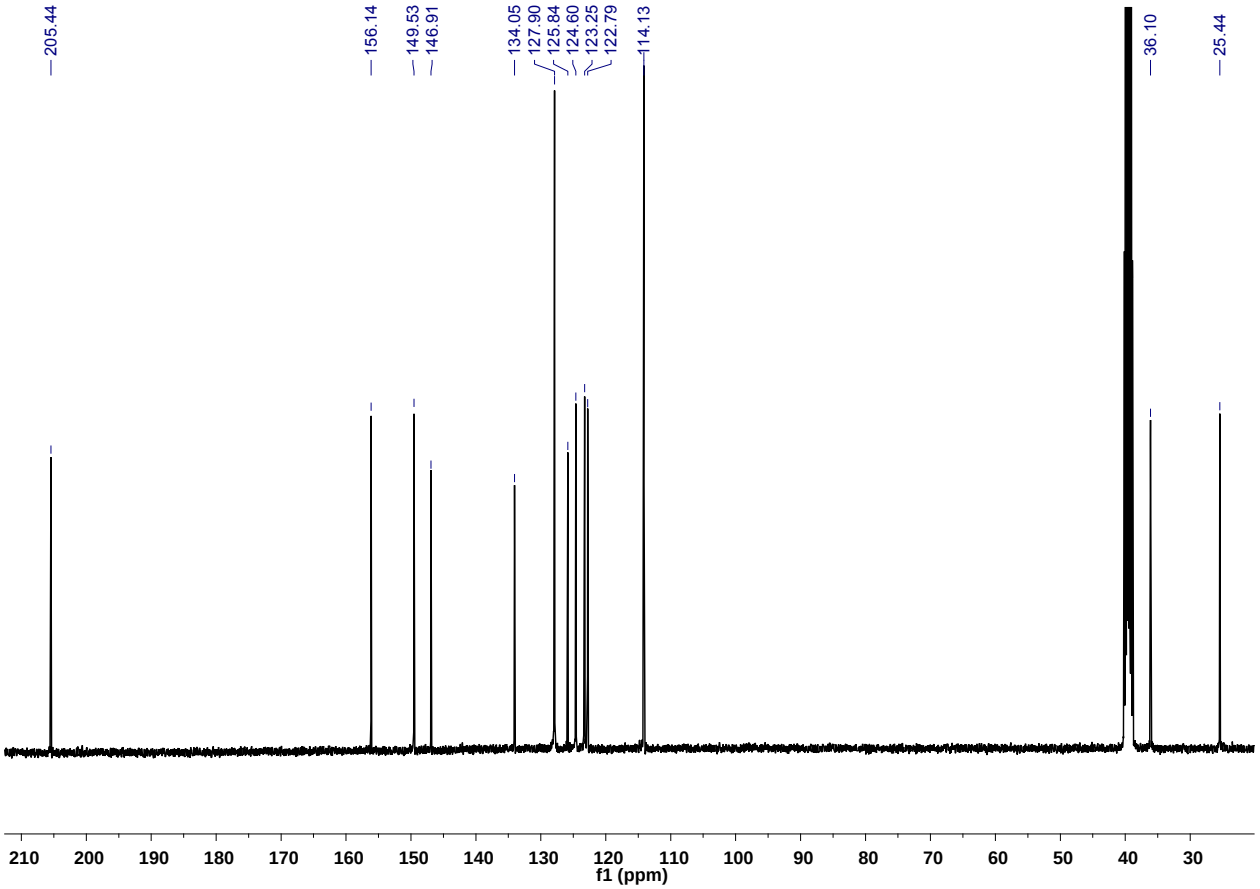
MS (EI) m/z (%): 224 (M^++1, 17), 223 (M^+, 100), 195 (18), 167 (8), 152 (9), 139 (4), 117 (7), 96 (3).

HRMS (EI): calcd. for $C_{15}H_{13}NO$: 223.0997 (M+); found: 223.0997.

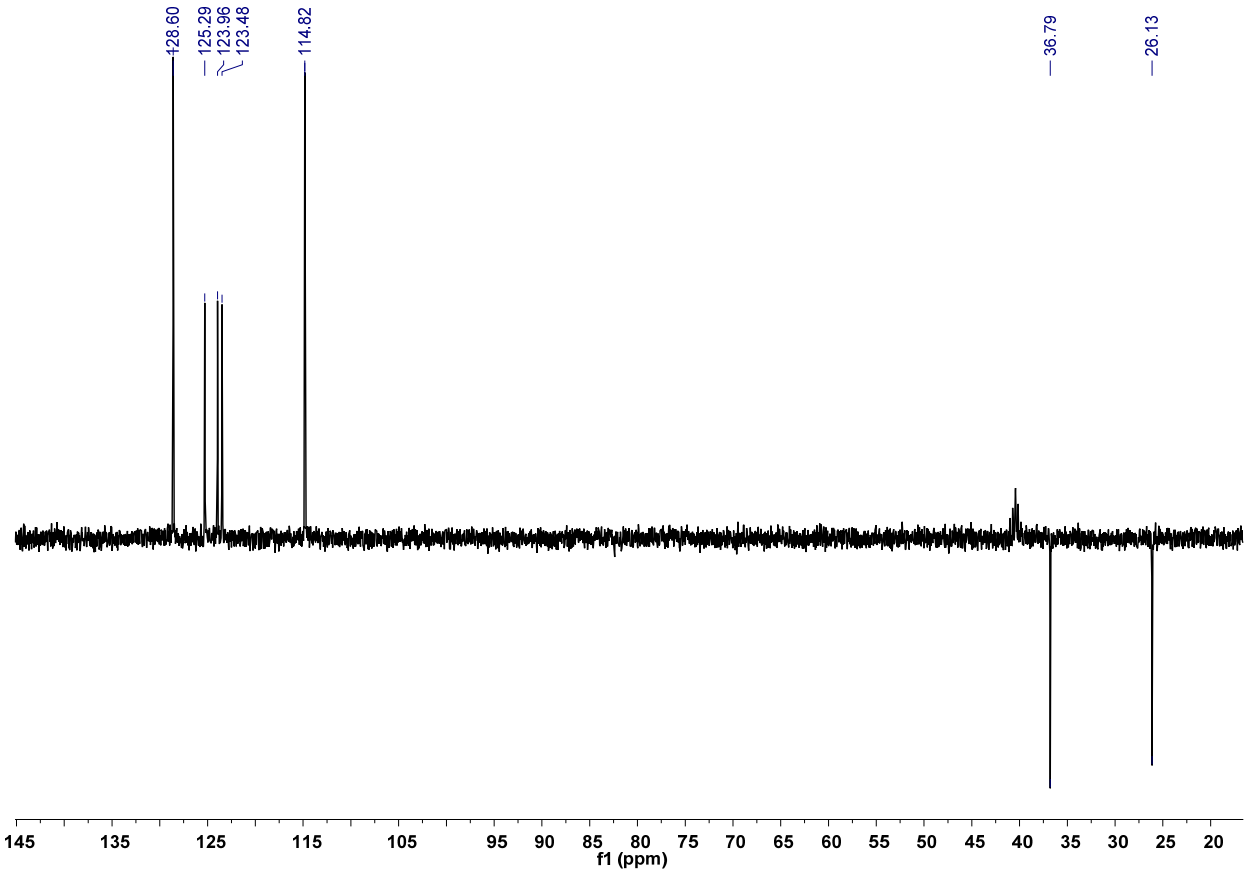
Anal. Calcd for $C_{15}H_{13}NO$: C, 80.69; H, 5.87; N, 6.27. Found: C: 80.44; H: 5.95; N: 6.11.



^{1}H-NMR spectrum (DMSO-d_6, 400 MHz) of 5-(4-aminophenyl)indan-1-one

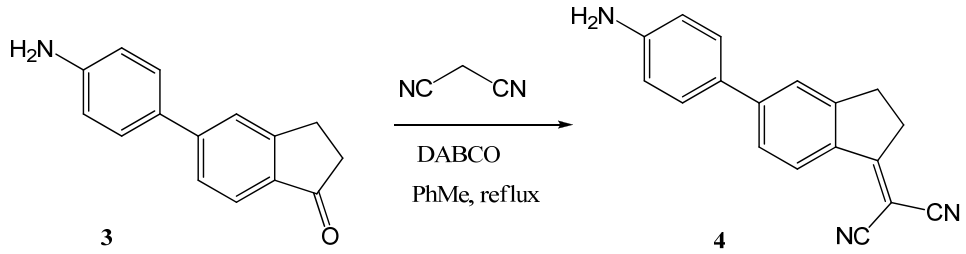


^{13}C-NMR spectrum (DMSO-d_6, 100 MHz) of 5-(4-aminophenyl)indan-1-one



DEPT-NMR spectrum (DMSO-d_6, 100 MHz) of 5-(4-aminophenyl)indan-1-one

Synthesis of 1-dicyanomethylene-5-(4-aminophenyl)indane 4



Malononitrile (50 mg 0.76 mmol) and DABCO (30 mg, 0.23 mmol) were added under nitrogen to 5-(4-aminophenyl)indan-1-one **3** (50 mg, 0.23 mmol) dissolved in toluene (10 mL). The mixture was heated under reflux for 2 h and then added to water (30 mL), stirred for 5 minutes and extracted with dichloromethane (5 x 20 mL). The combined organic extracts were dried (Na_2SO_4) and the solvent evaporated under reduced pressure. The crude product was purified by flash chromatography (silica, 3 x 30 cm), CH_2Cl_2, to get 1-dicyanomethylene-5-(4-aminophenyl)indene **4** as an orange solid (59 mg, 95 %), mp 320-322 ºC (decomp).

IR (KBr, cm^{-1}): 3491 and 3382 (NH_2), 2221 (CN), 1627, 1600 and 1561 (C=C).

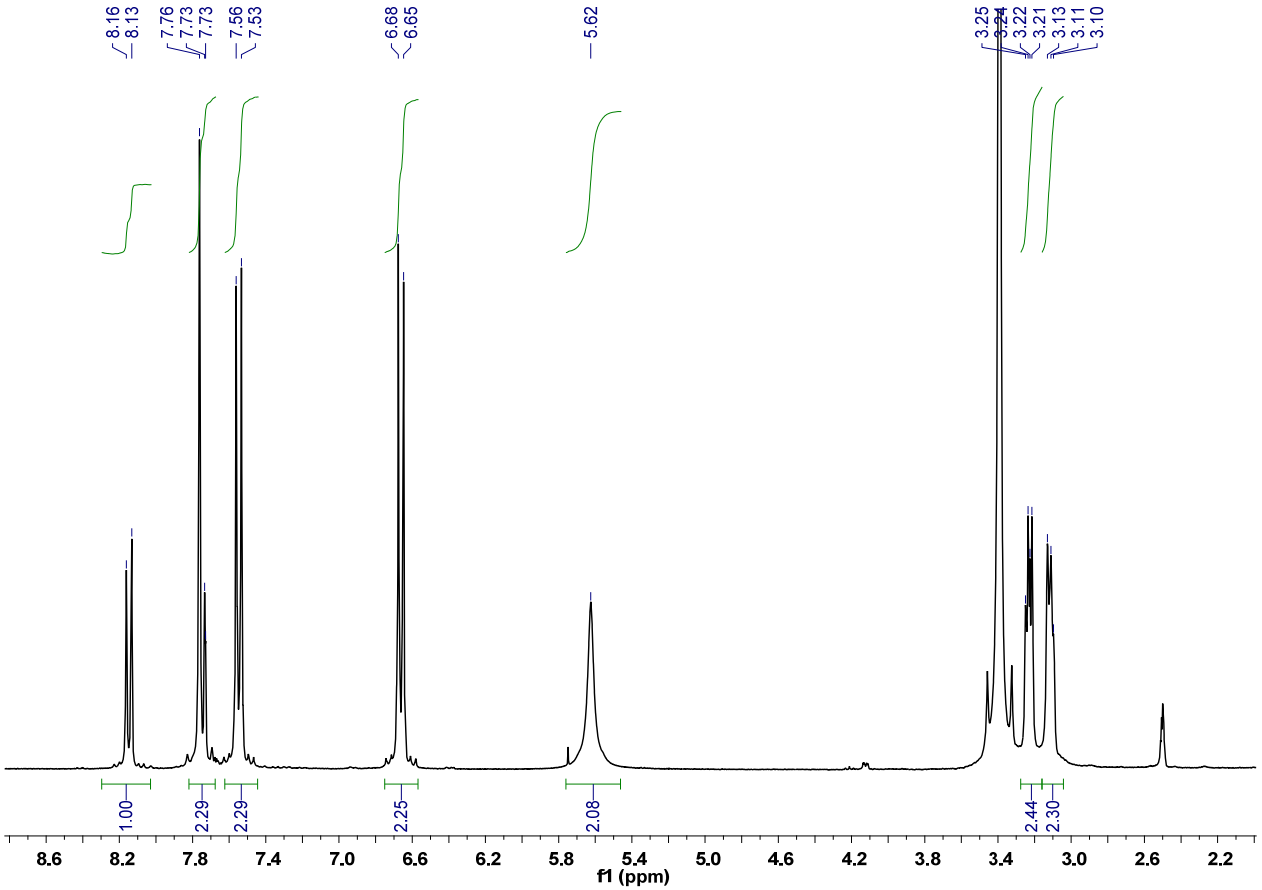
^{1}H NMR (DMSO-d_6, 300MHz): 8.14 (d, J = 9 Hz, 1H, ArH), 7.75 (m, 2H, ArH), 7.54 (d, J = 9 Hz, 2H, ArH), 6.66 (d, J = 9 Hz, 2H, ArH), 5.62 (s, br, exch, 2H, NH_2), 3.23 (m, 2H, CH_2), 3.23 (m, 2H, CH_2).

^{13}C NMR (DMSO-d_6, 75 MHz): 179.83, 156.43, 150.35, 147.42, 132.53, 128.20 (CH), 125.34 (CH), 124.90 (CH), 124.72, 121.69 (CH), 114.36 (CN), 114.15 (CH), 113.95 (CN), 69.96 (C(CN)$_2$), 34.66 (CH_2), 29.41 (CH_2).

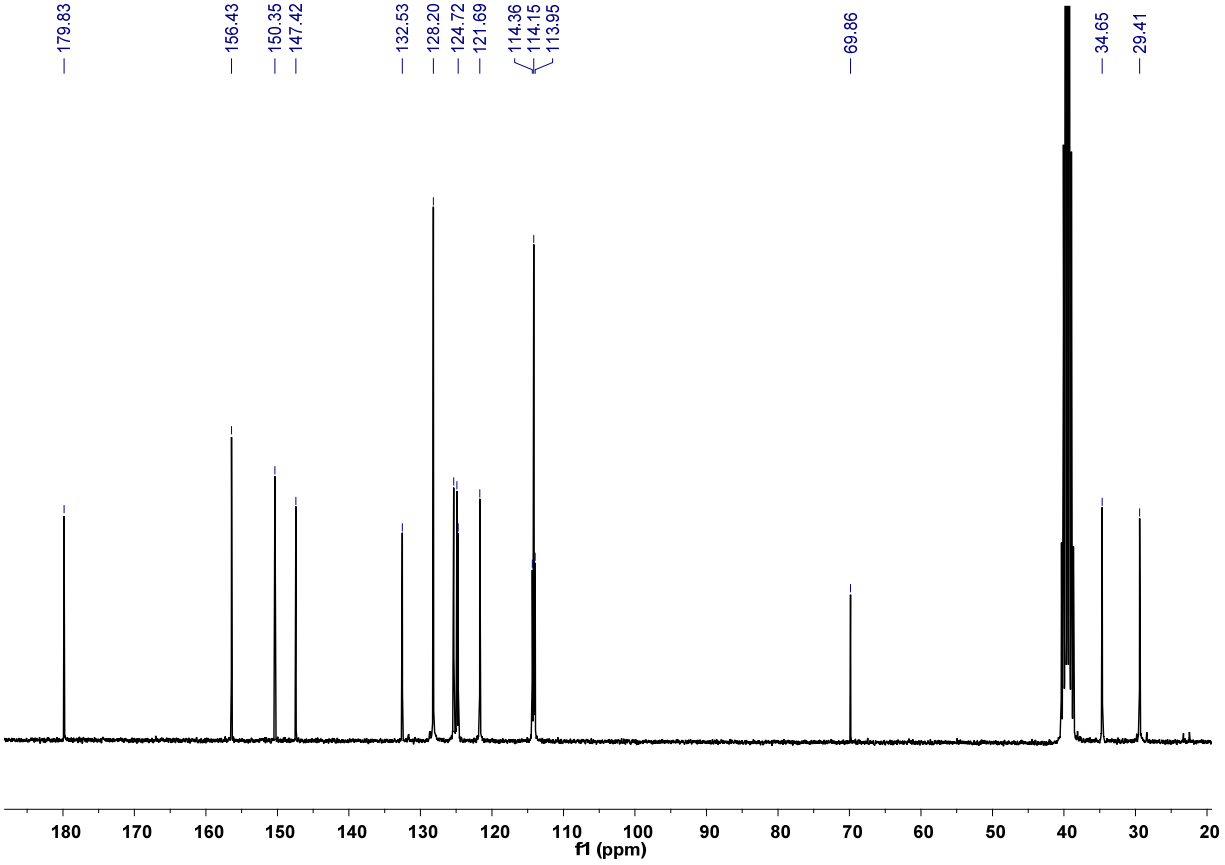
MS (EI) m/z (%): 272 (M^++1, 20), 271(M^+, 100), 243 (3), 214 (2), 206 (1), 204 (2), 189 (2), 135 (2).

HRMS (EI): calcd. for $C_{18}H_{13}N_3$: 271.1109 (M+); found: 271.1108

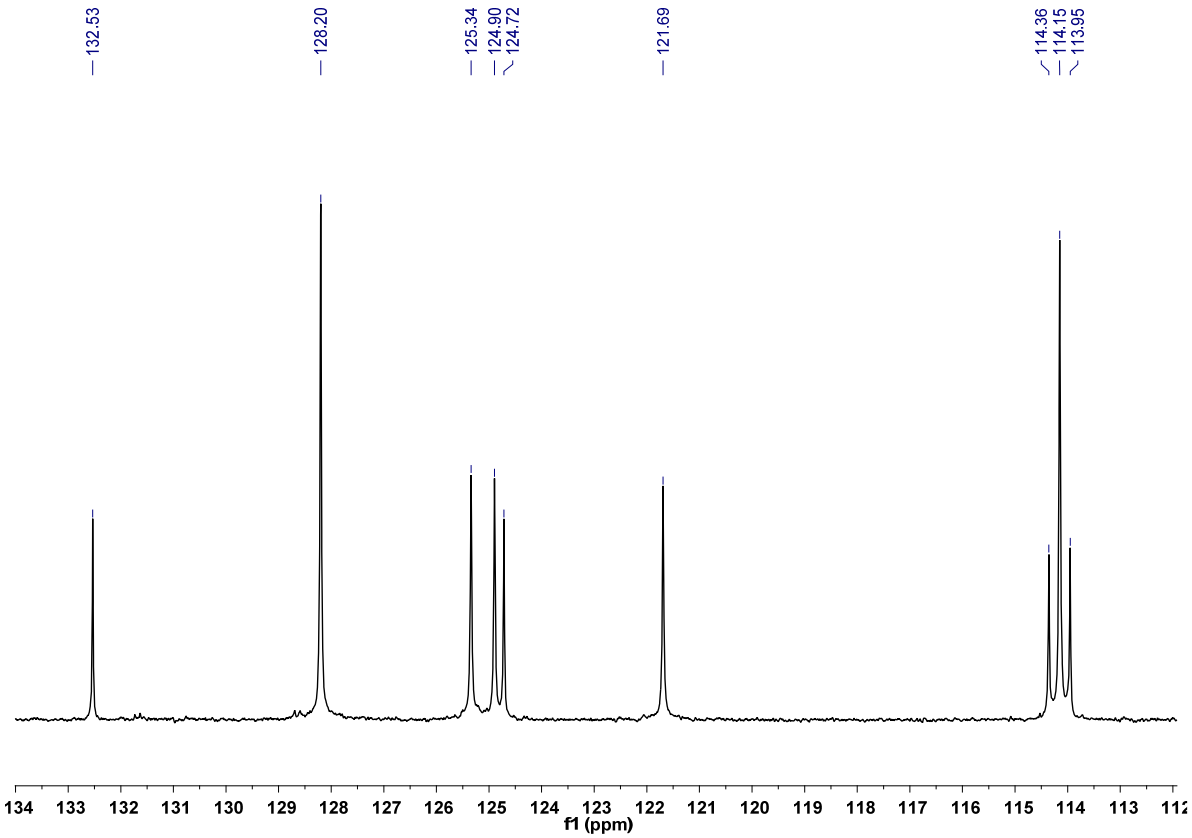
Anal. Calcd for $C_{18}H_{13}N_3$: C, 79.68; H, 4.83; N, 15.49. Found: C: 79.77; H: 4.97; N: 15.35.



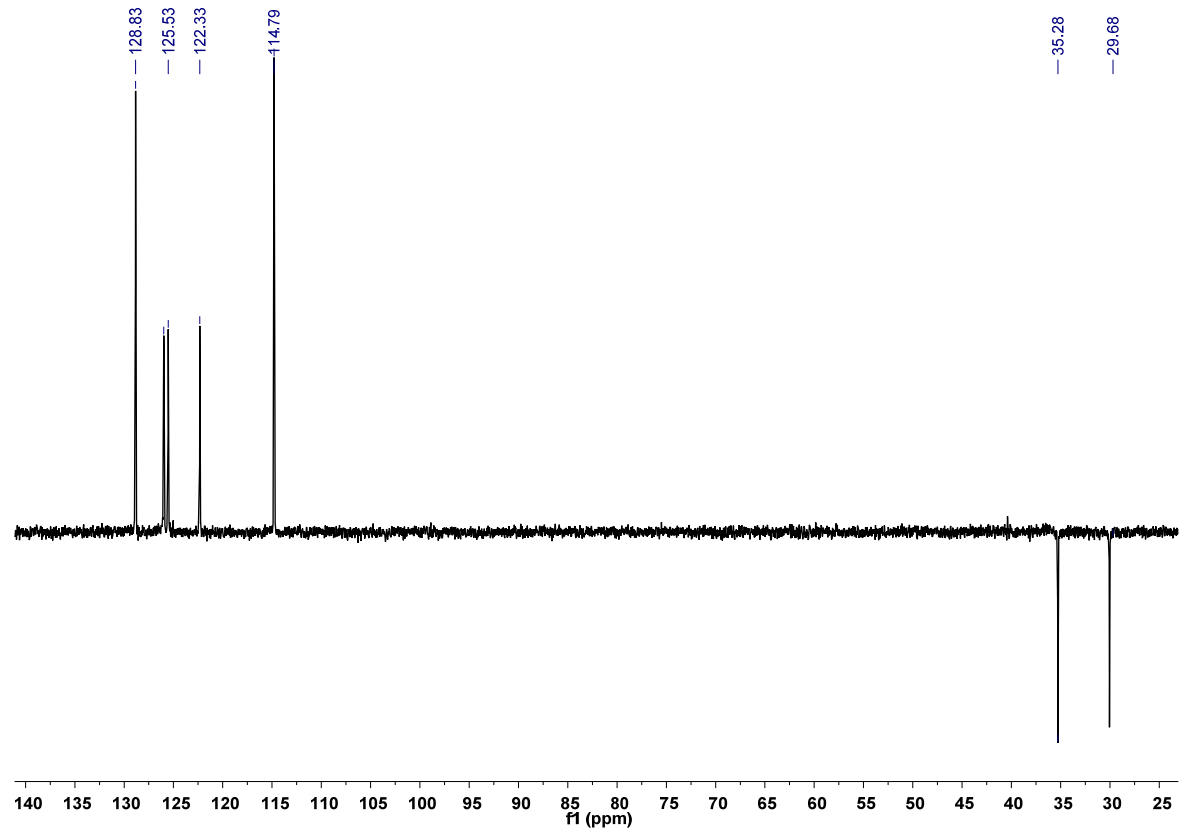
^{1}H NMR spectrum (DMSO-d_6, 300 MHz) of 1-dicyanomethylene-5-(4-aminophenyl)indane



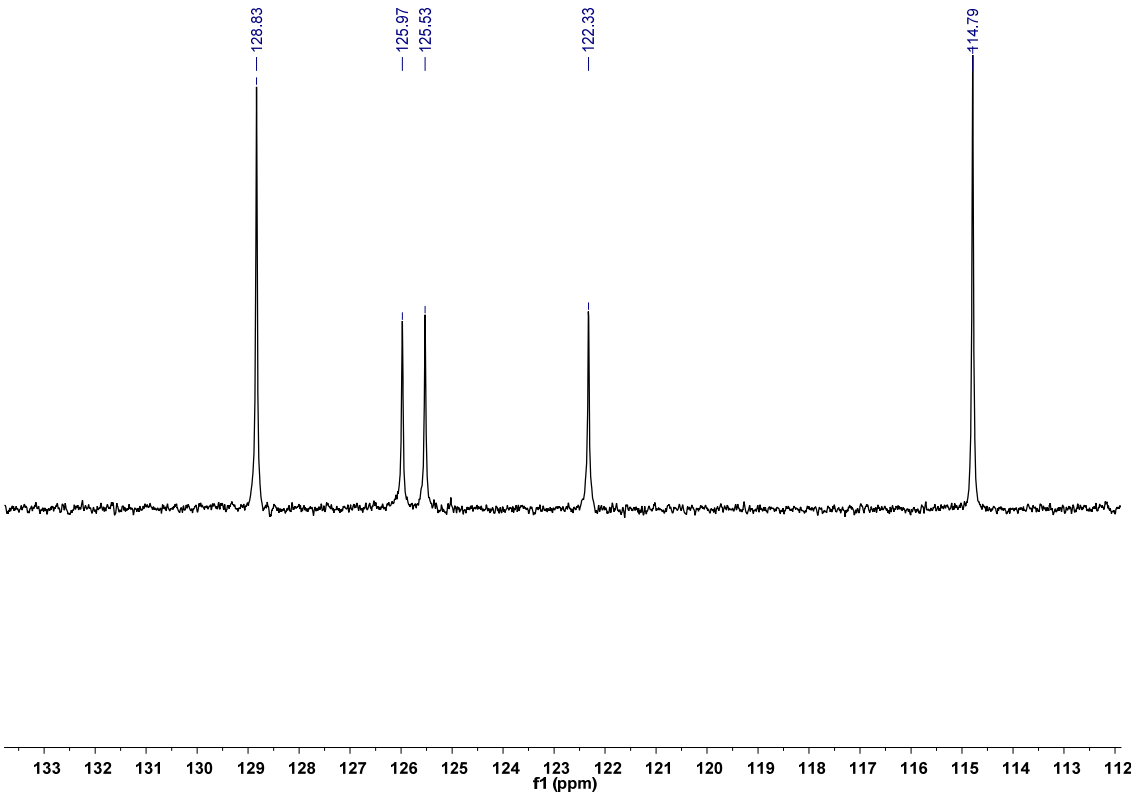
^{13}C NMR spectrum (DMSO-d_6, 75 MHz) of 1-dicyanomethylene-5-(4-aminophenyl)indane



Expansion of the ^{13}C NMR spectrum (DMSO-d_6, 75 MHz) of 1-dicyanomethylene-5-(4-aminophenyl)indane

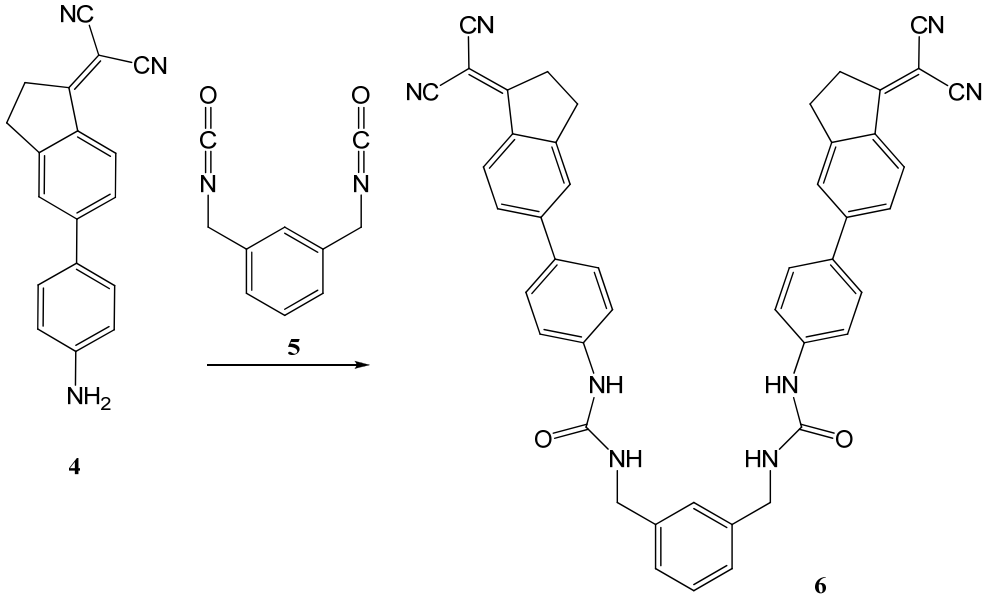


DEPT-NMR spectrum (DMSO-d_6, 75 MHz) of 1-dicyanomethylene-5-(4-aminophenyl)indane



Expansion of the DEPT-NMR spectrum (DMSO-d_6, 75 MHz) of 1-dicyanomethylene-5-(4-aminophenyl)indane

Synthesis of 1,3-bis[4-(1-dicyanomethyleneindan-5-yl)phenylureidomethyl)benzene 6



1-Dicyanomethylene-5-(4-aminophenyl)indane **4** (100 mg, 0.38 mmol) was added to *m*-xylylene diisocyanate **5** (15 μL, 0.095 mmol) dissolved in $CHCl_3$ (20 mL) and the mixture was stirred at room temperature for 24 h. Then a second portion of *m*-xylylene diisocyanate (15 μL, 0.095 mmol) was added and the mixture was stirred for additional 24 h. The solid formed after this time was filtered off, washed with $CHCl_3$ (2 portions of 2 mL) and dried under reduced pressure. Compound **6** was obtained as a yellow solid (104 mg, 75%), mp 212-214ºC.

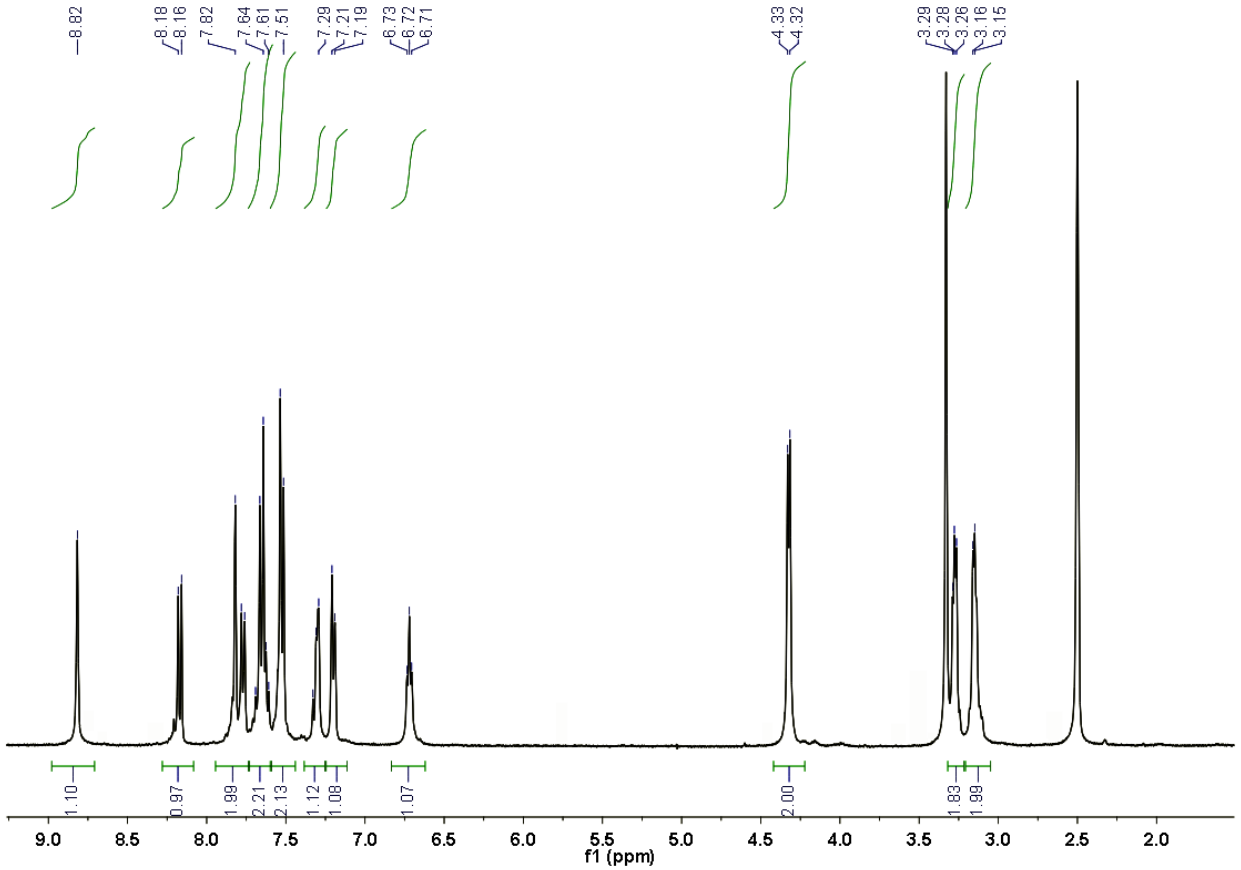
IR (KBr, cm^{-1}): 3328 (br, NH), 2221(C≡N), 1658, 1604 and 1557 (C=O), 1320, 1219, 1184.

^{1}H NMR (DMSO-d_6, 400MHz) 8.82 (s, 2H, NH) 8.17 (d, *J* = 6 Hz, 2H, ArH), 7.82 (s, 2H, ArH), 7.79 (d, *J* = 6 Hz, 2H, ArH), 7.65 (d, *J* = 6 Hz, 4H, ArH), 7.52 (d, *J* = 6 Hz, 4H, ArH), 7.29 (m, 2H, ArH), 7.20 (m, 2H, ArH), 6.71 (t, *J* = 6 Hz, 2H, NH), 4.32 (d, *J* = 6 Hz, 4H, CH_2) , 3.28 (m, 4H, CH_2), 3.15 (m, 4H, CH_2).

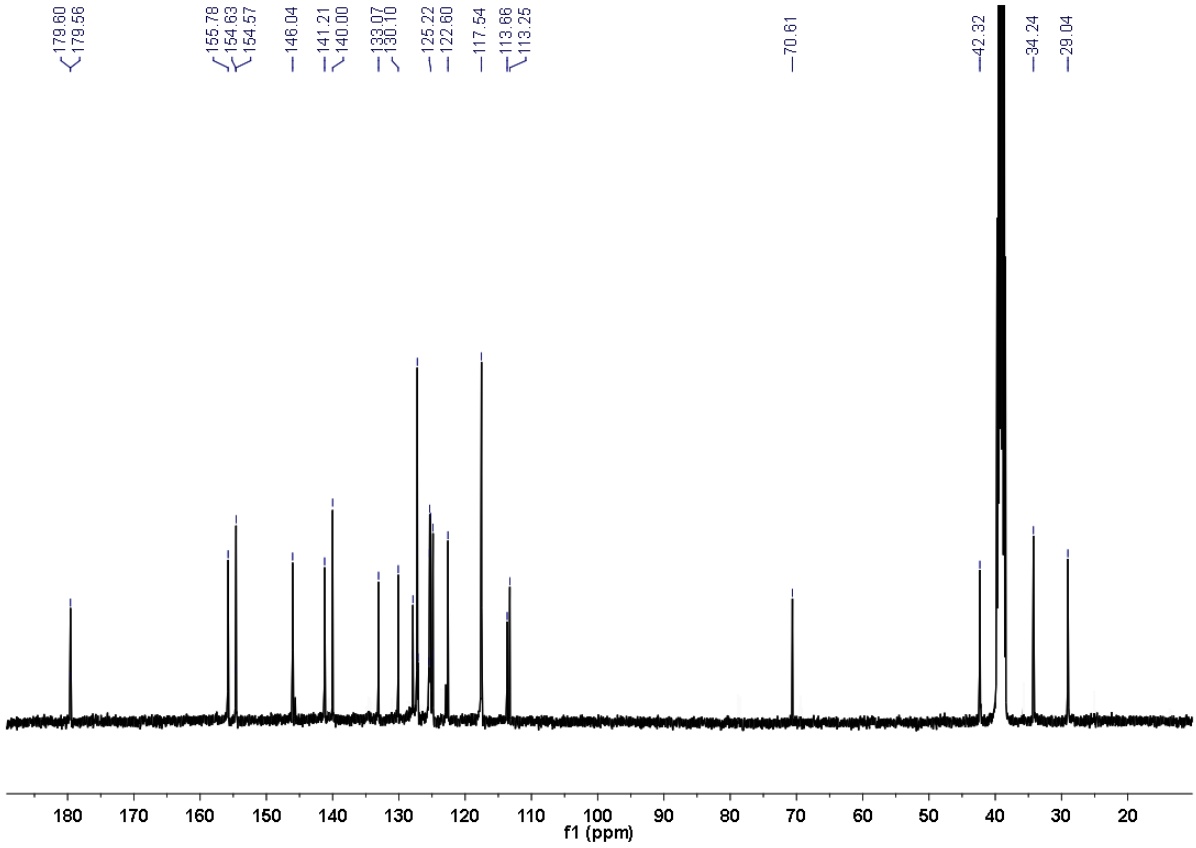
^{13}C NMR (DMSO-d_6, 100MHz) 179.60, 155.78, 154.63, 146.04, 141.21, 140.00, 133.07, 130.10, 128.22 (CH), 127.58 (CH), 125.72, 125.67 (CH), 125.55 (CH), 125.22 (CH), 122.60 (CH), 117.54 (CH), 113.66 (CN), 113.25 (CN), 70.61, 42.32 (CH_2), 34.24 (CH_2), 29.04 (CH_2).

MS (FAB) m/z (+): 731.40 (M^++1). ESI MS m/z (%):753.28 (M+Na^+), 731.29 (M^++1)

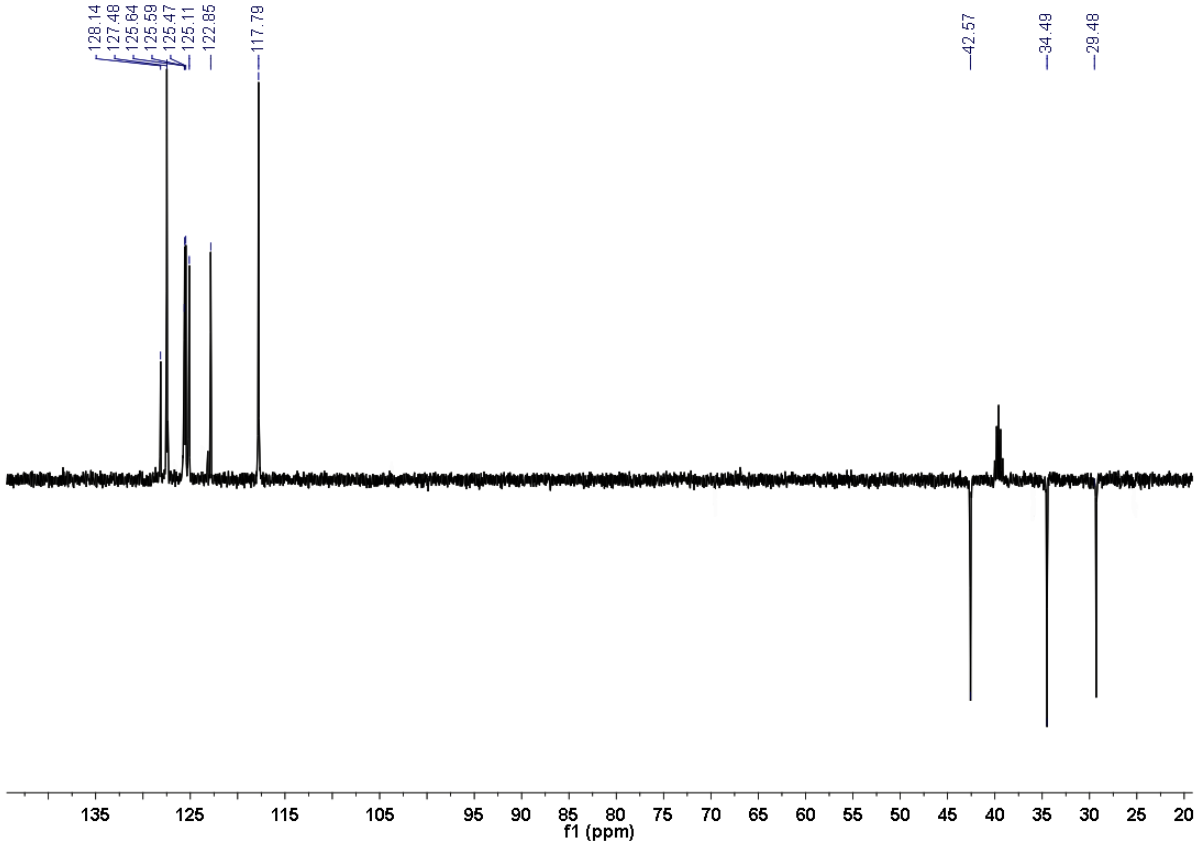
Anal. Calcd for $C_{46}H_{34}N_8O_2$: C, 75.60; H, 4.69; N, 15.33. Found: C, 75.42; H, 4.84; N, 15.09.



^{1}H NMR spectrum (DMSO-d_6, 400 MHz) of 1,3-bis[4-(1-dicyanomethyleneindan-5-yl)phenylureidomethyl)benzene

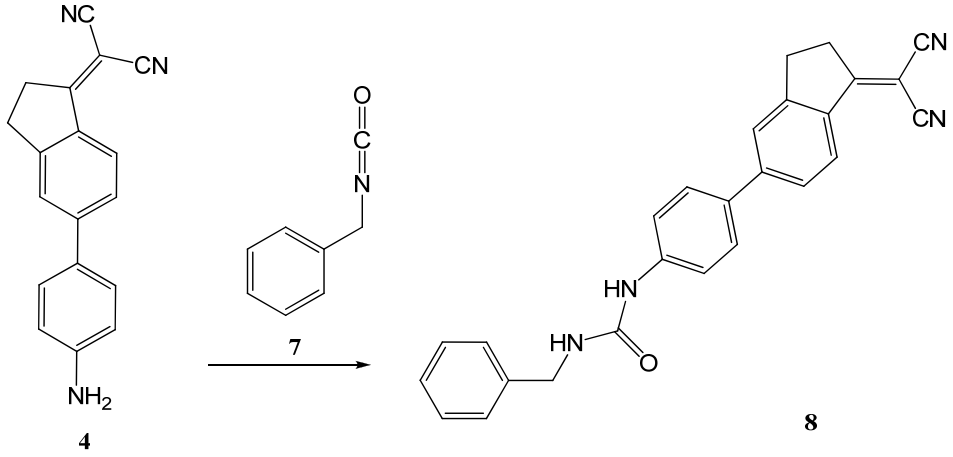


^{13}C NMR spectrum (DMSO-d_6, 100 MHz) of 1,3-bis[4-(1-dicyanomethyleneindan-5-yl)phenylureidomethyl)benzene



DEPT-NMR spectrum (DMSO-d_6, 100 MHz) of 1,3-bis[4-(1-dicyanomethyleneindan-5-yl)phenylureidomethyl)benzene

Synthesis of 1-benzyl-3-[4-(1-(dicyanomethyleneindan-5-yl)phenyl]urea 8:



1-Dicyanomethylene-5-(4-aminophenyl)indane **4** (50 mg, 0.18 mmol) was added to benzyl isocyanate **7** (25 mg, 0.18 mmol) dissolved in $CHCl_3$ (10mL) and the mixture was stirred at room temperature for 48 h. Hexane (2 mL) was then added and the solid formed was filtered off, washed with $CHCl_3$ (2 portions of 2 mL) and dried. Compound **8** was obtained as a yellow solid (56 mg, 75%), mp 189-190 ºC.

IR (KBr, cm^{-1}) 3336 (br, NH), 2221 (C≡N), 1639 and 1565 (C=O), 1328, 1227.

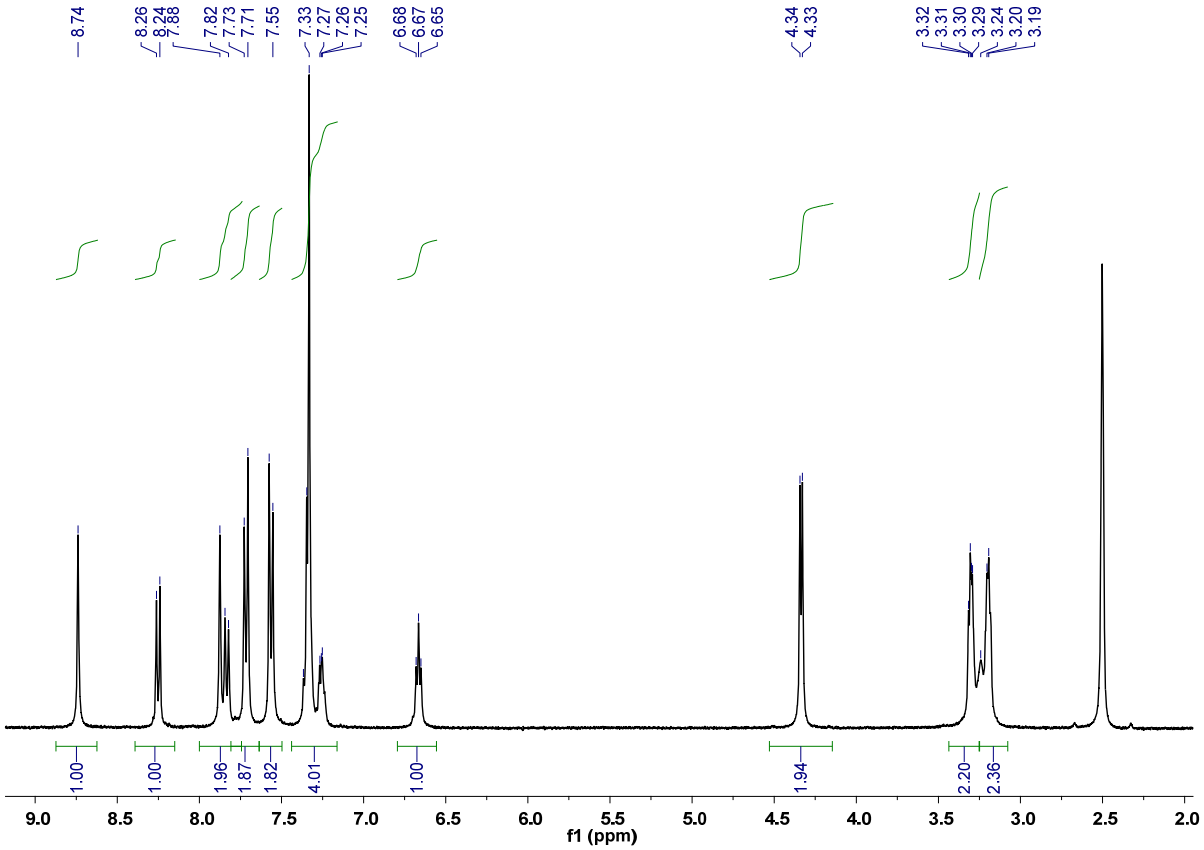
^{1}H NMR (DMSO-d_6 , 400MHz): 8.74 (s, 1H, NH) 8.25 (d, J = 8 Hz, 1H, ArH), 7.87 (s, 1H, ArH), 7.83 (d, J = 8 Hz, 1H, ArH), 7.71 (d, J = 8 Hz, 2H, ArH), 7.56 (d, J = 8 Hz, 2H, ArH), 7.34-7.32 (m, 4H, ArH), 7.25 (m, 1H, ArH), 6.66 (t, J = 6 Hz, 1H, NH), 4.33 (d, J = 6 Hz, 2H, CH_2), 3.30 (m, 2H, CH_2), 3.19 (m, 2H, CH_2).

^{13}C NMR (DMSO-d_6, 100MHz) 179.68, 155.96, 154.82, 146.36, 141.44, 139.96, 133.40, 130.55, 128.08, 127.44, 126.96, 126.57, 125.63, 125.17, 122.87 (CH_{Ar}), 117.85, 113.81 (CN), 113.40 (CN), 71.00, 42.69 (CH_2), 34.50 (CH_2), 29.28 (CH_2).

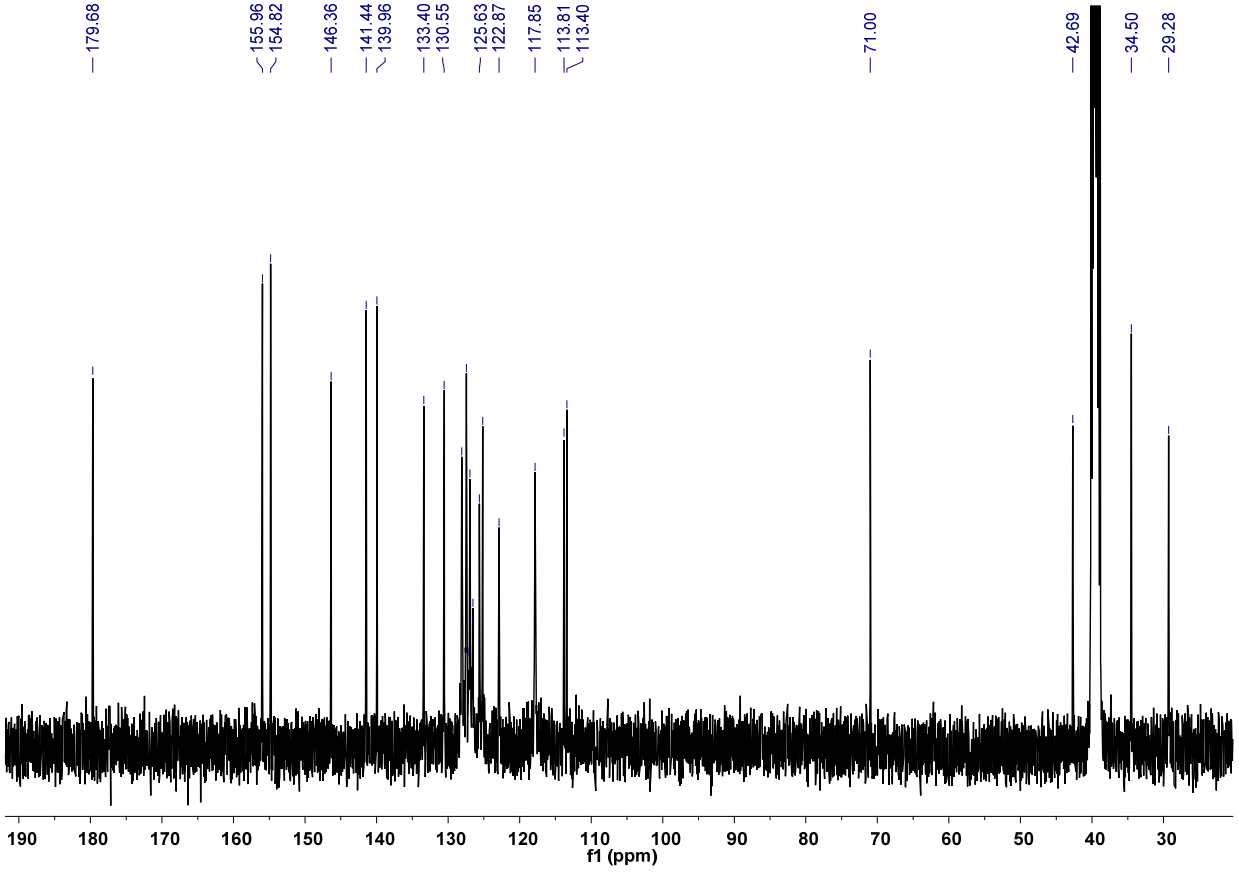
MS (FAB) m/z (+): 405.06 (M^++1). MS (EI) m/z (%): 404 (M^+, 10), 297 (95), 271 (100).

HRMS (EI): calcd. for $C_{26}H_{20}N_4O$: 404.1637 (M^+); found: 404.1633.

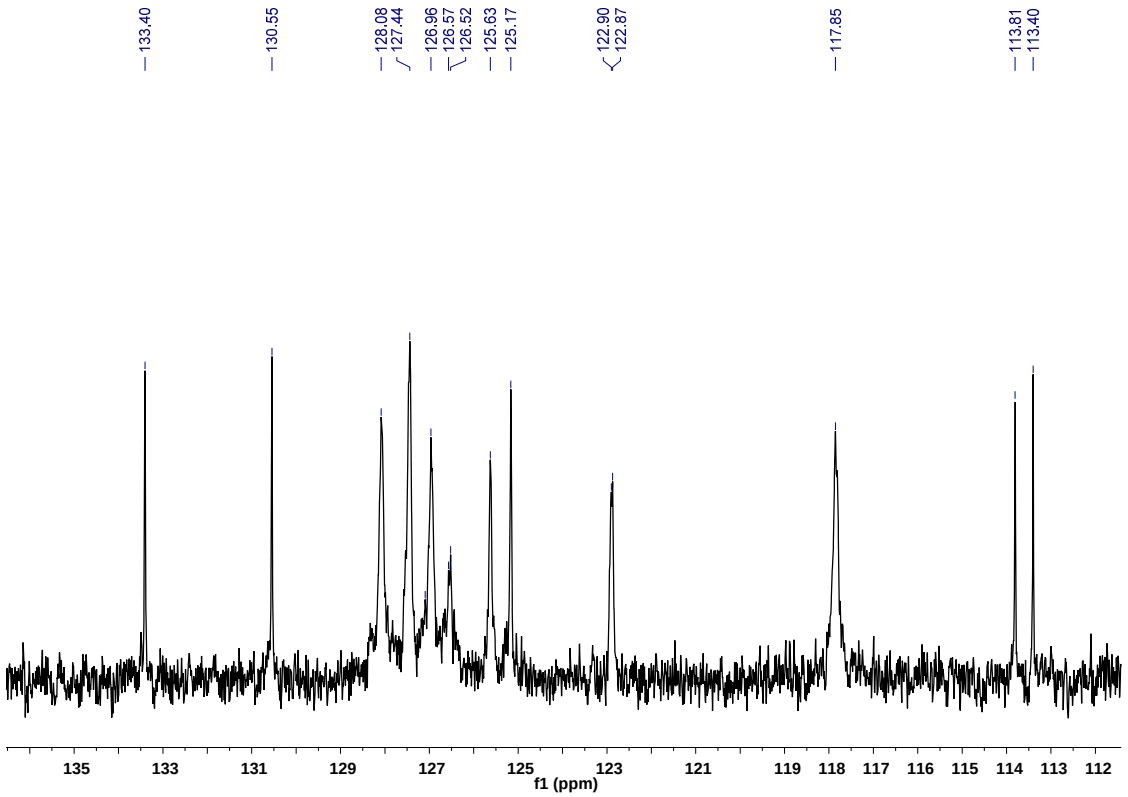
Anal. Calcd for $C_{26}H_{20}N_4O$: C, 77.21; H, 4.98; N, 13.85. Found: C, 77.06; H, 5.12; N, 13.67.



[1]H NMR spectrum (DMSO-d_6, 400 MHz) of 1-benzyl-3-[4-(1-(dicyanomethyleneindan-5-yl)phenyl]urea



[13]C NMR spectrum (DMSO-d_6, 100 MHz) of 1-benzyl-3-[4-(1-(dicyanomethyleneindan-5-yl)phenyl]urea

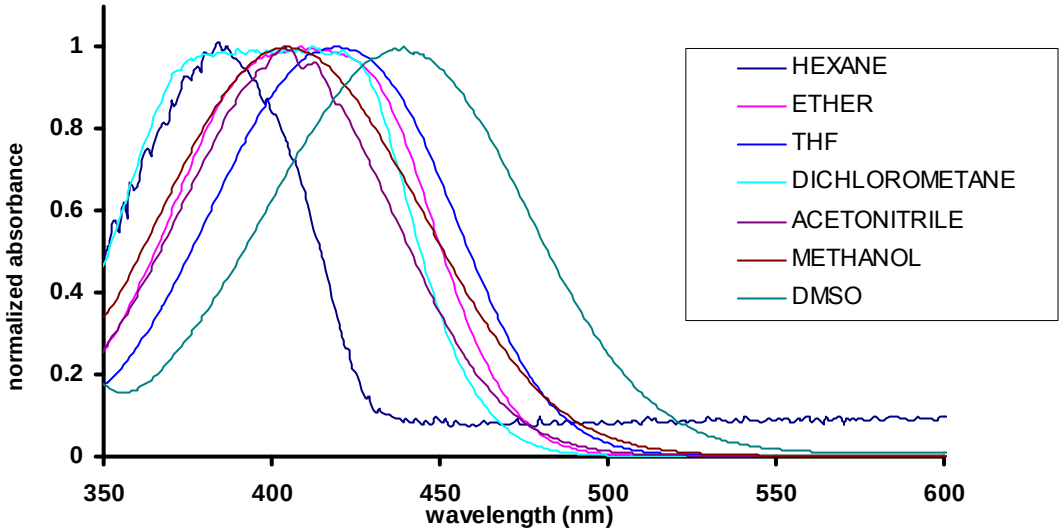


An expansion of the ^{13}C NMR spectrum (DMSO-d_6, 100 MHz) of 1-benzyl-3-[4-(1-(dicyanomethyleneindan-5-yl)phenyl]urea

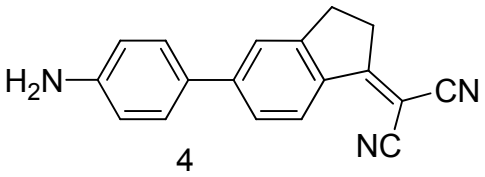
Solvatochromism of 1-dicyanomethylene-5-(4-aminophenyl)indane 4:

(1) (2) (3) (4) (5) (6) (7)

10^{-4} M solutions of 4 in (1) Hexane, (2) Et_2O, (3) THF, (4) CH_2Cl_2, (5) MeCN, (6) MeOH, (7) DMSO



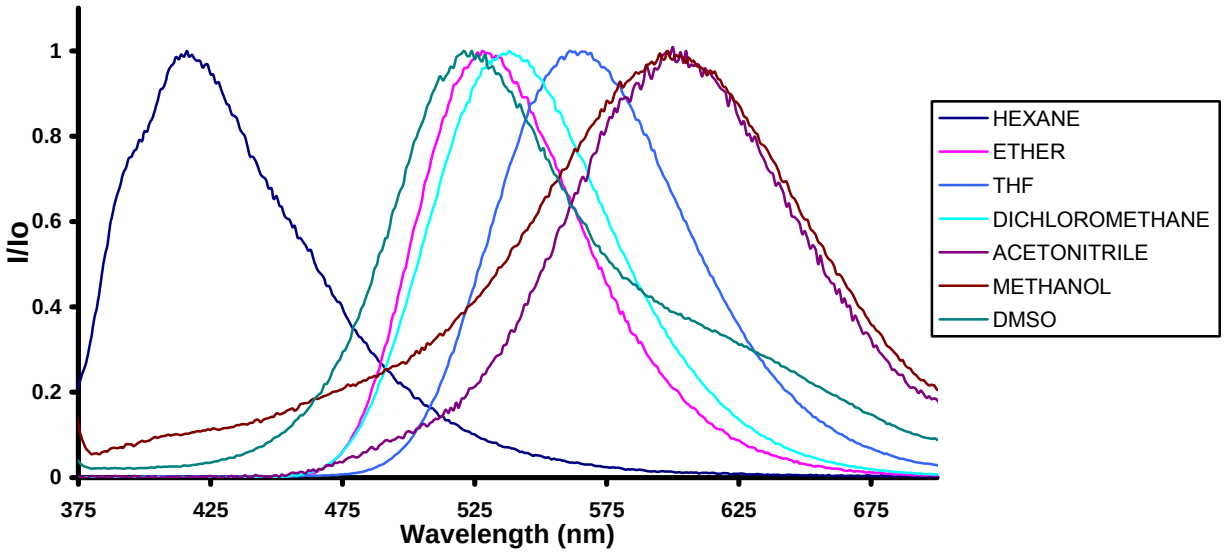
Normalized absorbance spectra of 10^{-4} M solutions of 4 in Hexane, Et_2O, THF, CH_2Cl_2, MeCN, MeOH, DMSO



4

(1) (2) (3) (4) (5) (6) (7) λ_{exc} **= 366 nm**

10^{-4} M solutions of 4 in (1) Hexane, (2) Et_2O, (3) THF, (4) CH_2Cl_2, (5) MeCN, (6) MeOH, (7) DMSO



Normalized fluorescente spectra of 10^{-4} M solutions of 4 in Hexane, Et_2O, THF, CH_2Cl_2, MeCN, MeOH, DMSO; λ_{exc} = 366 nm.

Behavior of 1,3-bis[4-(1-dicyanomethyleneindan-5-yl)phenylureidomethyl)benzene 6 in MeCN and DMSO in the presence of common anions:

Ref. F^- Cl^- Br^- I^- BzO^- NO_3^- $H_2PO_4^-$ HSO_4^- AcO^- CN^- SCN^-

10^{-4} M Solutions of **6** in MeCN +2 equivalents of anion: F^-, Cl^-, Br^-, I^-, BzO^-, NO_3^-, $H_2PO_4^-$, HSO_4^-, AcO^-, CN^-, SCN^-

Ref. F^- Cl^- Br^- I^- BzO^- NO_3^- $H_2PO_4^-$ HSO_4^- AcO^- CN^- SCN^-

10^{-4} M Solutions of **6** in MeCN +2 equivalents of anion: F^-, Cl^-, Br^-, I^-, BzO^-, NO_3^-, $H_2PO_4^-$, HSO_4^-, AcO^-, CN^-, SCN^-; λ_{exc} = 366nm

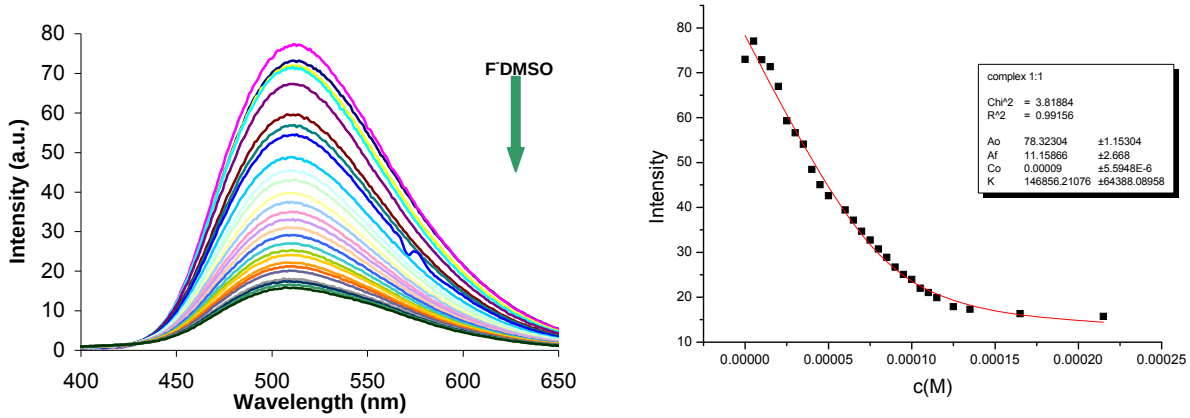
Ref. F^- Cl^- Br^- I^- BzO^- NO_3^- $H_2PO_4^-$ HSO_4^- AcO^- CN^- SCN^-

10^{-4} M Solutions of **6** in DMSO +2 equivalents of anion: F^-, Cl^-, Br^-, I^-, BzO^-, NO_3^-, $H_2PO_4^-$, HSO_4^-, AcO^-, CN^-, SCN^-

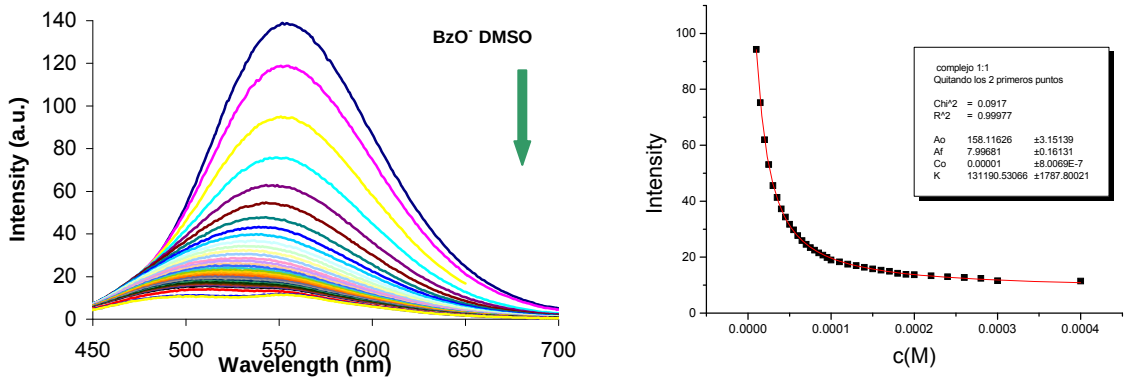
Ref. F^- Cl^- Br^- I^- BzO^- NO_3^- $H_2PO_4^-$ HSO_4^- AcO^- CN^- SCN^-

10^{-4} M Solutions of **6** in DMSO +2 equivalents of anion: F^-, Cl^-, Br^-, I^-, BzO^-, NO_3^-, $H_2PO_4^-$, HSO_4^-, AcO^-, CN^-, SCN^-; λ_{exc} = 366 nm

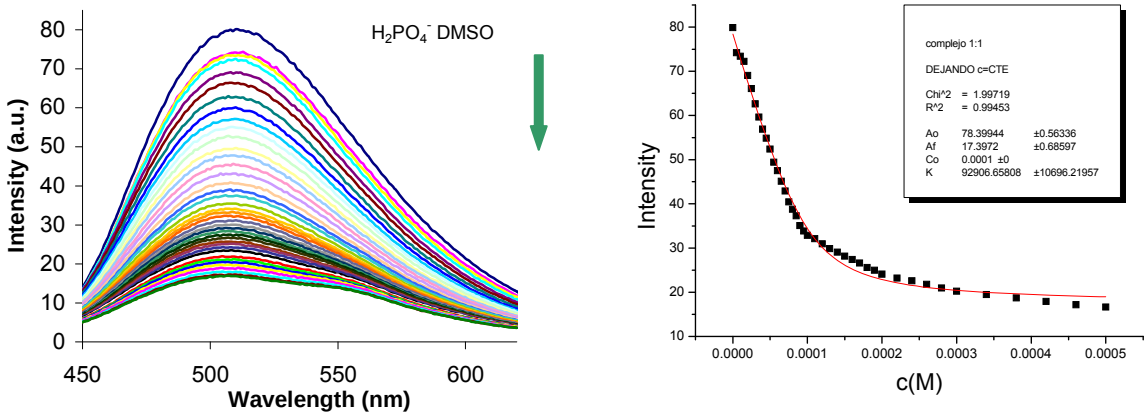
Fluorimetric titrations of 1,3-bis[4-(1-dicyanomethyleneindan-5-yl)phenylureidomethyl)benzene 6 in DMSO with common anions:



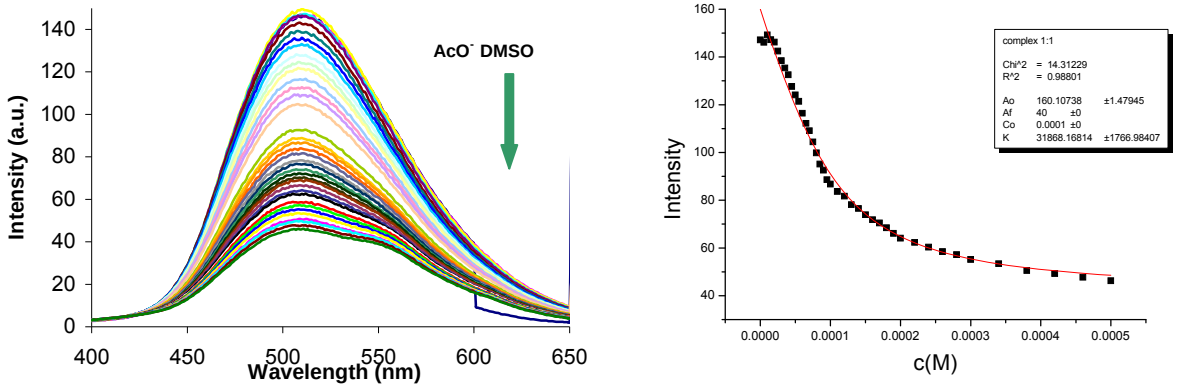
Left: Emission spectra for the fluorimetric titration of **6** (10^{-4} M in DMSO) with F^- ($\lambda_{excitation}$ = 290 nm). Right: Titration profile fitted to a 1:1 model (λ_{max} = 515 nm) for the titration of **6** with F^-



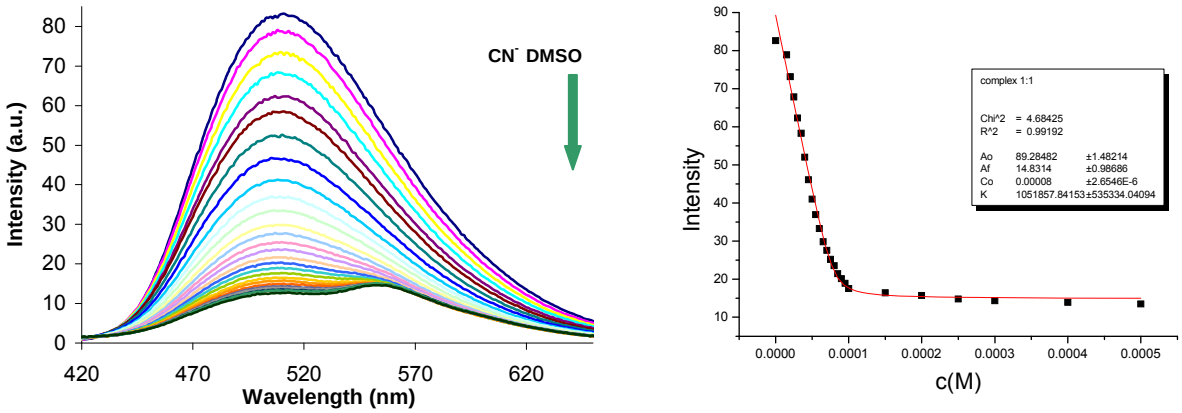
Left: Emission spectra for the fluorimetric titration of **6** (10^{-4} M in DMSO) with BzO^- ($\lambda_{excitation}$ = 396 nm). Right: Titration profile fitted to a 1:1 model (λ_{max} = 555 nm) for the titration of **6** with BzO^-



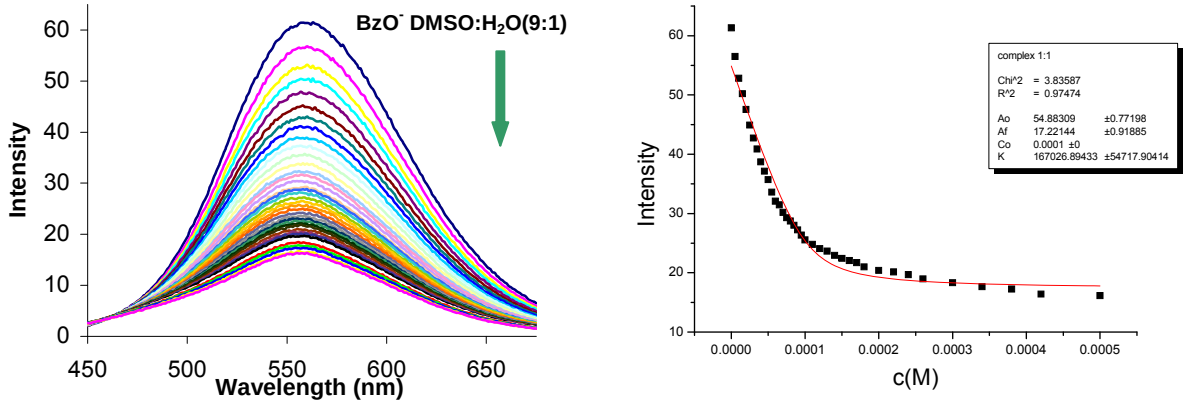
Left: Emission spectra for the fluorimetric titration of **6** (10^{-4} M in DMSO) with $H_2PO_4^-$ (λ_{exc} = 290 nm). Right: Titration profile fitted to a 1:1 model (λ_{max} = 515 nm) for the titration of **6** with $H_2PO_4^-$



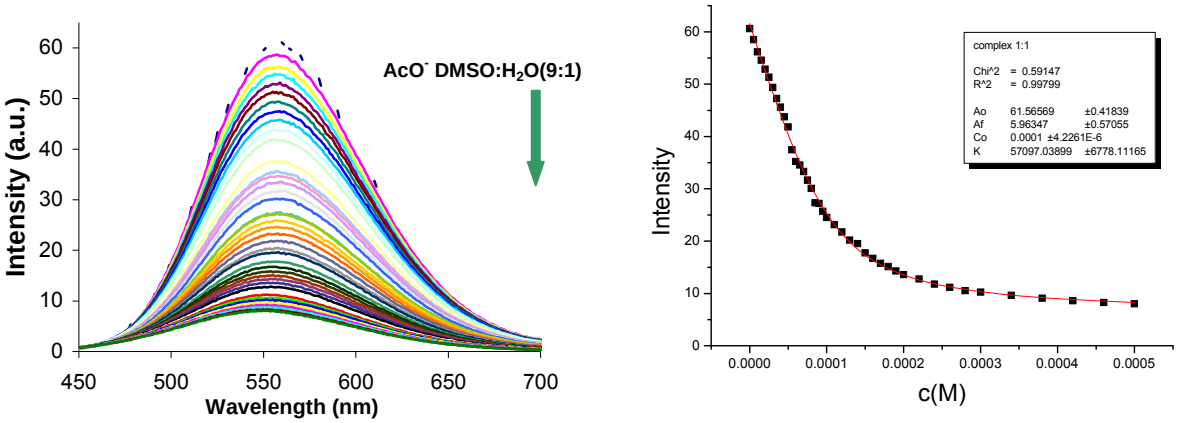
Left: Emission spectra for the fluorimetric titration of **6** (10^{-4} M in DMSO) with AcO^- (λ_{exc} = 290 nm). Right: Titration profile fitted to a 1:1 model (λ_{max} = 515 nm) for the titration of **6** with AcO^-



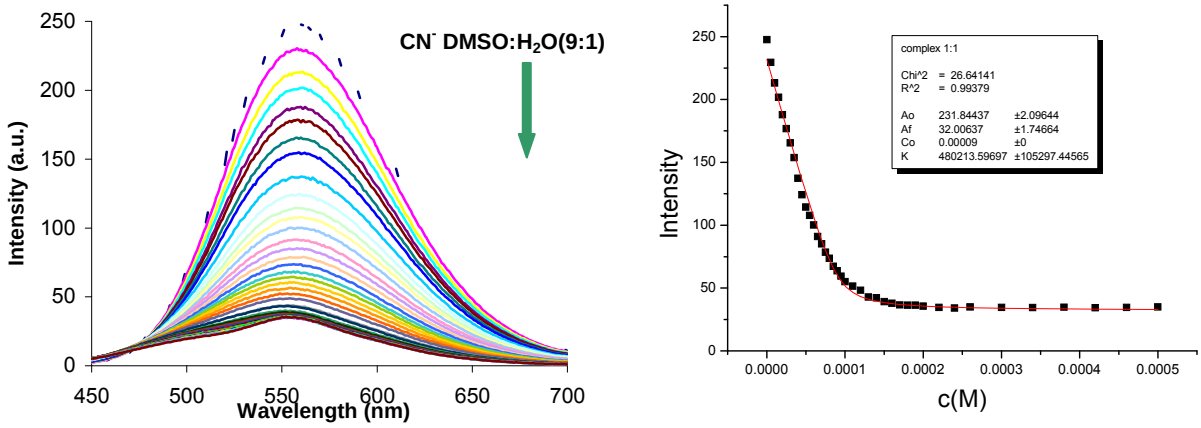
Left: Emission spectra for the fluorimetric titration of **6** (10^{-4} M in DMSO) with CN^- (λ_{exc} = 290 nm). Right: Titration profile fitted to a 1:1 model (λ_{max} = 515 nm) for the titration of **6** with CN^-



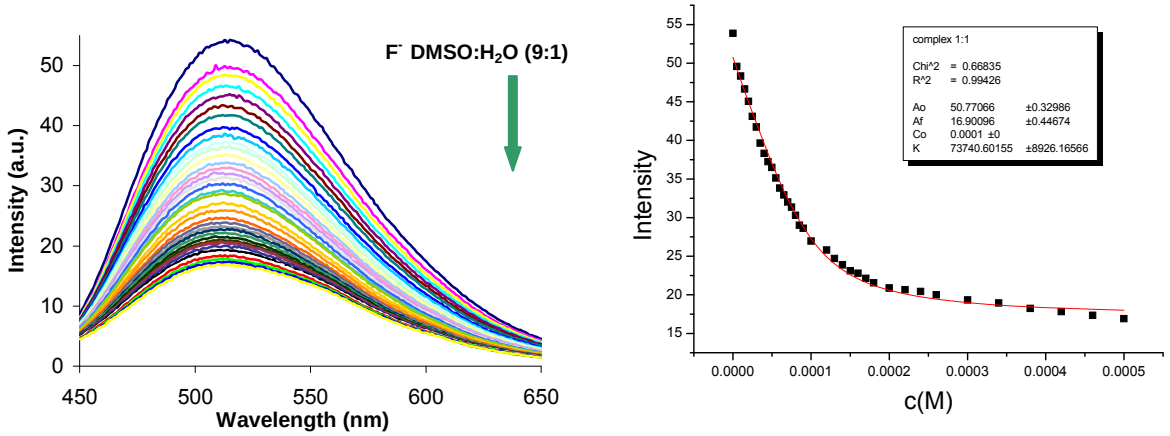
Left: Emission spectra for the fluorimetric titration of **6** (10^{-4} M in DMSO:H_2O (9:1)) with BzO^- (λ_{exc} = 396 nm). Right: Titration profile fitted to a 1:1 model (λ_{max} = 555 nm) for the titration of **6** with BzO^-



Left: Emission spectra for the fluorimetric titration of **6** (10^{-4} M in DMSO:H_2O (9:1)) with AcO^- (λ_{exc} = 396 nm). Right: Titration profile fitted to a 1:1 model (λ_{max} = 555 nm) for the titration of **6** with AcO^-



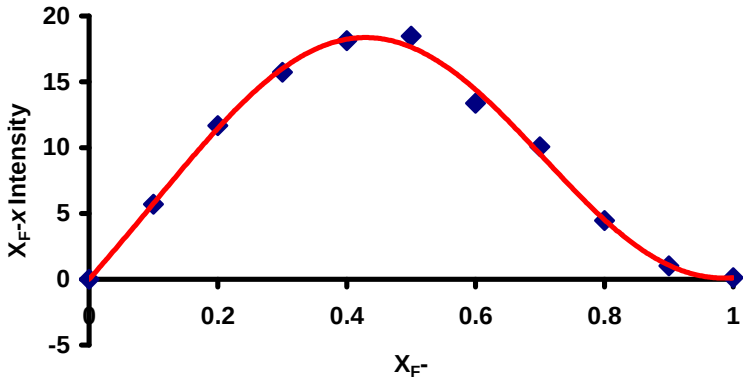
Left: Emission spectra for the fluorimetric titration of **6** (10^{-4} M in DMSO:H_2O (9:1)) with CN^- (λ_{exc} = 396 nm). Right: Titration profile fitted to a 1:1 model (λ_{max} = 555 nm) for the titration of **6** with CN^-



Left: Emission spectra for the fluorimetric titration of **6** (10^{-4} M in DMSO:H_2O (9:1)) with F^- (λ_{exc} = 396 nm). Right: Titration profile fitted to a 1:1 model (λ_{max}= 555 nm) for the titration of **6** with F^-

Job's plot analysis of fluorescence titrations of 6 with F^-

Job's plot analysis of fluorescence titrations of **6** with F^- revealed a maximum at a 50% mole fraction, in accord with the proposed 1:1 binding stoichiometry



Job's plot analysis of fluorescence titrations of **6** with F^- in MeCN (λ_{exc} = 396 nm) (λ_{em} = 557 nm)

Complexation constants for compound 6 and common anions

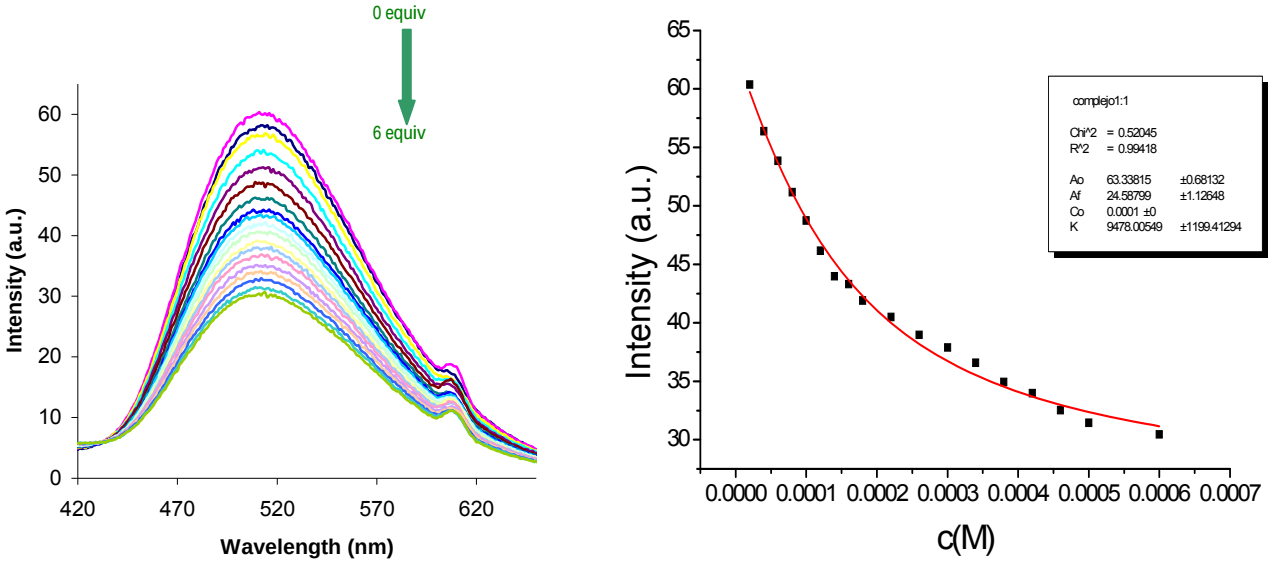
Anions	Complex	$logK_1$	R^2
BzO^-	1:1	5.118 ± 0.006	0.999
AcO^-	1:1	4.503 ± 0.023	0.988
CN^-	1:1	6.022 ± 0.179	0.992
F^-	1:1	5.169 ± 0.158	0.991
$H_2PO_4^-$	1:1	4.968 ± 0.047	0.994

Complexation constants for compound 6 in DMSO and common anions

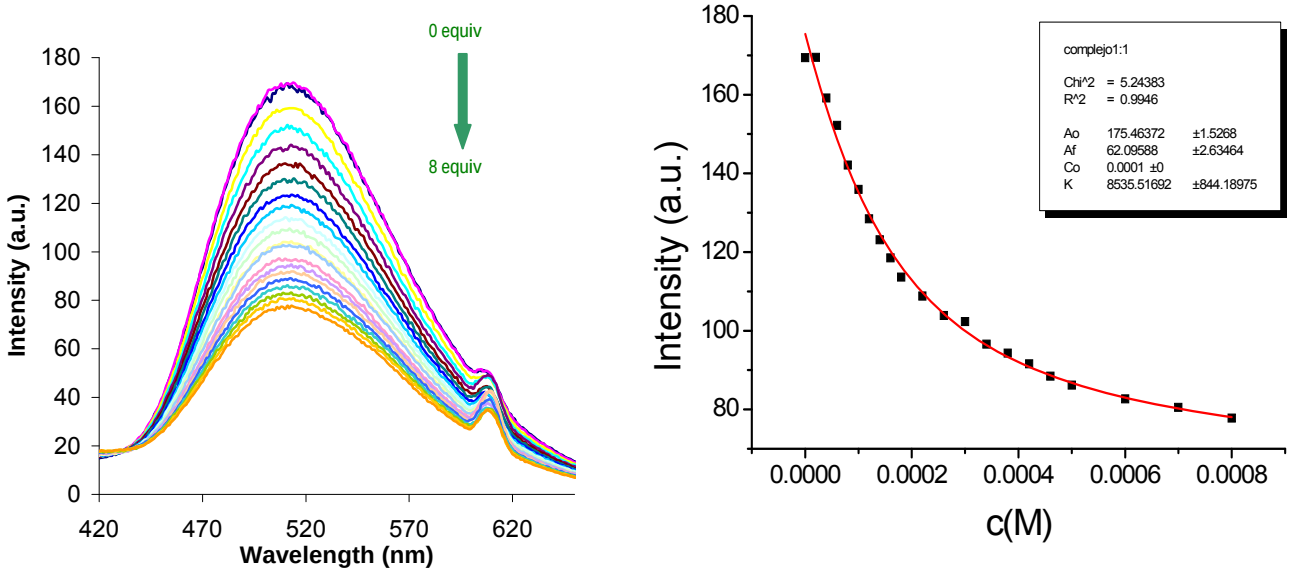
Anions	Complex	$logK_1$	R^2
BzO^-	1:1	5.118 ± 0.006	0.999
AcO^-	1:1	4.503 ± 0.023	0.988
CN^-	1:1	6.022 ± 0.179	0.992
F^-	1:1	5.169 ± 0.158	0.991
$H_2PO_4^-$	1:1	4.968 ± 0.047	0.994

Complexation constants for compound 6 in DMSO:H_2O (9:1) and common anions

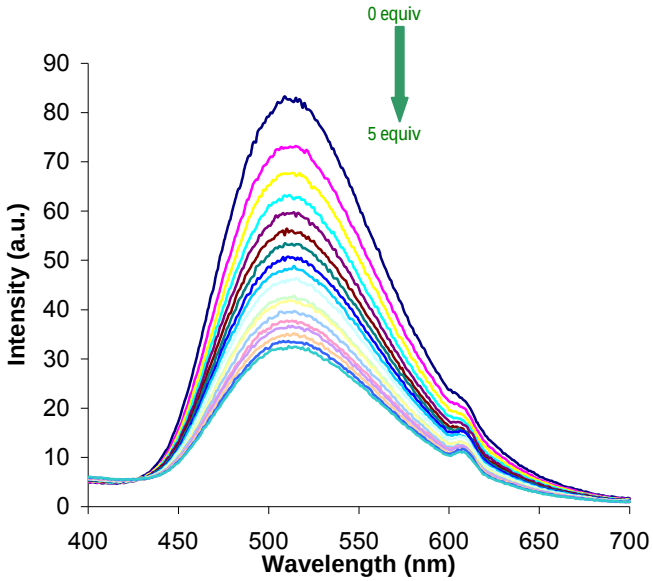
Fluorimetric titrations of 1,3-bis[4-(1-dicyanomethyleneindan-5-yl)phenylureidomethyl)benzene 6 in DMSO with α-aminoacids:

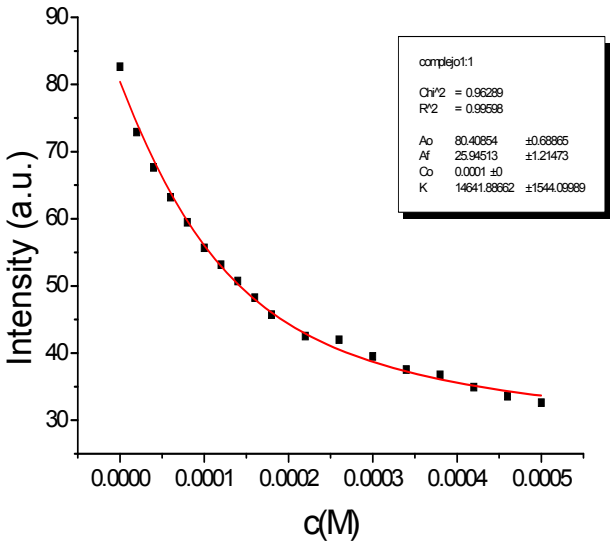


Left: Emission spectra for the fluorimetric titration of **6** (10^{-4} M in DMSO) with *L*-arginine in water (λ_{exc} = 306 nm). Right: Titration profile fitted to a 1:1 model (λ_{max} = 511 nm) for the titration of **6** with *L*-arginine

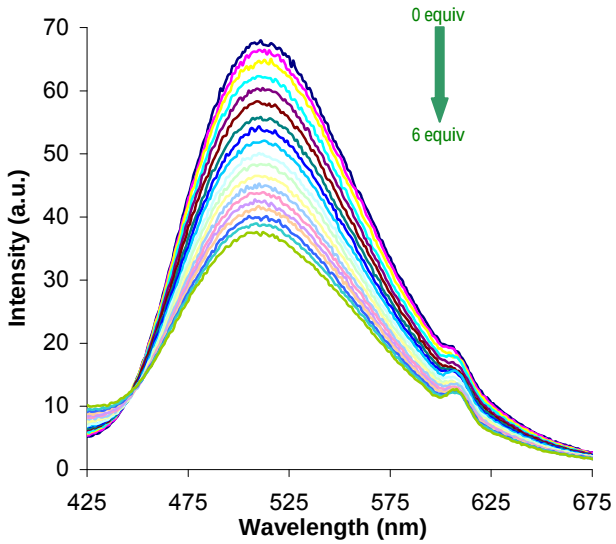


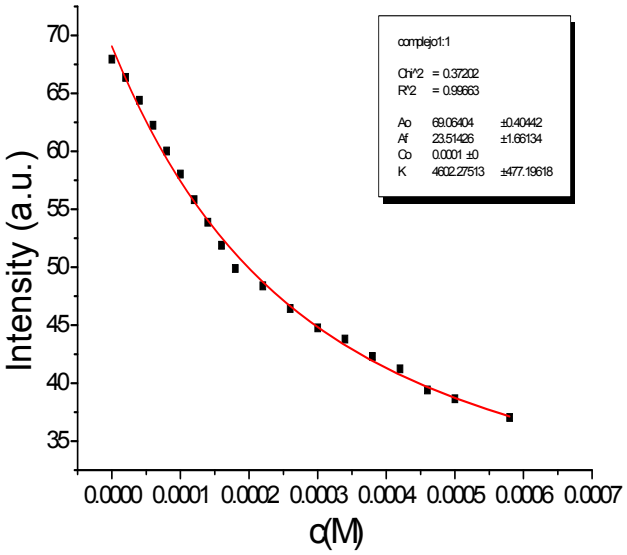
Left: Emission spectra for the fluorimetric titration of **6** (10^{-4} M in DMSO) with *L*-asparagine in water (λ_{exc} = 306 nm). Right: Titration profile fitted to a 1:1 model (λ_{max} = 511 nm) for the titration of **6** with *L*-asparagine.





Left: Emission spectra for the fluorimetric titration of **6** (10^{-4} M in DMSO) with *L*-glutamine in water (λ_{exc} = 306 nm). Right: Titration profile fitted to a 1:1 model (λ_{max} = 511 nm) for the titration of **6** with *L*-glutamine.

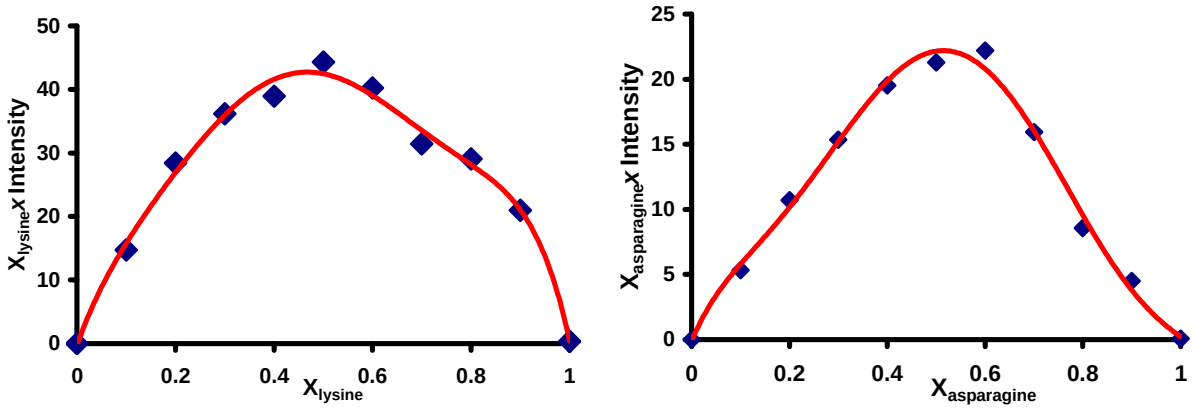




Left: Emission spectra for the fluorimetric titration of **6** (10^{-4} M in DMSO) with *L*-lysine in water (λ_{exc} = 306nm). Right: Titration profile fitted to a 1:1 model (λ_{max} = 511 nm) for the titration of **6** with *L*-lysine

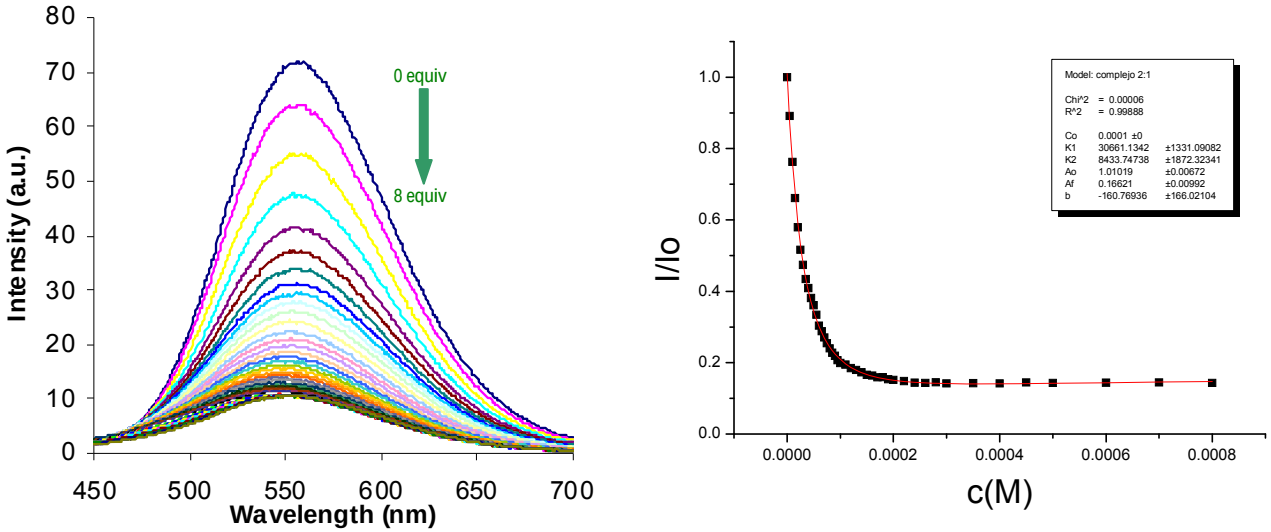
Job's plot analysis of fluorescence titrations of 6 with *L*-lysine and *L*-asparagine

Job's plot analysis of fluorescence titrations of **6** with *L*-lysine and *L*-asparagine revealed maximum peaks at 50% mole fraction, in accord with the proposed 1:1 binding stoichiometry

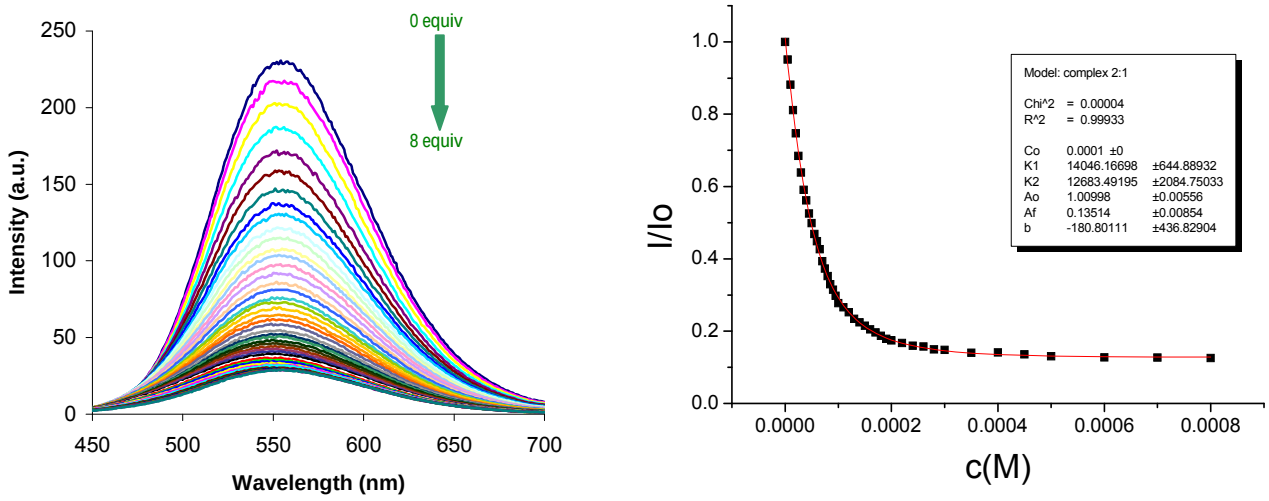


Left: Job's plot analysis of fluorescence titrations of **6** (10^{-4} M in DMSO) with *L*-lysine in water (λ_{exc} = 350 nm) (λ_{em} = 515 nm). Right: Job's plot analysis of fluorescence titrations of **6** (10^{-4} M in DMSO) with *L*-asparagine in water (λ_{exc} = 396 nm) (λ_{em} = 557 nm)

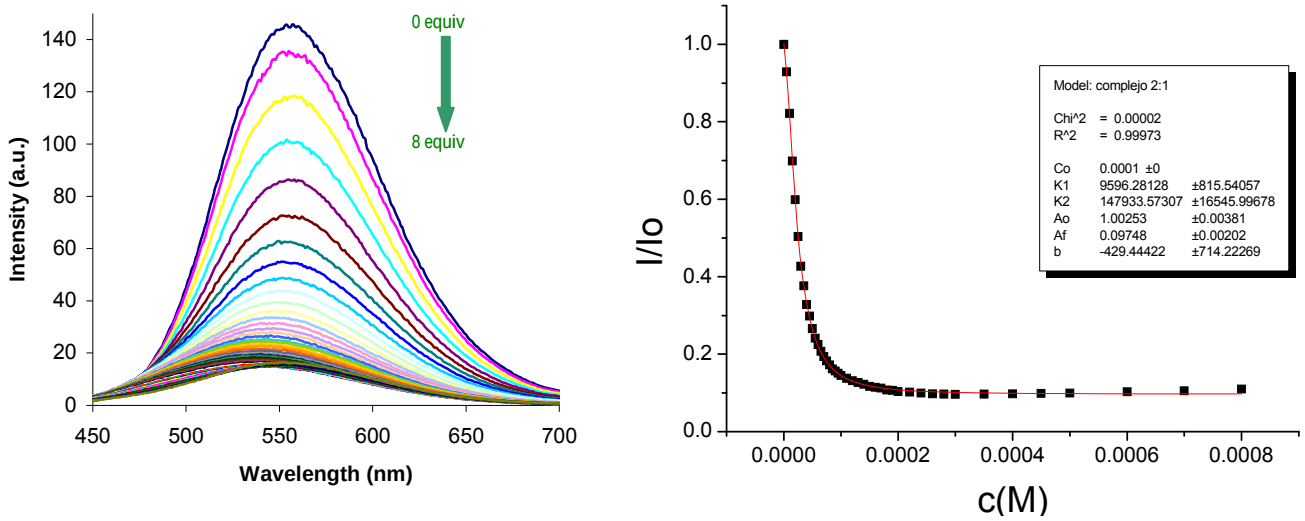
Fluorimetric titrations of 1,3-bis[4-(1-dicyanomethyleneindan-5-yl)phenylureidomethyl)benzene 6 in DMSO with ω-aminoacids:



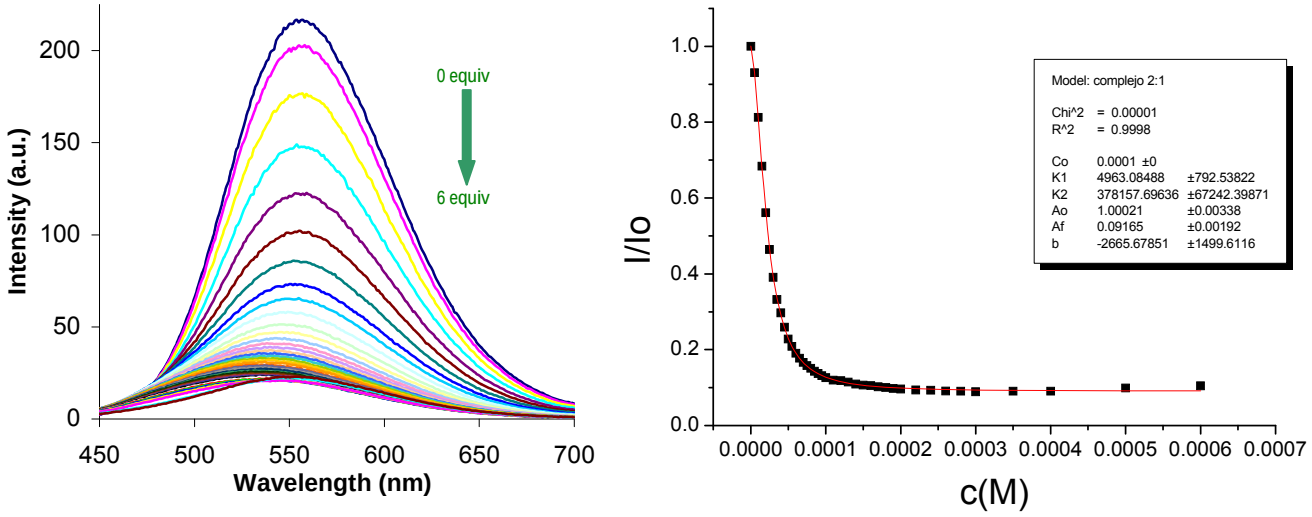
Left: Emission spectra for the fluorimetric titration of **6** (10^{-4}M in DMSO) with β-alanine in water (λ_{exc}= 396 nm). Right: Titration profile fitted to a 2:1 model (λ_{max} = 562 nm) for the titration of **6** with β-alanine.



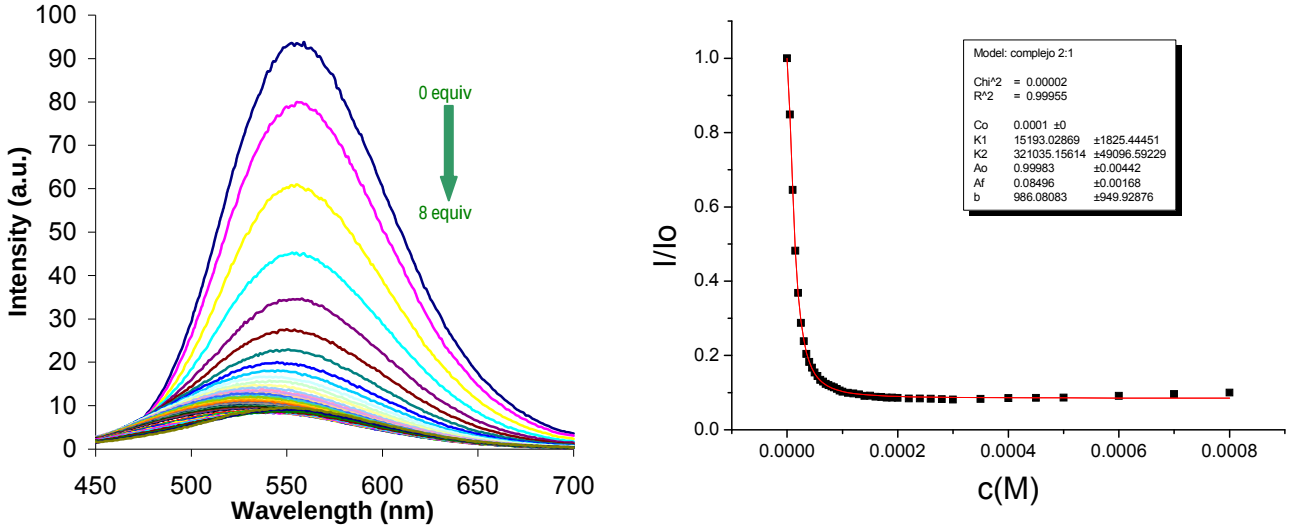
Left: Emission spectra for the fluorimetric titration of **6** (10^{-4}M in DMSO) with γ-aminobutiric acid in water (λ_{exc} = 396 nm). Right: Titration profile fitted to a 2:1 model (λ_{max} = 562 nm) for the titration of **6** with γ-aminobutiric acid.



Left: Emission spectra for the fluorimetric titration of **6** (10^{-4}M in DMSO) with 5-aminovaleric acid in water (λ_{exc} = 396 nm). Right: Titration profile fitted to a 2:1 model (λ_{max} = 562 nm) for the titration of **6** with 5-aminovaleric acid.



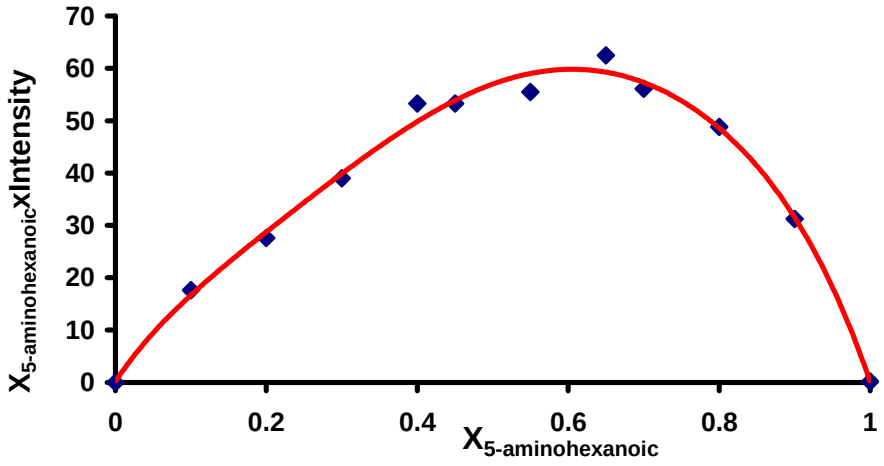
Left: Emission spectra for the fluorimetric titration of **6** (10^{-4}M in DMSO) with 6-aminohexanoic acid in water (λ_{exc} = 396 nm). Right: Titration profile fitted to a 2:1 model (λ_{max}= 562 nm) for the titration of **6** with 6-aminohexanoic acid.



Left: Emission spectra for the fluorimetric titration of **6** (10^{-4}M in DMSO) with 7-aminoheptanoic acid in water (λ_{exc} = 396 nm). Right: Titration profile fitted to a 2:1 model (λ_{max} = 562 nm) for the titration of **6** with 7-aminoheptanoic acid.

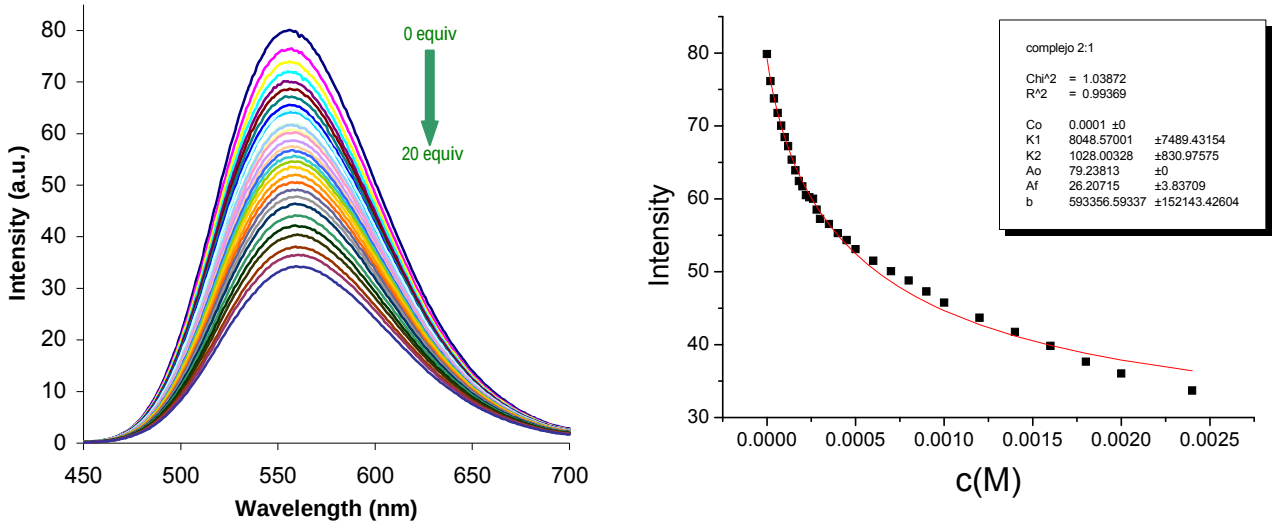
Job's plot analysis of fluorescence titrations of 6 with 6-aminohexanoic acid

Job's plot analysis of fluorescence titrations of **6** with 6-aminohexanoic acid revealed a maximum at 66% mole fraction, in accord with the proposed 2:1 binding stoichiometry

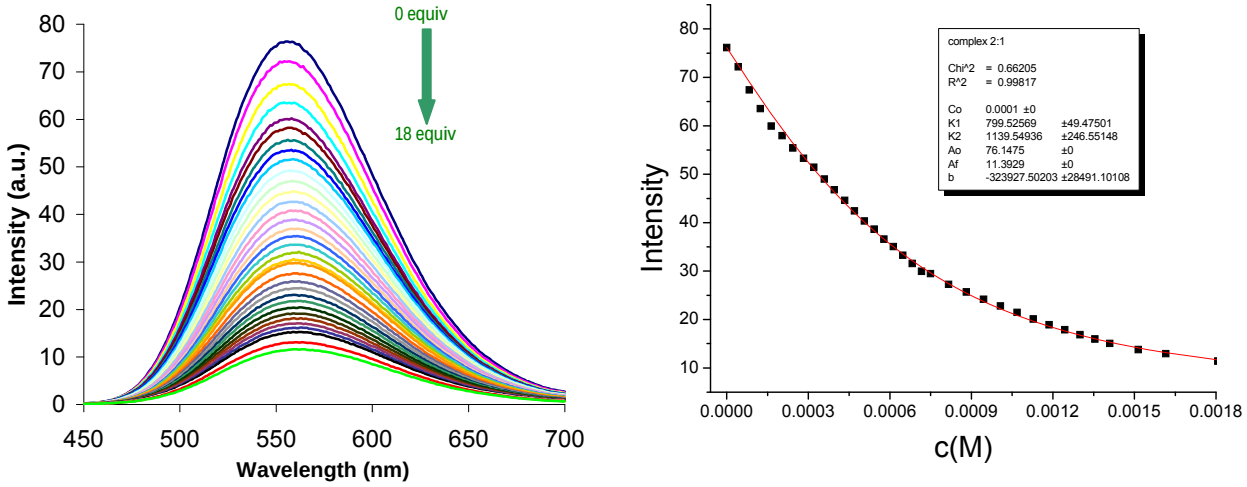


Job's plot analysis of fluorescence titrations of **6** (10^{-4} M in DMSO) with 6-aminohexanoic acid in water (λ_{exc} = 350 nm) (λ_{em} = 515 nm).

Fluorimetric titrations of 1,3-bis[4-(1-dicyanomethyleneindan-5-yl)phenylureidomethyl)benzene 6 in DMSO with gabapentin and pregabalin in water:



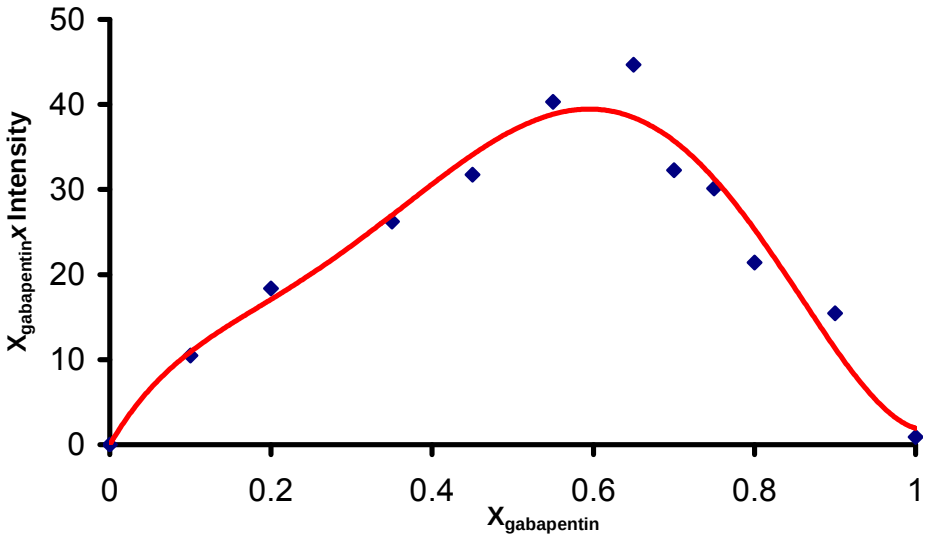
Left: Emission spectra for the fluorimetric titration of **6** (10^{-4}M in DMSO) with gabapentin in water (λ_{exc} = 428 nm). Right: Titration profile fitted to a 2:1 model (λ_{max} = 555 nm) for the titration of **6** with gabapentin.



Left: Emission spectra for the fluorimetric titration of **6** (10^{-4}M in DMSO) with pregabalin in water (λ_{exc} = 426 nm). Right: Titration profile fitted to a 2:1 model (λ_{max}= 554 nm) for the titration of **6** with pregabalin.

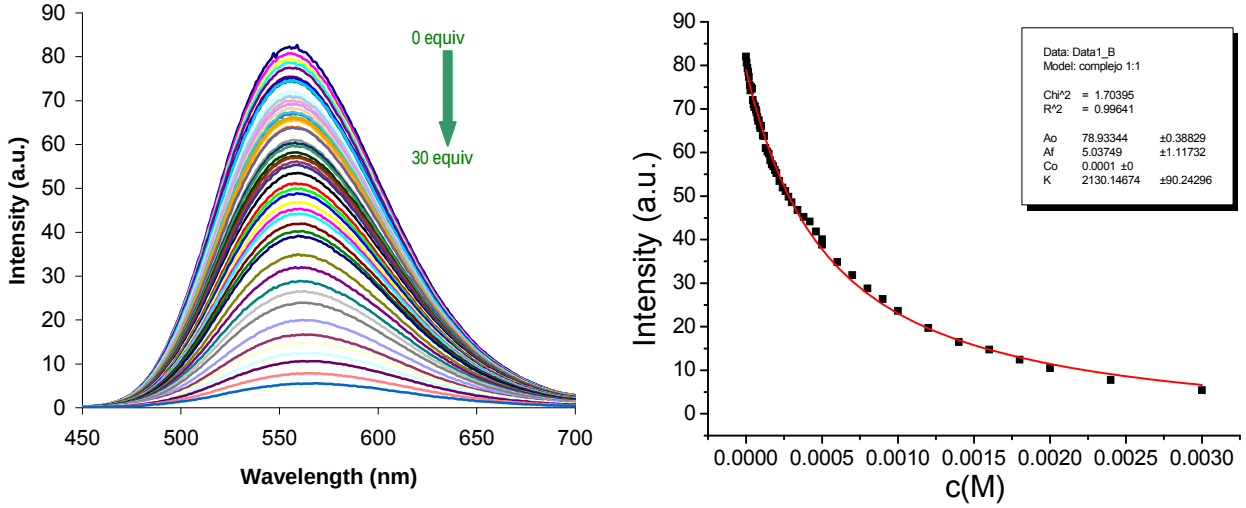
Job's plot analysis of fluorescence titrations of 6 with gabapentin

Job's plot analysis of fluorescence titrations of **6** with gabapentin acid revealed a maximum at 66% mole fraction, in accord with the proposed 2:1 binding stoichiometry

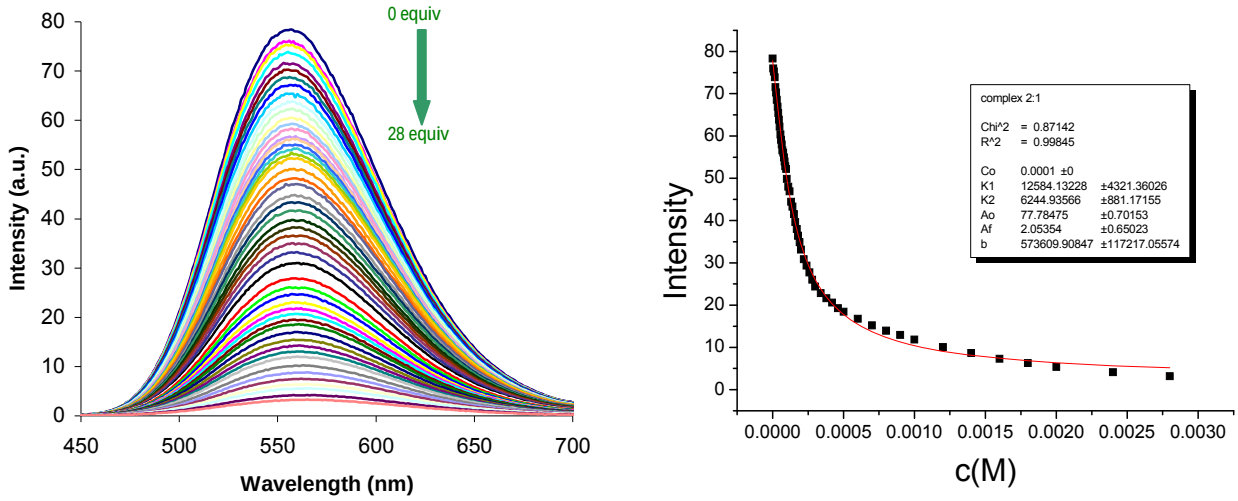


Job's plot analysis of fluorescence titrations of **6** (10^{-4} M in DMSO) with gabapentin in water (λ_{exc} = 350 nm) (λ_{em} = 515 nm).

Fluorimetric titrations of 1,3-bis[4-(1-dicyanomethyleneindan-5-yl)phenylureidomethyl)benzene 6 in DMSO with creatine and *L*-carnitine:



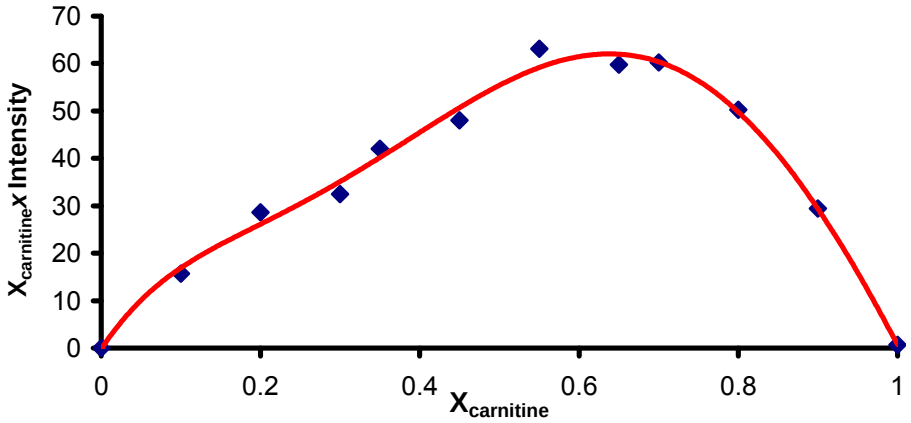
Left: Emission spectra for the fluorimetric titration of **6** (10^{-4}M in DMSO) with creatine in water (λ_{exc} = 428 nm). Right: Titration profile fitted to a 1:1 model (λ_{max}= 557 nm) for the titration of **6** with creatine.



Left: Emission spectra for the fluorimetric titration of **6** (10^{-4} M in DMSO) with *L*-carnitine in water (λ_{exc}= 426 nm). Right: Titration profile fitted to a 2:1 model (λ_{max} = 555 nm) for the titration of **6** with *L*-carnitine.

Job's plot analysis of fluorescence titrations of 6 with *L*-carnitine

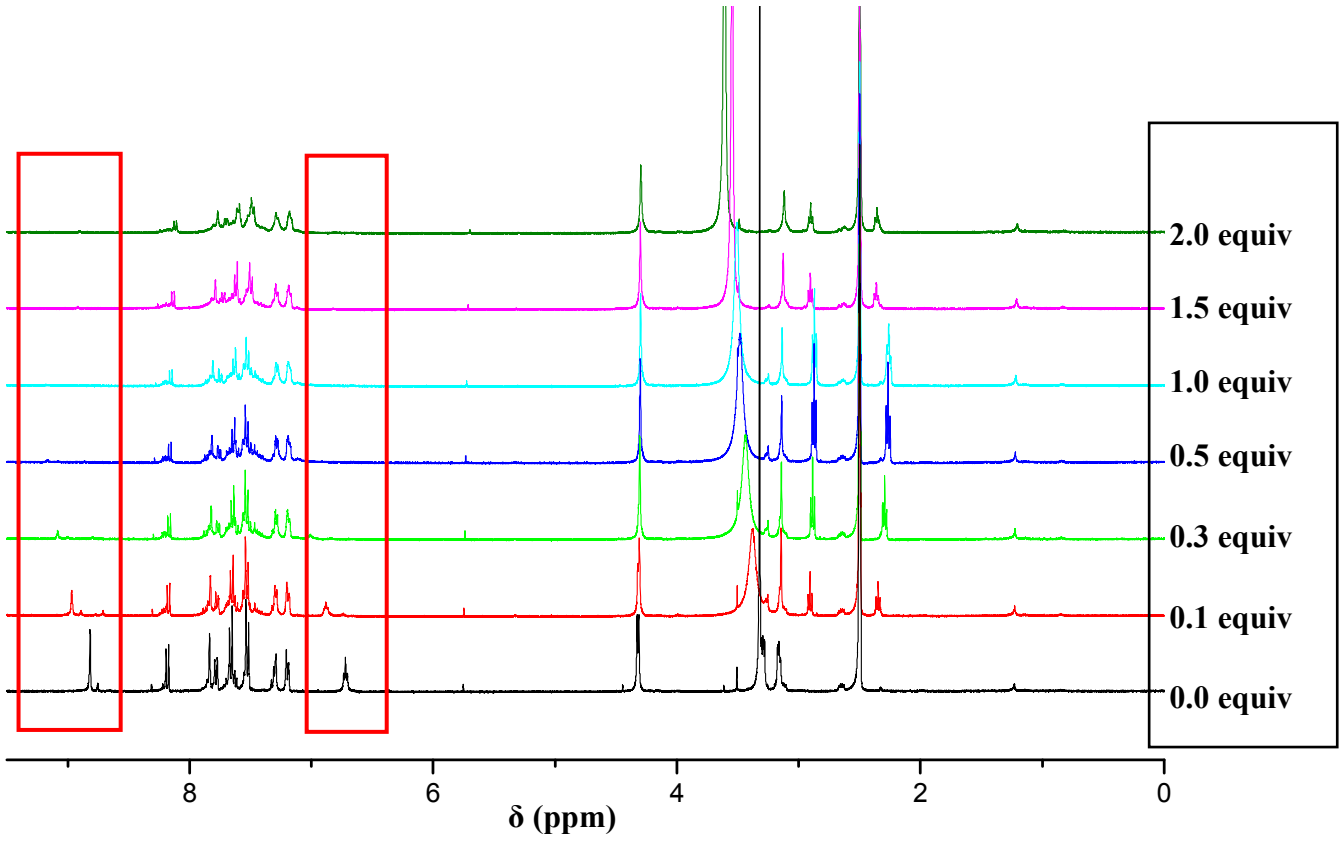
Job's plot analysis of fluorescence titrations of **6** with *L*-carnitine revealed a maximum at a 66% mole fraction, in accord with the proposed 2:1 binding stoichiometry



Job's plot analysis of fluorescence titrations of **6** (10^{-4} M in DMSO) with *L*-carnitine in water (λ_{exc} = 350 nm) (λ_{em} = 515 nm).

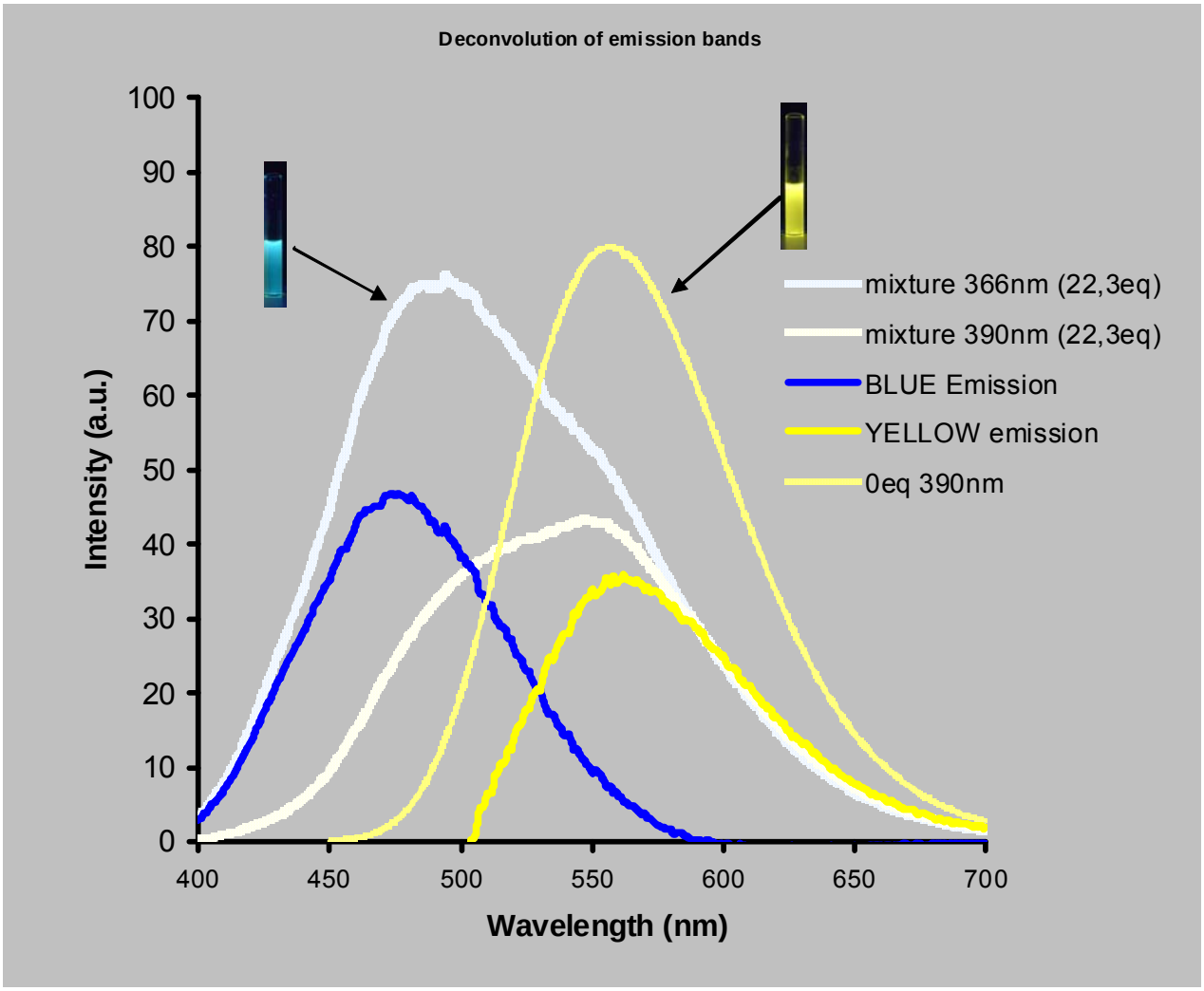
^{1}H NMR titration experiments of 1,3-bis[4-(1-dicyanomethyleneindan-5-yl)phenylureidomethyl)-benzene 6 (10^{-1} M solution in DMSO) with β-alanine in DMSO.

By addition of increasing amounts of β-alanine, the two urea NH signals at δ 8.8 and 6.7 move to lower field and progressively disappear as the H-bonds are formed. The aromatic signals become more complicate but their chemical shifts remain unchanged.



^{1}H NMR titration of 6 (10^{-1} M solution in DMSO-d_6) with β-alanine in DMSO-d_6.

The blue emission: deconvolution of the emission bands after titration:



0 eq. 390 nm: the original fluorescence spectrum at λ_{exc} = 390 nm of **6** (10^{-4} M in DMSO).

Mixture 390 nm (22.3 equiv): Fluorescence spectrum at λ_{exc} = 390 nm after addition of 22.3 equivalents of *L*-carnitine to the previous solution.

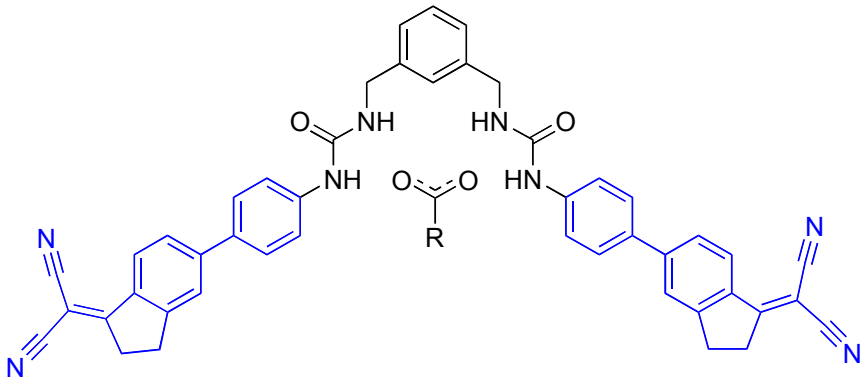
Mixture 366 nm (22.3 equiv): Fluorescence spectrum at λ_{exc} = 366 nm of the same mixture used in the acquisition of the previous spectrum.

BLUE Emission: Fluorescence spectrum of the blue emission obtained by substraction of the yelow emission spectrum at λ_{exc} = 390 nm from the bluish emission spectrum obtained at λ_{exc} = 366 nm from the mixture of **6** and 22.3 equivalents of the analite (λ_{max} = 475 nm).

YELLOW Emission: Fluorescence spectrum of the yellow emission obtained by substraction of the blue emission spectrum at λ_{exc} = 366 nm from the emission spectrum obtained at λ_{exc} = 366 nm from the mixture of **6** and 22.3 equivalents of the analite.

Calculations:

All calculations were performed with the Gaussian 03 set of programs using the ONIOM method.[1] Geometry optimizations were conducted using a two-level ONIOM procedure[2, 3] in which the hybrid functional B3LYP[4, 5] along with the basis set 6-31G+(d,p) was used as the higher level of theory and the semiempirical AM1 model[6] was used as the lower level. After the optimization, analytical computations of the Hessian matrix were performed to compute the nature of the located minima on the potential energy surface. The real and model systems used in the two-layer ONIOM(MO:MO) calculations are shown in the figure.



Computational Model
B3LYP/AM1

References:

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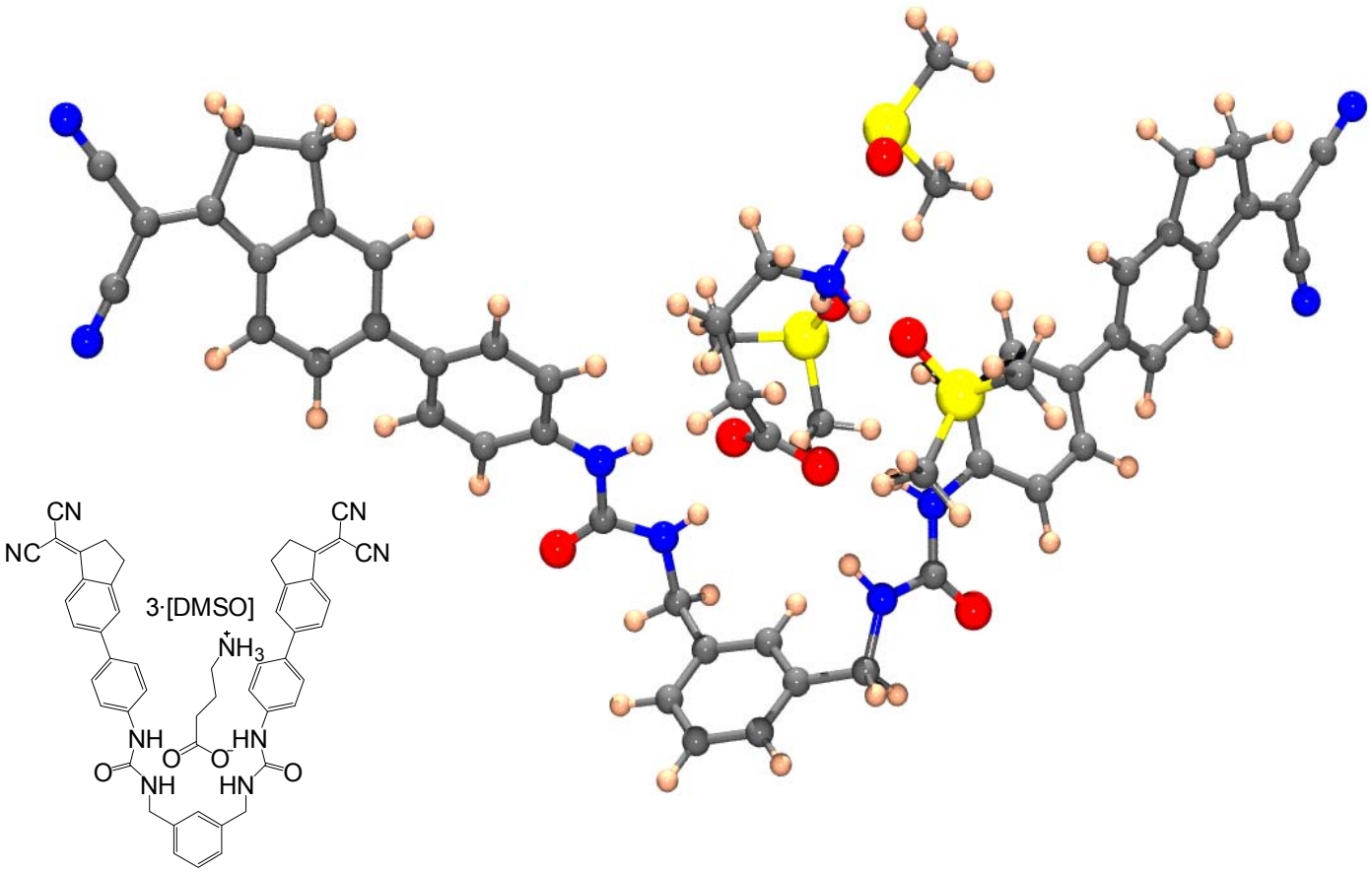
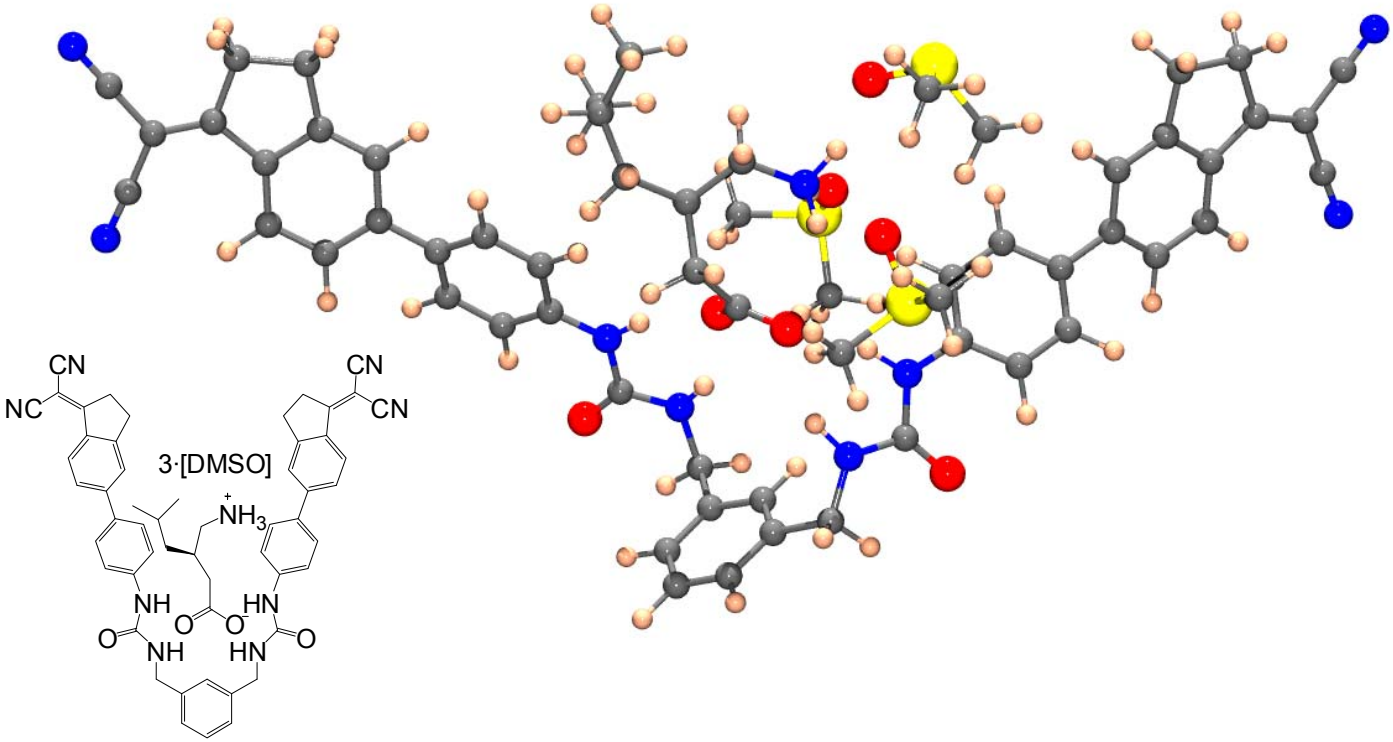


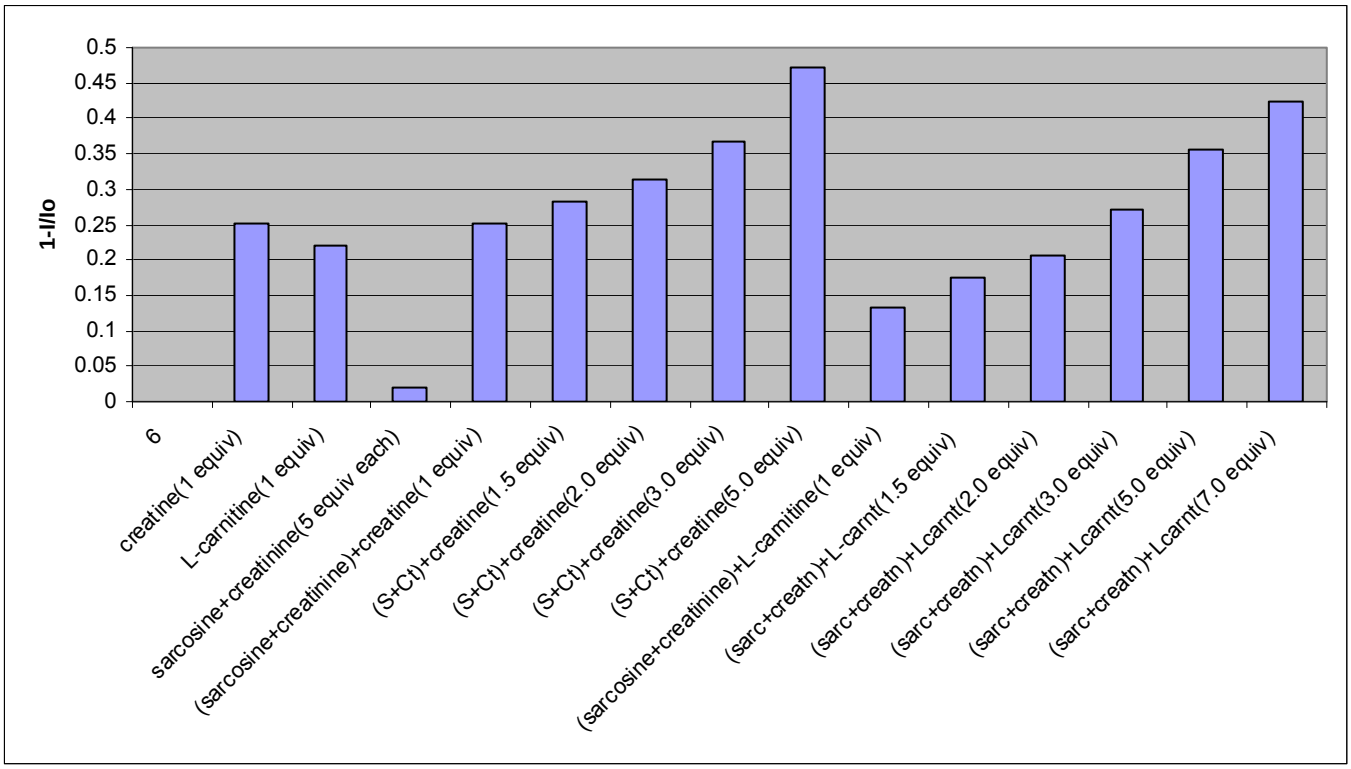
Optimized geometry of the **6**:GABA·3DMSO complex at the ONIOM (B3LYP/6-31G*:AM1) in GAUSSIAN 03.

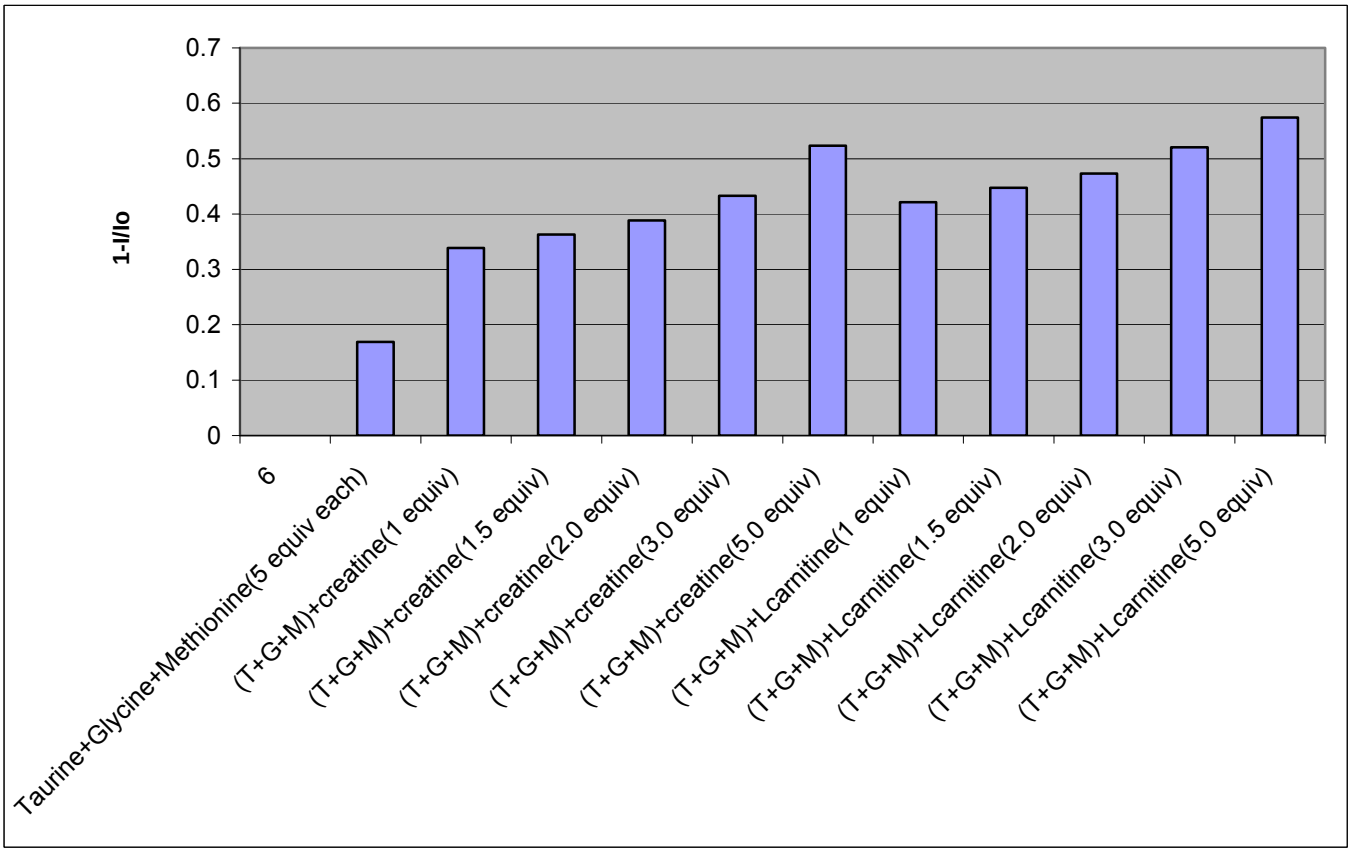


Optimized geometry of the **6**:PREGABALIN·3DMSO complex at the ONIOM (B3LYP/6-31G*:AM1) in GAUSSIAN 03.

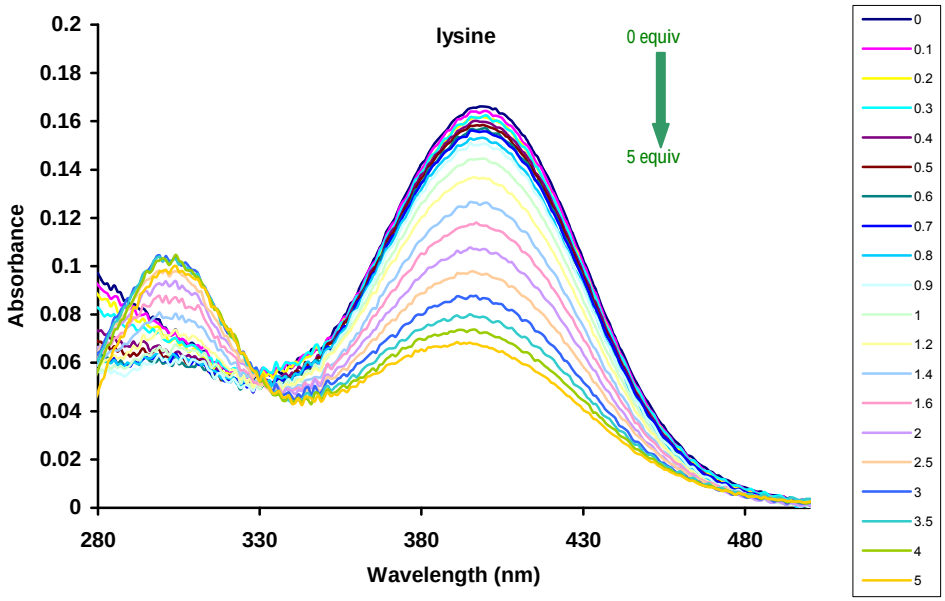
INTERFERENCE EXPERIMENTS:

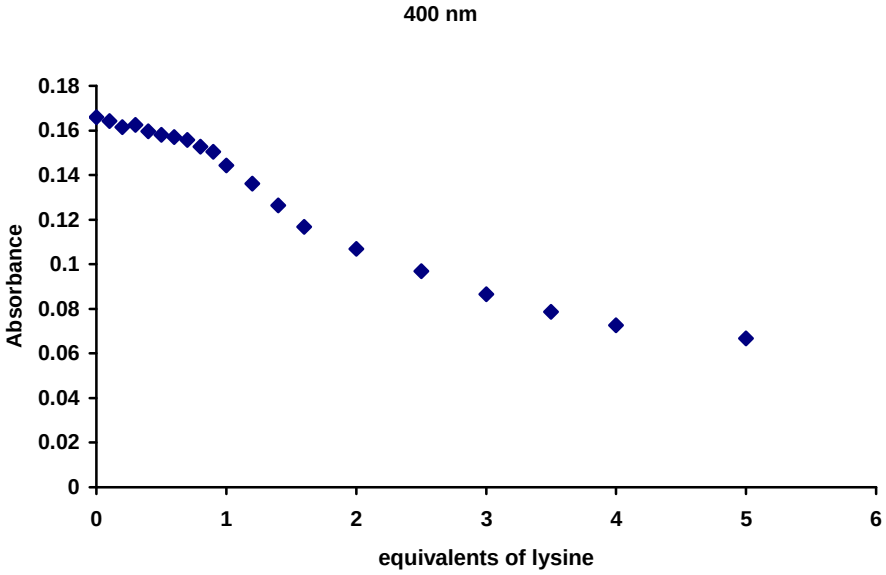
We realized some interference experiments by adding 5 equivalents of interference compounds and then succesive equivalents of the measured analyte (0.5 equiv, 1 equiv, 2 equiv, 3 equiv, 5 equiv, 7 equiv) and represented in the plots the variation of the fluorescence intensity in each case.





Ultraviolet-visible titration of 1,3-bis[4-(1-dicyanomethyleneindan-5-yl)phenylureidomethyl)-benzene 6 in DMSO with *L*-lysine in water:





Up: UV-visible absorption spectra for the colorimetric titration of **6** (10^{-5} M in DMSO) with *L*-lysine in water. Down: Titration profile of the λ_{max} = 400 nm for the UV-visible titration of **6** with *L*-lysine

Titration Materials and Methods.

5×10^{-2} M, 5×10^{-3} M, 5×10^{-4} M solutions of every salt or aminoacid were prepared, then a 10^{-4} M solution of the compound under study was prepared. For qualitative experiments, 2 mL solution of the compound under study were measured and the corresponding amount of salt or aminoacid was added by micropipette.

Eppendorf Research micropipette characteristics:

MODEL	Ep T.I.P.S.	Volume	Systematic error of measurement	Random error of measurement (CV)
2 - 20µL	2 - 200	2 µL	± 5.0 %	≤ 1.5 %
		10 µL	± 1.2 %	≤ 0.6 %
		20 µL	± 1.0 %	≤ 0.3 %
10 - 100µL	2 - 200	10 µL	± 3.0 %	≤ 1.0 %
		50 µL	± 1.0 %	≤ 0.3 %
		100 L	± 0.8 %	≤ 0.2 %
100 - 1000µL	50 - 1000	100 µL	± 3.0 %	≤ 0.6 %
		5000 µL	± 1.0 %	≤ 0.2 %
		1000 µL	± 0.6 %	≤ 0.2 %
500 - 5000µL	100 - 5000	500 µL	± 2.4 %	≤ 0.6 %
		2500 µL	± 1.2 %	≤ 0.25 %
		5000 µL	± 0.6 %	≤ 0.15 %

Quantitative studies:

As a general procedure, to a 5000 µl solution of complex, the corresponding amount (µL) of solution of the corresponding salt (5×10^{-2} M, 5×10^{-3} M, 5×10^{-4} M solutions) was added, using the minor amount of solvent.

A table, corresponding to the number of equivalents and the corresponding added volumes is included:

Vtotal (µL)	*equiv*	*C*
5000	0	0
5005	0.05	4.995E-06
5010	0.1	9.98E-06
5015	0.15	1.4955E-05
5020	0.2	1.992E-05
5025	0.25	2.4876E-05
5030	0.3	2.9821E-05
5035	0.35	3.4757E-05
5040	0.4	3.9683E-05
5045	0.45	4.4599E-05
5050	0.5	4.9505E-05

Vtotal (µL)	*equiv*	*C*
5055	0.55	5.4402E-05
5060	0.6	5.9289E-05
5065	0.65	6.4166E-05
5070	0.7	6.9034E-05
5075	0.75	7.3892E-05
5080	0.8	7.874E-05
5085	0.85	8.3579E-05
5090	0.9	8.8409E-05
5095	0.95	9.3229E-05
5100	1	9.8039E-05
5105	1.05	0.00010284
5110	1.1	0.00010763
5115	1.15	0.00011241
5120	1.2	0.00011719
5125	1.25	0.00012195
5130	1.3	0.00012671
5135	1.35	0.00013145
5140	1.4	0.00013619
5145	1.45	0.00014091
5150	1.5	0.00014563
5155	1.55	0.00015034
5160	1.6	0.00015504
5165	1.65	0.00015973
5170	1.7	0.00016441
5180	1.8	0.00017375
5190	1.9	0.00018304
5200	2	0.00019231
5210	2.1	0.00020154
5220	2.2	0.00021073
5230	2.3	0.00021989
5240	2.4	0.00022901
5250	2.5	0.0002381
5260	2.6	0.00024715
5270	2.7	0.00025617
5280	2.8	0.00026515
5300	3	0.00028302

Vtotal (µL)	*equiv*	*C*
5320	3.2	0.00030075
5340	3.4	0.00031835
5360	3.6	0.00033582
5380	3.8	0.00035316
5400	4	0.00037037
5405	4.5	0.00041628
5410	5	0.00046211
5415	5.5	0.00050785
5420	6	0.00055351
5430	7	0.00064457
5440	8	0.00073529
5450	9	0.00082569
5460	10	0.00091575