Conformational control of HCl co-transport: imidazole functionalised isopphtalamide vs. 2,6-dicarboxamidopyridine

Philip A. Gale,[*a] Joachim Garric,[a] Mark E. Light,[a] Beth A. McNally,[b] and Bradley D. Smith [*b]

a School of Chemistry, University of Southampton, Southampton, SO17 1BJ, UK. Fax: 44 2380596805; Tel: 44 23 80593332; E-mail: philip.gale@soton.ac.uk
b Department of Chemistry and Biochemistry, University of Notre Dame, IN 46556, USA. Fax: 1 574 631 6652; Tel: 1 574 631 8632; E-mail: smith.115@nd.edu

Synthesis of methyl 6-((1-methyl-1H-imidazol-2-yl)methylcarbamoyl)pyridine-2-carboxylate :

6-(Methoxycarbonyl)pyridine-2-carboxylic acid (1.60 g, 8.8 mmol, 1.0 equiv.) was activated by reaction with thionyl chloride (30.00 mL, 439.0 mmol, 50 equiv.) at 90°C. The solution was heated at reflux for 30 minutes, then the thionyl chloride removed under vaccum. The solid was dissolved in dry dichloromethane, triethylamine (2.45 mL, 17.5 mmol, 2.0 equiv.) and (1-methyl-1H-imidazol-2-yl)methanamine (1.170 g, 10.6 mmol, 1.2 equiv.) were added to the solution. The reaction mixture was stirred at room temperature for 12 hours. After hydrolysis the solution was washed with water. The organic phase was dried over $MgSO_4$, and concentrated. The residue was purified by column chromatography on silica using a mixture CH_2Cl_2/MeOH (93/7) to give 1.56 g (65% yield) of methyl 6-((1-methyl-1H-imidazol-2-yl)methylcarbamoyl)pyridine-2-carboxylate as a white powder. ^{1}H NMR ($CDCl_3$, 300 MHz) : δ = 3.71 (3H, s), 3.99 (3H,s), 4.77 (2H; d; *J*= 6.0 Hz), 6.84 (1H, s), 6.97 (1H,s), 7.99 (1H, t, *J*= 7.8Hz), 8.21(1H, dd, *J*= 7.8, 1.2 Hz), 8.35 (1H, dd, *J*= 7.8, 1.2 Hz), 8.64 (1H, br). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 32.9, 35.4, 52.8, 121.7, 125.4, 127.4, 127.8, 138.5, 144.4, 146.8, 149.6, 163.3, 164.9. IR ν_{max} = 3424, 3295, 2954, 1731, 1667, 1531, 1280, 754 cm^{-1}. Anal: Calcd for $C_{13}H_{14}N_4O_3$ + 0.25 H_2O: C, 56.01; H, 5.24; N, 20.10; O, 18.65. Found C, 56.01; H, 5.07; N, 19.99.

Synthesis of N^2-(4-butylphenyl)-N^6-((1-methyl-1H-imidazol-2-yl)methyl)pyridine-2,6-dicarboxamide 3 :

Methyl 6-((1-methyl-1H-imidazol-2-yl)methylcarbamoyl)pyridine-2-carboxylate (1.52 g mg, 5.6 mmol, 1.0 equi.) was dissolved in 50 mL of a mixture MeOH/H_2O (4/1) then KOH (404 mg, 7.2 mmol, 1.3 equiv.) was added to the solution. The reaction mixture was stirred 12 hours at room temperature. Solvents were removed under vacuum and the white residue was activated by reaction with thionyl chloride (20.25 mL, 277.6 mmol, 50.0 equiv.) at 90°C. The solution was heated at reflux for 30 minutes, and then the thionyl chloride was removed under vaccum. The solid was dissolved in dry dichloromethane, and triethylamine (1.544 mL, 11.1 mmol, 2.00 equi.) and 4-butylaniline (3.500 mL, 22.2 mmol, 4.0 equi.) were added to the solution. The reaction mixture was stirred at room temperature for 12 hours. After hydrolysis the solution was washed with water. The organic phase was dried over $MgSO_4$, and concentrated. The residue was purified by column chromatography using a mixture CH_2Cl_2/MeOH (96/4) to give 1.57 g (72% yield) of compound **3** as a white powder.

^{1}H NMR ($CDCl_3$, 400 MHz) : δ = 0.93 (3H, t, *J*= 7.2 Hz), 1.30-1.40 (2H, m), 1.64-1.54 (2H, m), 2.58 (2H, d, *J*= 8.0 Hz), 3.63 (3H, s), 4.71 (2H, d, *J*= 5.6 Hz), 6.76 (1H, s), 6.87 (1H, s), 7.12 (2H, d, *J*= 8.0 Hz), 7.44 (2H, d, *J*= 8.0 Hz), 8.03 (1H, t, *J*= 8.0 Hz), 8.34 (1H, d, *J*= 8.0 Hz), 8.41 (1H, d, *J*= 8.0 Hz), 9.87-9.89 (2H, m). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 13.9, 22.3, 32.8, 33.6, 35.1, 35.5, 120.7, 121.9, 125.0, 125.2, 127.0, 128.6, 134.9, 139.0, 139.4, 144.9, 148.5, 149.2, 161.2 163.5. IR ν_{max} = 3312, 2931, 1682, 1544, 747 cm^{-1}. Anal: Calcd for $C_{22}H_{25}N_5O_2$: C, 67.50; H, 6.44; N, 17.89; O, 8.17. Found C, 67.43; H, 6.53; N, 17.87.

Synthesis of *N*^{1}-(4-butylphenyl)-*N*^{3}-(2-mercaptothiazolide)-isophthalamide :

N^1,*N*^{3}-Bis(2-mercaptothiazolides)-isophthalamide (500 mg, 1.4 mmol, 1.0 equiv.) was dissolved in 10mL of dry CH_2Cl_2 and then 4-butylaniline (214 µL, 1.4 mmol, 1.0 equiv.) was added to the solution. The reaction mixture was stirred 3 days at room temperature. The solution was washed with 1M NaOH aqueous solution (3x10 mL). The organic phase was dried over $MgSO_4$, and concentrated. The residue was purified by column chromatography using a mixture CH_2Cl_2/AcOEt (98/2) on silica to give 110 mg (20% yield) of *N*^{1}-(4-butylphenyl)-*N*^{3}-(2-mercaptothiazolide)-isophthalamide as a yellow powder. ^{1}H NMR ($CDCl_3$, 300 MHz) : δ = 0.93 (3H, t, *J*= 7.5 Hz), 1.30-1.42 (2H, m), 1.55-1.65 (2H, m), 2.60 (2H, t, *J*= 7.8 Hz), 3.50 (2H, t, *J*= 7.2 Hz), 4.57 (2H, t, *J*= 7.2 Hz), 7.18 (2H, d, *J*= 8.4 Hz), 7.50-7.54 (3H, m), 7.81-7.86 (2H, m), 8.01 (1H, d, *J*= 8.1Hz), 8.15 (1H, s). ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 13.0, 22.3, 29.7, 33.6, 35.1, 56.5, 120.4, 127.9, 128.9, 129.0, 131.1, 132.4, 134.2, 135.2, 135.4, 139.6, 164.5, 170.4, 202.3. IR ν_{max} = 3330, 3099, 2927, 1663, 1519, 1531, 643 cm^{-1}. . Anal: Calcd for $C_{21}H_{22}N_2O_2S_2$: C, 63.29; H, 5.56; N, 7.03; O, 8.03; S; 16.09. Found C, 63.42; H, 5.72; N, 7.09.

Synthesis of compound *N*^{1}-(4-butylphenyl)-*N*^{3}-((1-methyl-1H-imidazol-2-yl)methyl)isophthalamide 4 :

N^1-(4-Butylphenyl)-*N*^{3}-(2-mercaptothiazolide)-isophthalamide (100 mg, 2.5 mmol, 1.0 equi.) was dissolved in 10mL of dry CH_2Cl_2 then (1-methyl-1H-imidazol-2-yl)methanamine (56 mg , 5.0 mmol, 2.0 equi.) was added to the solution. The reaction mixture was stirred 3 days at room temperature. The solution was washed with 1M NaOH aqueous solution (3x10 mL). The organic phase was dried over $MgSO_4$, and concentrated. The residue was purified by column chromatography using a mixture CH_2Cl_2/AcOEt (93/7) on silica to give 90 mg (92% yield) of *N*^{1}-(4-butylphenyl)-*N*^{3}-((1-methyl-1H-imidazol-2-yl)methyl)isophthalamide **4** as a white powder.
^{1}H NMR ($CDCl_3$, 300 MHz) : δ = 0.95 (3H, t, *J*= 7.5 Hz), 1.34-1.41 (2H, m), 1.59-1.64 (2H, m), 2.61 (2H, d, *J*= 7.5 Hz); 3.51 (3H, s), 4.53 (2H, d, *J*= 5.1 Hz), 6.51 (1H, s), 6.90 (1H, s), 7.12-7.17 (3H, m), 7.54 (2H, d, *J*= 7.4 Hz), 7.68 (1H, d, *J*= 7.5 Hz), 7.76 (1H, d, *J*= 7.5 Hz), 8.12 (1H, s), 8.66 (1H, s), 10.98 (1H, br). ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 13.9, 22.2, 32.8, 33.8, 35.1, 36.0, 120.8, 121.6, 124.1, 126.2, 128.4, 128.8, 130.5, 131.2, 132.5, 136.3, 136.34, 138.7, 145.8, 166.4, 167.3. IR ν_{max} = 3248, 2925, 2850, 1667, 1531, 1531, 1322, 702 cm^{-1}. . Anal: Calcd for $C_{23}H_{26}N_4O_2$: C, 70.75; H, 6.71; N, 14.34; O, 8.19. Found C, 70.40; H, 6.75; N, 14.25.

Synthesis of compound N^2,N^6-bis(4-butylphenyl)pyridine-2,6-dicarboxamide 5 :

Isophthaloyl dichloride (1.00 g, 4.9 mmol, 1.0 equiv.) was dissolved in 50 mL of dry THF, triethylamine (2.73 mL, 19.6 mmol, 4.0 equiv.) and 4-butylaniline (2.31 mL, 14.7 mmol, 3.0 equiv.) were added to the solution. The reaction mixture was stirred at room temperature for 12 hours and the solution subsequently was washed with water. The organic phase was dried over $MgSO_4$, and concentrated. The residue was purified by column chromatography using a mixture CH_2Cl_2/MeOH (96/4) to give 1.610 mg (77% yield) of compound **3** as a white powder. ^{1}H NMR ($CDCl_3$, 300 MHz) : δ = 0.94 (6H, t, *J*= 7.2 Hz); 1.31-1.43 (4H, m), 1.56-1.66 (4H, m); 2.62 (4H; *J*= 7.5 Hz), 7.20 (4H, d, *J*= 7.4 Hz), 7.64 (4H, d, *J*= 7.4 Hz), 8.10 (1H, t, *J*= 7.5 Hz), 8.47 (2H, d, *J*= 7.5 Hz), 9.48 (s, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 13.9, 22.2, 33.6, 35.1, 120.2, 125.4, 129.1, 134.7, 139.5, 139.8, 149.1, 161.0. IR ν_{max} = 3299, 2927, 2856, 1661, 1522, 827 cm^{-1}. Anal: Calcd for $C_{27}H_{31}N_3O_2$: C, 75.5; H, 7.27; N, 9.78; O, 7.45. Found C, 75.55; H, 7.34; N, 9.80.

NMR spectra

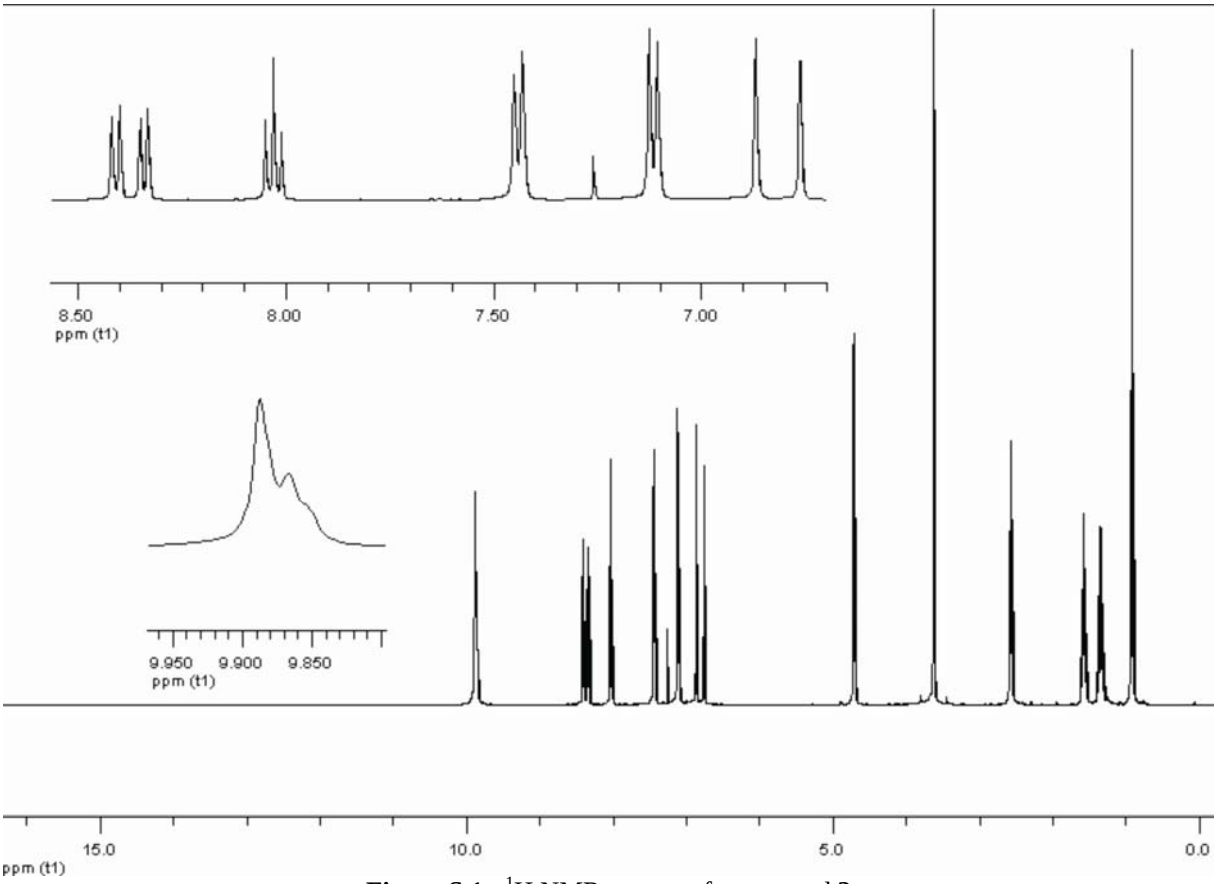

Figure S 1 : ^{1}H NMR spectra of compound **3**

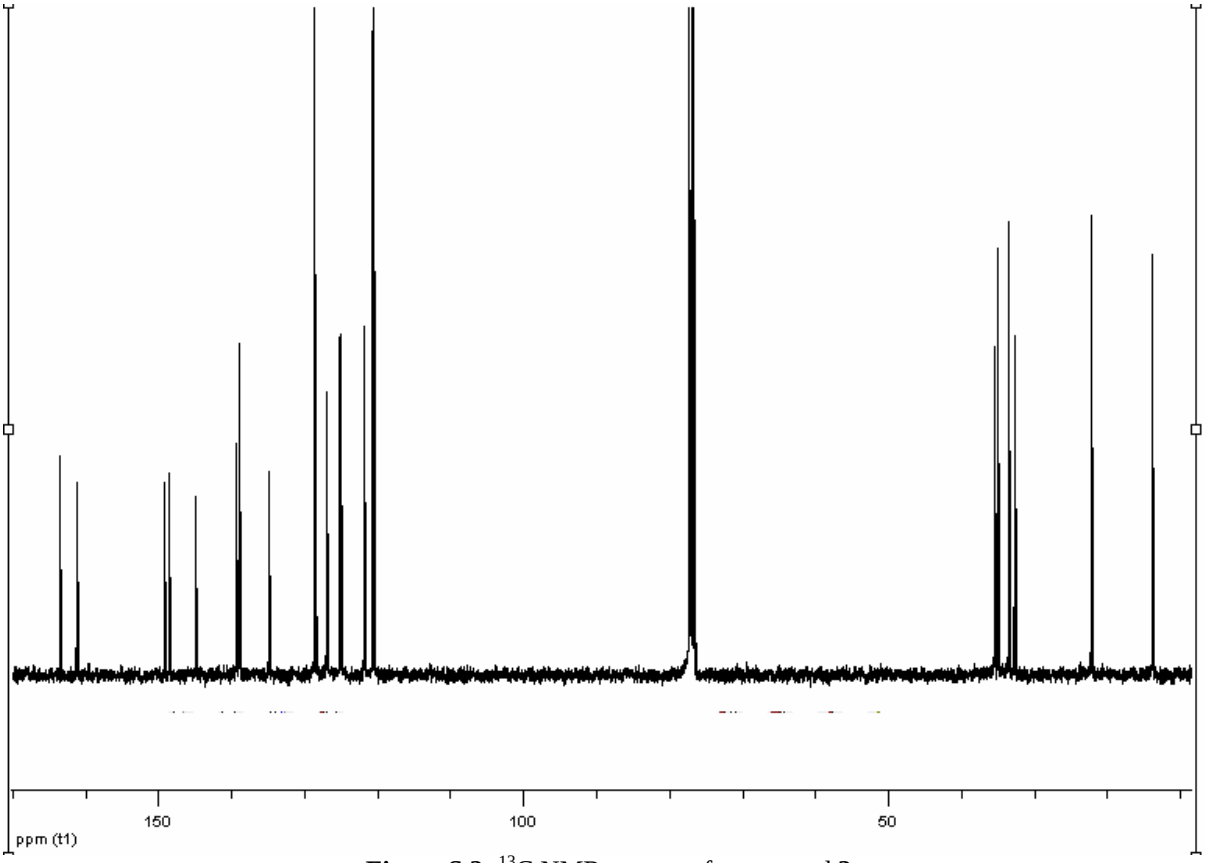

Figure S 2: ^{13}C NMR spectra of compound **3**

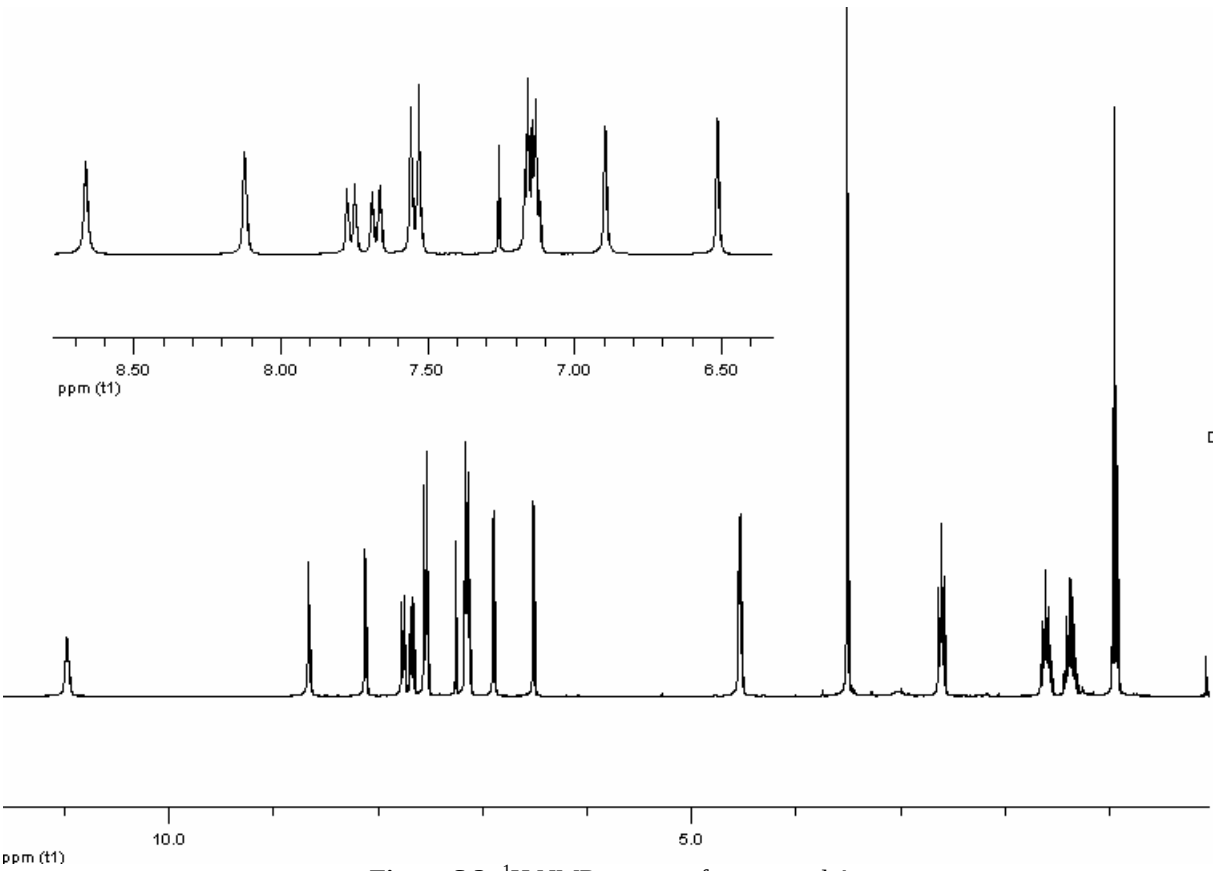

Figure S 3: ^{1}H NMR spectra of compound **4**

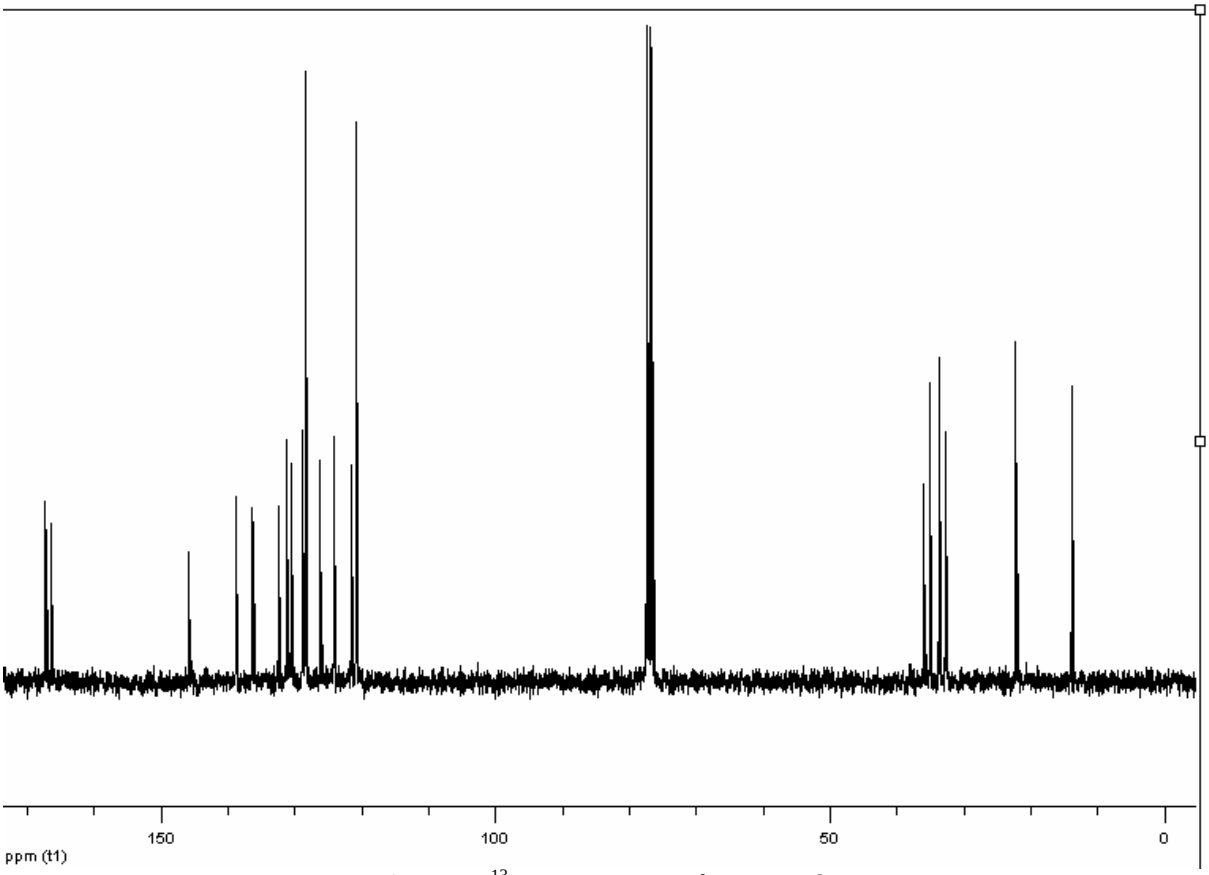

Figure S 4^{13}C NMR spectra of compound **4**

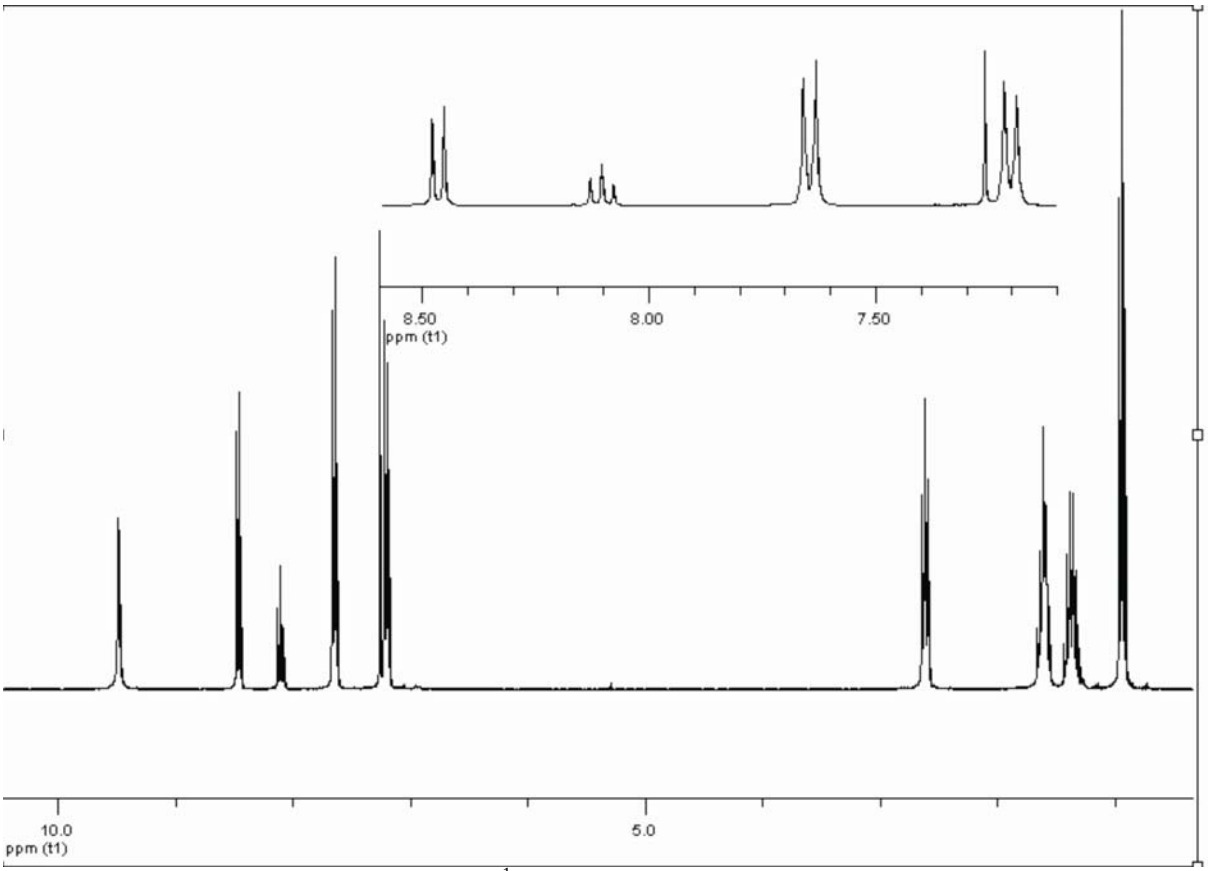

Figure S 5: ^{1}H NMR spectra of compound **5**

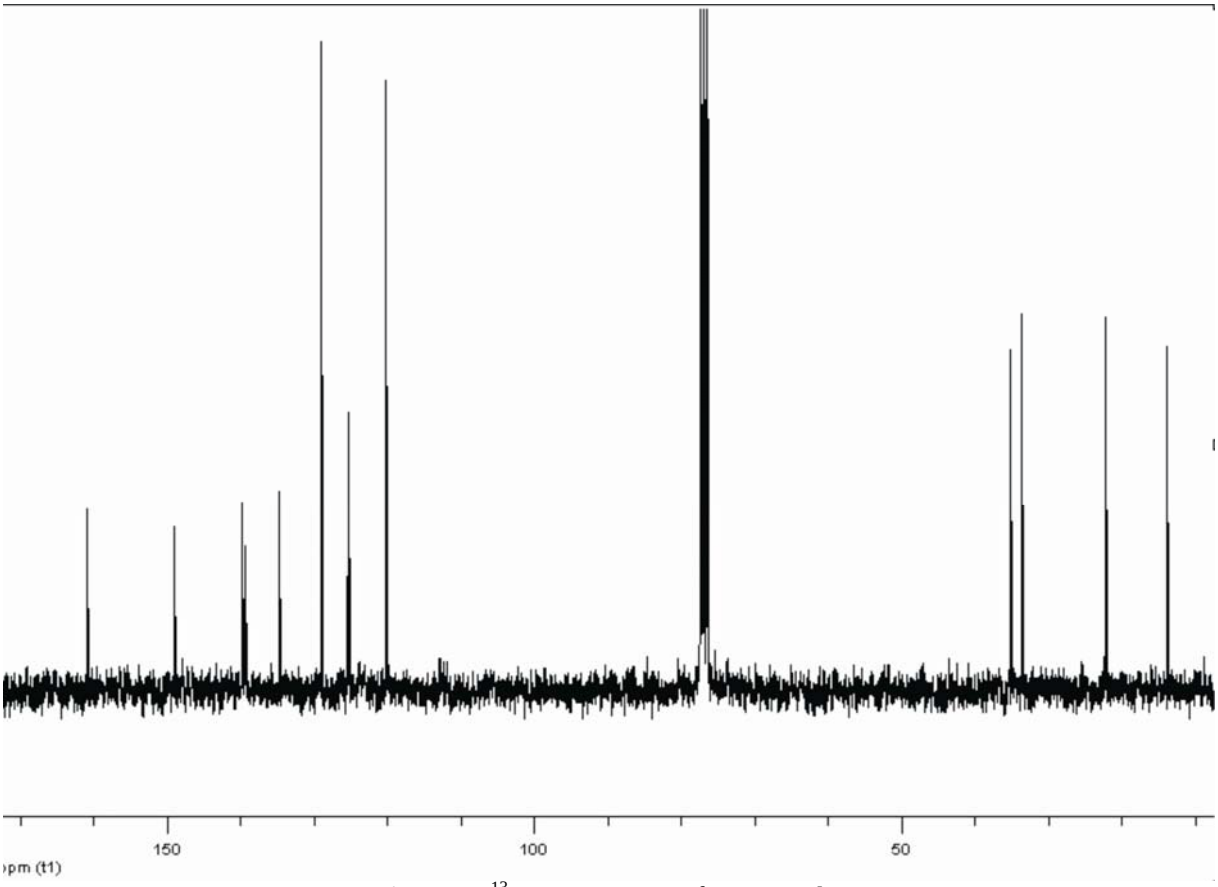

Figure S 6 ^{13}C NMR spectra of compound **5**

Binding studies:

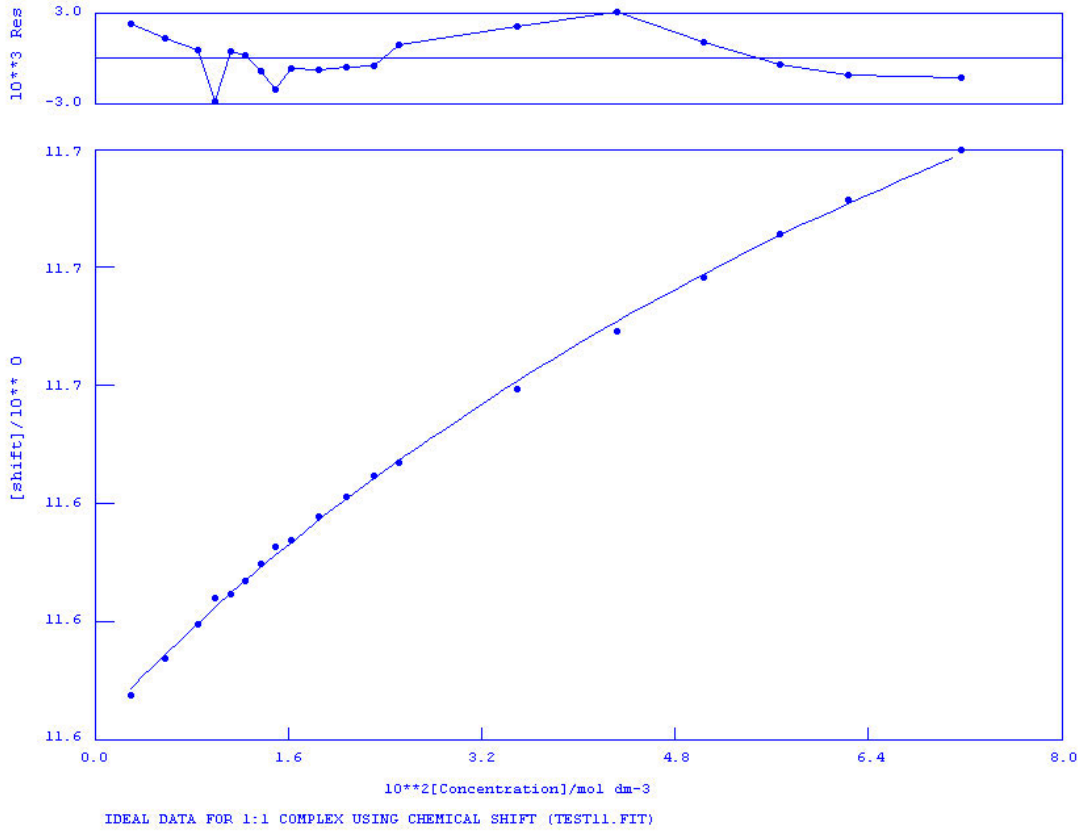

Figure S 7: Fit plot of NMR titration of compound **2** *vs* TBACl in DMSO-d_6

```
Calculations by WinEQNMR Version 1.20 by Michael J. Hynes
Program run at 15:38:10   on 01/29/2007

 IDEAL DATA FOR 1:1 COMPLEX USING CHEMICAL SHIFT (TEST11.FIT)
  Reaction:   M + L = ML
 FILE: TEST11.FIT
 IDEAL DATA: K1 = 63.091; DELTA M = 20.0; DELTA ML = 120.0
 File prepared by M. J. Hynes, October 22 2000

NO.  A   PARAMETER    DELTA     ERROR    CONDITION   DESCRIPTION
   1  1  9.18083E+00 2.000E-01 7.156E-01 1.599E+02    K1
   2  1  1.15555E+01 2.000E-01 1.012E-03 5.356E+00   SHIFT M
   3  1  1.19947E+01 1.000E+00 2.077E-02 1.290E+02    SHIFT ML

0RMS ERROR = 1.60E-03  MAX ERROR = 2.97E-03 AT OBS.NO. 15
 RESIDUALS SQUARED = 4.08E-05
 RFACTOR =     0.0126 PERCENT
```

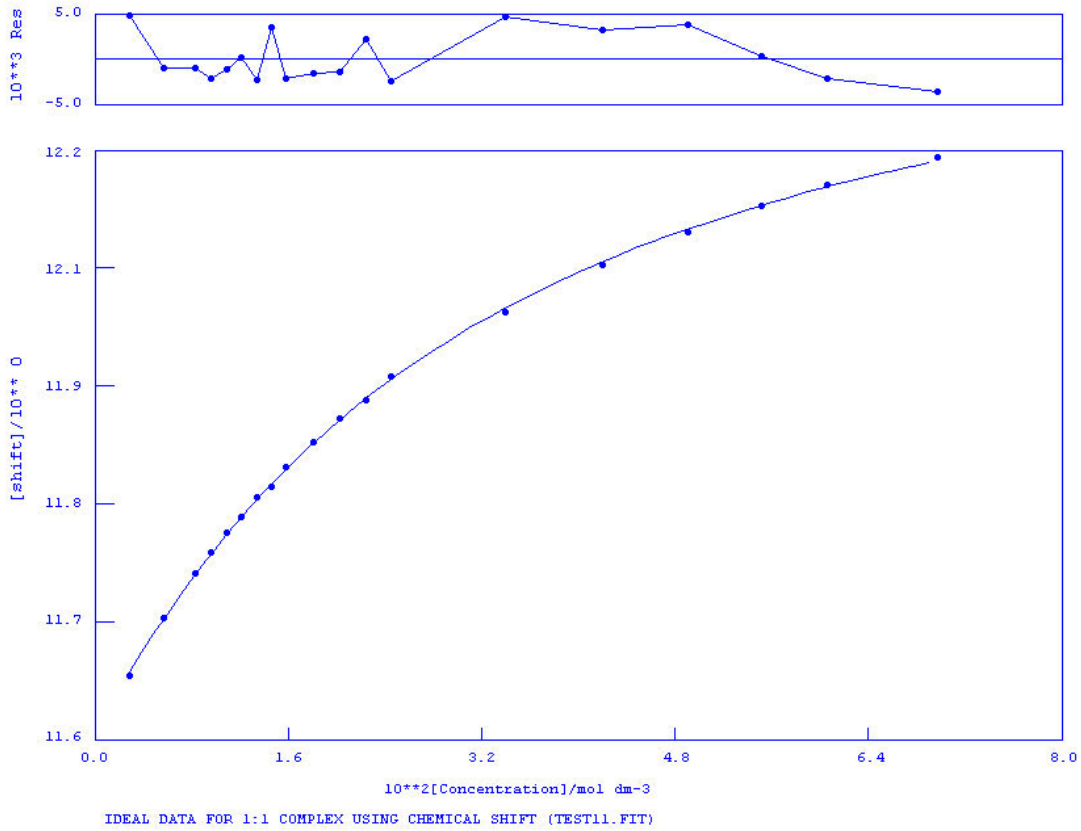

Figure S 8 : plot of NMR titration of compound **2**•HPF_6 *vs* TBACl in DMSO-d_6

```
Calculations by WinEQNMR Version 1.20 by Michael J. Hynes
Program run at 15:44:29   on 01/29/2007

 IDEAL DATA FOR 1:1 COMPLEX USING CHEMICAL SHIFT (TEST11.FIT)
  Reaction:   M + L = ML
 FILE: TEST11.FIT
 IDEAL DATA: K1 = 63.091; DELTA M = 20.0; DELTA ML = 120.0
 File prepared by M. J. Hynes, October 22 2000

NO.  A   PARAMETER    DELTA     ERROR    CONDITION   DESCRIPTION
   1  1  3.95559E+01 2.000E-01 1.112E+00 4.431E+01    K1
   2  1  1.15777E+01 2.000E-01 2.654E-03 6.610E+00   SHIFT M
   3  1  1.24337E+01 1.000E+00 7.728E-03 2.598E+01    SHIFT ML

0RMS ERROR = 2.86E-03  MAX ERROR = 4.64E-03 AT OBS.NO.  1
 RESIDUALS SQUARED = 1.31E-04
 RFACTOR =     0.0221 PERCENT
```

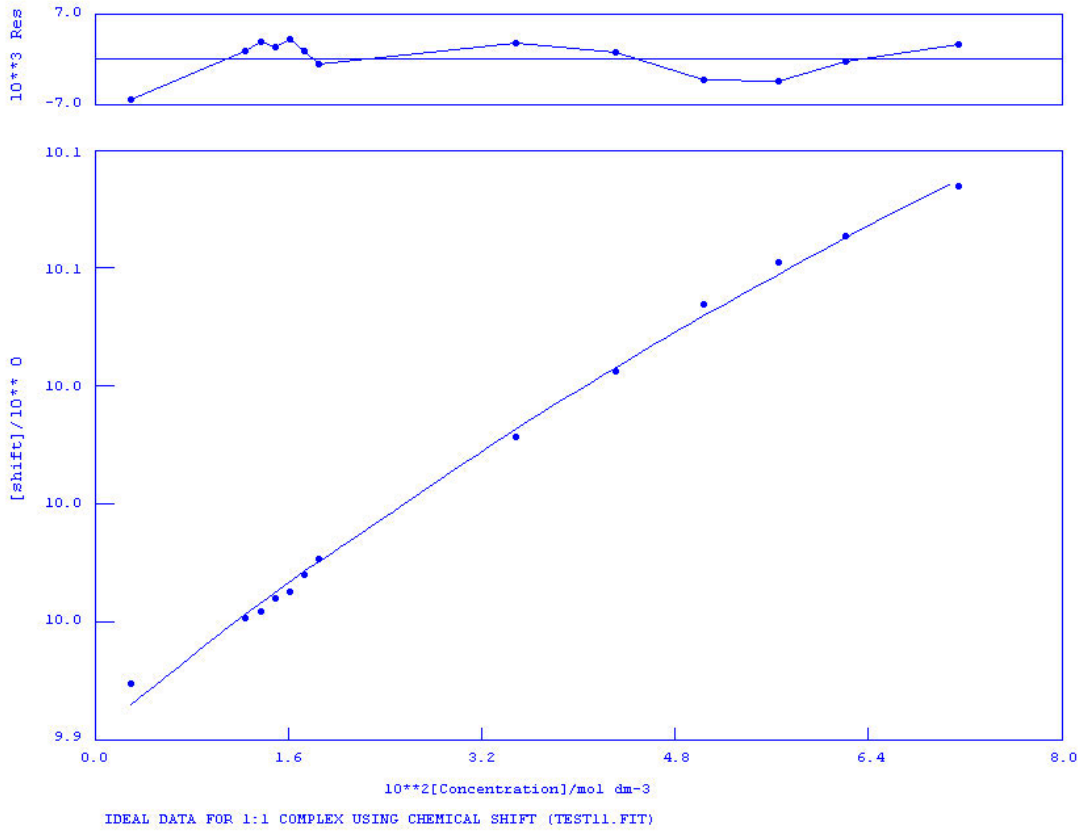

Figure S 9: Fit plot of NMR titration of compound **3** *vs* TBACl in DMSO-d_6

```
Calculations by WinEQNMR Version 1.20 by Michael J. Hynes
Program run at 17:39:09   on 02/13/2007

 IDEAL DATA FOR 1:1 COMPLEX USING CHEMICAL SHIFT (TEST11.FIT)
  Reaction:   M + L = ML
 FILE: TEST11.FIT
 IDEAL DATA: K1 = 63.091; DELTA M = 20.0; DELTA ML = 120.0
 File prepared by M. J. Hynes, October 22 2000

NO.  A   PARAMETER    DELTA     ERROR    CONDITION   DESCRIPTION
   1  1  4.15001E+00 2.000E-01 1.477E+00 9.436E+02    K1
   2  1  9.94260E+00 2.000E-01 2.690E-03 7.617E+00   SHIFT M
   3  1  1.06953E+01 1.000E+00 1.955E-01 8.392E+02    SHIFT ML

0RMS ERROR = 3.14E-03  MAX ERROR = 6.46E-03 AT OBS.NO.  1
 RESIDUALS SQUARED = 9.84E-05
 RFACTOR =     0.0274 PERCENT
```

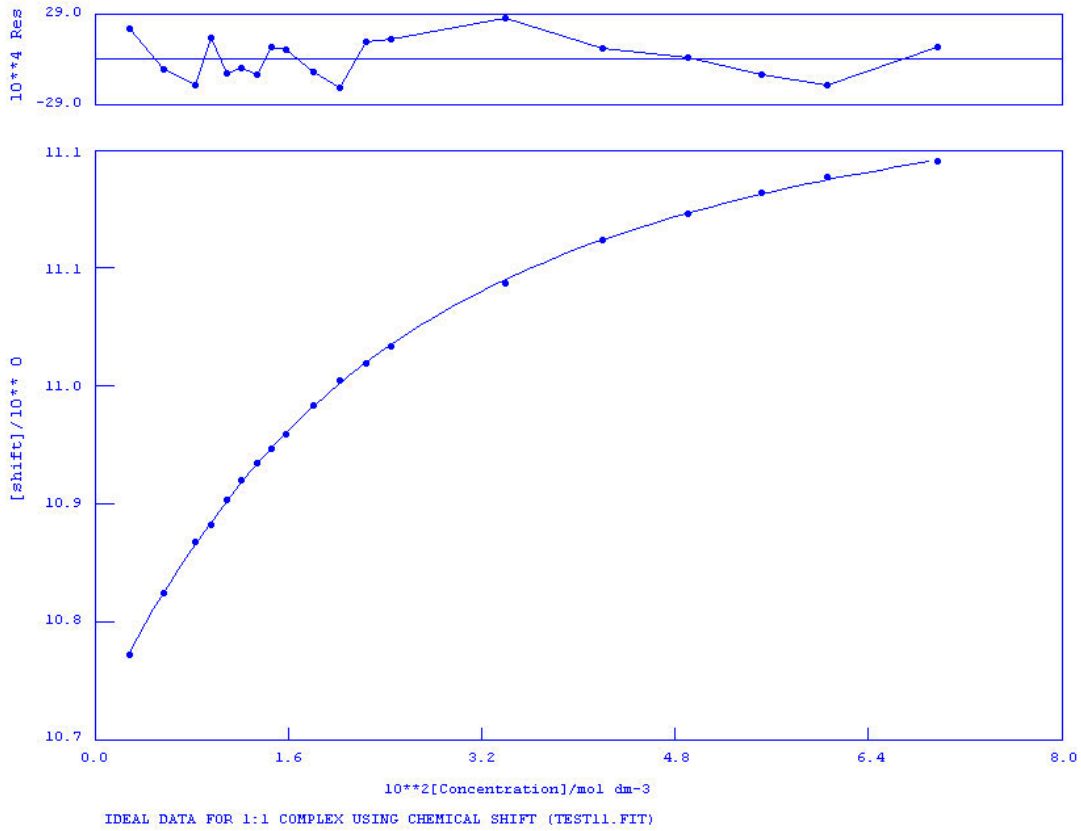

Figure S 10: plot of NMR titration of compound **3**•HPF_6 *vs* TBACl in DMSO-d_6

```
Calculations by WinEQNMR Version 1.20 by Michael J. Hynes
Program run at 14:57:26   on 01/29/2007

 IDEAL DATA FOR 1:1 COMPLEX USING CHEMICAL SHIFT (TEST11.FIT)
  Reaction:   M + L = ML
 FILE: TEST11.FIT
 IDEAL DATA: K1 = 63.091; DELTA M = 20.0; DELTA ML = 120.0
 File prepared by M. J. Hynes, October 22 2000

NO.  A   PARAMETER    DELTA     ERROR    CONDITION   DESCRIPTION
   1  1  5.89608E+01 2.000E-01 1.115E+00 3.232E+01    K1
   2  1  1.07340E+01 2.000E-01 1.429E-03 6.573E+00   SHIFT M
   3  1  1.12550E+01 1.000E+00 2.629E-03 1.709E+01    SHIFT ML

0RMS ERROR = 1.39E-03  MAX ERROR = 2.60E-03 AT OBS.NO. 14
 RESIDUALS SQUARED = 3.11E-05
 RFACTOR =     0.0117 PERCENT
```

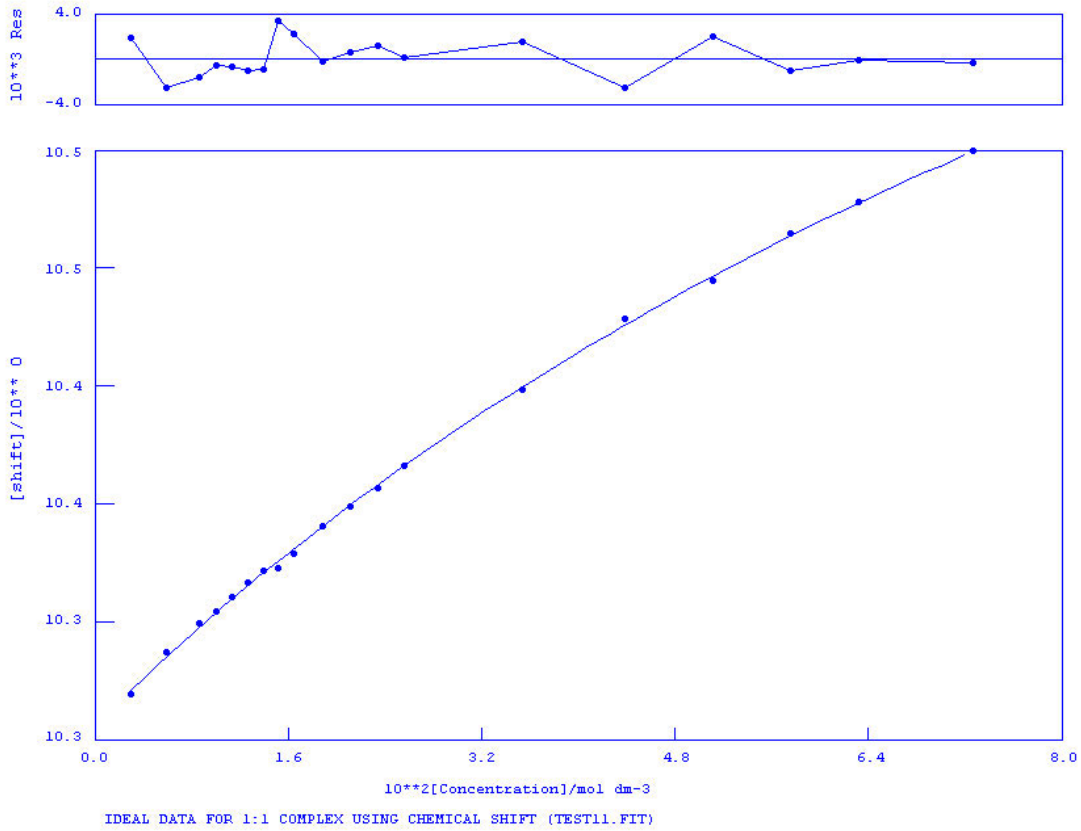

Figure S 11: Fit plot of NMR titration of compound **4** *vs* TBACl in DMSO-d_6

```
Calculations by WinEQNMR Version 1.20 by Michael J. Hynes
Program run at 18:46:36   on 01/31/2007

 IDEAL DATA FOR 1:1 COMPLEX USING CHEMICAL SHIFT (TEST11.FIT)
  Reaction:   M + L = ML
 FILE: TEST11.FIT
 IDEAL DATA: K1 = 63.091; DELTA M = 20.0; DELTA ML = 120.0
 File prepared by M. J. Hynes, October 22 2000

NO.  A   PARAMETER    DELTA     ERROR    CONDITION   DESCRIPTION
   1  1  7.78799E+00 2.000E-01 5.645E-01 2.620E+02    K1
   2  1  1.02810E+01 2.000E-01 1.217E-03 7.031E+00   SHIFT M
   3  1  1.09708E+01 1.000E+00 3.160E-02 2.113E+02    SHIFT ML

0RMS ERROR = 1.71E-03  MAX ERROR = 3.28E-03 AT OBS.NO.  8
 RESIDUALS SQUARED = 4.67E-05
 RFACTOR =     0.0151 PERCENT
```

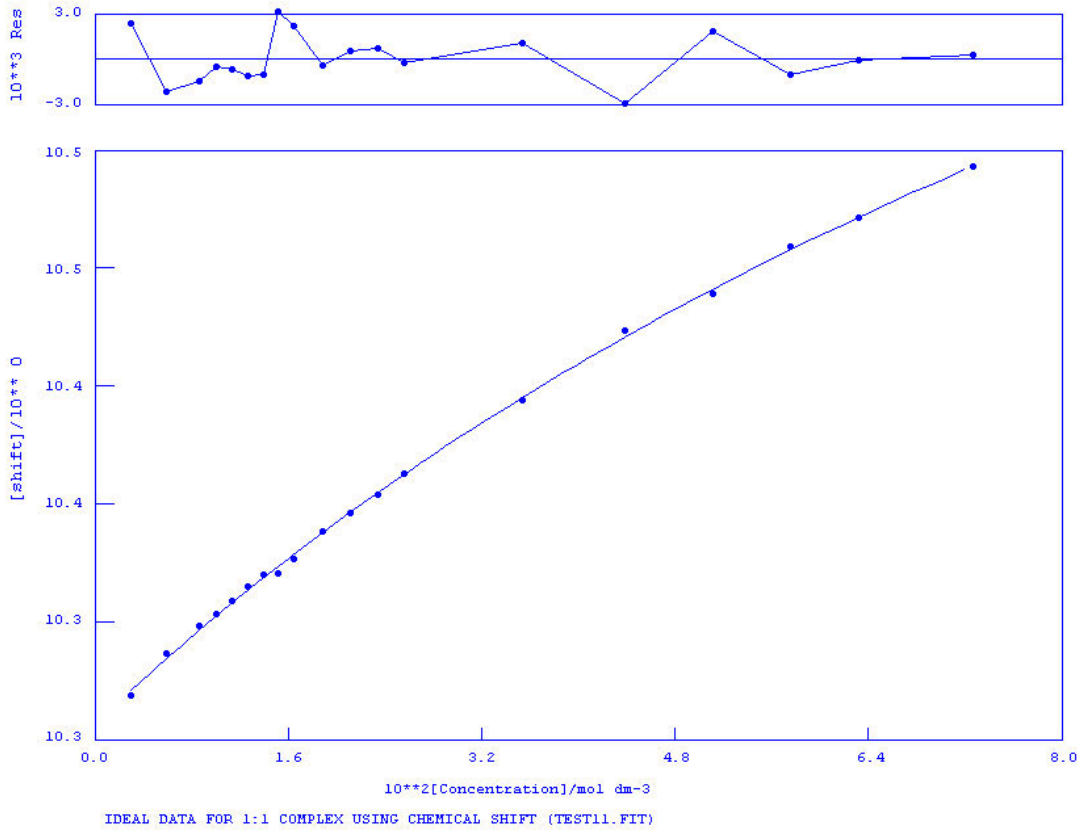

Figure S 12: plot of NMR titration of compound **4**•HPF_6 *vs* TBACl in DMSO-d_6

```
Calculations by WinEQNMR Version 1.20 by Michael J. Hynes
Program run at 18:40:48   on 01/31/2007

 IDEAL DATA FOR 1:1 COMPLEX USING CHEMICAL SHIFT (TEST11.FIT)
  Reaction:   M + L = ML
 FILE: TEST11.FIT
 IDEAL DATA: K1 = 63.091; DELTA M = 20.0; DELTA ML = 120.0
 File prepared by M. J. Hynes, October 22 2000

NO.  A   PARAMETER    DELTA     ERROR    CONDITION   DESCRIPTION
   1  1  7.35579E+00 2.000E-01 5.634E-01 2.925E+02    K1
   2  1  1.02818E+01 2.000E-01 1.258E-03 7.687E+00   SHIFT M
   3  1  1.09962E+01 1.000E+00 3.394E-02 2.350E+02    SHIFT ML

0RMS ERROR = 1.70E-03  MAX ERROR = 3.13E-03 AT OBS.NO.  8
 RESIDUALS SQUARED = 4.60E-05
 RFACTOR =     0.0150 PERCENT
```

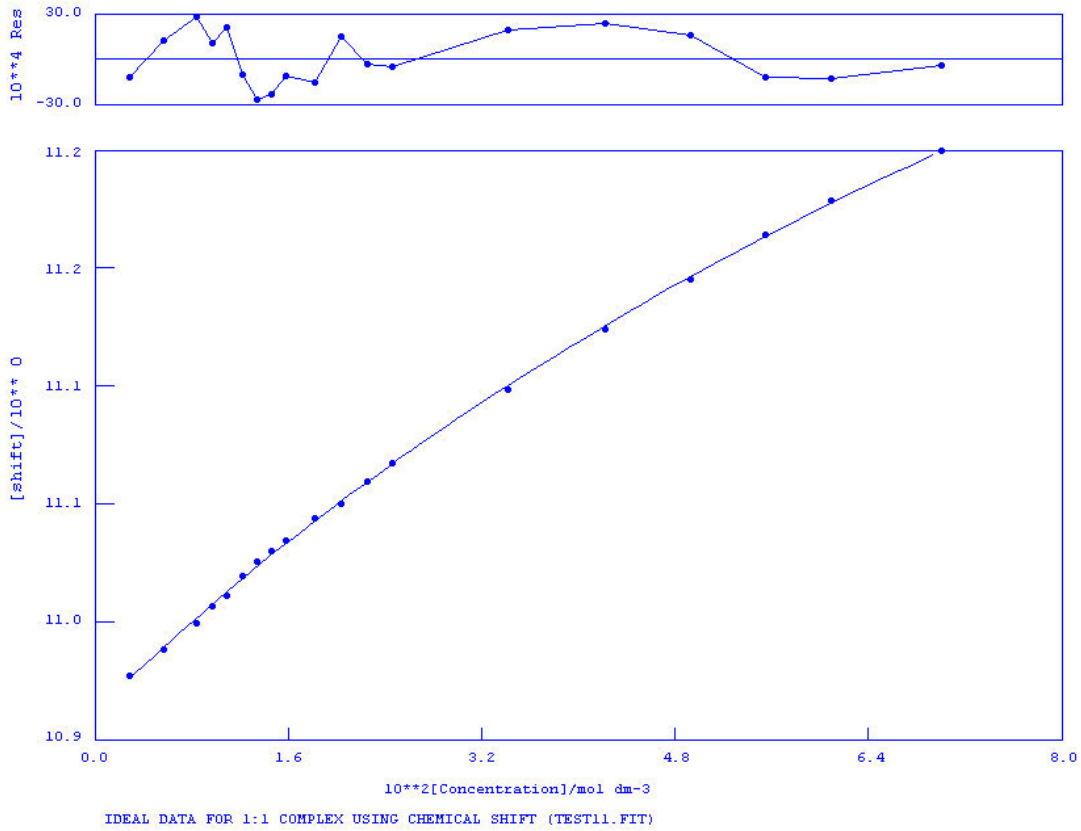

Figure S 13: Fit plot of NMR titration of compound **5** *vs* TBACl in DMSO-d_6

```
Calculations by WinEQNMR Version 1.20 by Michael J. Hynes
Program run at 18:24:01   on 01/31/2007

 IDEAL DATA FOR 1:1 COMPLEX USING CHEMICAL SHIFT (TEST11.FIT)
  Reaction:   M + L = ML
 FILE: TEST11.FIT
 IDEAL DATA: K1 = 63.091; DELTA M = 20.0; DELTA ML = 120.0
 File prepared by M. J. Hynes, October 22 2000

NO.  A   PARAMETER    DELTA     ERROR    CONDITION   DESCRIPTION
   1  1  7.22057E+00 2.000E-01 5.699E-01 4.560E+02    K1
   2  1  1.09451E+01 2.000E-01 1.464E-03 9.485E+00   SHIFT M
   3  1  1.18653E+01 1.000E+00 4.786E-02 3.717E+02    SHIFT ML

0RMS ERROR = 1.79E-03  MAX ERROR = 2.74E-03 AT OBS.NO.  7
 RESIDUALS SQUARED = 5.15E-05
 RFACTOR =     0.0149 PERCENT
```

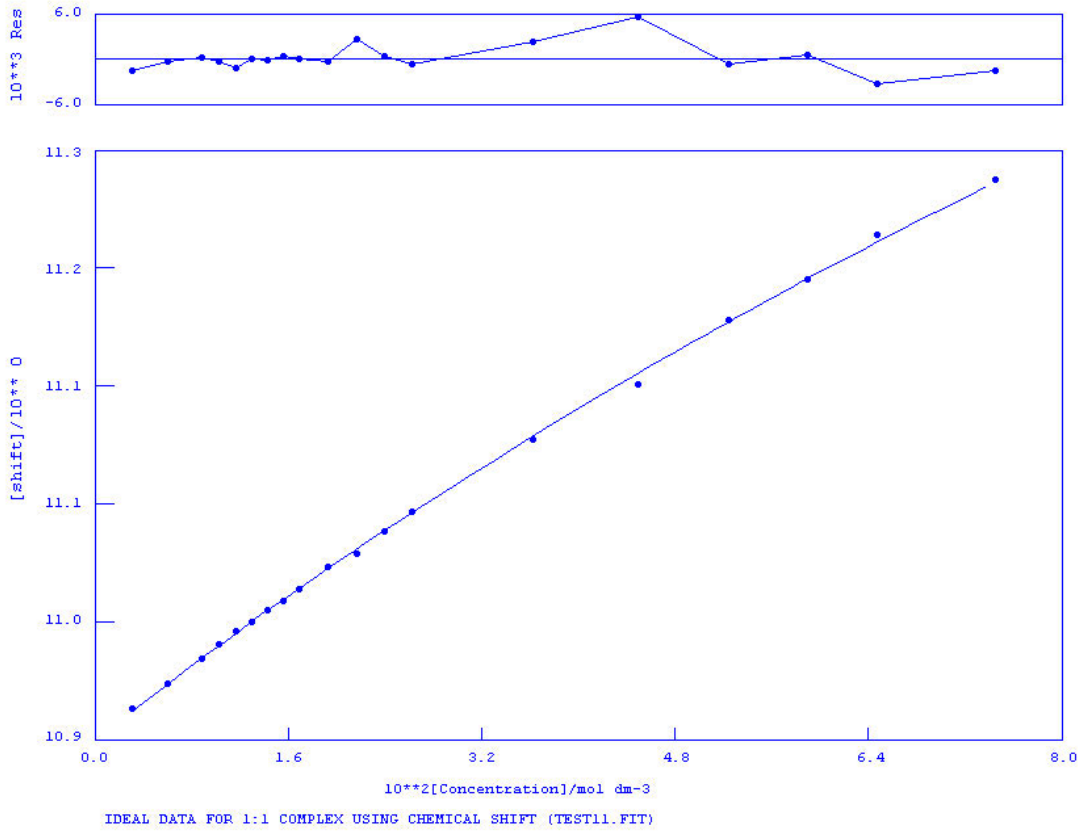

Figure S 14: plot of NMR titration of compound **5**•HPF_6 *vs* TBACl in DMSO-d_6

```
 Calculations by WinEQNMR Version 1.20 by Michael J. Hynes
Program run at 10:44:47   on 02/13/2007

 IDEAL DATA FOR 1:1 COMPLEX USING CHEMICAL SHIFT (TEST11.FIT)
  Reaction:   M + L = ML
 FILE: TEST11.FIT
 IDEAL DATA: K1 = 63.091; DELTA M = 20.0; DELTA ML = 120.0
 File prepared by M. J. Hynes, October 22 2000

NO.  A   PARAMETER    DELTA     ERROR    CONDITION   DESCRIPTION
   1  1  3.78485E+00 2.000E-01 4.381E-01 8.778E+02    K1
   2  1  1.09443E+01 2.000E-01 1.391E-03 7.916E+00   SHIFT M
   3  1  1.22869E+01 1.000E+00 1.188E-01 7.736E+02    SHIFT ML

0RMS ERROR = 1.97E-03  MAX ERROR = 5.46E-03 AT OBS.NO. 15
 RESIDUALS SQUARED = 6.22E-05
 RFACTOR =     0.0164 PERCENT
```