Towards extracellular Ca^{2+} sensing by MRI: synthesis and calcium-dependent ^{1}H and ^{17}O relaxation studies of two novel bismacrocyclic ligands

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Equations.

^{17}O NMR relaxation:

From the measured ^{17}O NMR relaxation rates of the paramagnetic solutions, $1/T_1$ and $1/T_2$, and of the acidified water reference, $1/T_{1A}$ and $1/T_{2A}$, one can calculate the reduced relaxation rates, $1/T_{1r}$, $1/T_{2r}$ (Eq. **[1]** and **[2]**), where $1/T_{1m}$, $1/T_{2m}$ are the relaxation rates of the bound water and $\Delta\omega_m$ is the chemical shift difference between bound and bulk water, τ_m is the mean residence time or the inverse of the water exchange rate k_{ex} and P_m is the mole fraction of the bound water.[1,2]

$$\frac{1}{T_{1r}} = \frac{1}{P_m}\left[\frac{1}{T_1} - \frac{1}{T_{1A}}\right] = \frac{1}{T_{1m} + \tau_m} + \frac{1}{T_{1OS}} \qquad \textbf{[1]}$$

$$\frac{1}{T_{2r}} = \frac{1}{P_m}\left[\frac{1}{T_2} - \frac{1}{T_{2A}}\right] = \frac{1}{\tau_m}\frac{T_{2m}^{-2} + \tau_m^{-1}T_{2m}^{-1} + \Delta\omega_m^2}{\left(\tau_m^{-1} + T_{2m}^{-1}\right)^2 + \Delta\omega_m^2} + \frac{1}{T_{2OS}} \qquad \textbf{[2]}$$

The terms $1/T_{1OS}$ and $1/T_{2OS}$ describe relaxation contributions from water molecules not directly bound to the paramagnetic centre. In previous studies it has been shown that ^{17}O outer sphere relaxation terms due to water molecules freely diffusing on the surface of Gd-polyaminocarboxylate complexes are negligible. For complexes with electronegative groups relaxation terms due to 2nd sphere water molecules can however be important for longitudinal relaxation $1/T_{1r}$ and have therefore to be included.

$$\frac{1}{T_{1r}} = \frac{1}{T_{1r}^{1st}} + \frac{1}{T_{1r}^{2nd}} = \frac{1}{T_{1m} + \tau_m} + \frac{1}{T_{1r}^{2nd}} \qquad \textbf{[3]}$$

$$\frac{1}{T_{2r}} = \frac{1}{T_{2r}^{1st}} = \frac{1}{\tau_m}\frac{T_{2m}^{-2} + \tau_m^{-1}T_{2m}^{-1} + \Delta\omega_m^2}{\left(\tau_m^{-1} + T_{2m}^{-1}\right)^2 + \Delta\omega_m^2} \qquad \textbf{[4]}$$

First sphere contribution to ^{17}O relaxation:

The ^{17}O longitudinal relaxation rates in Gd(III) solutions are the sum of the contributions of the dipole-dipole and quadrupolar (in the approximation developed by Halle for non-extreme narrowing conditions) mechanisms as expressed by Eq. **[6]-[7]**, where γ_S is the electron and γ_I is the nuclear gyromagnetic ratio (γ_S = 1.76×1011 rad·s^{-1}·T^{-1}, γ_I =-3.626×10^7 rad·s^{-1}·T^{-1}), r_{GdO} is the effective distance between the electron charge and the ^{17}O nucleus, I is the nuclear spin (5/2 for ^{17}O), χ is the quadrupolar coupling constant and η is an asymmetry parameter:

$$\frac{1}{T_{1m}} = \frac{1}{T_{1dd}} + \frac{1}{T_{1q}} \qquad \textbf{[5]}$$

$$\frac{1}{T_{1dd}} = \frac{2}{15}\left(\frac{\mu_0}{4\pi}\right)^2 \frac{\hbar^2\gamma_I^2\gamma_S^2}{r_{GdO}^6} S\left(S+1\right)\left[3J\left(\omega_I;\tau_{d1}\right) + 7J\left(\omega_S;\tau_{d2}\right)\right]; \quad J\left(\omega;\tau\right) = \frac{\tau}{1+\left(\omega\tau\right)^2} \qquad \textbf{[6]}$$

$$\frac{1}{\tau_{d1}} = \frac{1}{\tau_m} + \frac{1}{T_{1e}} + \frac{1}{\tau_{RO}}$$

$$\frac{1}{T_{1q}} = \frac{3\pi^2}{10}\frac{2I+3}{I^2\left(2I-1\right)}\chi^2\left(1+\frac{\eta^2}{3}\right)\left[0.2J_1\left(\omega_I;\tau_{RO}\right) + 0.8J_2\left(\omega_I;\tau_{RO}\right)\right]; \quad J_n\left(\omega;\tau\right) = \frac{\tau}{1+\left(n\omega\tau\right)^2} \qquad \textbf{[7]}$$

In the transverse relaxation the scalar contribution, $1/T_{2sc}$, is dominating, Eq. **[8]**. $1/\tau_{s1}$ is the sum of the exchange rate constant and the electron spin relaxation rate.

$$\frac{1}{T_{2m}} \cong \frac{1}{T_{2SC}} = \frac{S\left(S+1\right)}{3}\left(\frac{A}{\hbar}\right)^2 \tau_{S1} \qquad \textbf{[8]}$$

$$\frac{1}{\tau_{S1}} = \frac{1}{\tau_m} + \frac{1}{T_{1e}} \qquad \textbf{[9]}$$

Second sphere contribution to ^{17}O relaxation:

$$\frac{1}{T_1^{2nd}} \cong \frac{q^{2nd}}{q^{1st}} \frac{1}{T_{1m}^{2nd}} = \frac{q^{2nd}}{q^{1st}} \left(\frac{1}{T_{1dd}^{2nd}} + \frac{1}{T_{1q}^{2nd}} \right)$$

[10]

$$\frac{1}{T_{1dd}^{2nd}} = C_{dd}^{2nd,O} \left(\frac{3\tau_{d1}^{2nd,O}}{1+\left(\omega_I \tau_{d1}^{2nd,O}\right)^2} + \frac{7\tau_{d2}^{2nd,O}}{1+\left(\omega_S \tau_{d2}^{2nd,O}\right)^2} \right)$$

$$C_{dd}^{2nd,O} = \frac{2}{15} \left(\frac{\mu_0}{4\pi} \right)^2 \frac{h^2 \gamma_{^{17}O}^2 \gamma_S^2}{\left(r_{GdO}^{2nd}\right)^6} S(S+1)$$

[11]

$$\frac{1}{T_{1q}^{2nd}} = \frac{3\pi^2}{10} \frac{2I+3}{I^2(2I-1)} \chi^2 \left(1+\eta^2/3\right) \left(\frac{0.2\tau^{2nd,O}}{1+\left(\omega_I \tau^{2nd,O}\right)^2} + \frac{0.8\tau^{2nd,O}}{1+\left(2\omega_I \tau^{2nd,O}\right)^2} \right)$$

[12]

$$\frac{1}{\tau^{O,2nd}} = \frac{1}{\tau_g} + \frac{1}{\tau_1^O} \cong \frac{1}{\tau_1^O}$$

[13]

$$\frac{1}{\tau_{di}^{2nd,O}} = k_{ex}^{2nd} + \frac{1}{\tau^{O,2nd}} + \frac{1}{T_{ie}}$$

[14]

^{1}H NMRD:

The measured longitudinal proton relaxation rate, R_1^{obs} is the sum of a paramagnetic and a diamagnetic contribution as expressed in Eq. **[15]**, where r_1 is the proton relaxivity:

$$R_1^{obs} = R_1^d + R_1^p = R_1^d + r_1\left[Gd^{3+}\right]$$

[15]

The relaxivity is here given by the sum of inner sphere, second sphere and outer sphere contributions:

$$r_1 = r_{1is} + r_{1,2nd} + r_{1os}$$

[16]

Inner sphere ^{1}H relaxation:

The inner sphere term is given in Eq. **[17]**, where q^{1st} is the number of inner sphere water molecules.[3]

$$r_{1is} = \frac{1}{1000} \times \frac{q^{1st}}{55.55} \times \frac{1}{T_{1m}^H + \tau_m}$$

[17]

The longitudinal relaxation rate of inner sphere protons, $1/T_{1m}^H$ is expressed by Eq. **[18]**, where r_{GdH} is the effective distance between the electron charge and the ^{1}H nucleus, ω_I is the proton resonance frequency and ω_S is the Larmor frequency of the Gd(III) electron spin.

$$\frac{1}{T_{1m}^H} = \frac{2}{15} \left(\frac{\mu_0}{4\pi} \right)^2 \frac{\hbar^2 \gamma_I^2 \gamma_S^2}{r_{GdH}^6} S(S+1) \left[3J(\omega_I;\tau_{d1}) + 7J(\omega_S;\tau_{d2}) \right]$$

[18]

$$J(\omega;\tau_{di}) = \left(\frac{S^2 \tau_{dig}}{1+\omega^2 \tau_{dig}^2} + \frac{\left(1-S^2\right)\tau_{di}}{1+\omega^2 \tau_{di}^2} \right); \quad i=1,2$$

[19]

$$\frac{1}{\tau_{dig}} = \frac{1}{\tau_m} + \frac{1}{\tau_g} + \frac{1}{T_{ie}}\ ; \quad \frac{1}{\tau_{dig}} = \frac{1}{\tau_m} + \frac{1}{\tau^H} + \frac{1}{T_{ie}}\ ; \quad i=1,2$$

[20]

$$\frac{1}{\tau^H} = \frac{1}{\tau_g} + \frac{1}{\tau_1^H}$$

[21]

The spectral density functions are given by Eq. **[19]**.

Second sphere ^{1}H relaxation:

$$\mathrm{r}_1^{\mathrm{2nd}} = \frac{1}{1000} \times \frac{\mathrm{q}^{\mathrm{2nd}}}{55.55} \times \frac{1}{\mathrm{T}_{\mathrm{1dd}}^{\mathrm{2nd,H}} + \tau_m^{2nd}} \cong \frac{1}{1000} \times \frac{\mathrm{q}^{\mathrm{2nd}}}{55.55} \times \frac{1}{\mathrm{T}_{\mathrm{1dd}}^{\mathrm{2nd,H}}} \qquad \textbf{[22]}$$

$$\frac{1}{\mathrm{T}_{\mathrm{1dd}}^{\mathrm{2nd,H}}} = \mathrm{C}_{\mathrm{dd}}^{\mathrm{2nd,O}} \left(\frac{3\tau_{\mathrm{d1}}^{\mathrm{2nd,H}}}{1 + \left(\omega_{\mathrm{I}} \tau_{\mathrm{d1}}^{\mathrm{2nd,H}}\right)^2} + \frac{7\tau_{\mathrm{d2}}^{\mathrm{2nd,H}}}{1 + \left(\omega_{\mathrm{S}} \tau_{\mathrm{d2}}^{\mathrm{2nd,H}}\right)^2} \right) \qquad \textbf{[23]}$$

$$\mathrm{C}_{\mathrm{dd}}^{\mathrm{2nd,H}} = \frac{2}{15} \left(\frac{\mu_0}{4\pi} \right)^2 \frac{\mathrm{h}^2 \gamma_{^1\mathrm{H}}^2 \gamma_{\mathrm{S}}^2}{\left(\mathrm{r}_{\mathrm{GdO}}^{\mathrm{2nd}}\right)^6} \mathrm{S}(\mathrm{S}+1) \qquad \textbf{[24]}$$

$$\frac{1}{\tau_{\mathrm{di}}^{\mathrm{2nd,H}}} = \mathrm{k}_{\mathrm{ex}}^{\mathrm{2nd}} + \frac{1}{\tau^{\mathrm{H}}} + \frac{1}{\mathrm{T}_{\mathrm{ie}}} \qquad \textbf{[25]}$$

$$\frac{1}{\tau^{\mathrm{H}}} = \frac{1}{\tau_{\mathrm{g}}} + \frac{1}{\tau_{\mathrm{l}}^{\mathrm{H}}} \qquad \textbf{[26]}$$

Outer sphere ^{1}H relaxation:

The outer-sphere contribution can be described by Eq. **[27]** where N_A is the Avogadro constant, and J_{os} is its associated spectral density function.[4,5]

$$r_{1os} = \frac{32 N_A \pi}{405} \left(\frac{\mu_0}{4\pi} \right)^2 \frac{\hbar^2 \gamma_S^2 \gamma_I^2}{a_{GdH} D_{GdH}} S(S+1)\left[3J_{os}(\omega_I; T_{1e}) + 7J_{os}(\omega_S; T_{2e})\right] \qquad \textbf{[27]}$$

$$J_{os}(\omega, T_{je}) = \mathrm{Re}\left[\frac{1 + \frac{1}{4}\left(i\omega\tau_{GdH} + \frac{\tau_{GdH}}{Tje} \right)^{1/2}}{1 + \left(i\omega\tau_{GdH} + \frac{\tau_{GdH}}{T_{je}} \right)^{1/2} + \frac{4}{9}\left(i\omega\tau_{GdH} + \frac{\tau_{GdH}}{T_{je}} \right) + \frac{1}{9}\left(i\omega\tau_{GdH} + \frac{\tau_{GdH}}{T_{je}} \right)^{3/2}} \right]; \quad j = 1,2 \qquad \textbf{[28]}$$

$$\tau_{GdH} = \frac{a_{GdH}^2}{D_{GdH}} \qquad \textbf{[29]}$$

$\mathrm{a}_{\mathrm{GdH}}$ is the distance of closes approach and D_{GdH} is the diffusion coefficient for the diffusion of a water proton relative to the Gd(III) complex.

Electron spin relaxation:

The longitudinal and transverse electronic relaxation rates, $1/T_{1e}$ and $1/T_{2e}$ are described by Solomon-Bloembergen-Morgan theory modified by Powell (Eqs. **[30]-[31]**), where τ_V is the correlation time for the modulation of the zero-field-splitting interaction.

$$\left(\frac{1}{T_{1e}} \right)^{ZFS} = \frac{1}{25} \Delta^2 \tau_v \left\{ 4S(S+1) - 3 \right\} \left(\frac{1}{1 + \omega_S^2 \tau_v^2} + \frac{4}{1 + 4\omega_S^2 \tau_v^2} \right) \qquad \textbf{[30]}$$

$$\left(\frac{1}{T_{2e}} \right)^{ZFS} = \Delta^2 \tau_v \left(\frac{5.26}{1 + 0.372\omega_S^2 \tau_v^2} + \frac{7.18}{1 + 1.24\omega_S \tau_v} \right) \qquad \textbf{[31]}$$

Temperature dependences of water exchange rates and correlation times:

The exchange rates are supposed to follow the Eyring equation. In Eq. **[32]** $\Delta S^{\ddagger}$ and $\Delta H^{\ddagger}$ are the entropy and enthalpy of activation for the water exchange process, and k_{ex}^{298} is the exchange rate at 298.15 K. In Eq. **[33]** $\Delta H^{\ddagger 2nd}$ is the enthalpy of activation for the second sphere water exchange process and $k_{ex}^{2nd,298}$ is the corresponding exchange rate at 298 K.

$$k_{ex} = \frac{1}{\tau_m} = \frac{k_B T}{h} \exp\left\{ \frac{\Delta S^{\ddagger}}{\mathrm{R}} - \frac{\Delta H^{\ddagger}}{RT} \right\} = \frac{k_{ex}^{298} T}{298.15} \exp\left\{ \frac{\Delta H^{\ddagger}}{R} \left(\frac{1}{298.15} - \frac{1}{T} \right) \right\} \qquad \textbf{[32]}$$

$$k_{ex}^{2nd} = \frac{k_{ex}^{2nd,298}}{298.14} \exp\left\{ \frac{\Delta H^{\ddagger 2nd}}{T} \left(\frac{1}{298.15} - \frac{1}{T} \right) \right\} \qquad \textbf{[33]}$$

All correlation times and the diffusion constant are supposed to obey an Arrhenius law:

$$\tau = \tau^{298} \exp\left\{ \frac{E_a}{R} \left(\frac{1}{T} - \frac{1}{298.15} \right) \right\} \qquad \textbf{[34]}$$

$$D_{GdH} = D_{GdH}^{298} \exp\left\{ \frac{E_{GdH}}{R} \left(\frac{1}{298.15} - \frac{1}{T} \right) \right\} \qquad \textbf{[35]}$$

Table S1. Proton relaxivities (r_1 / mM^{-1}s^{-1}) of Gd_2L^1 in the absence of Ca^{2+}, c(Gd^{3+})=5.1 mM, pH=6.25, at 25 and 37°C.

ν (^{1}H)/MHz	15.5°C	25°C	37°C
0.010	10.0	7.45	5.89
0.0144	-	7.43	5.86
0.0208	-	7.46	5.84
0.0216	9.91	-	-
0.0298	-	7.47	5.87
0.0428	-	7.46	5.86
0.0465	9.88	-	-
0.0616	-	7.49	5.88
0.0887	-	7.47	5.85
0.10	9.85	-	-
0.127	-	7.50	5.89
0.183	-	7.45	5.80
0.216	9.83	-	-
0.264	-	7.44	5.85
0.379	-	7.40	5.81
0.465	9.35	-	-
0.546	-	7.30	5.76
0.784	-	7.27	5.74
1.00	9.14	-	-
1.13	-	7.11	5.65
1.62	-	6.92	5.45
2.15	8.65	-	-
2.34	-	6.72	5.29
3.36	-	6.40	5.08
4.64	7.80	-	-
4.83	-	6.19	4.83
6.95	-	5.98	4.62
10	7.56	5.72	4.24
12	-	5.64	4.12
14	-	5.59	-
16	-	5.56	4.01
18	-	5.55	-
20	-	5.74	4.09
30	-	5.94	4.14
40	-	5.93	4.10
60	-	5.95	4.07
200	-	5.14	3.95
500	-	3.40	-

Table S2. Proton relaxivities (r_1 / $mM^{-1}s^{-1}$) of Gd_2L^1 in the presence of Ca^{2+}, c(Gd^{3+})=3.2 mM, c(Ca^{2+})=4.4 mM, pH=7, at 15.5, 25 and 37°C.

ν (^{1}H)/MHz	15.5°C	25°C
0.010	11.56	8.10
0.0144	-	8.10
0.0208	-	8.10
0.0298	-	8.10
0.0398	11.01	-
0.0428	-	8.10
0.0616	-	8.10
0.0887	-	8.10
0.127	-	8.10
0.158	11.17	-
0.183	-	8.10
0.264	-	8.00
0.379	-	8.00
0.546	-	7.97
0.784	-	7.82
1.13	-	7.65
1.62	-	7.44
2.34	-	7.16
2.51	9.49	-
3.36	-	6.88
4.83	-	6.56
6.95	-	6.40
10	8.7	6.19
11.5	-	6.08
13.2	-	5.97
14	-	-
15.2	-	5.97
16	-	-
17.0	-	-
17.4	-	5.95
20	-	6.13
30	-	6.39
40	-	6.38
60	-	6.31
200	-	5.76
500	-	3.98

Table S3. Variable temperature reduced transverse and longitudinal ^{17}O relaxation rates of Gd_2L^1 in the absence of Ca^{2+}, c(Gd^{3+})=51.8 mM, pH=7, $P_m=4.00\cdot10^{-4}$ at 11.75 T. Reference was acidified H_2O, pH=3.4.

t / °C	T / K	1000/T / K^{-1}	P_m	T_1 (Gd)/s	T_1 (ref)/s	T_2(Gd)/s	T_2(ref)/s	ln(1/T_{1r})	ln(1/T_{2r})
3.75	276.9	3.61	4.00E-04	2.93E-03	3.65E-03	2.09E-03	3.29E-03	12.03	12.98
10.45	283.6	3.53	4.00E-04	3.74E-03	4.51E-03	2.02E-03	4.66E-03	11.64	13.46
16.65	289.8	3.45	4.00E-04	4.26E-03	5.66E-03	2.04E-03	5.70E-03	11.88	13.57
25.45	298.6	3.35	4.00E-04	5.65E-03	7.06E-03	2.36E-03	6.74E-03	11.39	13.44
35.45	308.6	3.24	4.00E-04	7.03E-03	8.92E-03	2.75E-03	8.88E-03	11.23	13.35
46.75	319.9	3.13	4.00E-04	9.07E-03	1.16E-02	3.42E-03	1.18E-02	10.99	13.16
59.25	332.4	3.01	4.00E-04	1.20E-02	1.45E-02	4.83E-03	1.43E-02	10.47	12.75
70.55	343.7	2.91	4.00E-04	1.45E-02	1.75E-02	6.37E-03	1.72E-02	10.27	12.42

Table S4. Variable temperature reduced transverse and longitudinal ^{17}O relaxation rates of Gd_2L^1 in the presence of 1M Ca^{2+}, c(Gd^{3+})=45.6 mM, pH=7, $P_m=5.41\cdot10^{-4}$ at 11.75 T. Reference was acidified 1M $CaCl_2$.

t / °C	T / K	1000/T / K^{-1}	P_m	T_1 (Gd)/s	T_1 (ref)/s	T_2(Gd)/s	T_2(ref)/s	ln(1/T_{1r})	ln(1/T_{2r})
4.25	277.4	3.60	5.41E-04	2.56E-03	4.14E-03	1.36E-03	4.08E-03	12.38	13.71
10.55	283.7	3.52	5.41E-04	2.86E-03	5.06E-03	1.39E-03	4.93E-03	12.25	13.77
17.95	291.1	3.44	5.41E-04	3.78E-03	6.16E-03	1.46E-03	5.97E-03	12.09	13.77
24.85	298.0	3.36	5.41E-04	4.05E-03	7.24E-03	1.45E-03	6.76E-03	11.94	13.81
34.15	307.3	3.25	5.41E-04	5.23E-03	9.16E-03	1.67E-03	8.94E-03	11.76	13.71
50.35	323.5	3.09	5.41E-04	6.98E-03	1.29E-02	1.95E-03	1.17E-02	11.44	13.58
65.25	338.4	2.96	5.41E-04	8.85E-03	1.66E-02	2.48E-03	1.59E-02	11.16	13.35

Table S5. Relaxometric Ca^{2+} titration of Gd$_2$L^1 at 25°C, pH 7 and 11.75 T.

c(Gd^{3+}) / mM	c(Ca^{2+}) / mM	c(complex) / mM	n(Ca^{2+}):n(complex)	T_1 / ms	r_1 / mmol^{-1}s^{-1}
7.4	0	3.7	0.00	39.67	3.36
7.05	0.88	3.525	0.25	40.90	3.42
6.73	1.67	3.365	0.50	42.51	3.44
6.43	2.4	3.215	0.75	43.97	3.48
6.08	3.28	3.04	1.08	46.04	3.51
5.77	4.06	2.885	1.41	48.31	3.52
5.62	8.9	2.81	3.17	48.92	3.57
5.41	15.72	2.705	5.81	49.90	3.64
5.22	22.05	2.61	8.45	51.35	3.66
4.99	43.56	2.495	17.46	53.03	3.71
4.77	63.23	2.385	26.51	53.40	3.85
4.58	81.27	2.29	35.49	56.16	3.81
4.4	97.88	2.2	44.49	58.63	3.79
4.07	127.44	2.035	62.62	63.36	3.79
3.79	152.96	1.895	80.72	67.45	3.82
3.552	175.22	1.776	98.66	71.79	3.82
3.24	203.73	1.62	125.76	77.92	3.85

Table S6. Relaxometric Ca^{2+} titration of Gd$_2$L^2 at 25°C, pH 7 and 11.75 T.

c(Gd^{3+}) / mM	c(Ca^{2+}) / mM	c(complex) / mM	n(Ca^{2+}):n(complex)	T_1 / ms	r_1 / mmol^{-1}s^{-1}
4.03	0	2.015	0	91.51	2.62
3.78	1.15	1.89	0.61	94.04	2.72
3.56	2.16	1.78	1.21	99.00	2.73
3.224	3.68	1.612	2.28	104.7	2.85
3.06	4.43	1.53	2.90	113.5	2.76
2.91	5.10	1.455	3.51	117.3	2.80
2.63	6.40	1.315	4.87	128.0	2.83
2.52	14.3	1.26	11.35	131.1	2.88
2.33	28.2	1.165	24.21	140.5	2.89
2.16	61.9	1.08	57.31	151.5	2.89

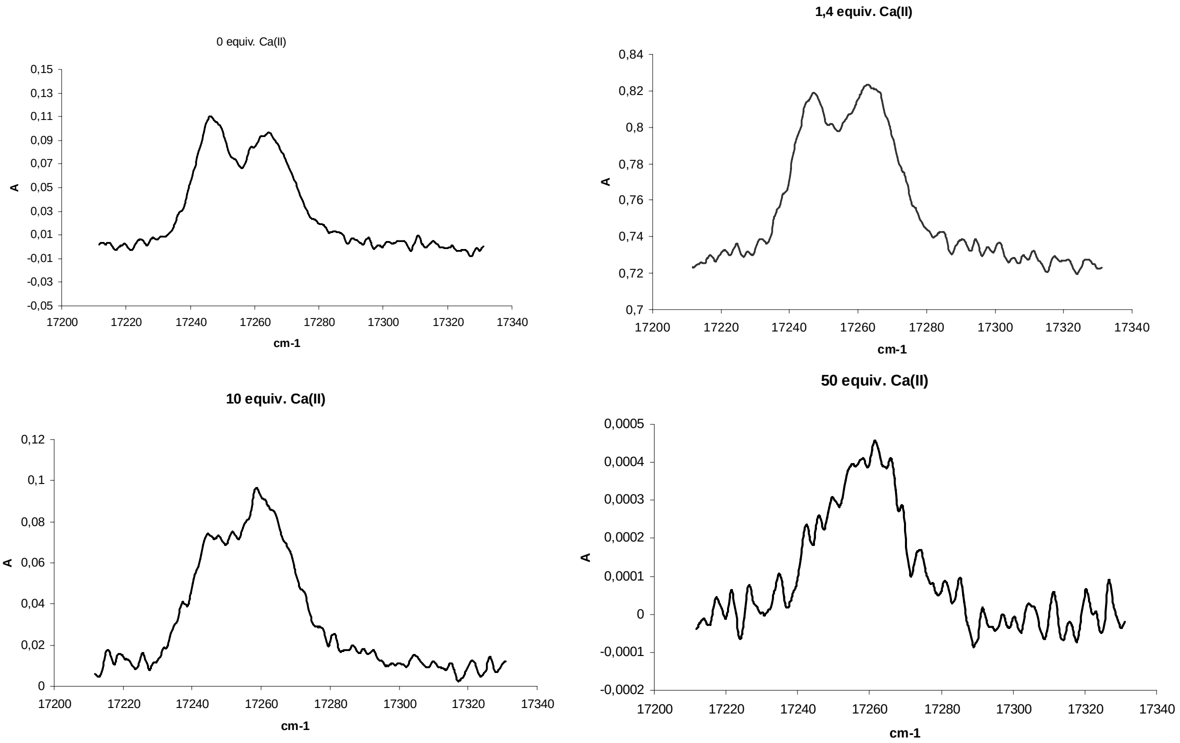
Table S7. Relaxometric Mg^{2+} titration of Gd_2L^1 at 25°C, pH 7 and 11.75 T.

c(Gd^{3+}) / mM	c(Mg^{2+}) / mM	c(Ca^{2+}) / mM	c(complex) / mM	n(Mg^{2+}):n(complex)	n(Ca^{2+}):n(complex)	r_1 / mmol^{-1}s^{-1}
2.60	0	0	1.30	0.00	0.00	3.35
2.57	1.21	0	1.29	0.94	0.00	3.37
2.54	2.38	0	1.27	1.87	0.00	3.37
2.48	4.65	0	1.24	3.75	0.00	3.34
2.42	6.82	0	1.21	5.64	0.00	3.41
2.34	9.88	0	1.17	8.44	0.00	3.36
2.26	12.74	0	1.13	11.27	0.00	3.51
2.17	16.28	0	1.09	15.00	0.00	3.45
2.08	19.54	0	1.04	18.79	0.00	3.43
2.00	22.55	0	1.00	22.55	0.00	3.47
1.96	22.12	1.86	0.98	22.57	1.90	3.54
1.93	21.71	3.66	0.97	22.50	3.79	3.53
1.86	20.94	7.05	0.93	22.52	7.58	3.59
1.79	20.21	10.21	0.895	22.58	11.41	3.70
1.73	19.54	13.16	0.865	22.59	15.21	3.71
1.68	18.91	15.92	0.840	22.51	18.95	3.76
1.625	18.32	18.51	0.813	22.55	22.78	3.78
1.58	17.76	20.94	0.790	22.48	26.51	3.81
1.51	16.99	24.3	0.755	22.50	32.19	3.86
1.42	16.06	28.39	0.710	22.62	39.99	3.92
1.30	14.655	34.55	0.650	22.55	53.15	3.90

Table S8. Luminescence lifetimes measurements on Eu_2L^1 at 25°C, pH neutral, and the calculated hydration numbers q.

n(Ca^{2+}):n(complex)	τ_{H2O} / ms	τ_{D2O} / ms	q
0	0.325	0.407	0.44
0	0.327	0.408	0.43
0	0.329	0.409	0.41
0	0.327	0.411	0.45
0	0.329	0.408	0.40
2	0.346	0.471	0.63
2	0.348	0.471	0.60
2	0.347	0.472	0.61
2	0.346	0.469	0.61
2	0.345	0.470	0.63
10	0.389	0.595	0.77
10	0.388	0.591	0.76
10	0.390	0.594	0.76
10	0.390	0.591	0.74
10	0.389	0.591	0.76
20	0.423	0.661	0.72
20	0.424	0.665	0.73
20	0.423	0.661	0.72
20	0.419	0.661	0.75
20	0.420	0.659	0.73
40	0.461	0.729	0.66
40	0.461	0.730	0.66
40	0.462	0.729	0.65
40	0.462	0.729	0.65
40	0.459	0.728	0.67
80	0.484	0.772	0.62
80	0.483	0.775	0.63
80	0.485	0.774	0.62
80	0.485	0.774	0.62
80	0.486	0.772	0.62

Figure S1. Variable Ca^{2+} concentration UV-Vis studies on Eu_2L^1 at 50°C.

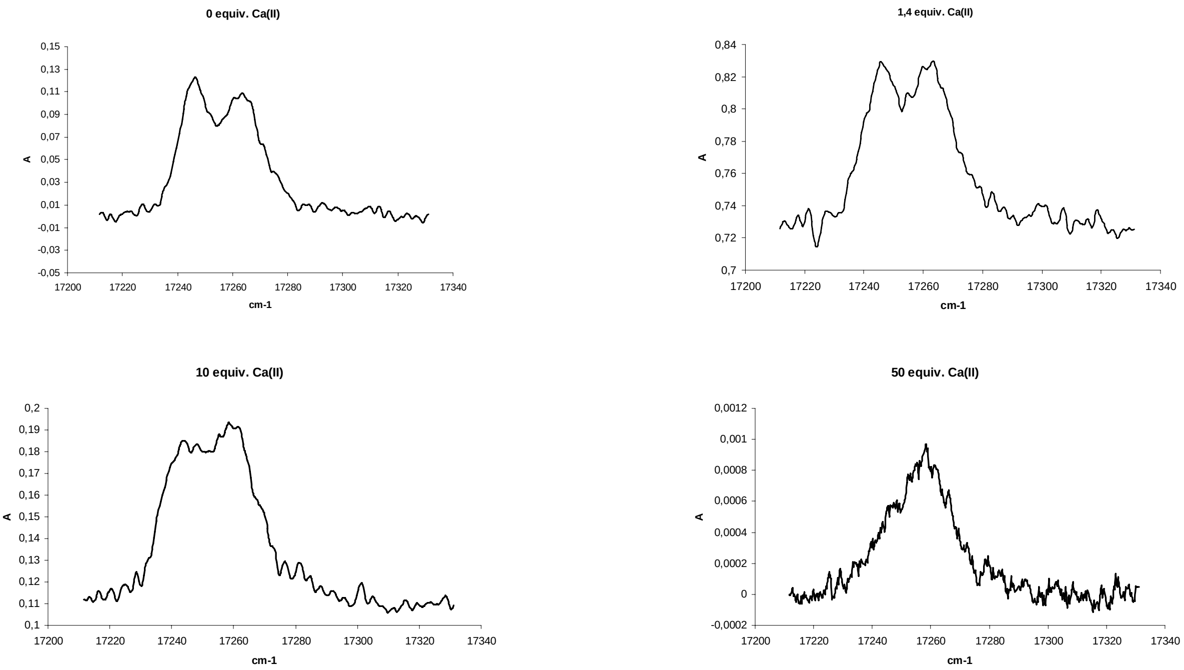

0 equiv. Ca(II)

1,4 equiv. Ca(II)

10 equiv. Ca(II)

50 equiv. Ca(II)

Figure S2. Variable Ca^{2+} concentration UV-Vis studies on Eu_2L^1 at 37°C.


0 equiv. Ca(II)

1,4 equiv. Ca(II)

10 equiv. Ca(II)

50 equiv. Ca(II)

Figure S3. Variable Ca^{2+} concentration UV-Vis studies on Eu_2L^1 at 25°C.

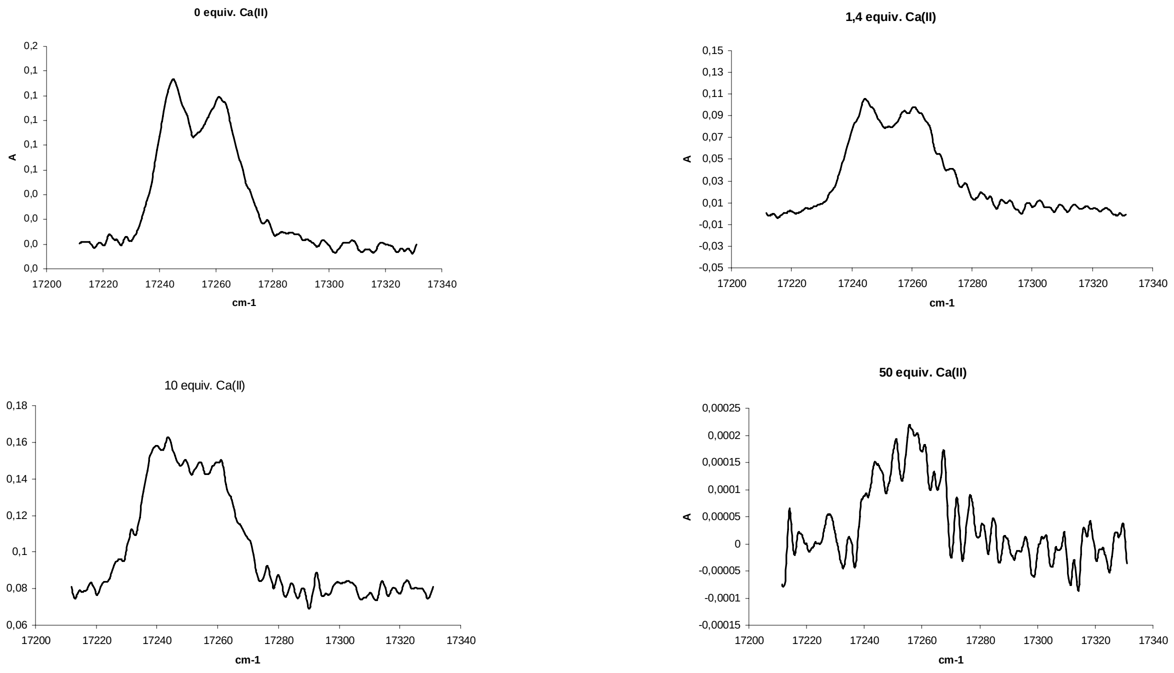


Figure S4. Variable Ca^{2+} concentration UV-Vis studies on Eu_2L^1 at 15.5°C.

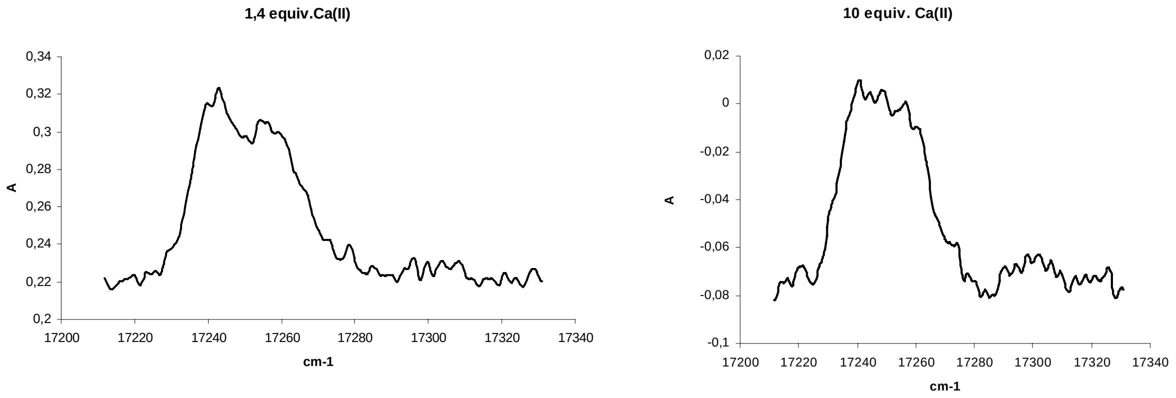


Table S9. Fitted parameters of Gd_2L^1 in the absence of Ca^{2+}. The underlined parameters were fixed during the fitting.

Parameter	Gd_2L^1
k_{ex}^{298} [10^6 s^{-1}]	2.4±0.2
$\Delta H^\ddagger$ [kJ mol^{-1}]	43.6±3.3
$\Delta S^\ddagger$ [J $mol^{-1}K^{-1}$]	+23.5
$A/\hbar$ [10^6 rad s^{-1}]	$\underline{-3.8}$
τ_{RO}^{298} [ps]	349±47
E_R [kJ mol^{-1}]	24±1
τ_V^{298} [ps]	20.6±2.7
E_V [kJ mol^{-1}]	$\underline{1}$
Δ^2[10^{20} s^{-2}]	0.46±0.10
D^{298}_{GdH} [$10^{-10}m^2s^{-1}$]	25±3
E_{DGdH} [kJ mol^{-1}]	30±2
δg_L^2 [10^{-1}]	2.7±0.7
$\tau_{RH}^{298}/\ \tau_{RO}^{298}$	0.76±0.12
r_{GdO} [Å]	$\underline{2.5}$
r_{GdH} [Å]	$\underline{3.1}$
$r_{GdHouter}$ [Å]	$\underline{3.6}$
$\chi(1+\eta^2/3)^{1/2}$ [MHz]	$\underline{7.58}$
q	$\underline{0.4}$
q_{2nd}	$\underline{1}$
$\tau_M{}^{298}{}_{2nd}$ [ps]	$\underline{50}$
$\Delta H^{298}{}_{2nd}$ [kJ mol^{-1}]	$\underline{35}$
$r^{2nd}{}_{GdH}$ [Å]	$\underline{3.5}$
$r^{2nd}{}_{GdO}$ [Å]	$\underline{4.1}$

Table S10. Fitted parameters of Gd_2L^1 in the presence of 1M Ca^{2+}. The underlined parameters were fixed during the fitting.

Parameter	$Gd_2L^1 + Ca^{2+}$
k_{ex}^{298} [10^6 s^{-1}]	7.5±1.6
$\Delta H^{\ddagger}$ [kJ mol^{-1}]	$\underline{43.6}$
$\Delta S^{\ddagger}$ [J $mol^{-1}K^{-1}$]	+33.0
$A/\hbar$ [10^6 rad s^{-1}]	$\underline{-3.8}$
τ_{RO}^{298} [ps]	1152±243
E_R [kJ mol^{-1}]	21±6
τ_V^{298} [ps]	0.13±0.02
E_V [kJ mol^{-1}]	$\underline{1}$
Δ^2[10^{20} s^{-2}]	0.50±0.05
δg_L^2 [10^{-2}]	$\underline{2.1}$
r_{GdO} [Å]	$\underline{2.5}$
r_{GdH} [Å]	$\underline{3.1}$
$r_{GdHouter}$ [Å]	$\underline{3.5}$
$\chi(1+\eta^2/3)^{1/2}$ [MHz]	$\underline{7.58}$
q	$\underline{0.7}$
q_{2nd}	$\underline{1}$
$\tau_M{}^{298}{}_{2nd}$ [ps]	$\underline{50}$
$\Delta H^{298}{}_{2nd}$ [kJ mol^{-1}]	$\underline{35}$
$r^{2nd}{}_{GdH}$ [Å]	$\underline{3.5}$
$r^{2nd}{}_{GdO}$ [Å]	$\underline{4.1}$

Figure S5. IR spectrum of the ligand L^1.

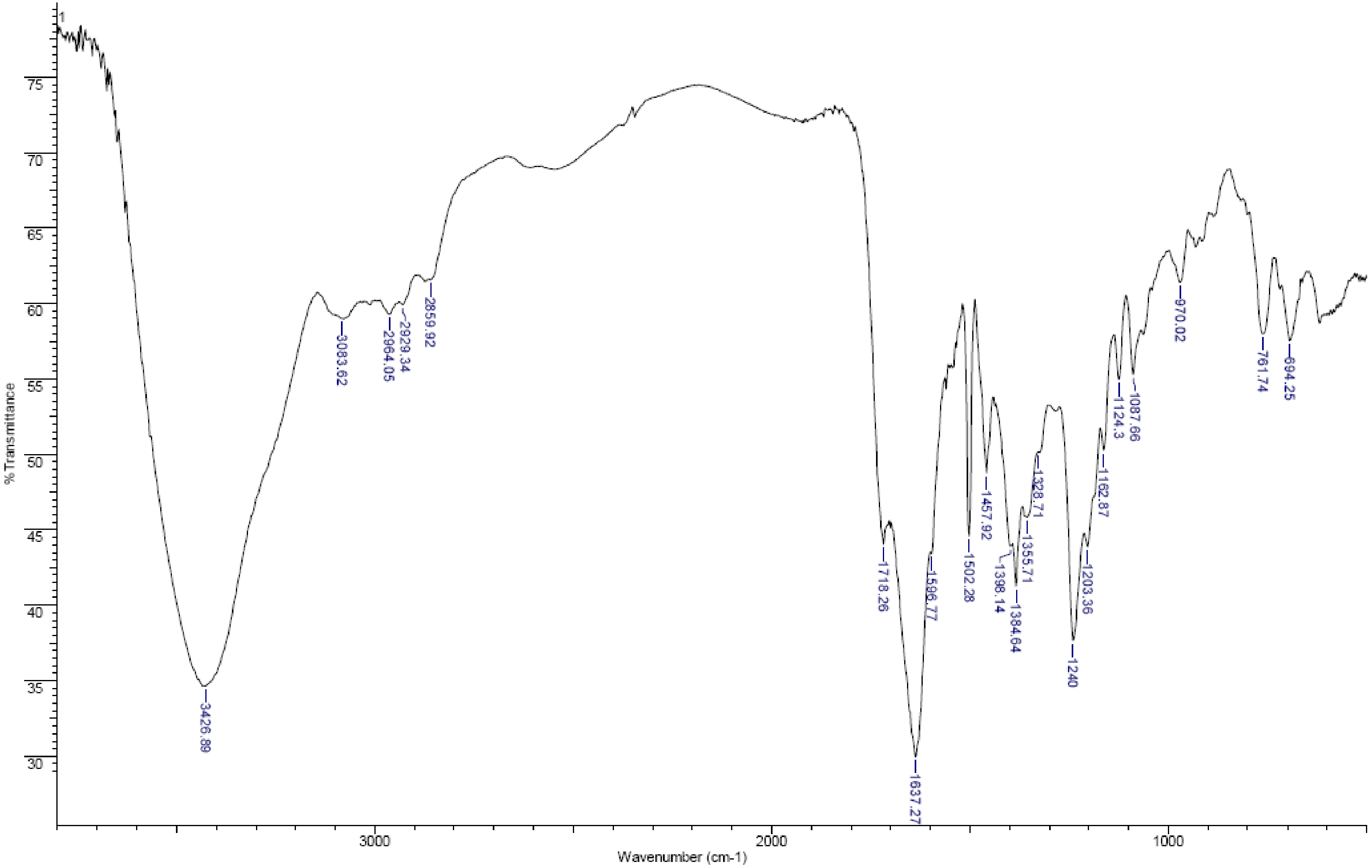


Figure S6. IR spectrum of Gd_2L^1 complex.

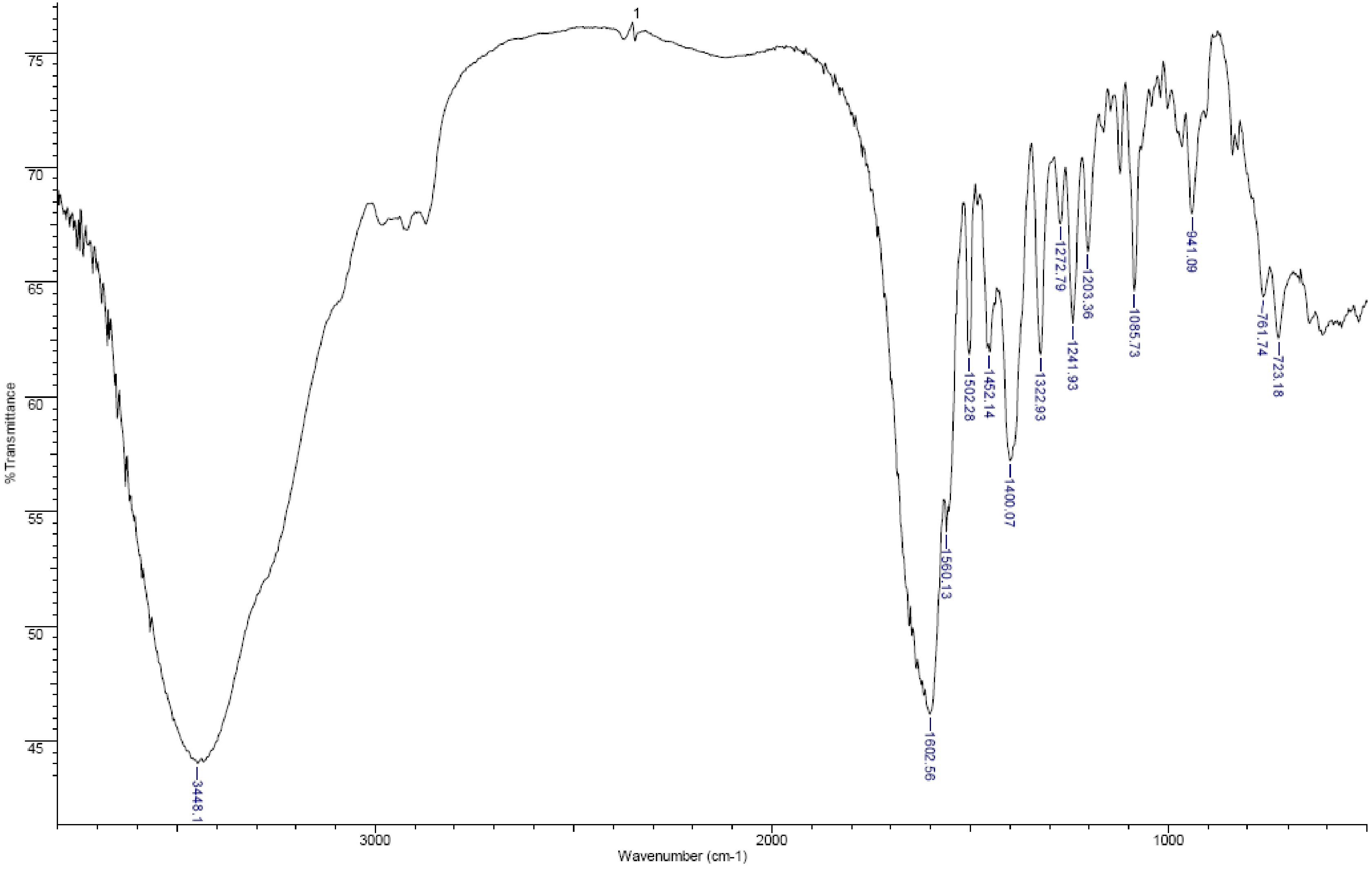


Figure S7. ^{1}H NMR spectrum of the compound **6a**.

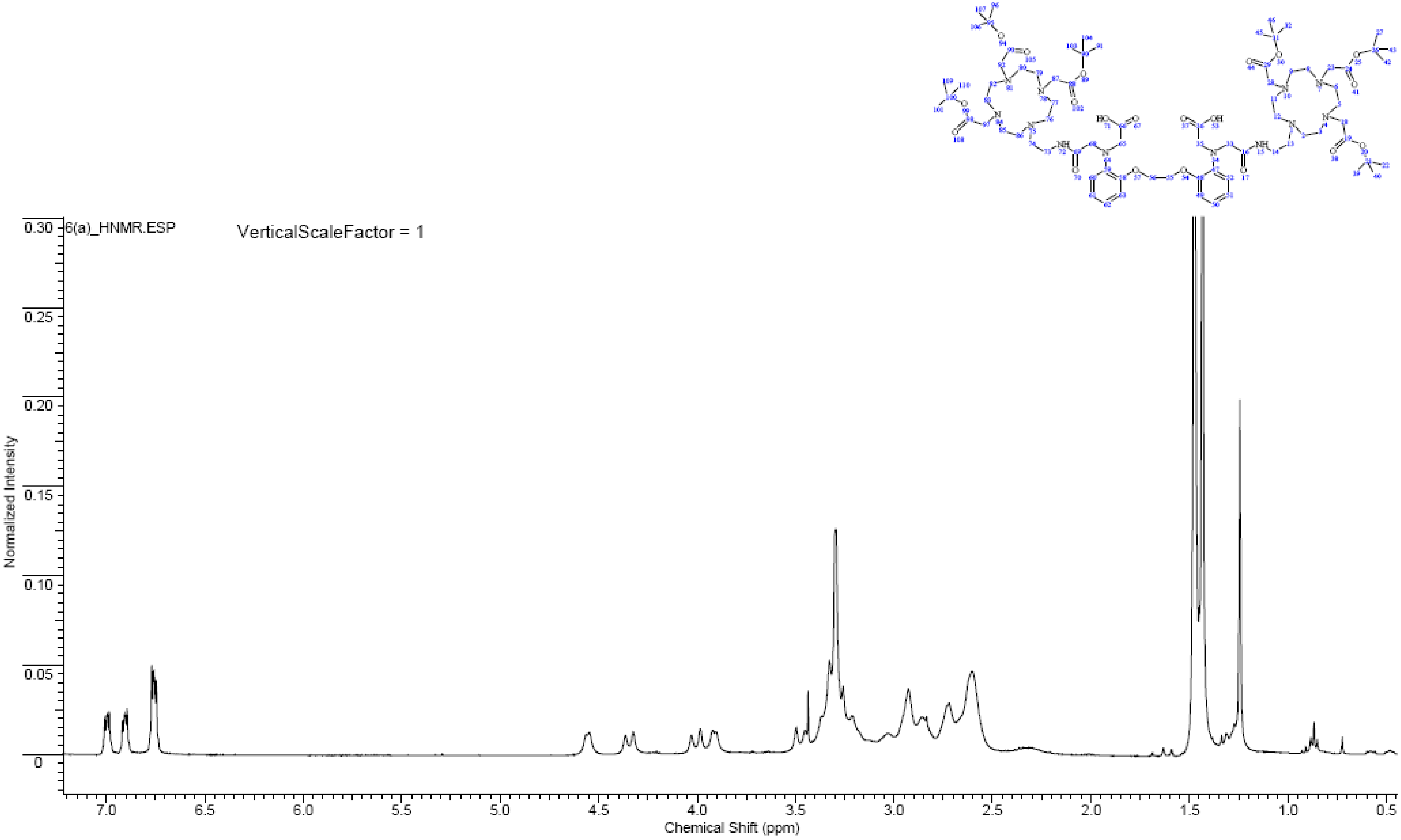
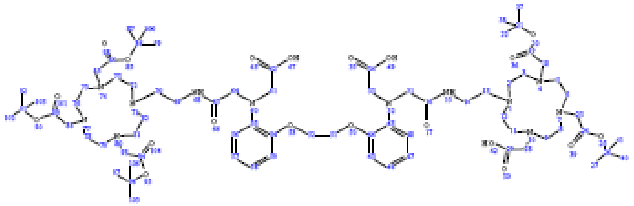


Figure S8. ^{13}C NMR spectrum of the compound **6a**.



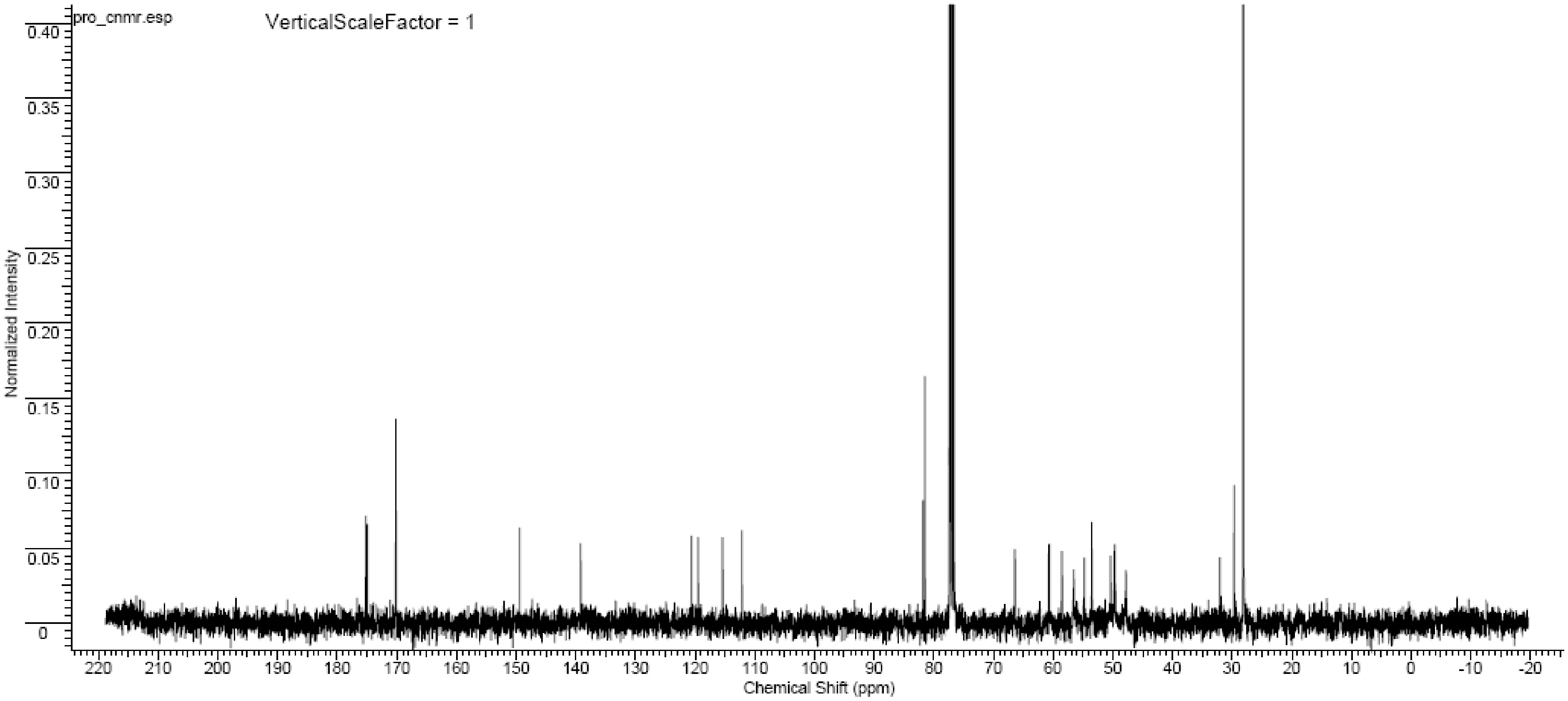


Figure S9. ^{1}H NMR spectrum of the ligand L^1.

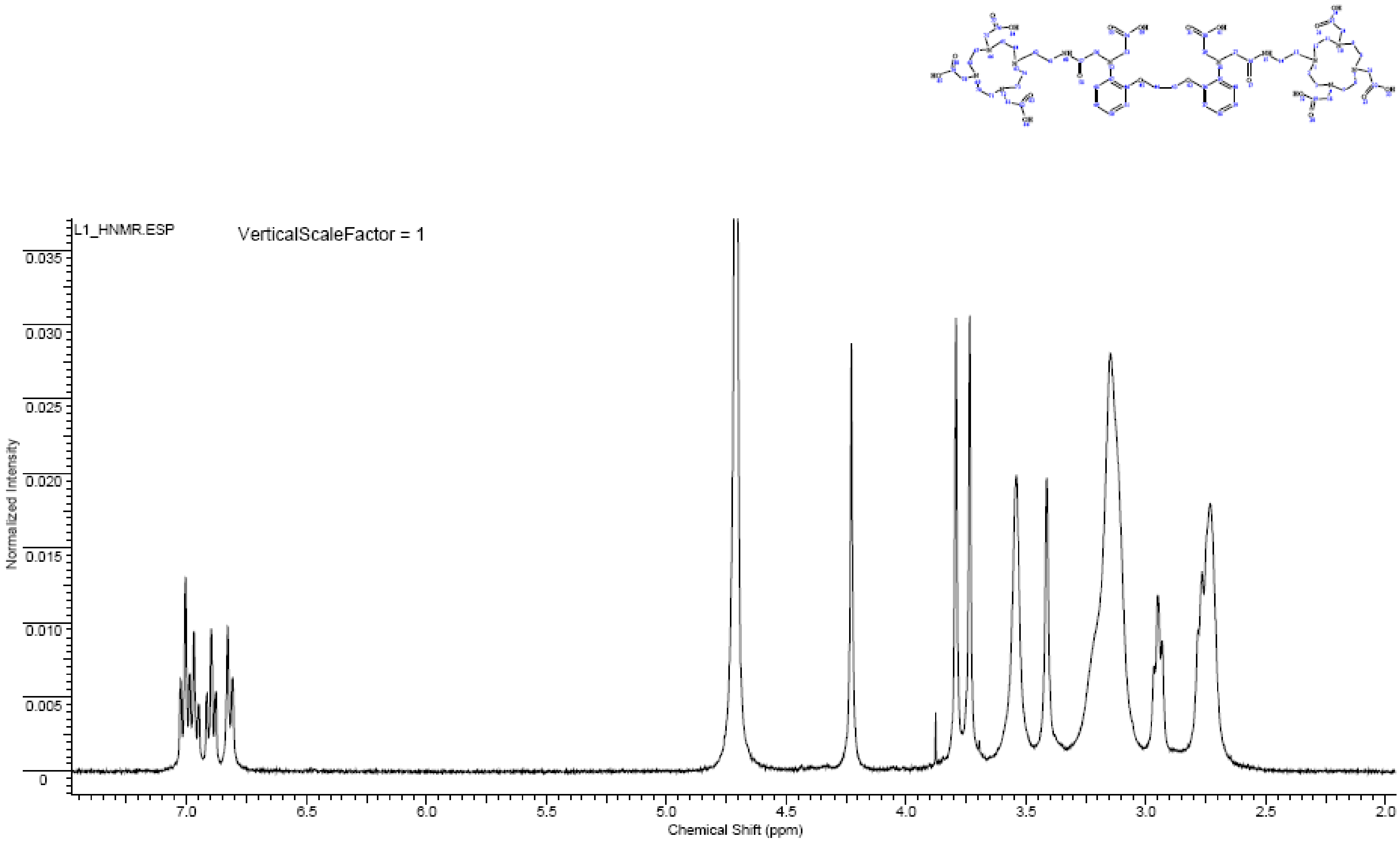
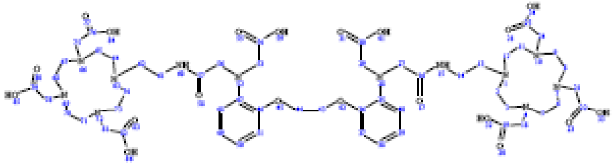


Figure S10. ^{13}C NMR spectrum of the ligand L^{1}.



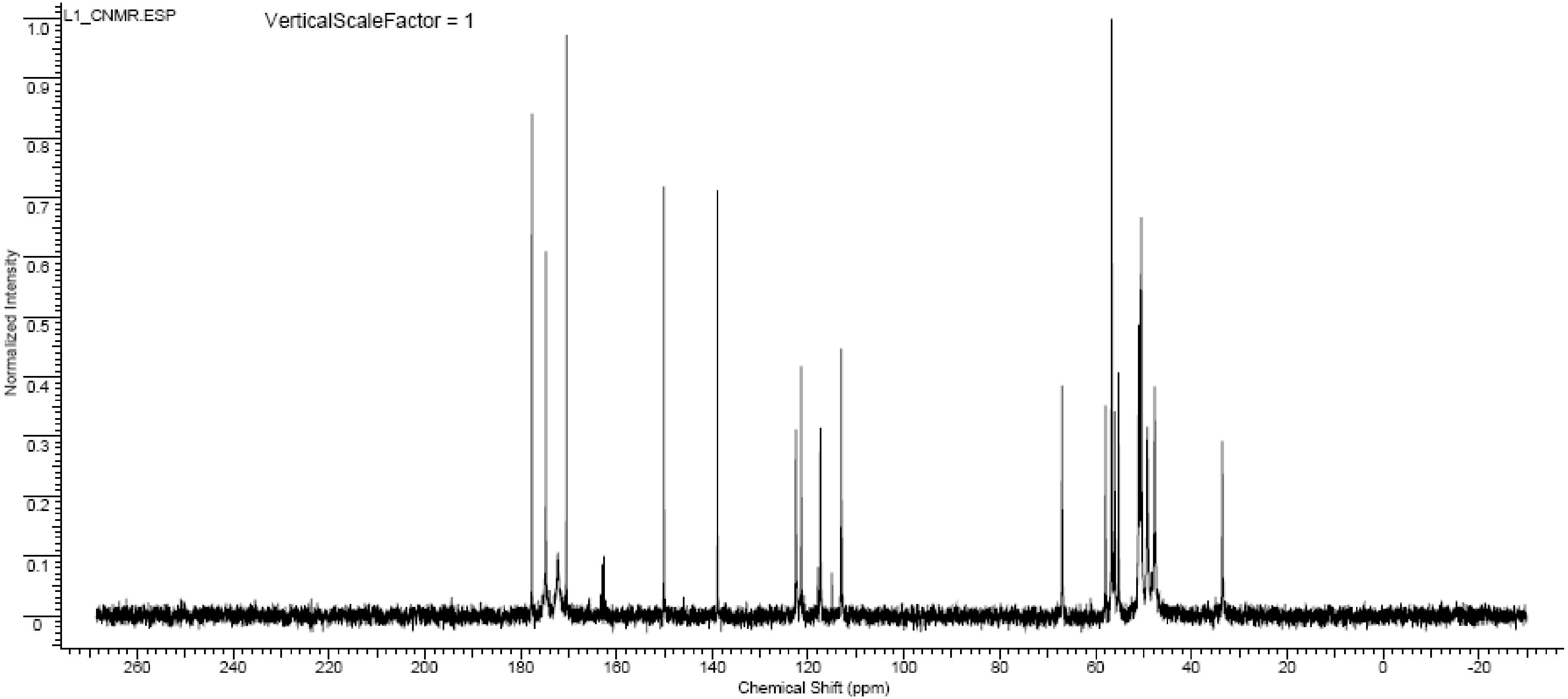


Figure S11. ^{1}H NMR spectrum of the ligand L^2.

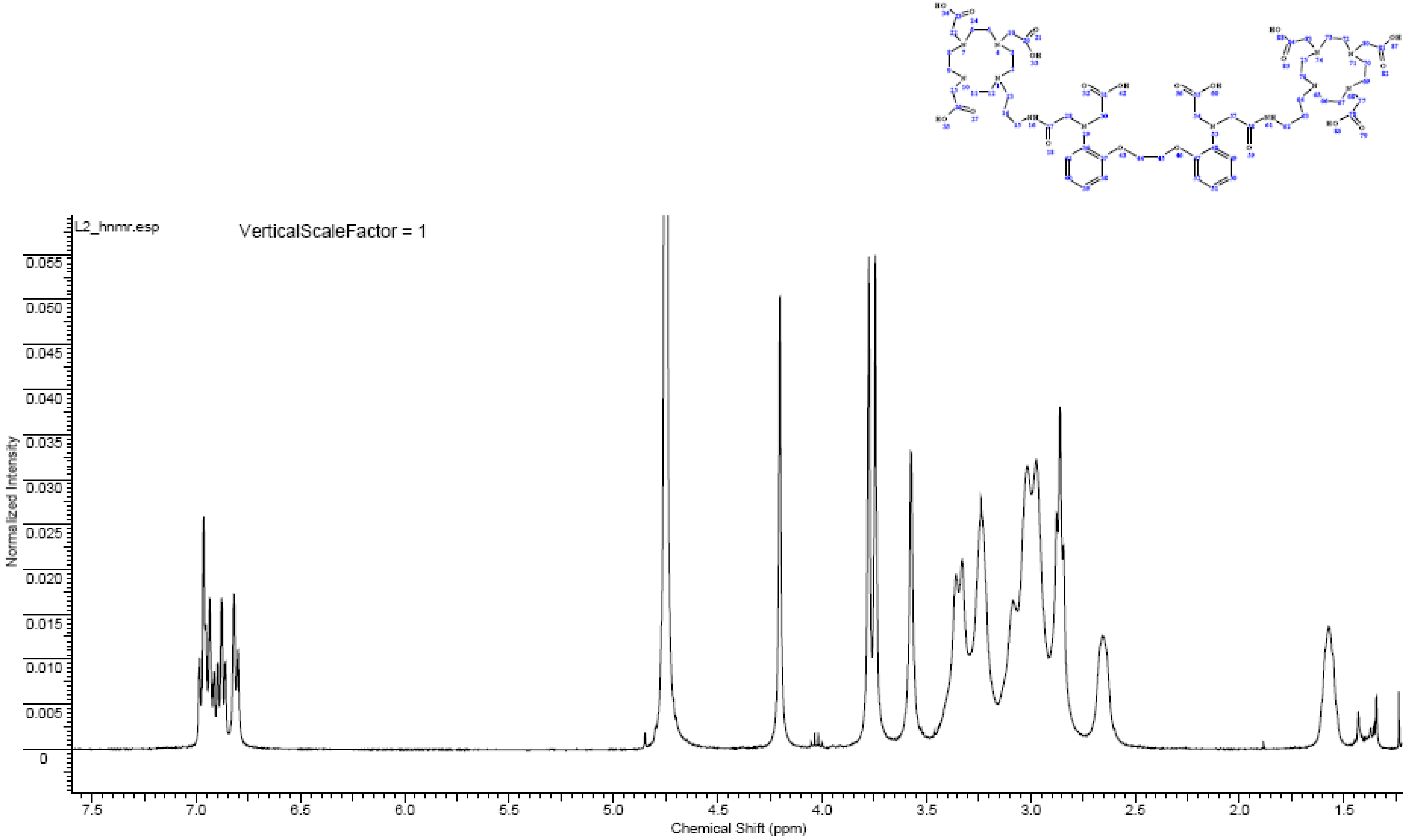


Figure S12. ^{13}C NMR spectrum of the ligand L^2.

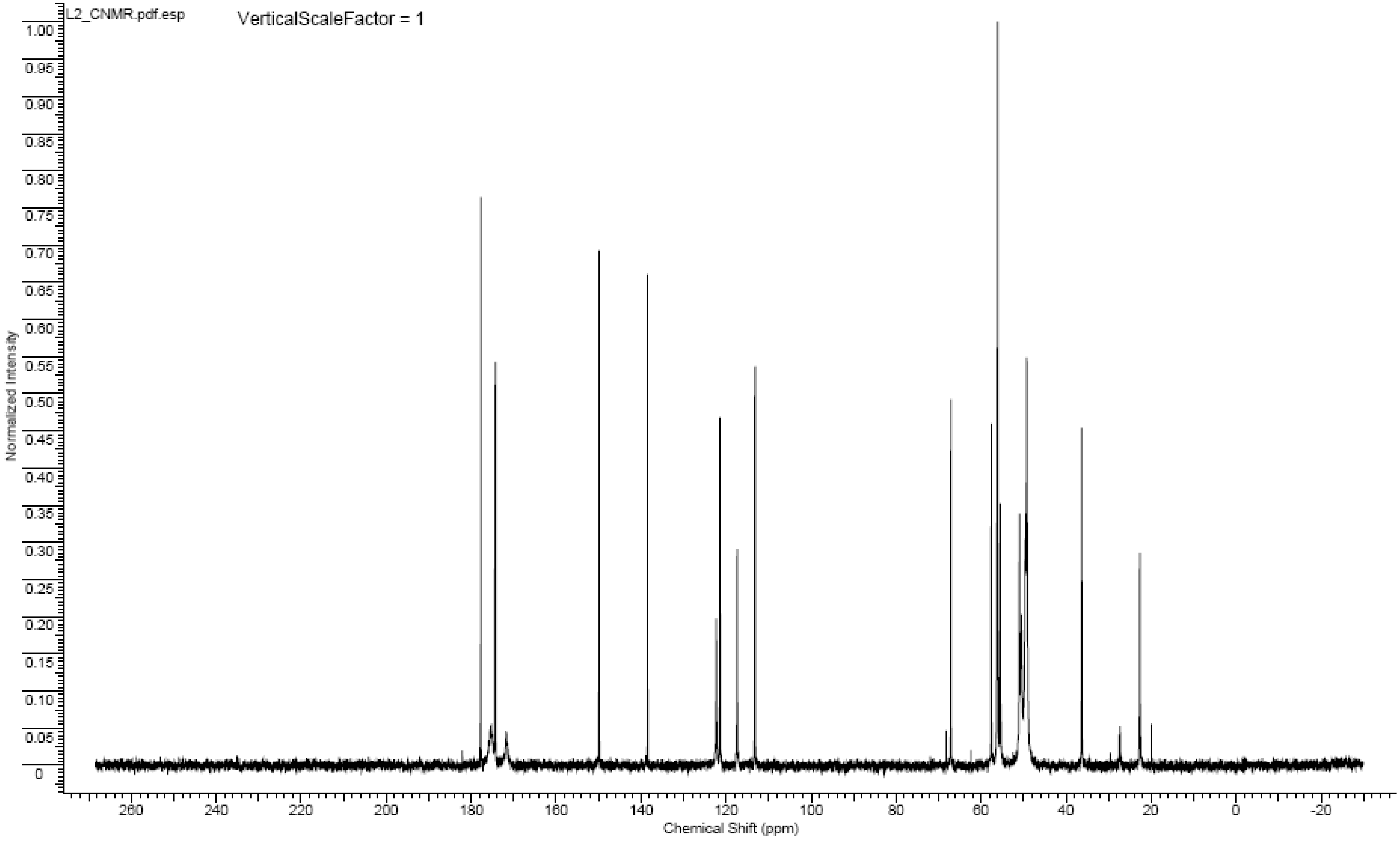


Figure S13. ^{1}H-^{1}H COSY spectrum of the compound 6a.

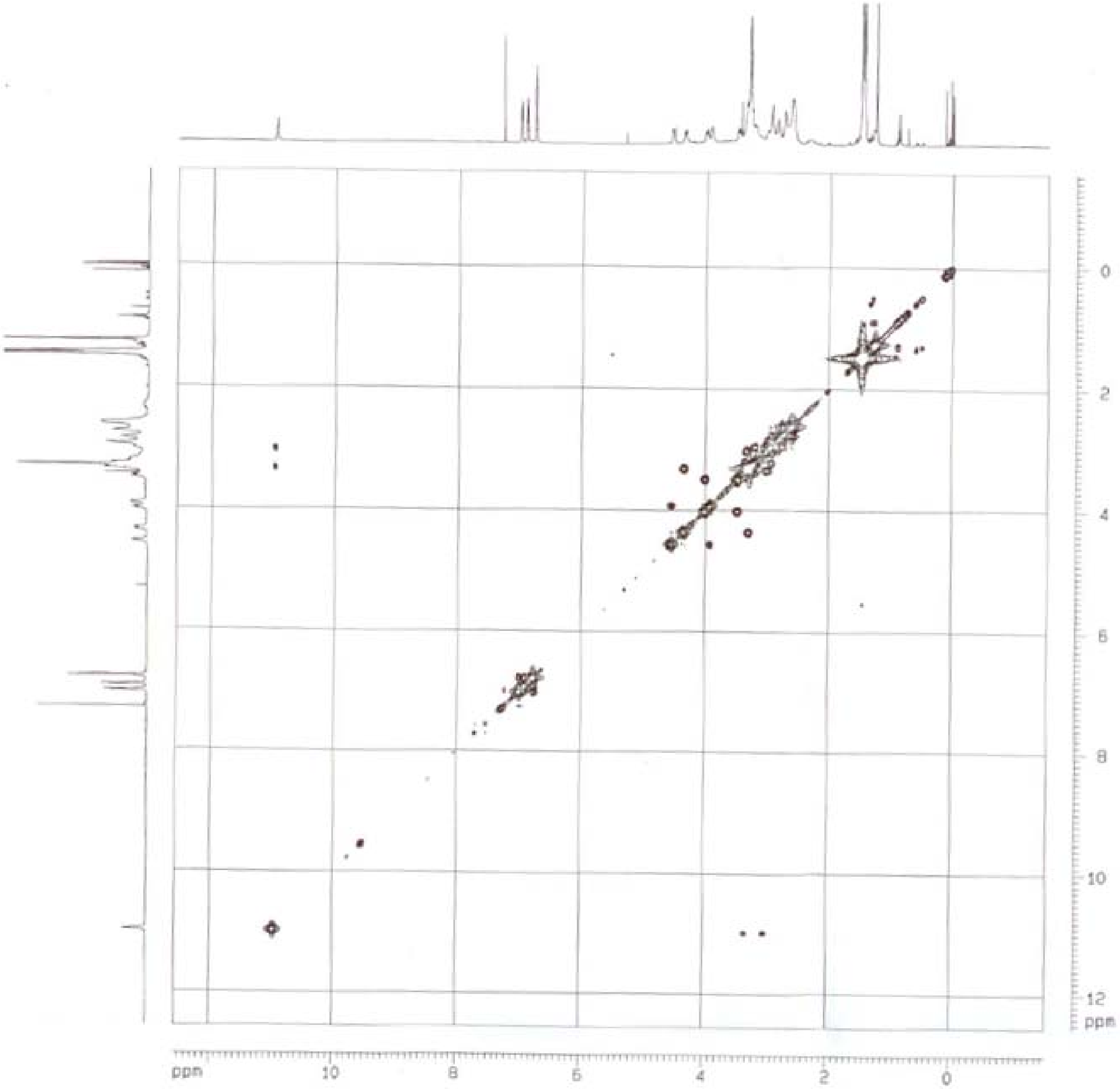
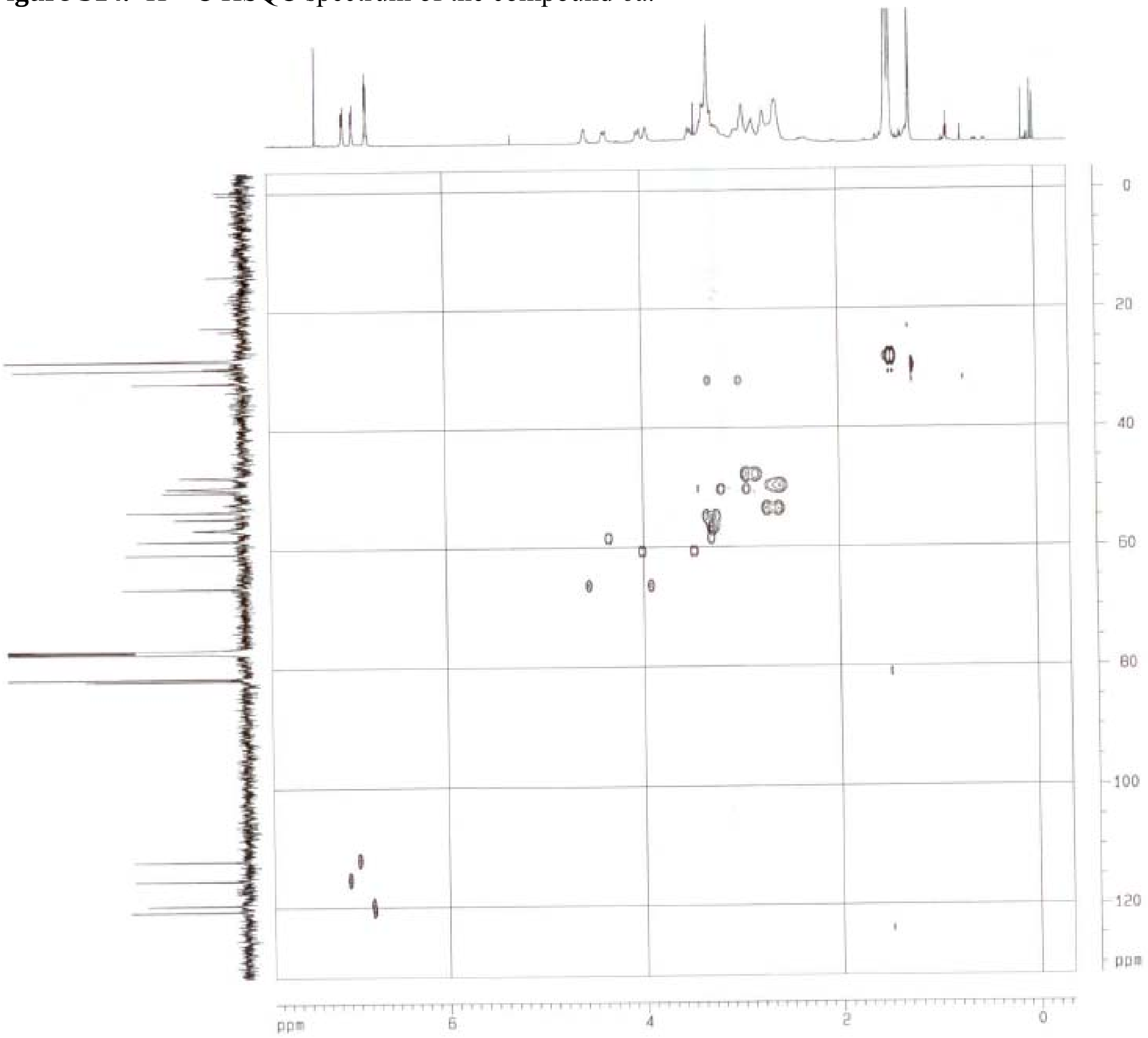


Figure S14. ^{1}H-^{13}C HSQC spectrum of the compound 6a.



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