Supporting Information

A Novel Ratiometric Emission probe for Ca^{2+} in living cells

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Content

1. Calculation of dissociation constants

1.1. Dissociation constant based on ratiometric probe with stoichiometric ratio 1:1

$$\mathrm{ML} \rightleftharpoons \mathrm{M} + \mathrm{L}$$

$$K_d = \frac{[L]\times[M]}{[ML]} \quad \Rightarrow \quad \frac{K_d}{[M]} = \frac{[L]}{[ML]} \tag{1}$$

The fluorescence intensities (F_1 and F_2) measured at two emission wavelengths and an excitation wavelength can be determined by the following equations:

$$F_1 = S_{f1}[L] + S_{b1}[ML]$$

$$F_2 = S_{f2}[L] + S_{b2}[ML]$$

where S_f and S_b are the fluorescence intensity coefficients of Ca^{2+}-free and Ca^{2+}-bound probe, respectively. Then,

$$R = \frac{F_1}{F_2} = \frac{S_{f1}[L] + S_{b1}[ML]}{S_{f2}[L] + S_{b2}[ML]}$$

For the free ligand and full complexation, R is as follows:

$$R_{\min} = \frac{S_{f1}}{S_{f2}}$$

$$R_{\max} = \frac{S_{b1}}{S_{b2}}$$

Thus, $$\frac{R - R_{\min}}{R_{\max} - R} = \frac{[ML]}{[L]} \times \frac{S_{b2}}{S_{f2}} \tag{2}$$

Substitution of Eq.1 into 2,

$$\frac{R - R_{\min}}{R_{\max} - R} \times \frac{S_{f2}}{S_{b2}} = \frac{[M]}{[K_d]}$$

$$\log\left(\frac{R - R_{\min}}{R_{\max} - R} \times \frac{S_{f2}}{S_{b2}}\right) = \log M - \log K_d \tag{3}$$

In this work, free [Ca^{2+}] levels were controlled by Ca^{2+}/EGTA buffer and calculated according to the previous reports.[1,2]

1.2. Dissociation constant based on intensity-probe

$$\mathrm{M}_n\mathrm{L} \rightleftharpoons n\mathrm{M} + \mathrm{L}$$

$$K_d = \frac{([L]_0 - [M_nL]) \times [M]^n}{[M_nL]} \quad (4)$$

$$\frac{[M_nL]}{[L]_0 - [M_nL]} = \frac{[M]^n}{K_d} \quad (5)$$

where, $F_0 = F_{\max} = S_f[L]_0$, $F_{\min} = S_b[M_nL] = S_b[L]_0$

$$F = S_f([L]_0 - [M_nL]) + S_b[M_nL] \quad (6)$$

Thus,

$$\frac{F - F_{\min}}{F_{\max} - F} = \frac{(S_f - S_b)([L]_0 - [M_nL])}{(S_f - S_b)[M_nL]} = \frac{([L]_0 - [M_nL])}{[M_nL]} = \frac{K_d}{[M]^n} \quad (7)$$

Combining Eq. 5, 6 and 7, k_d can be obtained:

$$\log \frac{F_{\max} - F}{F - F_{\min}} = n \log M - \log K_d \quad (8)$$

where $F_{\max}$ is the fluorescence intensity of free probe, $F_{\min}$ is the fluorescence intensity of OXD-BAPTA with saturated Mg^{2+}, and F is the fluorescence intensity of OXD-BAPTA at various concentrations of Mg^{2+}. In this work, free [Mg^{2+}] levels were controlled by Mg^{2+}/EGTA buffer and calculated according to the previous reports.[1,2]

2. Determination of fluorescence quantum yield

Fluorescence quantum yield was measured by a standard method in air-equilibrated sample at room temperature. The fluorescence quantum yield was determined by using quinine bisulfate in 0.050 M H_2SO_4 (Φ = 0.546) as reference.[3,4]

$$\Phi_{\mathrm{sam}} = \Phi_{\mathrm{ref}} \frac{I_{sam}}{I_{ref}} \cdot \frac{A_{ref}}{A_{sam}} \left(\frac{n_{sam}}{n_{ref}}\right)^2$$

where Φ is the fluorescence quantum yield, I is the integrated emission intensity, A is the absorbance, and n is the refractive index. The subscripts $_{sam}$ and $_{ref}$ stand for sample and reference, respectively.

3. Supplementary figures

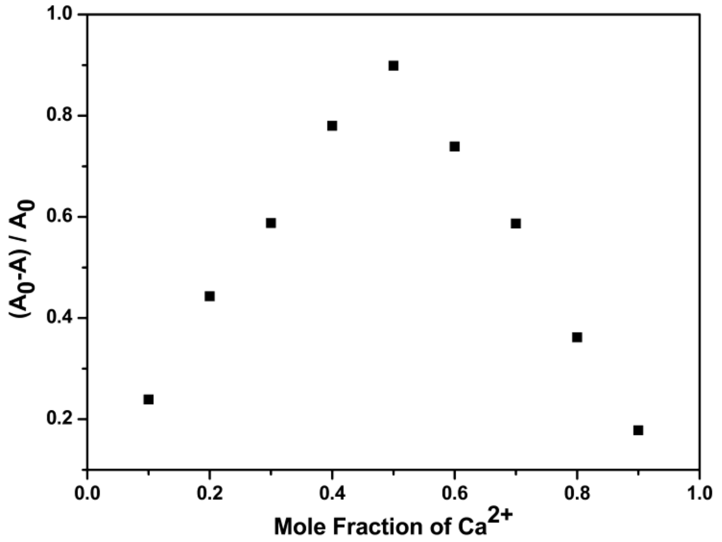


Fig. S1 Job plot for determination of the stoichiometry of OXD-BAPTA-Ca^{2+} complex. The total concentration of OXD-BAPTA and Ca^{2+} was maintained as 10.0 μM in 50 mM HEPES containing 100 mM KCl and 10 mM EGTA at pH 7.2. The plot of $(A_o - A)/A_o$ against the mole fraction of Ca^{2+}, where A_o and A are the absorbances of OXD-BAPTA in the absence and presence of Ca^{2+}.

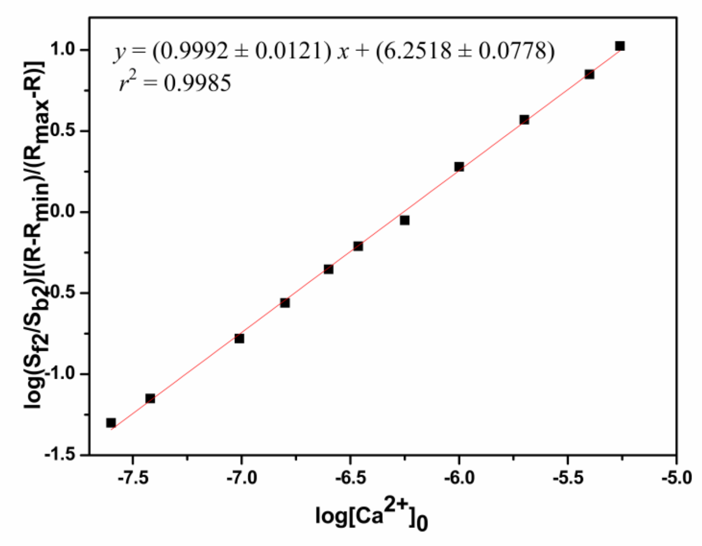


Fig. S2 Hill plot for the complexation of 1.0 μM OXD-BAPTA with free Ca^{2+} (0.0–11.1 μM) in 50 mM HEPES containing 100 mM KCl and 10 mM EGTA at pH 7.2. The excitation wavelength is 380 nm.

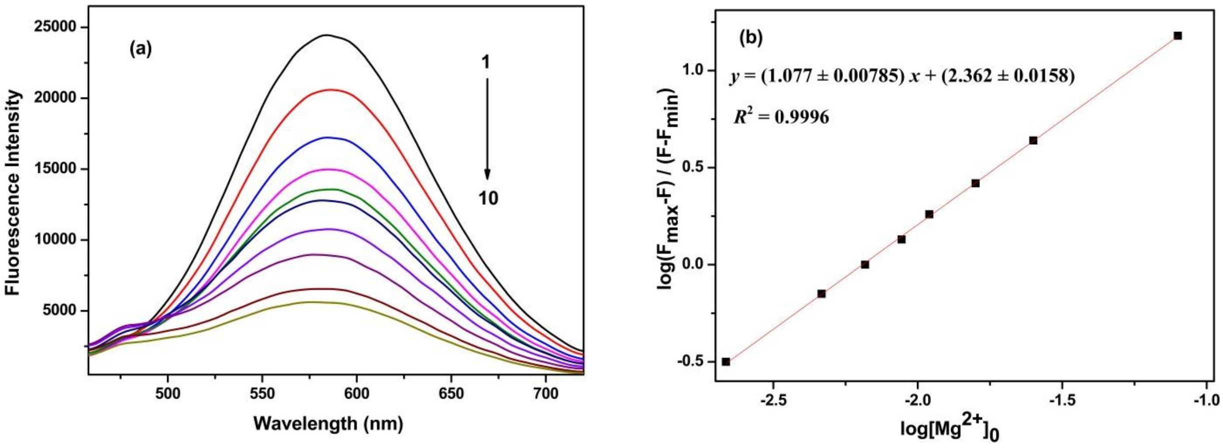


Fig. S3 (a) Fluorescence emission spectra of 1.0 μM OXD-BAPTA upon the addition of various concentrations of Mg^{2+} (1–10: 0.0, 2.17, 4.63, 6.57, 8.80, 11.0, 15.6, 24.7, 79.4, and 125.0 mM) in 50 mM HEPES buffer solution containing 10 mM EGTA and 100 mM KCl at pH 7.2. (b) Hill plot for the complexation of OXD-BAPTA with free Mg^{2+} (0.0–125 mM). The excitation wavelength is 380 nm.

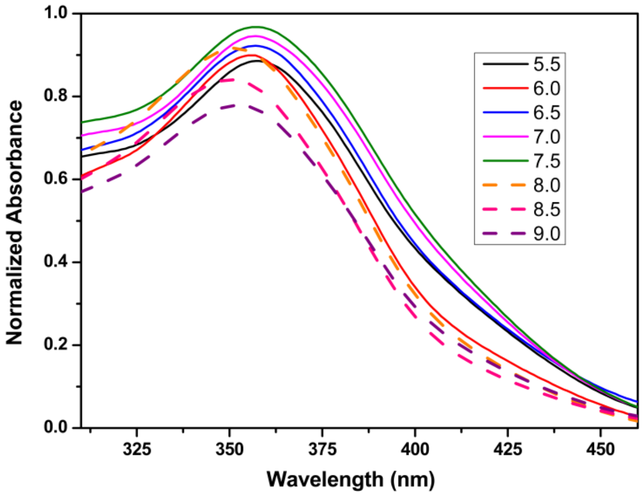
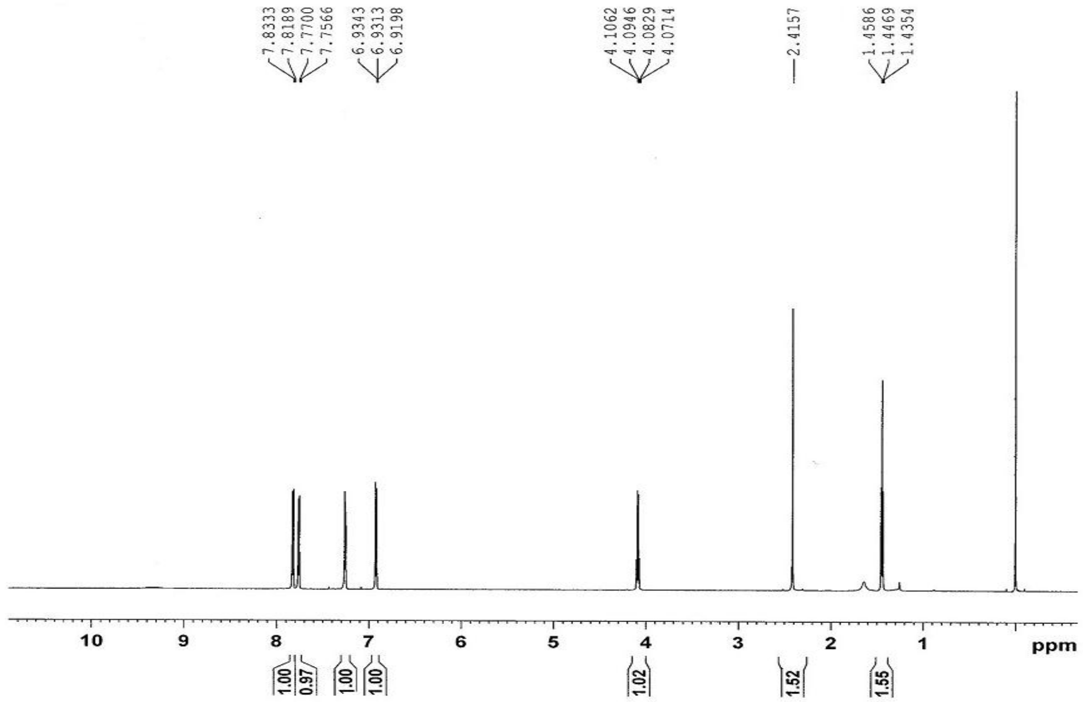


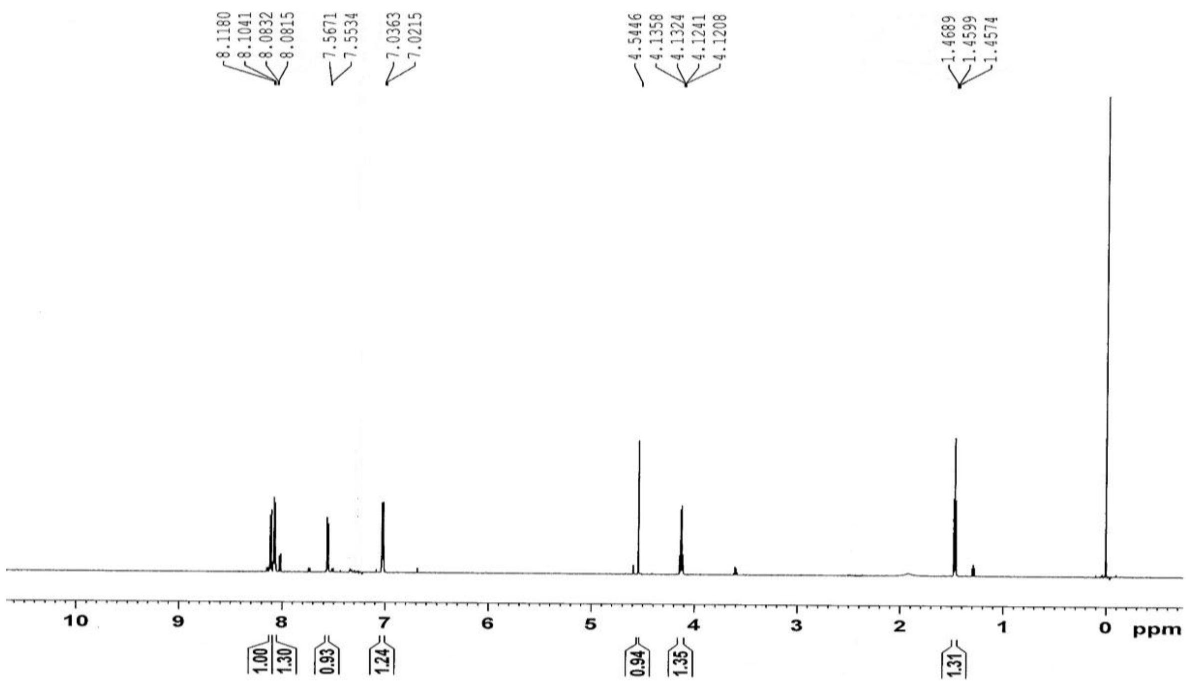
Fig. S4 Effect of pH on the absorption spectra of 10 μM OXD-BAPTA in 50 mM HEPES containing 100 mM KCl. The absorbance increases with pH from 5.5 to 7.5 and then decreases from 8.0 to 9.0.

4. NMR spectra and MALDI-TOF MS

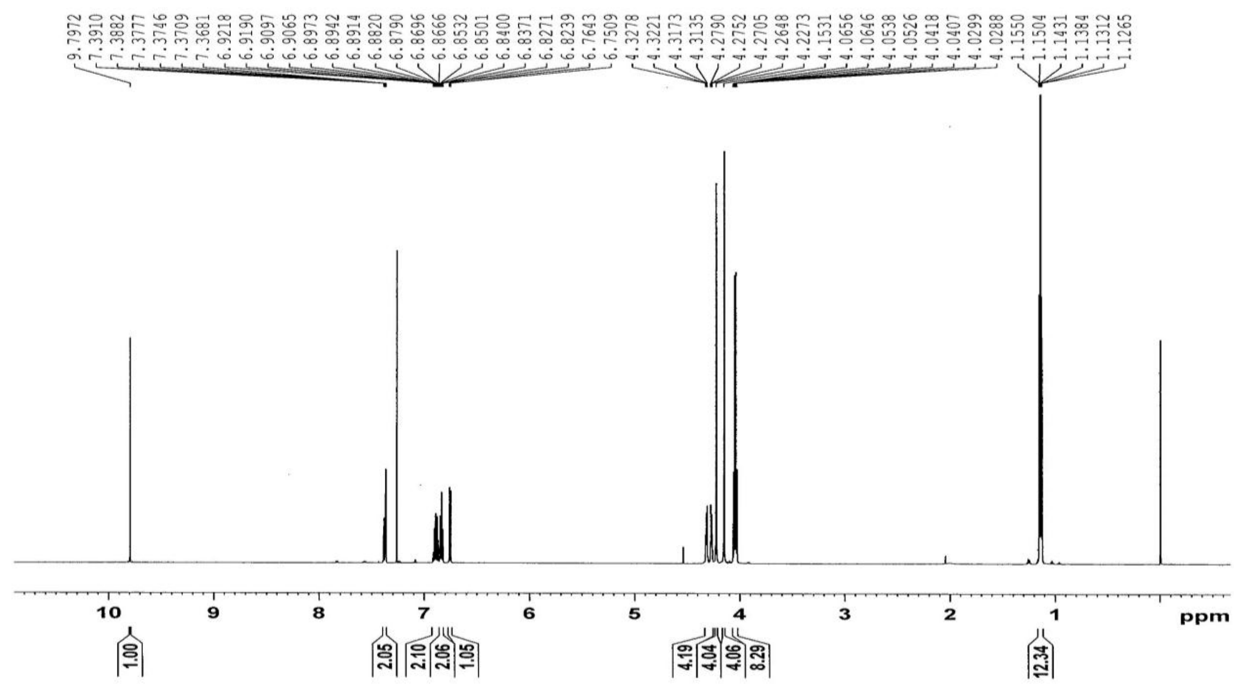
4.1. ^{1}H NMR spectrum of 2-(4-ethoxyphenyl)-5-(4-methyl phenyl)-1,3,4-oxadiazole in $CDCl_3$.



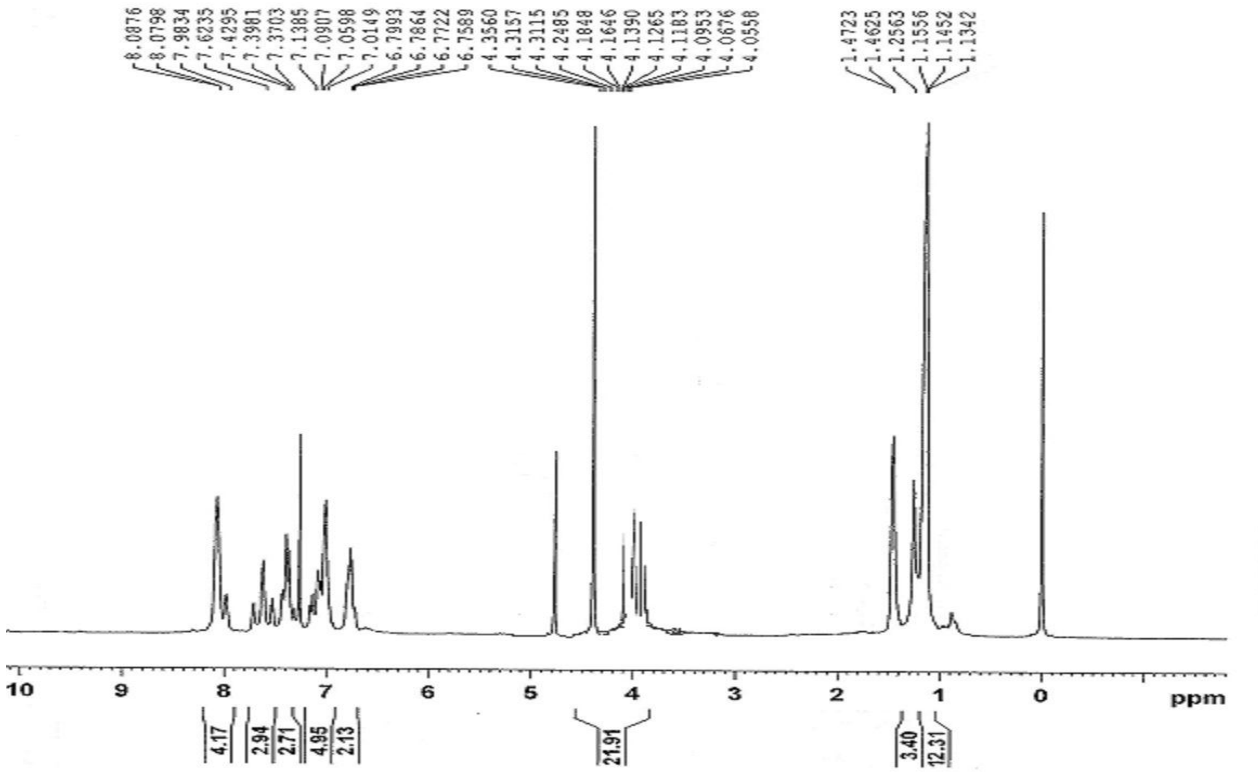
4.2. ^{1}H NMR spectrum of 2-[(4-bromomethyl)phenyl]-5-(4-ethoxy phenyl)-1,3,4-oxadiazole in $CDCl_3$.



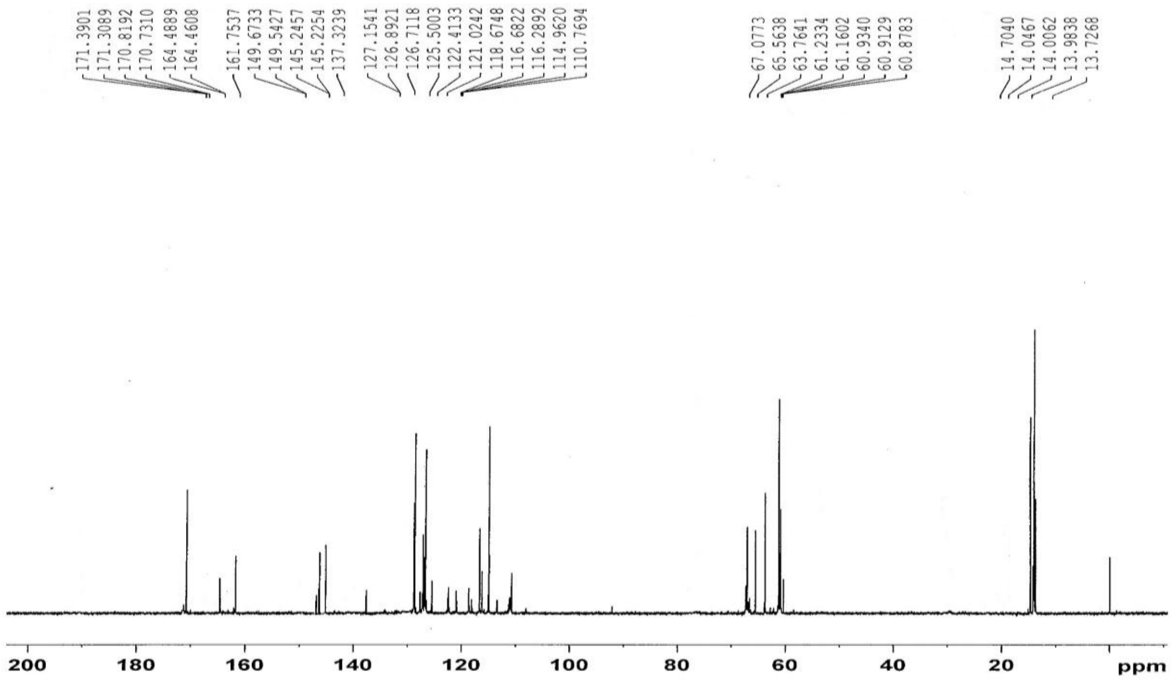
4.3. [1]H NMR spectrum of 5-formyl-BAPTA-tetraethyl ester in $CDCl_3$.



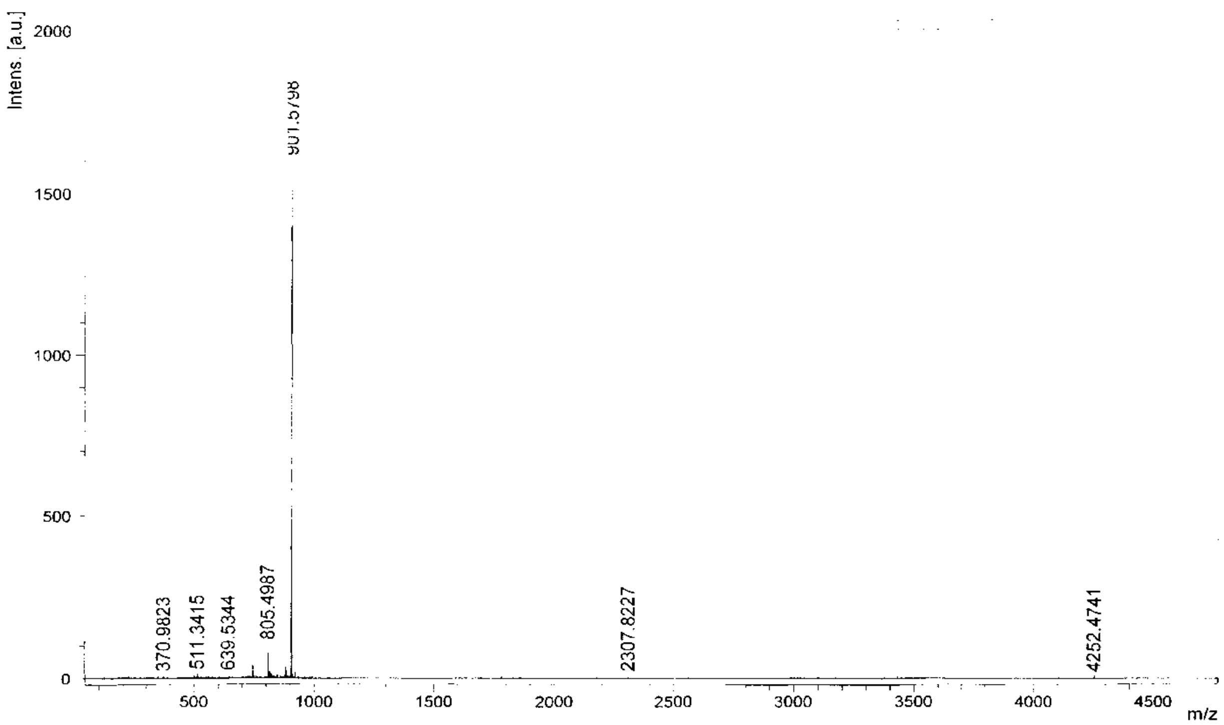
4.4. [1]H NMR spectrum of OXD-BAPTA-ester in $CDCl_3$.



4.5. ^{13}C NMR spectrum of OXD-BAPTA-ester in $CDCl_3$.



4.6. MALDI-TOF MS of OXD-BAPTA-ester



5. References

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