Synthesis of water soluble Terp-ligands for bioassay and bioimaging

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ABSTRACT

Pyrophosphate (PPi) anions play an important role in many biological and cellular metabolic processes. PPi concentration is a potentially useful indicator of metabolic function and diseases such as ATP hydrolysis and calcium pyrophosphate dihydrate crystal deposition disease.

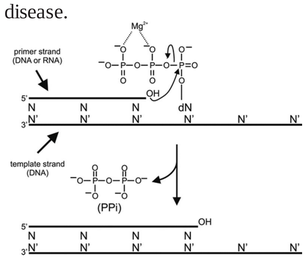
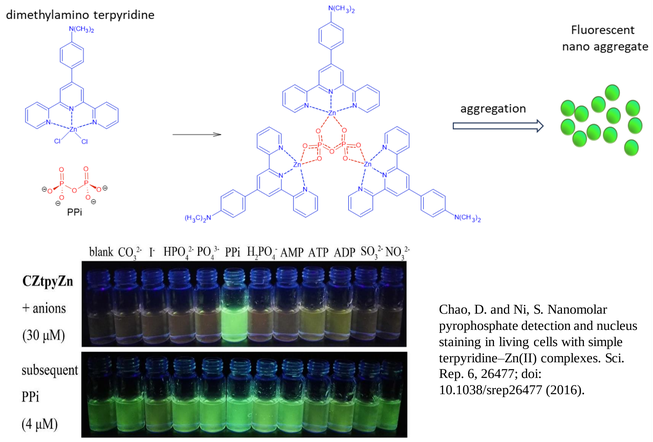


Figure 1. DNA polymerase catalyze the formation of a phosphodiester bond (PPi-PPi) between the incoming deoxynucleotide triphosphate(dNTP) and the terminal primer nucleotide with the release of a pyrophosphate (PPi) group.

Molecular Life Sciences. Julin, D.A. (2018). pp 611-623

Terpyridine(**L**)-Zn(II) complexes with dimethylamino[1] groups are known to selectively bind to PPi and form fluorescent nano-aggregates. **L**-Zn(II) coordinates with PPi in a 3:1 ratio. The maximum fluorescent intensity occurred when the mole ration of 3:1 of **L**-$ZnCl_2$:1 PPi, and respectively. It was found that **L**-$ZnCl_2$ complexes bind specifically with PPi and form fluorescent nanoaggregates which coordinate in a 3:1 ratio with the **L**-$ZnCl_2$ complexes.



Chao, D. and Ni, S. Nanomolar pyrophosphate detection and nucleus staining in living cells with simple terpyridine–Zn(II) complexes. Sci. Rep. 6, 26477; doi: 10.1038/srep26477 (2016).

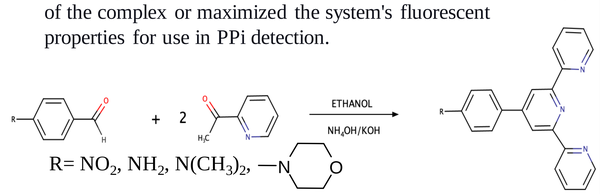
Figure 2 and 3. Diagram of the $N(CH_3)_2$-$ZnCl_2$ complexes forming nanoaggregates, and photography of $N(CH_3)_2$,-$ZnCl_2$ complexes in the Prescence of PPi underneath a 365nm UV lamp.

The goal of this project is to find selective and sensitive probes for PPi detection for diagnostic applications at the nanomolar level in water utilizing **L**-Zn(II) complexes. This research will use other groups in place of the $N(CH_3)_2$ group and see how PPi binding, and fluorescence is affected.

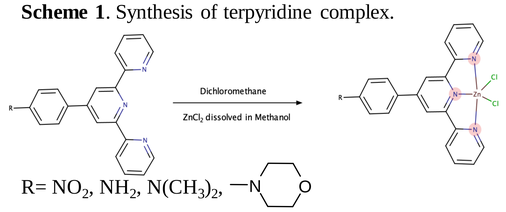
A variety of **L** and their **L**-Zn(II) complexes were synthesized and characterized by ^1H and C^{13} NMR, FTIR, UV-Visible, and fluorescence spectroscopy. A fluorescence titration for each **L**-$ZnCl_2$ complex was performed with varying PPi concentration. The titration will allow determination of the mole ratio of **L**: PPi complexes formed. This work should allow us to find the optimal terpyridine ligand structure for PPi detection.

METHODS

The synthesis of the terpyridine and **L**-ZnCl2 complexes is repetitive. The ligands synthesized varied only in the presences of an **R**-group whose nature ether improved the water solubility of the complex or maximized the system's fluorescent properties for use in PPi detection.



Scheme 1. Synthesis of terpyridine complex.



Scheme 2. Synthesis of terpyridine complex.

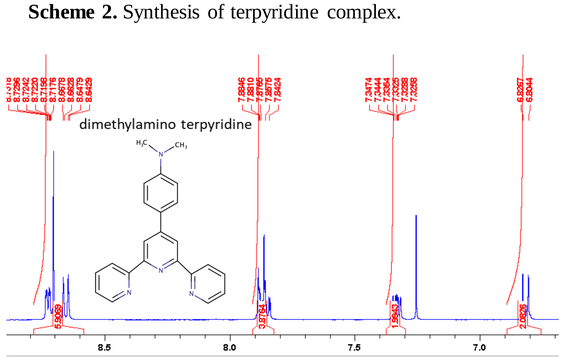


Figure 4. ^1H NMR of $N(CH_3)_2$ terpyridine.

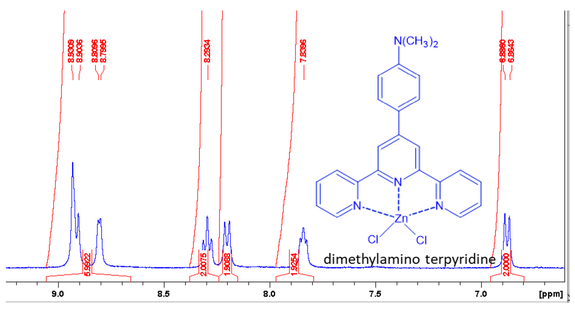
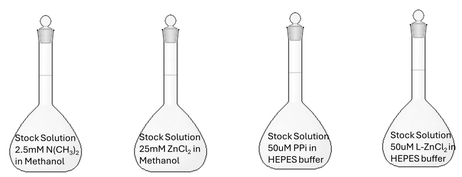


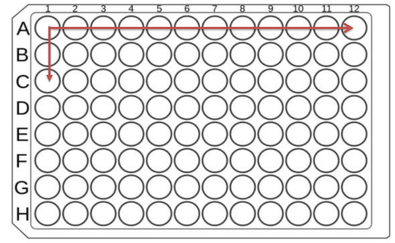
Figure 5. ^1H NMR of $N(CH_3)_2$-$ZnCl_2$ terpyridine.

For the titration study a series of stock solution of the **L** , $ZnCl_2$ and PPi is made up in HEPES buffer (pH 7.4). To prepare the **L**-$ZnCl_2$ solution $ZnCl_2$(25mM) 100uL and 1mL **L**(2.5mM) diluted with 50 mL HEPES buffer (pH 7.4) then sonicated for 5 minutes.



Well No.	1	2	3	4	5	6	7	8	9	10	11	12
50uM PPi:	0uL	30uL	60uL	75uL	90uL	120uL	150uL	180uL	210uL	240uL	270uL	300uL
50uM **L**-$ZnCl_2$	300uL	270uL	240uL	225uL	210uL	180uL	150uL	120uL	90uL	60uL	30uL	0uL
Mole fraction PPi	0	0.1	0.2	0.25	0.3	0.40	0.5	0.6	0.7	0.8	0.9	1.00

A 96 well plate(max volume is 300uL) is prepared with 50uM **L**-$ZnCl_2$ and 50uM PPi solution with varying volumes in replicates of three.



RESULTS

Figure 6 and 7. A fluorescence spectra of $N(CH_3)_2$-$ZnCl_2$ (50uM) upon addition on PPi excitation 440nm reading emission 500-700nm. March 6th, 2024. Job plot of binding study between PPi and $N(CH_3)_2$-$ZnCl_2$.

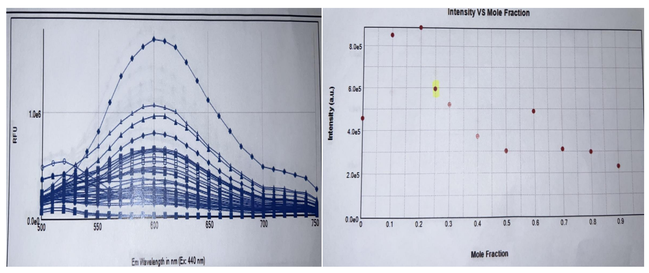


Figure 8 and 9. A fluorescence spectra of $N(CH_3)_2$-$ZnCl_2$ (50uM) upon addition on PPi excitation 440nm reading emission 500-700nm. March 7th , 2024. Job plot of binding study between PPi and $N(CH_3)_2$-$ZnCl_2$.

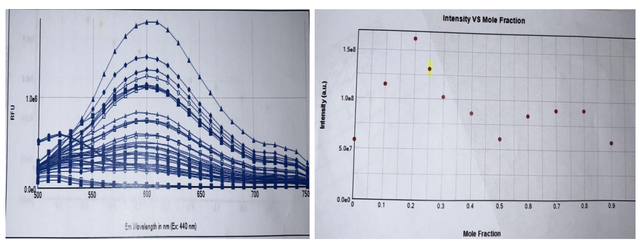


Figure 10 and 11. A fluorescence spectra of $N(CH_3)_2$-$ZnCl_2$ (50uM) upon addition on PPi excitation 440nm reading emission 500-700nm. March 8th , 2024. Job plot of binding study between PPi and $N(CH_3)_2$-$ZnCl_2$.

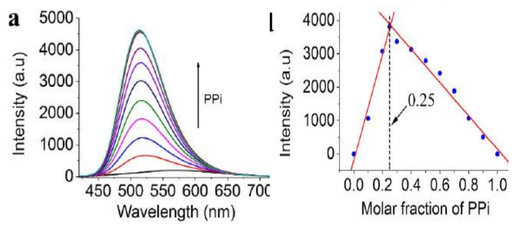


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Figure 12 and 13. A fluorescence spectra of CZtpyZn (50uM) upon addition on PPi excitation 440nm reading emission 500-700nm, and Job plot of binding study between PPi and CZtpyZn.

CONCLUSIONS

Some complications that occur in the research:

- Overtime the sample in solution and solid form deteriorate and experience photobleaching. To fix that issue is to store in dark conditions and make solutions fresh before making any sort of measurements.
- Also, PPi anion is unstable in aqueous solution and hydrolyze back into inorganic phosphate. To fix that issue we make the PPi solution fresh before making any sort of measurements.

$$P_2O_7^{4-} + H_2O \rightarrow 2\,HPO_4^{2-}$$

The HPO_4^{2-} binds with zinc complex and decreases fluorescence

- Another possible issue that might be occurring is the formation of the nanoaggregates are not forming instantly.

References

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4 a) J. Phys. Org. Chem. 2012, 25 754–759. Ting-Ting Wang. Facile synthesis and photochromic properties of diarylethene-containing terpyridine and its transition metal (Zn2+/Co2+/Ru2+) complexes. b) New Journal of Chemistry (2019), 43(48), 19355-19364, Dong, Chuan a Self-assembly and photo-responsive behavior of bis-terpyridyl Eu -complex. C)

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