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Supporting information for article:

Crystal engineering of an adenine–decavanadate molecular device towards label-free chemical sensing and biological screening

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I. Development and optimization of a synthesis method for compound 1

Details of all experiments have been tabulated here. For simplification, our method for the synthesis of adeninium decavanadate (reported method in the manuscript) has been referred to as "**method A**".

- *Solvothermal condition experiments:*

Test code	Variation	Result	Description
011	**Method A** at room temp*	green precipitation	-
012	**Method A** under reflux conditions	green precipitation	-
013-015	**Method A** at 100, 90 & 80ºC solvothermal conditions	mixture of green precipitations and gel	By decreasing the reaction temperature percentage of the gel product was increased. At 80 ºC the sole product was a green metallogel which is the subject of another ongoing research in our group.
016	**Method A** + at 120ºC solvothermal conditions	same as main reaction	Yield of the reaction didn't increase in comparison with 110 ºC

* Adenine was dissolved in hot deionized water after which the adenine solution was cooled to RT. The BTC and $VOSO_4$ were both added and mixed for 4 hours at RT.

As described in **method A**, upon completion of the hydrothermal reaction at 110 ºC for 72h and then 4ºC/h cooling rate to RT, the resulting solution contained yellow crystals of adeninium decavanadate. In brief, according to the above table, by altering the temperature parameter of the solvothermal conditions, we didn't obtain adeninium decavanadate: $[AdH]_6[V_{10}O_{28}].4(H_2O)$.

- *1,3,5-benzenetricarboxylic acid extra reagent experiments:*

Test code	Variation	Result	Description
021	**Method A** without BTC	orange solution	Slow evaporation of the resulted solution didn't give any crystals even until very small

			volumes remains clear (negligible colloidal orange precipitates were observed).
022	**021** + at 120ºC solvothermal conditions	orange solution	-
023	**021**+ reactants' concentrations×2	orange solution	same as **021** test results
024	**021**+ reactants' concentrations×3	orange solution	same as **021** test results
025-026	**Method A** (BTC=0.2 & 0.15 mmol)	same as main reaction + white precipitations	By increasing the BTC concentration the yields of the reactions were equal in comparison with the main reaction but some white precipitates appeared.
027-028	**Method A** (BTC=0.1 & 0.05 mmol)	Same as main reaction (less crystals)	By increasing the BTC concentrations yield of the reaction was dropped drastically.

As described in **method A**, a small amount of colloidal green precipitates was formed shortly after addition of vanadyl sulfate ($VOSO_4$) that certainly confirms the presence of V^{+3}. Upon completion of the hydrothermal reaction, the resulting solution contained yellow crystals of adeninium decavanadate, alongside the green precipitate (small amount).

But when the reaction was carried out in the absence of BTC, only a clear yellow solution (no yellow crystals nor green precipitations) were obtained, the resulting solution was subjected to slow evaporation but no crystals were grown and only in the last few drops of solution a negligible amount of colloidal orange precipitates was obtained (test code 021).

Thus, it could be concluded that V^{+4} (vanadyl sulfate) in the presence of BTC, during a redox reaction under hydrothermal condition, leads to the formation of a green precipitate (containing V^{+3}) and adeninium decavanadate crystals (containing V^{+5}).

- *NH_4VO_3 (vanadium cheaper salt) experiments:*

Test code	**Variation**	**Result**	**Description**

031	**Method A +** salt NH_4VO_3 was used instead of $VOSO_4$	green solution	At the beginning orange solution and at the end of the reaction green solution appeared.
032	**031** without BTC	mixture of white and green precipitations	-
033	**031** + 2<pH<3**	colorless solution	colorless solution with small amounts of white precipitates
034	NH_4VO_3+ 2<pH<3+ without BTC+ without DMF +not under solvothermal conditions***	orange poor-quality crystals	XRD pattern of obtained powder was different from adeninium decavanadate XRD pattern

** NH_4VO_3 was used instead of $VOSO_4$ as cheaper reactant and the 2<pH<3 was adjusted by dilute HCl
*** Sánchez-Lara, E., Treviño, S., Sánchez Gaytán, B. L., Sánchez-Mora, E., Castro, M. E., Meléndez-Bustamante, F. J., ... & González-Vergara, E. (2018). *Frontiers in chemistry*, **6**, 402.

- *DMF key factor:*

Test code	Variation	Result	Description
041	**Method A** without DMF solvent	Mixture of white and green precipitates.	-

- *pH key factor*

Test code	Variation	Result	Description
051--55	**Method A** (pH=6, 6.3, 6.5, 6.8 & 7)	same as main reaction	Yield of the reactions were almost equal to the main reaction
056-057	**method A** (pH= 5.8 & 7.3)	Same as the main reaction (less crystals)	Yield of the reactions were dropped drastically.

In summary, according to the results of the above tests, it appears that all the components $VOSO_4$, BTC, DMF, and hydrothermal conditions at 110°C for three days are critical for the production of the reported adeninium decavanadate compound.

II. Compound 1 bio-sensing feasibility

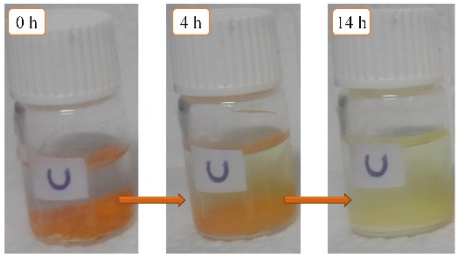

Figure S1: Bio-sensing feasibility of the compound **1** after (a) 0 h; (b) 4 h and (c) 14 h.

- *Compound **1** optical assessments in solid-state:*
- *Sample preparation:* Appropriate amount of crashed crystals (3 mg) of compound **1** transferred into an vessel, which then charged with 3ml of nucleic acid solutions (3M) in phosphate-buffered saline (PBS) solution, pH=7.4. The interaction between **1** and nucleic acids were monitored by UV-visible and fluorescence measurements and XRD patterns approved this changes.

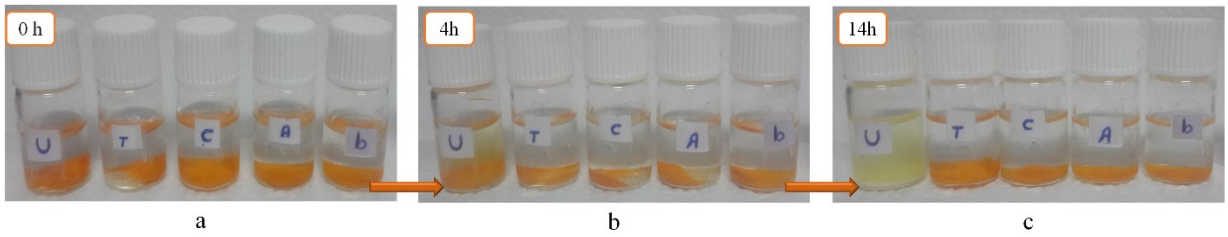

Figure S2: Crashed crystals of compound **1** incubated with diffident nucleic acids (U: Uracil, T: Thymine, C: Cytosine, G: Guanine, A: Adenine and B: blank), after (a) 0 h; (b) 4 h and (c) 14 h.

III. Compound 1 optical assessments in solution form:

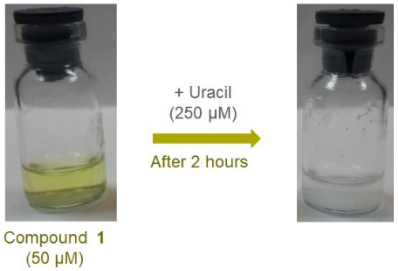

Figure S3: Feasibility of the bio-sensing of compound **1** in solution form.

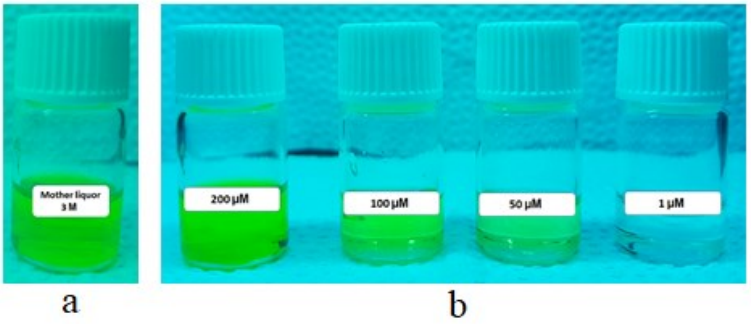

Figure S4: (a) Mother liquor of compound **1** (3 M), (b) Compound **1** samples to find optimum concentration through optical assessments

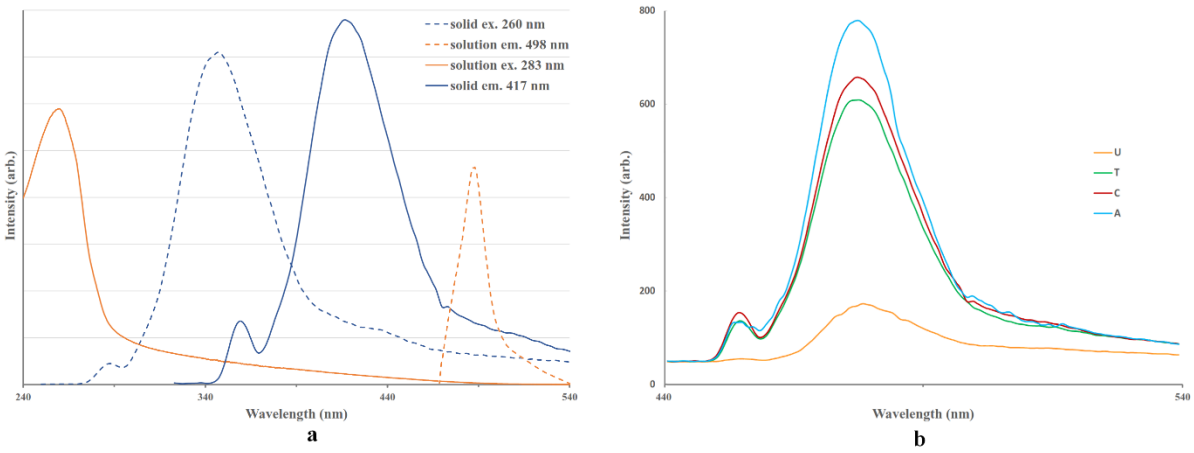

Figure S5: a) Fluorescent spectrum of compound **1** in solid state and solution form; and b) Fluorescent intensities of compound **1** solution in the presence of nucleobases (U, T, C and A)

IV. Biological assays

- *Sample preparation for biological assay:* Mother liquor of compound **1** (3M) for UV-Visible and fluorescent data collection was prepared by DMSO solvent after 3 hours stirring at ambient temperature.
- *Sample preparation to find optimum concentration:* Then to find optimum concentration for optical biological assessments, diluted samples of **1** (1, 50, 100, 150, 200 μM) were prepared over adding proper amount of fresh phosphate-buffered saline (PBS) solution (pH=7.4) to the mother liquor.
- *Sample preparation for Nucleic acid sensing study:* For biosensing comparison between solid and solution forms' of compound **1,** Nucleic acid solutions (250 μM) were incubated with **1** (50 μM) for 4 hours at RT in total 3ml PBS. Similar to solid-state optical assessments, the interaction between **1** and the nucleic acids were monitored by fluorescent optical measurements at 283 nm excitation wavelength (see section 3.3.1) (Fig. S5b).
- *Sample preparation for Hydrolysis rate study:* Hydrolysis rate of the compound **1** containing 50 μM (see section 3.2.2) have been evaluated *vs* time (from fresh and after 2, 6, 9, 12 and 24 hours after preparation).
- *Cell culture:* Human mammary carcinoma MDA-MB-231 and MCF7 cell lines were purchased from National Cell Bank of Iran (Pasteur Institute, Iran). Both cell lines were fed with RPMI-1640 (Gibco). This culture medium was supplemented with 10% FBS (Gibco) and 1% antibiotics (100 U/ml penicillin and 100μg/ml streptomycin). Cells were maintained at 37°C in a humidified incubator containing 5% CO_2 and were passaged using trypsin/EDTA (Sigma-Aldrich) and phosphate-buffered saline (PBS) solution.
- *Anticancer study:* The cytotoxicity of the compound **1** in solution was evaluated by 3-(4,5- dimethylthialzol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. MDA-MB-231 and MCF7 cells were seeded into 96-well plates at a density of $1.5x10^4$ per well in 200 μl of media and grown overnight. The cells were incubated with various concentrations (3.125/6.25/12.5/25/50/100/200μM) of compound **1** for 24 h at 37°C under 5% CO_2. Following this incubation, cells were incubated in medium containing

0.5 mg/ml of MTT (Sigma-Aldrich) for 4 h. The medium was discarded, and the precipitated formazan violet crystals were dissolved in 100 µL of DMSO to solubilize the formazan. After shaking the plate for 20 min, the absorbance of the sample was measured at 570nm using an ELISA reader (Model wave xs2 Biotek, USA). The absorbance of dissolved formazan in the visible region correlates with the number of intact active cells.

- *Cell permeability:* Internalization of the compound **1** was visualized using the fluorescence microscope (Nikon, TS-100) equipped with an appropriate filter set. MDA-MB-231 and MCF7 cells (1×10^4 cells/well) were seeded in 6-well plates (or $60mm^2$ plate) overnight before experiments. Compound **1** were added into the incubation medium at concentration of 50µM for 1h incubation in 5% CO_2 at 37°C. Both cells were washed twice with phosphate-buffered saline (PBS). Microscopic images in the green channel for detection of the compound **1** and in the bright-field were obtained by fluorescence microscopy.

Table S1. Internal angles (°) at the N atoms of adeniniums (AdH^+1, AdH^+2, AdH^+3)

Nitrogen atom	Angle	Degree (°)
N8	C7-N8-C9	124.2(2)
N18	C17-N18-C19	122.2(2)
N28	C27-N28-C29	121.6(2)
N4	C3-N4-C5	106.59(19)
N14	C13-N14-C15	106.52(19)
N24	C23-N24-C25	106.31(19)
N6	C5-N6-C7	112.4(2)
N16	C15-N16-C17	112.3(2)
N26	C25-N26-C27	111.7(2)
N2	C1-N2-C3	103.81(19)
N12	C11-N12-C13	104.13(19)
N22	C21-N22-C23	103.96(18)

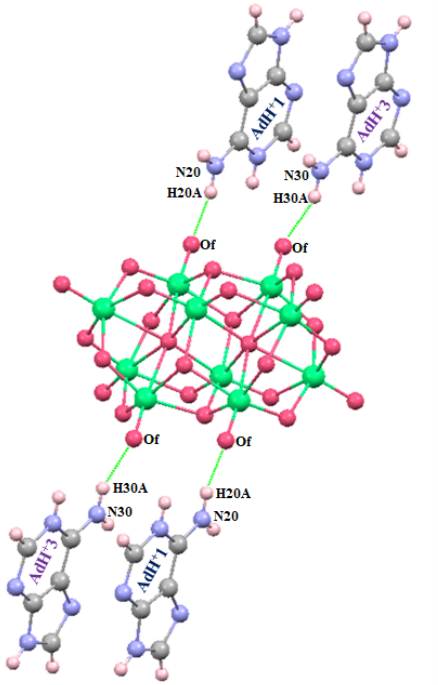

Figure S6: Decavanadate and adeniniums interactions