

Synthesis of fluorescent organic-lanthanide probe dedicated to novel SARS-COV-2 & Influenza non-antigen diagnostic test

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CHEMICAL DEVELOPMENT & PRODUCTION

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DESCRIPTION

The development of detection methods that give results as good as reverse transcriptase polymerase chain reaction (RT-PCR) at a cost and analysis time similar to antigenic tests is a major challenge. Based on a method developed by Nyanguile *et al.*¹, the aim is to produce a reactive conjugate ligand specific to virus and enabling FRET analysis.

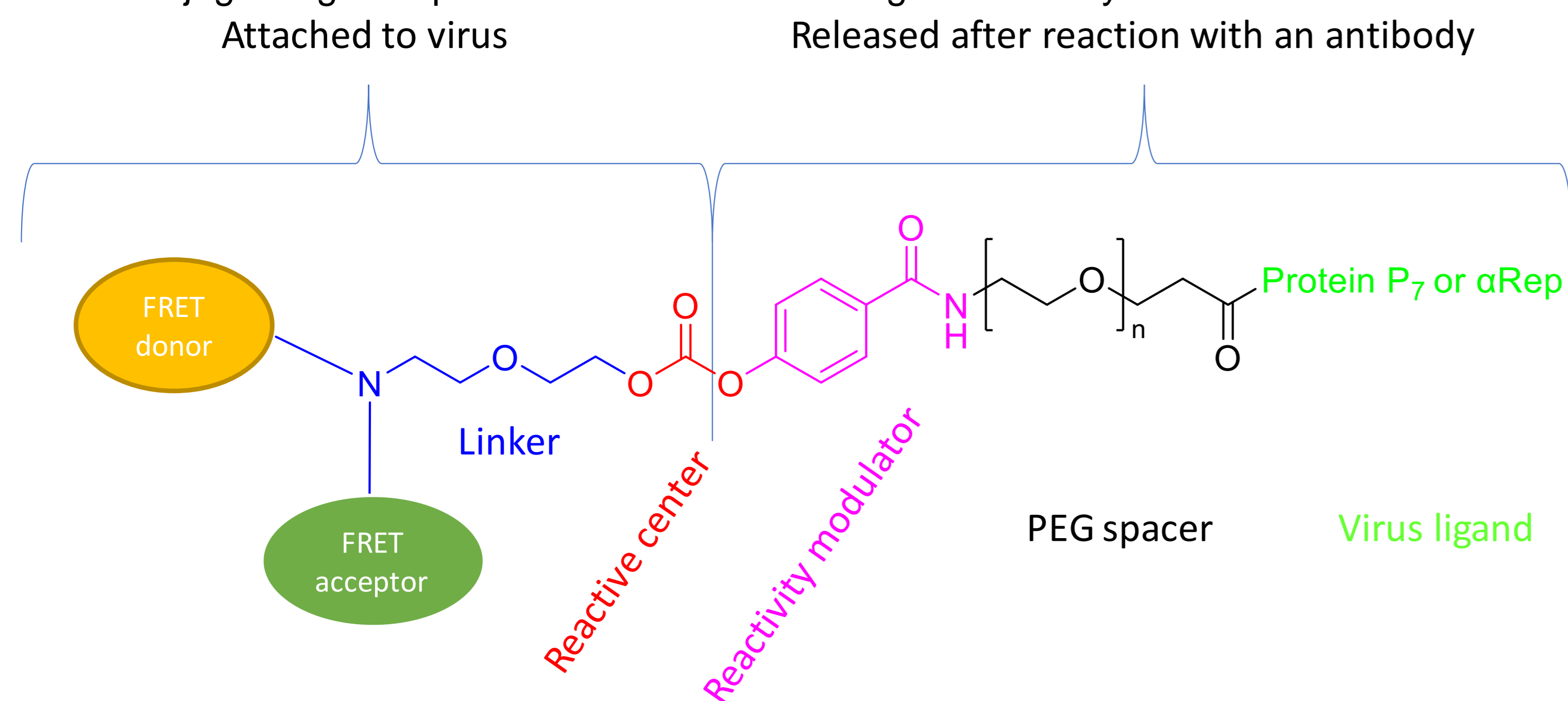
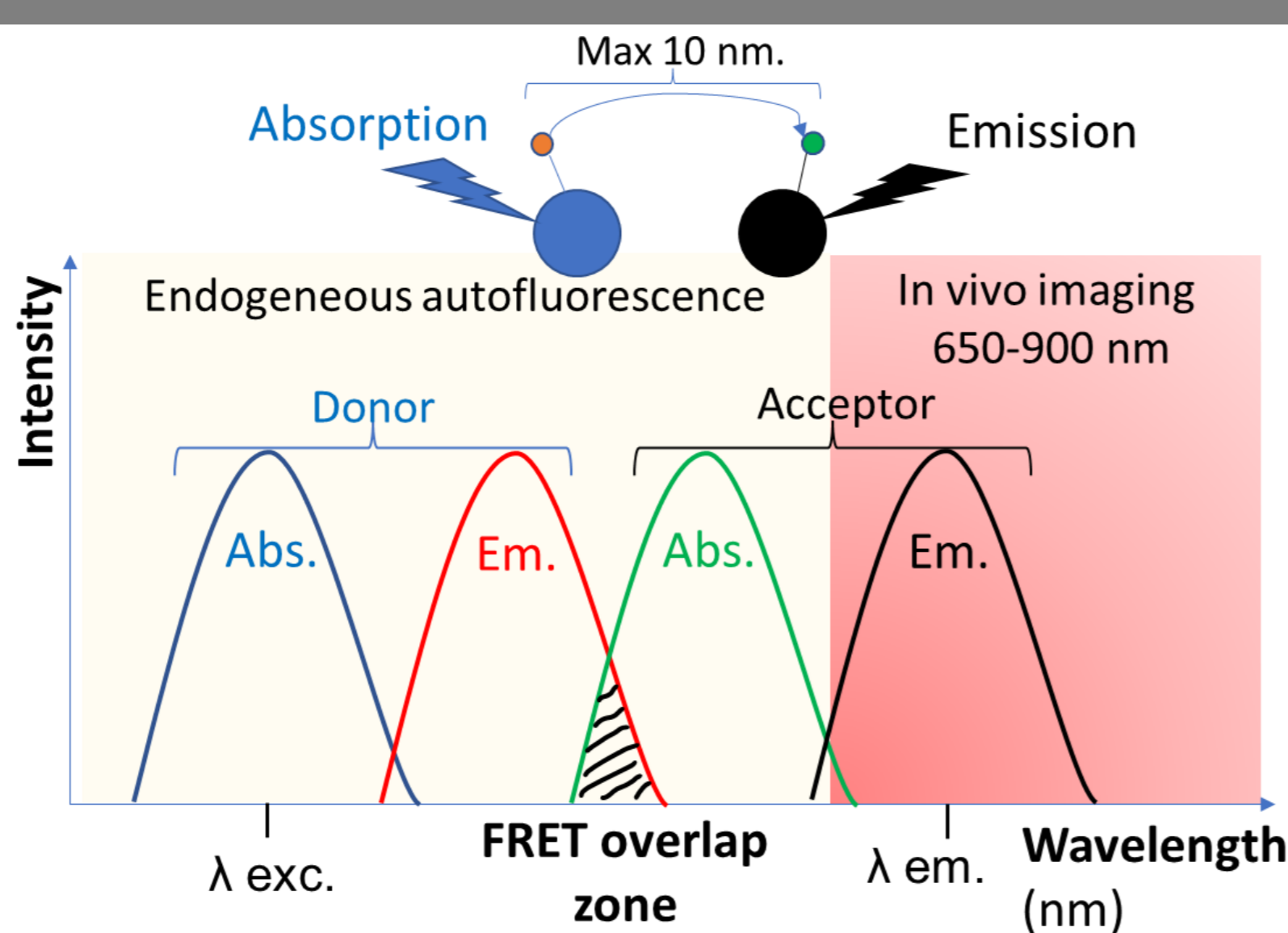


Figure: Ligand reactive conjugate

FRET limitation

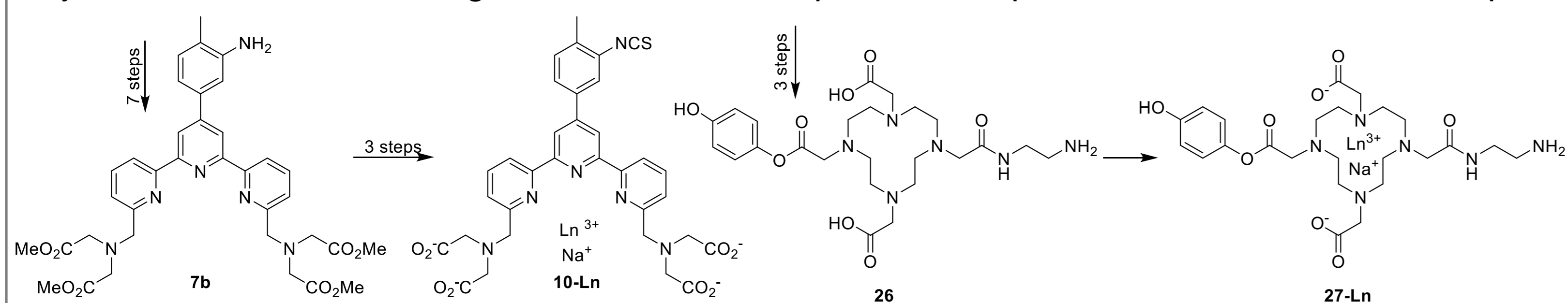
Limitation concerning FRET donor and acceptor are given by FRET concept and endogenous autofluorescence. Overlap of donor emission and acceptor absorption as well as analysis >650 nm is therefore necessary.

Figure: FRET concept limitation



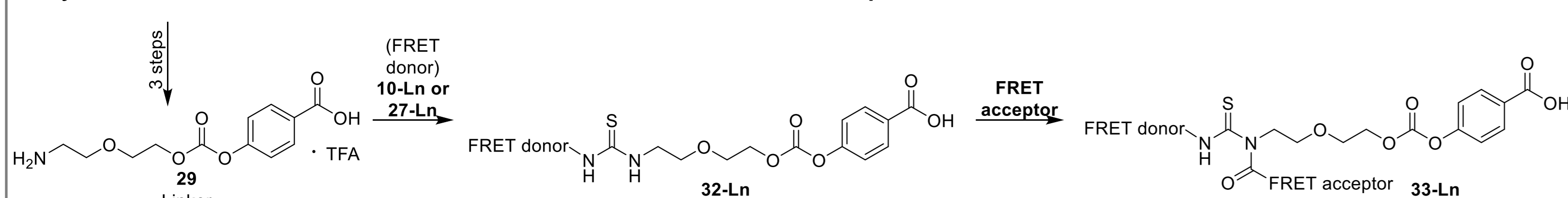
OBJECTIFS

-Synthesis of FRET donor ligand **7b** & **26** and respective europium, terbium, samarium complex.



Scheme: FRET donor ligands & complexes to synthesise

-Synthesis of linker and FRET donor and FRET acceptor attachment



Scheme: Linker synthesis & FRET attachment

CONCLUSION

- Complexes synthesis were achieved allowing confirmation of expected luminescent properties
- **10-Eu** present the best luminescent properties to perform the detection of virus using the developed method.
- **27-Ln** present too weak metal emission and strong ligand emission from 300 to 700 nm → Get ligand **26** with better purity to confirm emission issue
- Linker **31** synthesis was achieved
- Linker, FRET acceptor & FRET donor were not able to bind together → Need two primary amine or a thiocyanate function (NCS) and a primary amine
- Complex **47-Ln** which was left aside to prioritize other complexes synthesis should allowed linking of linker & FRET acceptor thanks to NCS and NH₂ function

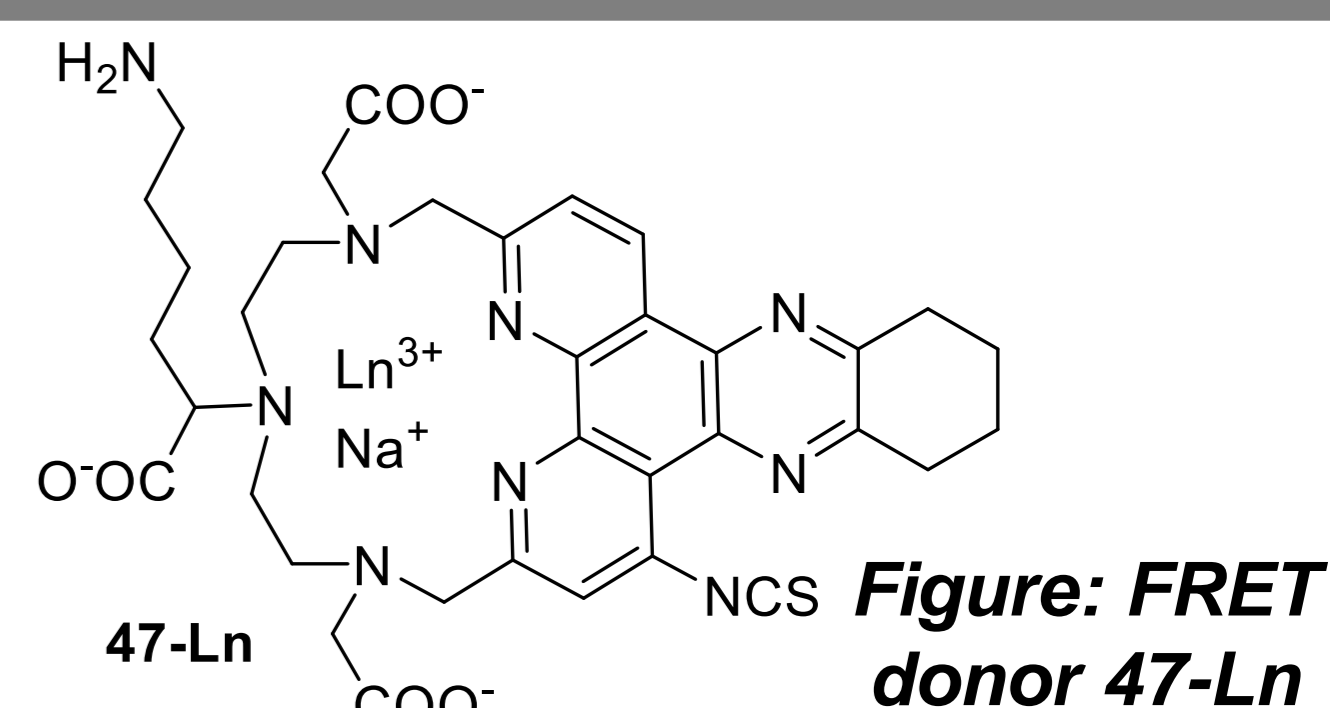


Figure: FRET donor 47-Ln

RESULTS

Compound **10-Ln** & **27-Ln** were synthesized with europium, samarium and terbium metal center. Except **10-Tb**, every obtained complexes were luminescent. **10-Eu** however, shows a much stronger visual intensity confirmed by fluorescence analysis.

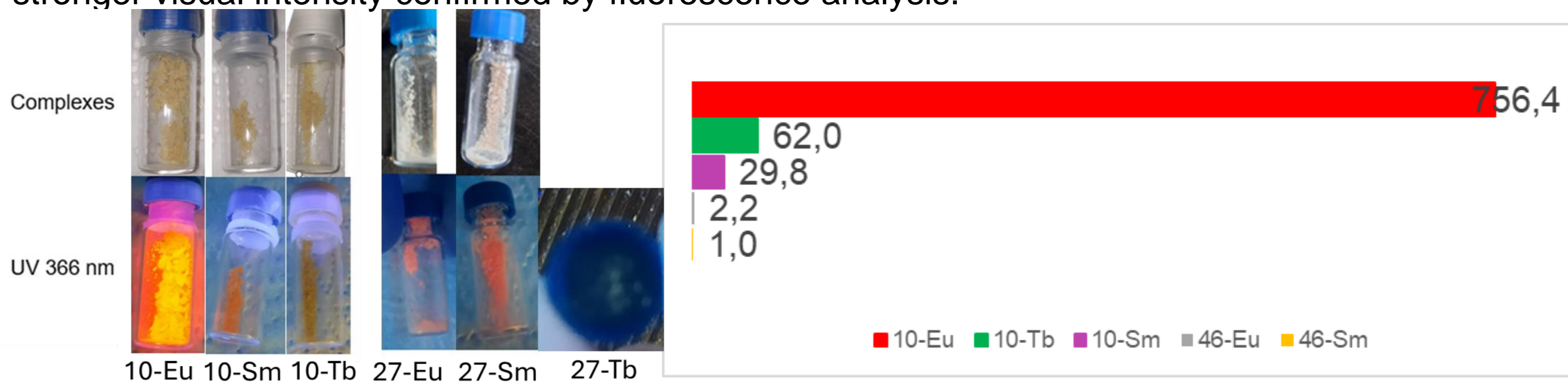
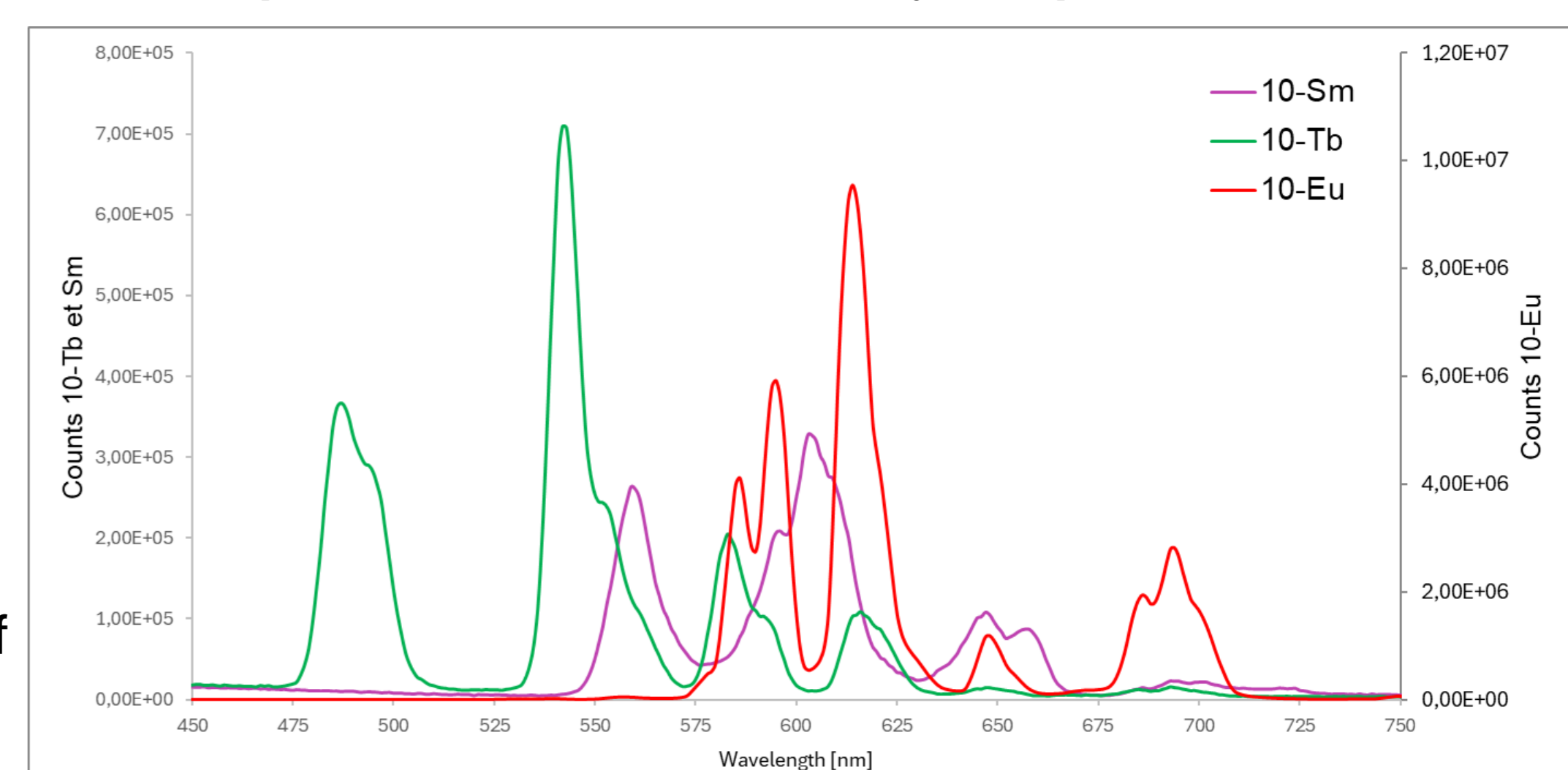


Figure: Visible emission of complexes and relative intensity comparison

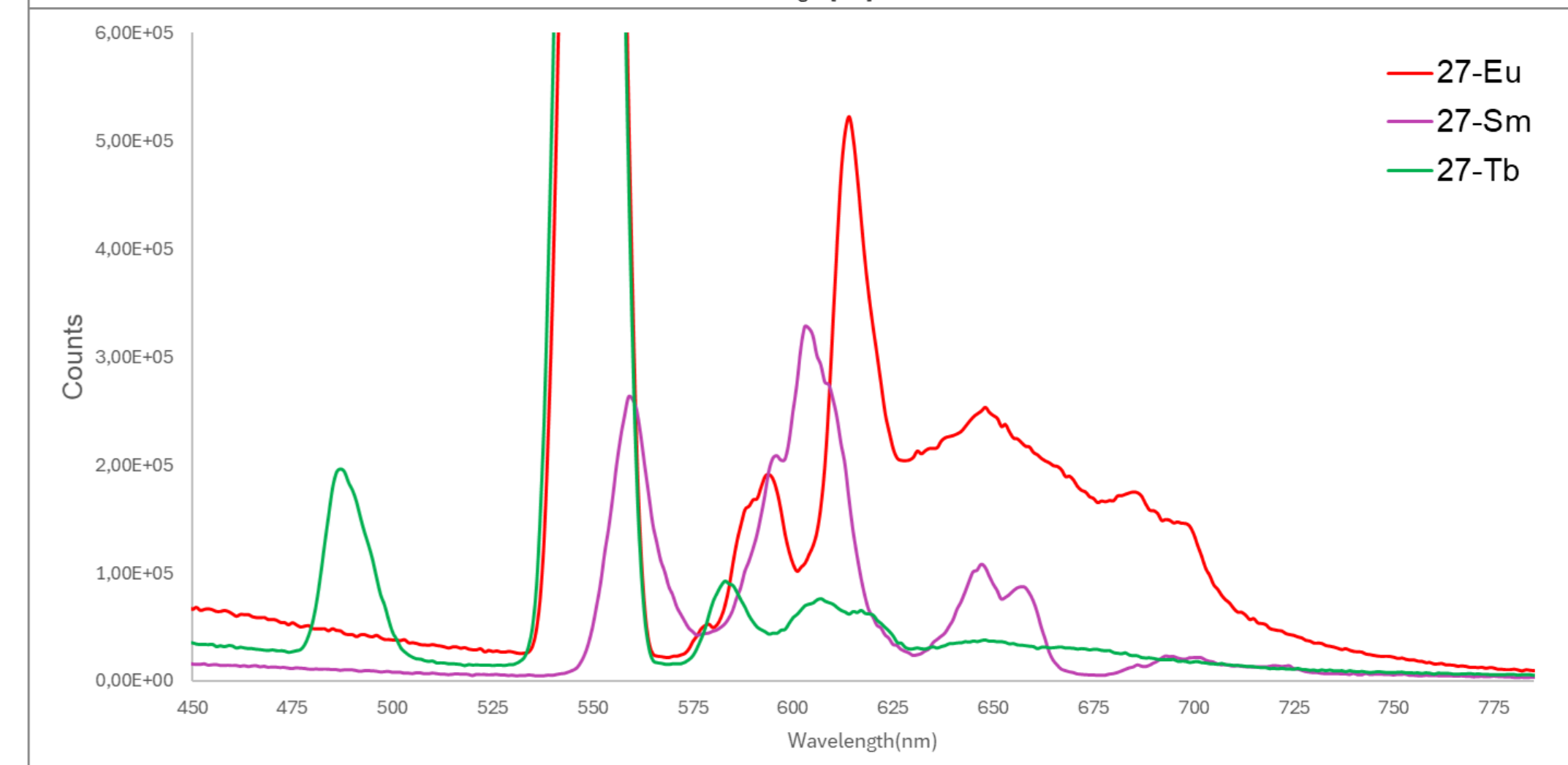
Fluorescence analysis confirm that every of the six complexes are emitting, despite **10-Tb** emission is not visible. It confirmed that **10-Eu** emit much more than other complex.



The fluorescence of complex **27-Ln** is mainly hidden behind that of the ligand. Despite a much lower emission than **10-Ln** complexes, **27-Ln** ligands can still activate the luminescence of the metal center.

Low emission intensity + wide emission range → **27-Ln** poor candidate for our FRET analysis.

Figure: Fluorescence analysis of complexes 10-Ln & 27-Ln



Based on fluorescence, Cyanine5 (Cy5) was chosen as FRET acceptor. Absorption 500 to 700 nm and emission 640 to 750 nm → Avoid endogenous autofluorescence

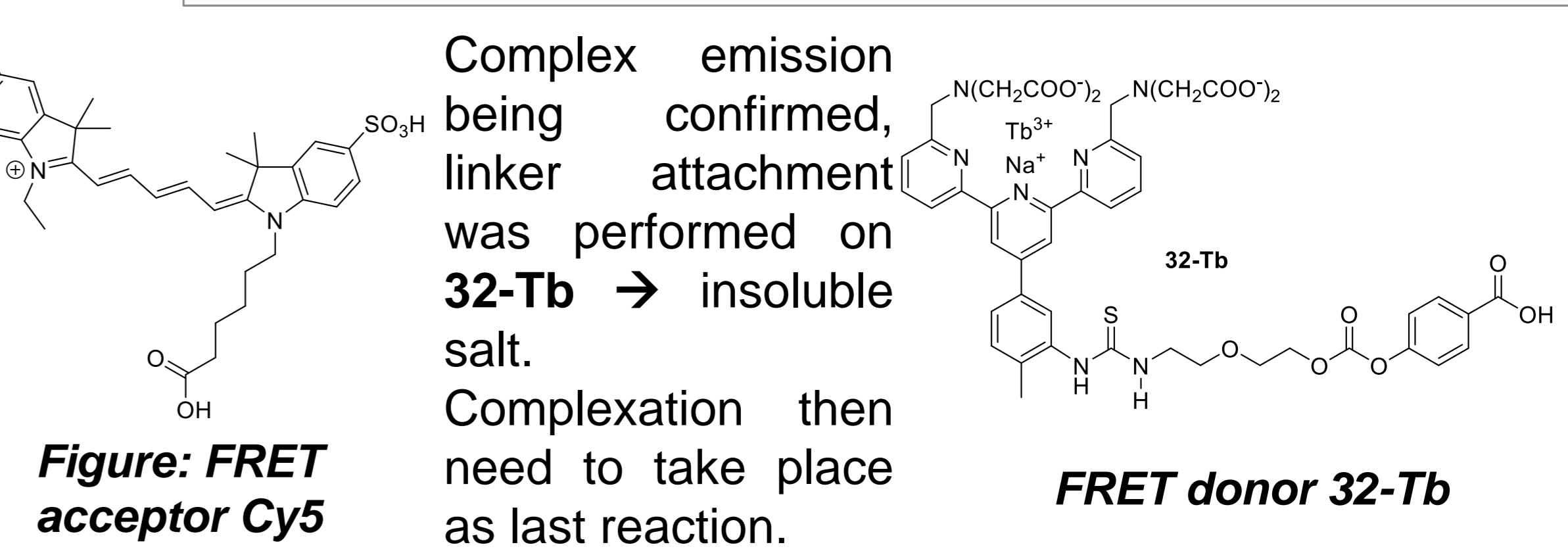
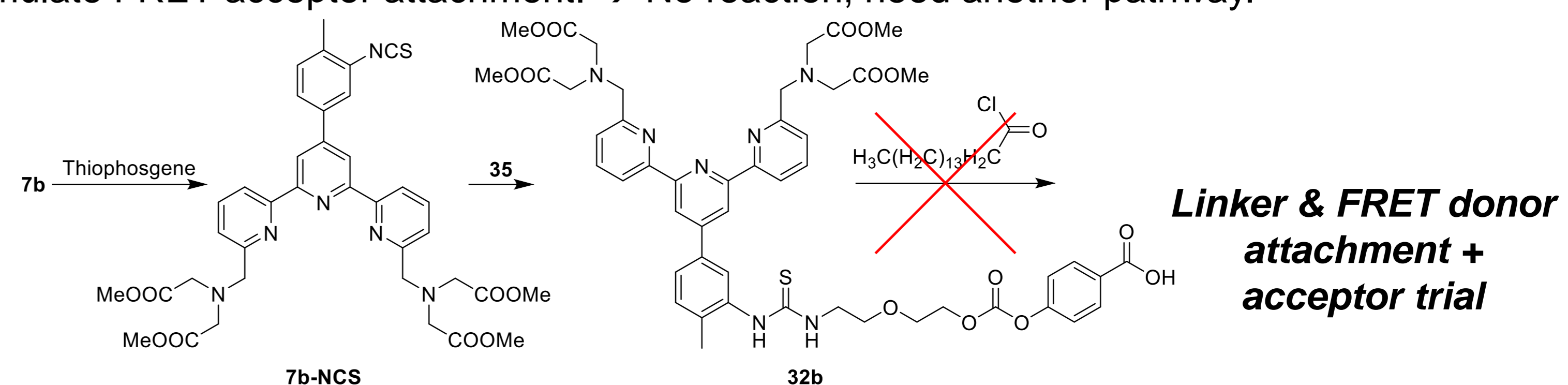


Figure: FRET acceptor Cy5

FRET donor 32-Tb

Complex emission being confirmed, linker attachment was performed on **32-Tb** → insoluble salt. Complexation then need to take place as last reaction.

Due to FRET acceptor (Cyanine5) high cost (8000 CHF/g), trial was made using palmitoyl chloride to simulate FRET acceptor attachment. → No reaction, need another pathway.



Linker & FRET donor attachment + acceptor trial