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Reviewer's report on doctoral dissertation by Marco Farinone entitled *Pyrrole/Pyridine Based Chromophores - Design, Synthesis and Post-Synthetic Reactivity*

The research work described by Marco Farinone in his thesis was guided under guidance of prof. Miłosz Pawlicki. The topic of the work is well suited with previous efforts by Pawlicki's group and broadens the area of research. The aims of the work are focused on three main topics, meaning *a*) the design and synthesis of BODIPY-carrying molecules responsive to external stimulus, *b*) synthetic join of the BODIPYs with amino acids and peptides to use those efficient fluorophores as labelling moieties *c*) the design and synthesis of larger macrocycles for their implementation in peptides. Except for the rational design, synthesis, separation and purification the photophysical properties of resulting molecules were measured in order to test the usefulness of new dyes in selected fields of science and to deeply understand their properties before further functionalization. The current report is divided into sections describing science standing behind the research, main comments related to scientific part and main selected issues, and minor comments mentioning other points that should not be left without a feedback.

The described syntheses of substrates for their use in next steps are, in general, successful. Obtained molecules are well characterized and their structure was confirmed without problems. On the other hand, a number of attempts for the synthesis of fluorophore-amino acids joined molecules were guided and a number of them were not successful. That shows the fluorophre-labelled biomolecules are not as easy to obtain as one would expect from their structure designed on a paper. However, it is necessary to mention that Marco Farinone have a significant contribution to the field of the synthesis of fluorophores and their functionalization. Also, he studied new compounds in very systematic way. The text of the thesis is written with a clear language and at every step the reasoning in understandable. Moreover, the protocols described in text are at a good detail level allowing to repeat experiments by others. All protocols are well referenced and, if it was necessary, for some of them a short information is added to further supplement previous methods.

Major comments

1. I'd like to comment on the synthesis of compound **148** presented on page 84. The subjected compound was prepared using **147** as a substrate. In fact, the reference for the synthesis of **147** [ref. 102, *Eur. J. Inorg. Chem.* (2014) **5**, 888-895] describes the synthesis with the use of benzyl alcohol and thionyl chloride. In that way the dimethoxy benzyl chloride was obtained with satisfactory yield. If so, I'd like to know how the product **148** was obtained, according to ref. 101 [*ACS Med. Chem. Lett.* (2015) **6** 902-907] from the benzyl chloride, while **148** is a bromide (anion exchange reaction?).

2. The definition of **tautomerism**[1] was incorrectly used in the thesis (examples below). Please, refer to the definition published by IUPAC in the Compendium of Chemical Terminology[2] and interesting essay by Alvarez [*Angew. Chem. Int. Ed.* (2012) **51** 590–600] focused on proper usage of arrows.

    - The discussion of **tautomerism** in molecule **96** is not needed as there is no other basic center in the molecule that could be protonated by the intramolecular proton shift. On the other hand, the discussion on tautomerism in **99** is fully justified.
    - In Scheme 4.12 resonance forms for *meso*-substituted BODIPY are shown, but incorrectly labelled as **tautomers** even when one note that Author labelled those as *amine-like* or *imine-like* tautomers. Still those are mesomeric structures, not tautomers. It is worth noting that **tautomerism** is joined with the change of bonding and shift of electrofuge or nucleofuge within the molecule. Thus, using the definition of **tautomerism** in the light of dynamic exchange observed by VT NMR is also not correct. Based on provided NMR data, recorded at variable temperature, I'd rather tell the rotation around C-N bond is limited at lowered temperatures.
    - The section entitled *Tautomeric Equilibrium Analysis with* ^{13}C *Chemical Shifts* should be changed to *Analysis of* ^{13}C *Chemical Shifts*. The use of **tautomerism** here is not correct.
    - Again, the term of **tautomerism** is used not correctly on page 140, where the results of DFT calculations are commented. The reference to Scheme 4.12 commenting on *tautomeric equilibria* is wrong due to two reasons *a*) the Scheme 4.12 does not show tautomerism and *b*) even if Scheme 4.12 shows the balance between two individuals being in equilibrium Author should use equilibrium arrows. The double headed arrow is reserved for mesomerism[3], which is not an equilibrium phenomenon.

3. An interesting feature is noticeable for NMR titration shown in Figure 4.11. There are signals that change their position in a regular fashion and those that act in opposite way. For example, two CH protons in the aromatic six-membered ring (most probably those labelled as 3^3 and 5^3) behaves differently. Proton labeled as 5^3, during the titration, shifts

[1] https://goldbook.iupac.org/terms/view/T06252
[2] 10.1351/goldbook
[3] https://goldbook.iupac.org/terms/view/M03845 and related definition of *delocalization* https://goldbook.iupac.org/terms/view/D01583

the signal from *ca.* 6.8 ppm to 6.5 ppm for 1:1 [TBAF]:[**101**] ratio, while proton 3^3 shifts from 7.0 ppm to 7.2 ppm for [TBAF]:[**101**] equal to 0.2 and then shifts back to higher field reaching value of 7.05 ppm for [TBAF]:[**101**] ratio equal 1. The similar may be observed for proton located initially at 6.5 ppm when compared to one at 7.25 ppm. How to explain that?

4. The sentence on page 137 telling *The presence of a nitrogen at the meso-position of bodipy from both aliphatic and aromatic amines, is a hipsochromic shift factor which can be assigned to strong disturbance of the delocalization within the pyrrole based chromophore.* is not precise.

   First, this is because it suggests the nitrogen atom is present at the *meso* position, while the carbon atom is there and the molecule is not aza-BODIPY derivative. In the current case the correct way of description of the structure is "The presence of amino group attached to meso-position / carbon in meso position...".

   Secondly, the observed absorption bands located at *ca.* 412nm for **124-127** are not surprising, while for those compounds the same chromophore is present in their structures. From the point of view of properties and topology of molecule I would not join their photophysics with *strong disturbance of the **delocalization** within the pyrrole based chromophore.* In my opinion, this is an oversimplification joining the blue-shift and the *disturbance of the delocalization.* It is quite easy to overthrow such hypothesis comparing a series of hydrocarbons in the order of benzene, naphtalane, anthracene, tetracene and so on. In the mentioned series, for sure, benzene molecule is the most aromatic as it is the model structure when one considers the definition of aromaticity/delocalization. In parallel its absorption spectrum in the most blue-shifted in the series. Moreover, even within the mentioned series (**124-127**) the absorption may be commented in the light of the geometry that changes from one molecule to another. This, most probably, is caused by the steric effect of the group attached directly to the amino nitrogen atom. In those molecules the long alkyl chain characterised by the presence of the tertiary carbon atom, instead of the methylene, yields, most probably, the twist around (*meso*)C-N bond in **125** and **126**.

5. The structure of **142** presented on page 150 as a product of the reaction of compound **141** with $CDCl_3$, and the associated data on page 83, is not correct. The spectrum shown in green in Figure 4.31 is in agreement with the spectrum of the substrate **137**. One can easily find it in commercial sources[4] recorded in the same solvent. While the substrate was observed after storing the sample in $CDCl_3$ solution for weeks the hydrolysis of the imine proceeded qualitatively. The other product of the hydrolysis, 3,5-dimethoxyaniline, is not visible in Figure 4.31 just because the values of the chemical shifts for that compound are below 6 ppm[5] while the NMR spectrum (Figure 4.31) starts at 6.8 ppm. I'd be happy to see the ratio of integrals for signals at 8.1 ppm (d) and 8.2 ppm (d) as a function of time. In my opinion, the spectrum marked in blue color represents the mixture of *ca.* 90% of **141** and *ca.* 10% of **137**.

[4] https://www.sigmaaldrich.com/deepweb/assets/sigmaaldrich/quality/spectra/379/770/FNMR000736.pdf
[5] https://www.sigmaaldrich.com/deepweb/assets/sigmaaldrich/quality/spectra/747/572/FNMR009941.pdf

6. I'd like Author to comment more the absorption spectra for **173** and **176** presented on page 179. In the text the *peaks* are listed: 460, 486 nm for compound number **173** and at 455, 481 nm for **176**, respectively. How those *peaks* are interpreted? Moreover, I do not agree that for the spectrum of **174** described *peaks* are not visible (grey spectrum, Figure 4.45). It looks like the FWHM for two constituents of the band is higher leading to their overlap.

Minor comments and issues

1. This is uncomfortable to see the equilibrium arrows at the beginning of thesis (Figure 1.1) representing mesomerism in benzene.

2. From time to time some informations in the text are unnecessarily repeated, for example, the mentioned imine form of the CN bond in compound **99** on page 103 or the whole sentence in procedure **d** on page 66.

3. Taking into account the atom numbering shown in Scheme 4.1 and the discussion of the solid state structures on page 104 it is mandatory to point out there is no C(5)-C(1) bond in structures **96** and **99**. When one refers to the supporting information files for publication *Org. Chem. Front.* (2019) **6**, 2825 it is clear that mentioned atoms should be C(5)-C(4) and C(5)-C(6). Moreover, there are two molecules of **96** in the unit cell, a feature not mentioned in the thesis.

4. In Figure 4.2 the crystal structure of compound **100** is shown, while below that figure there is a comment suggesting existence of strong hydrogen bond between NH donor from the pyrrole and the quinoline nitrogen atom. Moreover, the NMR spectrum (Figure 4.5) shows the NH protons in molecule **100** are located at *ca.* 10.4 and 9.2 ppm. Based on that I'd rather tell the hydrogen bonding is of medium energy, while the chemical shift comes from the inductive effect rather than the hydrogen bonding in five-membered quasi-ring with N–H···N interaction. For comparison, please, refer to the NH chemical shift in compound **101** that equals to 9.6 ppm and having no possibility to intramolecular hydrogen bonding.

5. It was not mentioned in the text, but the interesting feature of the emission spectra of compounds **101** and **102** is that for the later the shape of the emission spectra is much different from the former one. The clearly visible vibrational structure of the spectrum for **102**, washed out for **101**, resembles spectrum for classic hydrocarbon like anthracene. In fact, three fused rings in **101** and **102** have similar topology to anthracene, but for some reasons, the clearly differentiated vibrational features are not well resolved when the nitrogen atom in protonated in molecule **101**. How to explain that?

6. I believe the reference 135, by Pawlicki, cited on page 125 ("...1,2-di(1H-pyrrol-2-yl)ethane-1,2-dione framework.[135]") should be replaced by reference 91 (*J. Am. Chem. Soc.* (1999) **121**, 10438) by Sessler?

7. In tables presenting the results of ^{1}H NMR data in relation to those obtained by DFT calculations the correlation coefficients are missing. This applies for tables 4.1, 4.2, and 4.3.

8. There are typical editing errors as, for example, lack of nitrogen atoms in Scheme 4.27, 4.28, 4.29 (there are two schemes 4.29), 4.31.

It is a pleasure to comment on the scientific importance of the results presented in the thesis.

The molecules synthesized by Marco Farinone are, in general, quite compact structures, which connected with amino acid derivatives gave high fluorescence quantum yields. That gives the opportunity for their easy implementation in future research not only in the field of chemistry, but also in bioimaging. Also, some of molecules that are described in the thesis are capable for cation sensing, but this feature was not studied during the research. The largest molecules described are aromatic ones with their rings deviating from planarity. This kind of curved aromatic compounds are studied by a number of research groups as molecules useful in material science, including their application in OLED devices. One of the most important parts of the thesis, in my opinion, is the plausible mechanism of formation of compounds **173-175**. Taking into account detailed structural characterization of synthesized compounds, studying their photophysical properties and methods of the synthesis the thesis by Marco Farinone is a very good source of information about successes and failures in the narrow and demanding field of organic chemistry.

The research guided by Marco Farinone is at a very high level despite some errors in the text of the thesis. I'm fully convinced the thesis is complete and fulfils all necessary legal and habitual rules. In the light of the current legal order (Dz.U. 2020, 85) and with peace of mind I recommend proceeding the thesis towards public defence of doctorate.

Taking into account the quality of research that was published in well recognized journals I request for distinction of the thesis.

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