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nature portfolio

Peer Review File

Ortho-functionalized pyridinyl-tetrazines break the inverse correlation between click reactivity and cleavage yields in click-to-release chemistry

Corresponding Author: Dr MARC ROBILLARD

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The revised manuscript has addressed all of my previous concerns, and I believe it is now suitable for acceptance and publication.

Reviewer #2

(Remarks to the Author)
The authors have answered all comments of this reviewer. Before acceptance, it will be important to add some of the explanations provided in the rebuttal letter directly to the manuscript for clarity.

Reviewer #3

(Remarks to the Author)
The authors have addressed all the questions and points raised by this reviewer. Therefore, I recommend accepting this manuscript.

Reviewer #4

(Remarks to the Author)
Thank you very much for addressing all the points and making the corresponding changes to improve the quality of the work. Before accepting the paper, I have only two minimal suggestions.

- Include the definition of the abbreviation IEDDA (Inverse electron-demand Diels–Alder) when it is first mentioned in the text.

- Add the corresponding radio-TLC, SEC, SDS-PAGE performed as quality control of 125I-labeled ADC, together with the ones corresponding to 111In-labeled tetrazine probe

Rebuttal

Our comments in blue

REVIEWERS' COMMENTS:

Reviewer #1 (Remarks to the Author):

Thank you very much for addressing all the points and making the corresponding changes to improve the quality of the work. Before accepting the paper, I have only two minimal suggestions.

We thank reviewer 1

- Include the definition of the abbreviation IEDDA (Inverse electron-demand Diels–Alder) when it is first mentioned in the text.

This has been done

- Add the corresponding radio-TLC, SEC, SDS-PAGE performed as quality control of 125I-labeled ADC, together with the ones corresponding to 111In-labeled tetrazine probe

This has been added to the SI as Supplementary Fig 24

Reviewer #2 (Remarks to the Author):

The authors have addressed all the questions and points raised by this reviewer. Therefore, I recommend accepting this manuscript.
We thank reviewer 2

Reviewer #3 (Remarks to the Author):

The authors have answered all comments of this reviewer. Before acceptance, it will be important to add some of the explanations provided in the rebuttal letter directly to the manuscript for clarity.

We thank reviewer 3 and have added the following line to the start of the Modelling section in the SI (S8): “Internal hydrogen bonding and steric hindrance can be reliably evaluated using implicit solvation as these correspond to intramolecular properties. “

And we added ”indicative” before both mentions of “DFT calculations”. In the previous revision we had already removed the calculated energies and electron densities

With regard to the comment about the use of bis-phenyl-tetrazines : as mentioned in rebuttal the much less electron poor bis-phenyl-tetrazines are ca 50 fold less reactive and already give good release. To make this more clear in main text we edited the following sentence .

“So far, the IEDDA pyridazine elimination has been hampered by an inverse correlation between tetrazine reactivity and payload release yield, with the 100-fold less reactive alkyl or phenyl substituted tetrazines affording ca. 80% release (Figure 1)”.

Reviewer #4 (Remarks to the Author):

The revised manuscript has addressed all of my previous concerns, and I believe it is now suitable for acceptance and publication.

We thank reviewer 4