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I Bind It That Way – Bioorthogonal Unmasking of Pro-Fluorescent Quinone Methides

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Proof-of-concept studies for the bioorthogonally controlled generation of pro-fluorescent quinone-methides are presented here. The novel concept relies on a click-to-release tetrazine unit that chemically cages a pro-fluorescent quinone methide precursor. On a synthetically readily accessible model compound we demonstrate that IEDDA reaction of the tetrazine with a TCO leads to the liberation of a pro-fluorescent quinone methide, which is readily captured by nearby nucleophiles to form a fluorescent scaffold. Unlike in previous approaches to access bioorthogonally activatable fluorogenic probes, where the tetrazine unit acted as a quencher of fluorescence, the role of the tetrazine is redefined here. Such repurposed role of tetrazines is foreseen to address the limitations of tetrazine-quenched red/NIR excitable fluorogenic probes.

Introduction

Following and visualizing biological processes in their native environment has never been easier. The toolbox of exogenously applicable fluorescent probes is growing rapidly, offering diverse means for the selective tagging of biomolecules for various applications. To exploit the full potential of recent technological developments in the field of fluorescent microscopy, probes enabling improved S/N ratios are needed. Further requirements, such as the potential for the selective implementation have highlighted the importance of bioorthogonally applicable fluorogenic probes.[1] Among these probes, tetrazine modulated frames represent the most important group due to the excellent turn-on ratios and the fast kinetics of the involved inverse electron demand Diels-Alder (IEDDA) reaction. However, more and more applications require the use of such fluorogenic systems in the biologically more benign red range of the light spectrum. Unfortunately, tetrazine-based modulation of fluorescence dramatically decreases resulting in limited turn-on ratios in this region. Combination of tetrazine-quenching with further quenching mechanisms, i.e. polarity dependent structural changes,[2] intermolecular interaction controlled conformational changes[3] or polarity driven electronic changes[4] may result in improved fluorogenic behavior in case of red-excitable cores, however, these are still considered mediocre in terms of enhancement ratios. Since the quenching potential of tetrazines decreases dramatically toward the red edge of the spectrum, its role in the fluorescence turn-on process needs to be redefined. These suggest that a conceptually novel design strategy is needed to achieve improved, highly specific bioorthogonal reaction assisted fluorogenicity in the red range.

There are several examples for push-pull dyes, whose fluorescence is dependent on the presence/absence of a substituent on a key electron donor moiety, i.e. their fluorescence is disabled by derivatization of a phenolate or amino group.[5] Changes in the substitution pattern i.e., by chemical[6] or enzymatic[7] removal of the disabling substituent leads to restoration of fluorescence. With respect to trigger controlled substituent removal, click-to-release bioorthogonal reactions seem an ideal choice. The most notable examples for bioorthogonal chemistry related release platforms are click-to-release *trans*-cyclooctenes (rTCOs)[8] and click-to-release tetrazines (crTZs),[9] although other functions are also known.[10] Utilization of such bioorthogonal click-to-release motifs to induce removal of substituents from the key electron donor group of a push-pull fluorophore therefore keeps the high specificity of bioorthogonal (IEDDA) conjugation. At the same time, the role of the tetrazine is redefined in the process leading to increase of the fluorescent signal. Consequently, this design is conveniently applicable for red-shifted probes.[11] However, by its very nature, the click-to-release process leads to detachment of the fluorescent unit from the biomolecule of interest. To keep the released fluorescent probe covalently attached to the target biomolecule (i.e., protein), we foresee a design where the release process leads to the primary generation of a non-fluorescent, highly reactive electrophilic species prone to be captured by nucleophiles in the proximity of the IEDDA reaction.

Not only would capture of the primary species result in covalent bond formation but it also promotes the generation of a fluorescent push-pull chromophore, with intense fluorescence. Such masked electrophiles are well represented

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by quinone methides (QMs, Figure 1). Up to now, various dyes were developed exploiting *in situ* generated QMs.[12]

To address the aforementioned challenges, we propose a conceptually novel design for fluorogenic, bioorthogonally applicable probes. Our design involves a crTz linked directly or via a self-immolative linkage to a QM precursor through a key electron donor atom being part of a push-pull chromophore. We hypothesized that IEDDA reaction between such crTz-caged systems and a TCO triggers a reaction cascade, leading to the formation of the highly reactive, non-fluorescent QM moiety. Capture of the QM by a proximal nucleophilic motif (e.g., an amino acid side chain) results in covalent ligation of the dye core and simultaneous formation of the fluorescent label (Figure 1).

Herein we present the proof-of-concept studies of the proposed design. For the sake of synthetic ease, we chose a coumarin core as the push-pull chromophore to accommodate the masked QM motif.

Results and Discussion

Synthesis

For synthetic considerations, 4-methylumbelliferon was chosen as the core to validate our concept. Fluorogenic coumarins caged through their electron donor 7-amino group by a rTCO were already described in the literature.[13] Herein we wished to replace the TCO with a crTz unit as the methodology for TCO implementation into biomolecules is well-established. As for the crTz we followed the hints of Robillard and co-workers, who studied a set of crTzs in details.[9a] According to their results, isopropyl substituted crTzs showed enhanced release rates compared to phenyl congeners, although these latter ones displayed greater stability in solution. The extra methyl group at position R^2 (Scheme 1) facilitates the release by stabilizing positive charge developed upon carbamate release. As for the connection between the coumarin core and the crTZ, we reasoned that direct linkage via an ether bond or through a dimethyl ethylenediamine self-immolative linker are reasonable. Although release from ether bond is expected to be slower compared to the self-immolative linker,[8h,14] it results in the departure of the quinone methide species directly without the need for the self-immolation process from a freely diffusing linker-QM-precursor adduct. This could be crucial in terms of the capture step by a proximal nucleophile.

The methides are usually masked in the forms of mono-, difluoromethyl or ethyl carbamate functionalized methylenes.[15] Monofluoromethyl groups are characterized by faster fluoride elimination but decreased stability in physiological media compared to the difluoromethyl containing analogues,[16] which may lead to solvolysis resulting in the formation of an unreactive hydroxymethyl species. Such hydrolytic issues do not rise in case of ethyl carbamate masked methides.[16b] At the same time, the difluoromethyl moiety is prone to lose its second fluoride as well to yield an aldehyde by-product.[17] The relatively higher stability of the QM generated from the difluoromethyl group can lead to diffusion from the activation site, which compromises immediate QM capture by a proximal nucleophile.[18] At the same time, fluorovinylenes show higher reactivity against amine and thiol nucleophiles present in proteins, which may be crucial in the competitive reaction with water.[19]

In light of these considerations, we designed and synthesized a set of crTz caged probes (Scheme 1). Both the ether-linked and the self-immolative linker connected probes utilized hydroxymethyl coumarin **1**, which was accessed by a literature procedure from methylumbelliferone.[17a] In case of ether-linked probes, the hydroxymethyl moiety was protected with THP (**2**) and derivatized with bromo-tetrazines **3**–**6**[9,20] to yield **7**–**10**.[9b] The subsequent deprotection step afforded tetrazinylated coumarins **11**–**14**. In the final step, fluorination with diethylaminosulfur difluoride (DAST) or reaction with ethyl isocyanate provided target compounds **15**–**20**. We did not observe the formation of **21** and **22**. Incorporation of the dimethyl ethylenediamine self-immolative linker[21] (**23**) was carried out on the unsubstituted coumarin **1**, followed by the introduction of the respective leaving group. Hydroxymethyl- and hydroxyethyl tetrazines **29**–**32**[9,20] were activated with DSC (**34**–**37**) and then reacted with the deprotected coumarins (**27**, **28**) to yield probes **39**, **40**, **44** and **45**. Although formed, probes **41, 42, 46** and **47** decomposed quickly.

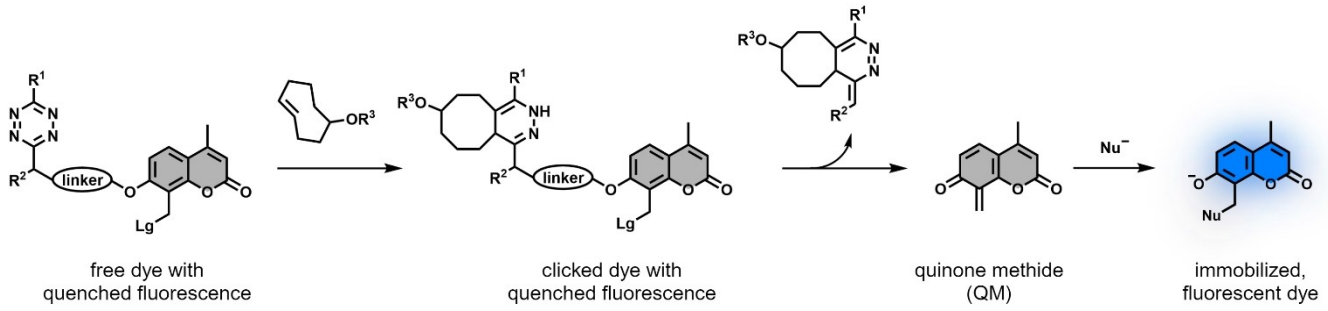

Figure 1. The immobilization process: the click reaction followed by elimination of the coumarin and the subsequent reaction of QM with nearby nucleophiles (Nu^-). Created with BioRender.com.

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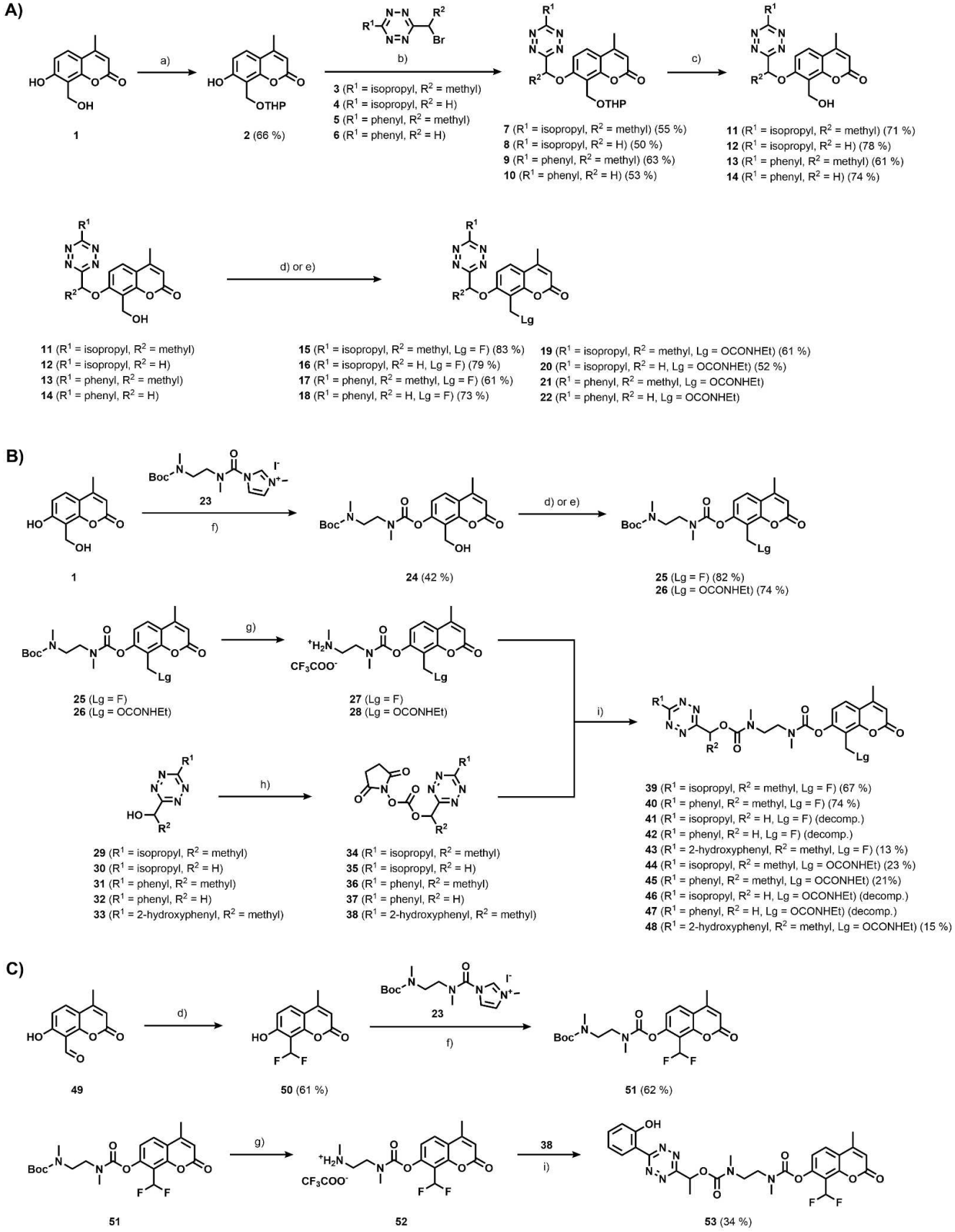

Scheme 1. Synthesis route of ether-linked (**A**, **15**–**22**) and self-immolative linker containing (**B**, **39**, **40**, **43**–**45**, **48** and **C**, **53**) probes. a) DHP, PPTS, dry DCM, 3 h, 25 °C; b) $KHCO_3$, 18-crown-6, acetone, 5 h, 25 °C; c) cc. HCl, EtOH/$CHCl_3$, 0.5 h, 25 °C; d) DAST, DCM, 15 min, 25 °C; e) ethyl isocyanate, TEA, dry DCM, 5 h, 25 °C; f) DIPEA, dry MeCN, 15 min, 82 °C; g) TFA, DCM, 10 min, 25 °C; h) DSC, DMAP, TEA, dry MeCN, 1 h, 25 °C; i) TEA, dry DCM, 1 h, 25 °C.

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Click-To-Release Measurements

With the target compounds in hand, we aimed to assess their click-to-release rates. To this end, we reacted the tetrazinylated probes with a TCO and followed subsequent release with LCMS. We applied a TCO-derivatized HaloTag substrate, HaloTCO, as reaction partner due to its favorable solubility in the applied LCMS compatible solvents (80% water, 20% acetonitrile at 37 °C). The HaloTCO was applied in five-fold molar excess (0.5 mM) relative to the probes (0.1 mM). Under these conditions, the generated QM is expected to react with water as the only nucleophile leading to the formation of **1**, thus the amount of **1** was monitored. The detailed release mechanism of ether-linked dyes and self-immolative linker-containing dyes is shown on representative examples in the Supporting Information (Schemes S5 and S6). These studies indicated instantaneous IEDDA reaction between the tetrazines and HaloTCO in most cases (Figures S3–S6). Subsequent release and conversion of the QM to **1** was followed at the absorption of the coumarin (**1**). Nor the quinone methide, nor the released, but self-immolative linker containing intermediates could be detected on LCMS. To compare release efficiencies for ether and self-immolative unit linked probes we determined the decomposition of the IEDDA products at 310 nm after 6 hours (Table 1).

Results indicate that better release yields were obtained in case of isopropyl tetrazine derivatized probes (**15**, **19**, **39**, **44**). The phenylated probes showed poor coumarin release, while no coumarin release was detected in case of **18** as its IEDDA product was oxidized to a non-releasing form (Scheme S7). We also studied the stability of two representative dyes in the presence of 5 equiv. GSH (pH = 7.4) in ammonium acetate/ammonium bicarbonate buffer (**15** and **44**, Figure S1). **44** was stable in the presence of GSH, while **15** started decomposing after 3 hours. Satisfyingly, no remarkable difference was observed between the dyes that only differed in the leaving group (**39** and **44**, **40** and **45**). Furthermore, we did not observe non-specific hydrolysis of fluorinated probes even after 24 hours.

To exploit the release-enhancing effect of hydroxyaryl-substituted tetrazine[22] and the superior stability of scaffolds linked via a self-immolative unit, we prepared probes **43** and **48** starting from precursor **33**. The synthesis of probes **43** and **48** was analogous to the previously described ones (Scheme 1, B). We also synthesized difluorinated probe **53** from aldehyde **49** (Scheme 1C).

We tested the reaction of **43**, **48** and **53** with HaloTCO under the same conditions as previously written. In case of these probes, fluorescence was also measured in PBS (c(dyes) = 1 μM; λ_{exc} = 365 nm) at the LCMS sampling points. **43** and **48** showed excellent release yields (~95% by both after 6 hours) and corresponding increase of fluorescence, moreover, they did not decompose in GSH (Figure S1). Difluorinated congener **53** on the other hand resulted in the corresponding aldehyde, **49** (Scheme S8). Figure 2 summarizes the sufficiently working probes and their conversion rate.

Table 1. Conversion of the IEDDA products. The LCMS measurements were conducted in water/acetonitrile mixture (80/20), c(probe) = 0.1 mM, c(HaloTCO) = 0.5 mM. The samples were kept at 37 °C.[a]

Probe	Conversion of the clicked product after 6 hours
15	97%
16	66%
17	29%
18	–[b]
19	95%
20	32%
39	85%
40	48%
43	96%
44	89%
45	33%
48	94%
53	–[c]

[a] Calculated from the absorption of the clicked product at 310 nm absorption. [b] No release. [c] Hydrolysis to aldehyde **49** occurred during release.

Studies on Elecrophile Capture

Probes **43** and **48** exhibited very similar click-to-release efficiencies regardless of possessing different methide masking moieties. We chose **43** for studying the electrophile capture step. The click-to-release process with HaloTCO was followed by LCMS in water/acetonitrile mixture (80/20) in the presence of 0.5 mM ethanolamine (**EA**, Figure S5), *N*-methylethanolamine (**NMEA**, Figure S4) or 2-mercaptoethanol (**ME**, Figure 2). As expected, the respective nucleophile-coumarin conjugates (**1-ME**, **1-NMEA**, **1-EA**) were detected with all nucleophiles, although formation of the respective **1-Nu** was not quantitative and the water adducts were also detected. **1-Nu**/**1** ratios were determined to be 90/10, 33/66 and 30/70 for **1-NMEA**, **1-ME** and **1-EA**, respectively.

Conclusions

Herein we provided the first proof-of-concept studies for a novel bioorthogonally driven fluorogenic tagging scheme, which may address the limitations of tetrazine-quenched red/NIR excitable fluorogenic probes. Our novel design concept involves a click-to-release tetrazine unit as the trigger that chemically cages a pro-fluorescent quinone methide precursor. Upon IEDDA reaction with a TCO a pro-fluorescent QM is generated, which is ready to be captured by nucleophilic amino acid side chains of the protein of interest. We have synthesized a set of model compounds to evaluate the viability of the design concept and studied the efficiency of the respective units. Results suggest that hydroxyphenyl derivatized crTz is the most suitable as the triggering unit, while the presence of a

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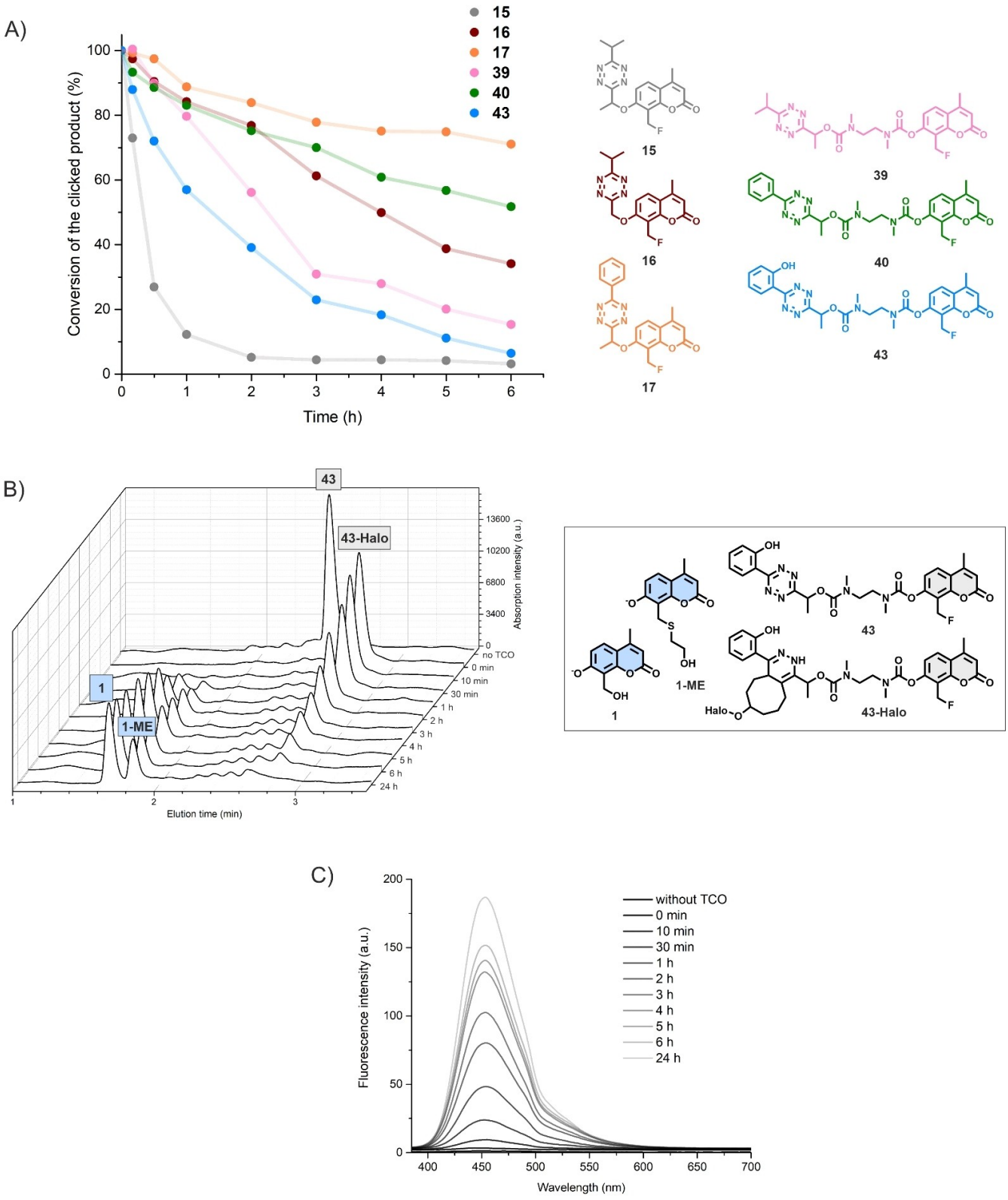

Figure 2. A) The conversion of the clicked product calculated from the absorption of the clicked product at 310 nm by the fluorinated dyes. B) The chromatograms (λ_{abs} = 310 nm; *c*(**43**) = 0.1 mM; *c*(ME) = 0.5 mM; *c*(HaloTCO) = 0.5 mM; water/acetonitrile - 80/20) obtained during the reaction of **43** with HaloTCO in the presence of **ME**. C) The increase in fluorescence during the reaction of **43** with HaloTCO (λ_{exc} = 365 nm; *c*(**43**) = 1 μM; *c*(ME) = 5 μM; PBS).

self-immolative linker between the crTz and the pro-fluorescent QM precursor is necessary for physiological stability. We also tested the capture of the generated pro-fluorescent QM by nucleophiles and found that their reaction indeed results in a fluorescent covalent nucleophile-probe adduct. These studies suggest that the proposed design concept is indeed viable and can be extended to red/NIR excitable cores. However, further studies are required to fine tune the reactivity of the generated quinone methide species in order to suppress competitive side reaction with water. Such studies are in progress in our laboratory and results will be reported in due course. Besides its importance in fluorogenic tagging schemes, the proposed design concept is also suitable to be applied to further photoresponsive compounds, such as $^1O_2$ sensitizers in photodynamic therapy (PDT) applications. Since this latter application does not require proximal capture of the QM, such bioorthogonally activatable singlet oxygen sensitizers offer cell specific intracellular activation of PDT agents, enabling a considerably improved localization precision of therapeutic drugs with minimalized side-effects. This approach can prevent a systemic photosensitivity as well as injury to healthy tissue in the proximity of the targeted cancer cells.

Experimental

General Procedure for 7–10

2 (1 eq), $KHCO_3$ (3 eq) and 18-crown-6 (0.25 eq) were dissolved in acetone. The solution was cooled to 0 °C and the tetrazine (**3**/**4**/**5**/**6**, 1.2 eq) dissolved in acetone was added dropwise. The reaction was stirred at room temperature until completion (6–24 hours). The solvent was evaporated under reduced pressure and the crude product was purified by preparative HPLC (H_2O:MeCN starting from 95 : 5–0 : 100 containing 0.1 % HCOOH).

General Procedure for 11–14

7/**8**/**9**/**10** (1 eq) was dissolved in the mixture of 3 cm^3 ethanol and 6 cm^3 chloroform. 0.1 cm^3 cc. HCl was added, then the solution was stirred at room temperature until completion (0.5–1 hour). The solvent was evaporated under reduced pressure. The crude product was purified by preparative HPLC (H_2O:MeCN starting from 95 : 5–0 : 100 containing 0.1 % HCOOH).

General Procedure for 15–18, 25 and 50

11/**12**/**13**/**14**/**24**/**49** (1 eq) was dissolved in dry DCM, then DAST (2.5 eq) was added dropwise to this solution, that was afterwards stirred at room temperature. After completion (15 minutes) 5 cm^3 water was added dropwise. The phases were separated, the aqueous phase was washed with DCM. The combined organic phases were dried on $MgSO_4$. The solvent was removed in vacuo, the crude product was purified by preparative HPLC (H_2O:MeCN starting from 95 : 5–0 : 100 containing 0.1 % HCOOH).

General Procedure for 19, 20 and 26

11/**12**/**24** (1 eq) was dissolved in dry DCM, then ethyl isocyanate (10 eq) and triethyl amine (5 eq) were added. The solution was stirred at room temperature overnight. After completion the solvent was removed in vacuo. The crude product was purified by preparative HPLC (H_2O:MeCN starting from 95 : 5–0 : 100 containing 0.1 % HCOOH).

General Procedure for 39, 40, 43–45, 48 and 53

29/**30**/**31**/**32**/**33** (1 eq), DSC (5 eq), DMAP (0.5 eq) and triethylamine (5 eq) were dissolved in dry acetonitrile and the mixture was stirred at room temperature. The reaction was monitored by TLC (DCM/methanol - 9/1). Upon completion (1–1.5 hours) the solvent was evaporated under reduced pressure; the residue was redissolved in ethyl acetate. The organic phase was washed with water five times, then with cc. $NaHCO_3$ solution also five times. The organic phase was dried on $MgSO_4$, then the solvent was evaporated in vacuo. The crude product (**34**–**38**) was used immediately without further purification. This crude product (**34**–**38**) was dissolved in dry DCM. Triethylamine was added (3 eq), then the solution of crude **27**/**28**/**52** (1 eq) in dry DCM was also added dropwise. The mixture was monitored by LCMS and was stirred at room temperature until completion (2–6 hours). Then the solvent was removed in vacuo. The crude product was purified by preparative HPLC (H_2O:MeCN starting from 95 : 5–0 : 100 containing 0.1 % HCOOH).

LC–MS Studies of the Click-To-Release Reactions

Stock solutions (c = 1 mM) were made in acetonitrile from **15**, **16**, **17**, **18**, **19**, **20**, **39**, **40**, **43**, **44**, **45**, **48** and **53**. Stock solution of HaloTCO was made in acetonitrile (c = 10 mM). Nucleophiles (NMEA, EA and ME) were dissolved in water (c = 5 mM). The LCMS measurements were conducted in water/acetonitrile mixture (80/20), c(**15**, **16**, **17**, **18**, **19**, **20**, **39**, **40**, **43**, **44**, **45**, **48** or **53**) = 0.1 mM, c(HaloTCO) = 0.5 mM. Nucleophile capture measurements were performed with NMEA, EA or ME (c = 0.5 mM). The samples were kept at 37 °C. Fluorescence was measured with excitation at 365 nm in PBS (pH = 7.4), c(**45** or **48**) = 1 μM; c(NMEA, EA or ME) = 5 μM; c(HaloTCO) = 5 μM.

Supporting Information Summary

Synthetic and experimental details, characterization data and additional references are provided in Supporting Information.[23]

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Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

Keywords: Click-to-release tetrazines · Fluorogenic probes · Quinone methides · Labeling · Masked electrophiles

[1] a) A. Herner, I. Nikič, M. Kállay, E. A. Lemke, P. Kele, *Org. Biomol. Chem.* **2013**, *11*, 3297–3306; b) A. Herner, G. Estrada Girona, I. Nikič, M. Kállay, E. A. Lemke, P. Kele, *Bioconjugate Chem.* **2014**, *25*, 1370–1374; c) L. G. Meimetis, J. C. T. Carlson, R. J. Giedt, R. H. Kohler, R. Weissleder, *Angew. Chem. Int. Ed.* **2014**, *53*, 7531–7534; d) A. Wieczorek, P. Werther, J. Euchner, R. Wombacher, *Chem. Sci.* **2017**, *8*, 1506–1510; e) E. Kozma, O. Demeter, P. Kele, *ChemBioChem* **2017**, *18*, 486–501; f) E. Kozma, P. Kele, *Top. Curr. Chem.* **2024**, *382*, 7.

[2] E. Kozma, G. Estrada Girona, G. Paci, E. A. Lemke, P Kele, *Chem. Commun.* **2017**, *53*, 6696–6699.

[3] A. Kormos, A. Egyed, J. M. Olvany, Á. Szatmári, A. Biró, Zs. Csorba, P. Kele, K. Németh, *Chemosensors* **2022**, *10*, 37.

[4] F. Liu, D. I. Danylchuk, B. Andreiuk, A. S. Klymchenko, *Chem. Sci.* **2022**, *13*, 3652–3660.

[5] W. Chyan, R. T. Raines, *ACS Chem. Biol.* **2018**, *13*, 1810–1823.

[6] a) Y. Li, K.-N. Wang, B. Liu, X.-R. Lu, M.-F. Li, L.-N. Ji, Z.-W. Mao, *Sens. Actuators B* **2018**, *255*, 193–202; b) X. Lin, Y. Hu, D. Yang, B. Chen, *Dyes Pigm.* **2020**, *174*, 107956; c) S. Y. Kim, J. Park, M. Koh, S. B. Park, J.-I. Hong, *Chem. Commun.* **2009**, 4735–4737; d) W.-H. Wu, X. Wang, L. Zong, D. Li, Y.-H Xiao, S.-H. Sui, J. Li, M. Liu, G.-Y. Chen, T. Luo, M. Liu, X.-M. Wang, Z.-G. Jiang, *J. Fluoresc.* **2021**, *31*, 141–149.

[7] a) Y. Li, H. Song, C. Xue, Z. Fang, L. Xiong, H. Xie *Chem. Sci.* **2020**, *11*, 5889–5894; b) Y. Zhang, X. Chen, Q. Yuan, Y. Bian, M. Li, Y. Wang, X. Gao, D. Su, *Chem. Sci.* **2021**, *12*, 14855–14862; c) Y.-X. Li, D.-T. Xie, Y.-X. Yang, Z. Chen, W.-Y. Guo, W.-C. Yang, *Molecules* **2022**, *27*, 4501.

[8] a) R. M. Versteegen, R. Rossin, W. ten Hoeve, H. M. Janssen, M. S. Robillard, *Angew. Chem. Int. Ed.* **2013**, *52*, 14112–14116; b) R. Rossin, S. M. J. van Duijnhoven, W. ten Hoeve, H. M. Janssen, L. H. J. Kleijn, F. J. M. Hoeben, R. M. Versteegen, M. S. Robillard, *Bioconjug. Chem.* **2016**, *27*, 1697–1706; c) R. Rossin, R. M. Versteegen, J. Wu, A. Khasanov, H. J. Wessels, E. J. Steenbergen, W. Ten Hoeve, A. H. A. M. Van Onzen, P. J. Hudson, M. S. Robillard, *Nat. Commun.* **2018**, *9*, 1484; d) Q. Yao, F. Lin, X. Fan, Y. Wang, Y. Liu, Z. Liu, X. Jiang, P. R. Chen, Y. Gao, *Nat. Commun.* **2018**, *9*, 5032; e) I. Khan, L. M. Seebald, N. M. Robertson, M. V. Yigit, M. Royzen, *Chem. Sci.* **2017**, *8*, 5705–5712; f) J. Li, S. Jia, P. R. Chen, *Nat. Chem. Biol.* **2014**, *10*, 1003–1005; g) M. Wilkowitsch, M. Haider, B. Sohr, B. Herrman, J. Klubnick, R. Weissleder, J. C. T. Carlson, H. Mikula, *J. Am. Chem. Soc.* **2020**, *142*, 19132–19141; h) R. M. Versteegen, W. Ton Hoeve, R. Rossin, M. A. R. de Geus, H. M. Janssen, M. S. Robillard, *Angew. Chem. Int Ed.* **2018**, *57*, 10494–10499.

[9] a) A. H. A. M. Van Onzen, R. M. Versteegen, F. J. M. Hoeben, I. A. W. Filot, R. Rossin, T. Zhu, J. Wu, P. J. Hudson, H. M. Janssen, W. ton Hoeve, M. S. Robillard, *J. Am. Chem. Soc.* **2020**, *142*, 10955–10963; b) Y. Wang, G. Shen, J. Lie, W. Mao, H. Sun, P. Feng, H. Wu, *Org. Lett.* **2022**, *24*, 5293–5297.

[10] a) M. Azoulay, G. Tuffin, W. Sallem, J.-C. Florent, *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3147–3149; b) J. M. Fairhall, J. C. Camilli, B. H. Gibson, S. Hook, A. B. Gamble, *Bioorg. Med. Chem.* **2021**, *46*, 116361; c) M. Xu, T. Deb, J. Tu, R. M. Franzini, *J. Org. Chem.* **2019**, *84*, 15520–15529; d) J. Tu, D. Svatunek, S. Parvez, A. C. Liu, B. J. Lewandowski, H. J. Eckvahl, R. T. Peterson, K. N. Houk, R. M. Franzini, *Angew. Chem. Int. Ed.* **2019**, *58*, 9043–9048.

[11] a) X. Zhang, H. Xu, J. Li, D. Su, W. Mao, G. Shen, L. Li, H. Wu, *Chem. Commun.* **2022**, *58*, 573–576; b) J. Tu, D. Svatunek, S. Parvez, H. J. Eckvahl, M. Xu, R. T. Peterson, K. N. Houk, R. M. Franzini, *Chem. Sci.* **2020**, *11*, 169–179.

[12] D. Kern, A. Kormos, *Chemosensors* **2023**, *11*, 155.

[13] X. Fan, Y. Ge, F. Lin, Y. Yang, G. Zhang, W. S. H. Ngai, Z. Lin, S. Zheng, J. Wang, J. Zhao, J. Li, P. R. Chen, *Angew. Chem. Int. Ed.* **2016**, *55*, 14046–14050.

[14] P. Keppel, B. Sohr, W. Kuba, M. Goldeck, P. Skrinjar, J. C. T. Carlson, H. Mikula, *ChemBioChem* **2022**, *23*, e202200363.

[15] a) Y. Li, C. Xue, Z. Fang, W. Xu, H. Xie, *Anal. Chem.* **2020**, *92*, 15017–15024; b) H. Ito, Y. Kawamata, M. Kamiya, K. Tsuda-Sakurai, S. Tanaka, T. Ueno, T. Komatsu, K. Hanaoka, S. Okabe, M. Miura, Y. Urano, *Angew. Chem. Int. Ed.* **2018**, *57*, 15702–15706; c) C.-H. Tai, C.-P. Lu, S.-H. Wu, L.-C. Lo, *Chem. Commun.* **2014**, *50*, 6116–6119; d) Y. Chen, C. Xue, J. Wang, M. Xu, Y. Li, Y. Ding, H. Song, W. Xu, H. Xie, *Chin. Chem. Lett.* **2022**, *33*, 1637–1642.

[16] a) J. Y. Hyun, S.-H. Park, C. W. Park, H. B. Kim, J. W. Cho, I. Shin, *Org. Lett.* **2019**, *21*, 4439–4442; b) H. Song, Y. Li, Y. Chen, C. Xue, H. Xie, *Chem. Eur. J.* **2019**, *25*, 13994–14002; c) Y. Li, H. Song, C. Xue, Z. Fang, L. Xiong, H. Xie, *Chem. Sci.* **2020**, *11*, 5889–5894.

[17] a) C.-H. Tsai, C.-P. Lu, S.-H. Wu, L.-C. Lo, *Chem. Commun.* **2014**, *50*, 6116–6119; b) J. Jiang, Q. Tan, S. Zhao, H. Song, L. Hu, H. Xie *Chem. Commun.* **2019**, *55*, 15000–15003.

[18] a) N. W. Polaske, B. D. Kelly, J. Ashworth-Sharpe, C. Bieniarz, *Bioconjugate Chem.* **2016**, *27*, 660–666; b) D. H. Kwan, H.-M. Chen, K. Ratananikom, S. M. Hancock, Y. Watanabe, P. T. Kongsaeree, A. L. Samuels, S. G. Withers, *Angew. Chem. Int. Ed.* **2011**, *50*, 300–303.

[19] W. Mao, L. Xia, Y. Wang, H. Xie, *Chem. Asian J.* **2016**, *11*, 3493–3497.

[20] X. Zhang, H. Xu, J. Li, D. Su, W. Mao, G. Shen, L. Li, H. Wu, *Chem. Commun.* **2022**, *58*, 573–576.

[21] C. Zoukimian, R. Béroud, D. Boturyn, *Org. Lett.* **2022**, *24*, 3407–3410.

[22] M. Wilkowitsch, W. Kuba, P. Keppel, B. Sohr, A. Löffler, S. Kronister, A. F. del Castillo, M. Goldeck, R. Dzijak, M. Rahm, M. Vrabel, D. Svatunek, J. C. T. Carlson, H. Mikula, *ChemRxiv* **2024**, DOI: doi:10.26434/chemrxiv-2024-gh8fz.

[23] D. R. Bender, D. Kanne, J. D. Frazier, H. Rapoport, *J. Org. Chem.* **1983**, *48*, 2709–2719.

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