A new azide staining reagent based on "click chemistry"†

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A new staining reagent was prepared and its ability to stain several azide-containing agents on TLC plates was determined.

A multiplicity of methods for the synthesis of organic azides for different purposes are known, but their TLC detection is sometimes still problematic. If the organic core of the azide compound does not bear UV active groups or functional groups allowing visualization on TLC plates with a fluorescent indicator, selective and effective staining will not be possible by common methods. In these cases, reaction control *via* TLC detection is difficult or impossible. Currently, there are only a few azide staining reagents available, *e.g.* molybdato phosphoric acid, "Seebach staining solution"‡ or a colorimetric test, which contains triphenyl phosphine.[1] The main disadvantage of using molybdato phosphoric acid as a staining reagent for azides is the lack of selectivity in terms of different functional groups. Molybdato phosphoric acid, as well as potassium permanganate, stains almost every functional group, hence a differentiation between several products or product and starting material by staining is not possible. The colorimetric test described by Finn and Punna comprises the reduction of azides to amines by means of triphenyl phosphine and can mainly be used for polymers and resins.[1] However, this triphenyl phosphine staining method bears considerable problems like the need of several ingredients to observe a positive staining, as well as the complexity of the procedure for staining the azide compounds on TLC plates.

The concept of click chemistry, in particular the Cu(I)-catalyzed Huisgen 1,3-dipolar cycloaddition reaction between a dipolarophile and a compound containing a 1,3-dipolar functional group, has become more and more attractive in the past few years,[2] *e.g.* in combinatorial drug research,[3] material science[4] and bioconjugate chemistry.[5] One 1,3-dipolar functional group that is suitable for use in click chemistry is the azide group, which has been under intense investigation as well.[6] To circumvent the outlined problems that emerge from conventional azide staining methods, we thought about utilizing click chemistry to stain organic azides. Consequently, we developed an azide-staining reagent based on click chemistry which is an interesting extension and a very useful advancement regarding the existing methods.

The new "click-staining-reagent" consisting of ethanol, propargylic alcohol (as a dipolarophile) and copper(I) is a fast and readily prepared mixture,§ which improves the TLC detection of azides. This azide staining reagent was tested with several different functionalized organic azides (Table 1). We observed that this "click-staining-reagent" only stains organic azides and amines. Sulfonyl azides do not react under these conditions and gave no visible spots (not shown). While amines lead to intense yellow spots, azides locally decolorize the TLC plate and give white spots on a slightly yellow background. The yellow background results from the propargylic alcohol that tints the TLC plate after heating with a heat gun and therefore makes the azide spot visible. To verify a selectivity with regard to azides (and amines) the staining reagent was also tested with compounds that bear functional groups other than azide groups (Table 2). We observed that neither aromatic or aliphatic alcohols nor halides or esters were stained by the "click-reagent". Thus, a high selectivity is given. Even though amines as well as azides are stained, the characteristic colour (amines tint strong yellow, while azides occur white) of each species allows their differentiation in mixtures (*e.g.* in a conversion of amines into azides) (for photographs, see Fig. 2 in the ESI†).

The supposed click reaction that takes place on the surface of the TLC plate seems to be very fast, as dipping the TLC plate into the "click-staining-reagent" for one second followed by heating with a heat gun is sufficient for most spots to appear. Furthermore, this high reaction rate implies that the spot on the TLC plate surface does not enlarge significantly when dipping the plate into the "click-staining-reagent" for short periods of time. Hence, a well-defined spot is visible after the staining procedure. The staining intensity can be enhanced, by dipping the TLC plate subsequently into "Seebach staining solution"; for photographs, see Fig. 1 in the ESI†.

As copper(I) could be oxidized to copper(II), which would certainly lead to inactivation of this "click-staining-reagent", we also investigated the storage stability of this mixture at room temperature. After a storage time of two months, the staining ability of the "click-staining-reagent" was only slightly reduced (leading to a negligible reduced staining intensity) compared to the use of a freshly prepared "click-staining-reagent". This observation suggests that oxidation of copper(I) occurs very slowly in this mixture.

The supposed mechanism of the described staining method is based on a 1,3-dipolar cycloaddition of the azide and the propargylic alcohol that leads to the corresponding triazole

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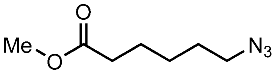
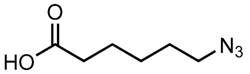
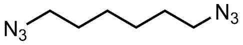
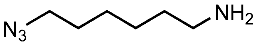
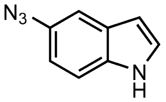
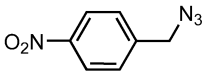
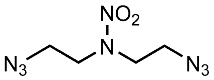
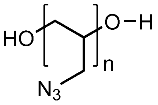
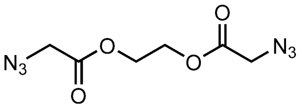
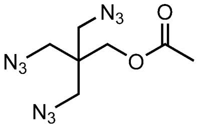
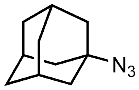
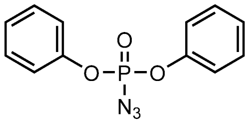
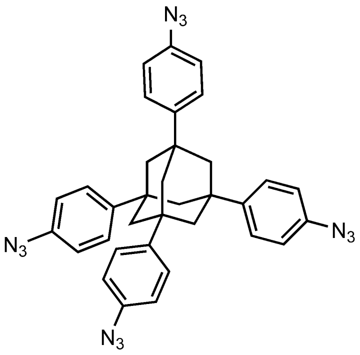
‡ "Seebach staining solution" is a mixture of molybdato phosphoric acid, cerium(IV)-sulfate tetrahydrate, sulfuric acid and water.

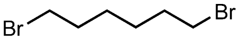
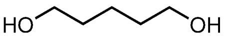
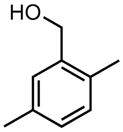
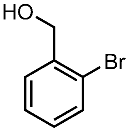
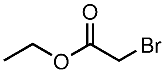
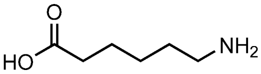
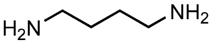
§ The "click-staining-reagent" is prepared by mixing 40 ml of ethanol, 40 ml of propargylic alcohol and a small amount (tip of a spatula) of copper(I)-bromide. No further additives are required.

Table 1 Examples of different azides and detection by the "click-staining-reagent"

Entry	Azide containing compound	Classical staining	Click-staining-reagent	Staining intensity[a]
1		None	Positive detection of azide group	s
2		None	Positive detection of azide group	m
3		Seebach staining reagent	Alternative method to classical staining	s
4		Ninhydrin staining reagent[b]	Alternative method to classical staining[c]	s (yellow)[d]
5		Seebach staining reagent	Alternative method to classical staining	s (red)
6		Seebach staining reagent[e]	Alternative method to classical staining	s
7		Seebach staining reagent[e]	Positive detection of azide group	s
8		Seebach staining reagent	Positive detection of azide group	s
9		Seebach staining reagent[e]	Positive detection of azide group	s
10		Seebach staining reagent[e]	Positive detection of azide group	s
11		Potassium permanganate[e]	Alternative method to classical staining	w
12		Potassium permanganate[e]	Alternative method to classical staining	s
13	Me_3SiN_3	Potassium permanganate[e]	Alternative method to classical staining	s
14		None[e]	Positive detection of azide group	w

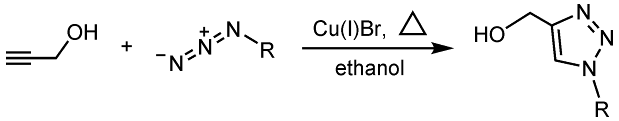
[a] Staining intensity: w = weak, m = medium, s = strong. [b] Detection with Seebach staining reagent also possible. [c] Staining with Seebach reagent possible after staining with "click-staining-reagent". [d] Free amines tint strong yellow. [e] Substance is also detectable in UV light on TLC plates with fluorescent indicator.

Table 2 Staining of azide-free compounds with the "click-staining-reagent"

Entry	Compound	Detection with the "click-staining-reagent"[a]
1		No staining
2		No staining
3		No staining
4		No staining
5		No staining
6		s[b]
7		s[b]

[a] Staining intensity: s = strong. [b] Free amines tint strong yellow.

(Scheme 1).[7] Presumably, the copper ion coordinates the azide as well as the propargylic alcohol when the TLC plate is dipped into the staining reagent. The remaining part of the TLC plate is only coated with propargylic alcohol and copper ions. Consequently, the click reaction only takes place in the small area where the azide is located. The rest of the TLC plate remains unaffected because of the lack of the required azide group. After heating, the occurring spot could have a white colour resulting from the formed triazole that occurs as a white spot in front of a slightly yellow background caused by heating propargylic alcohol.



Scheme 1 Possible reaction equation for the staining of organic azides on TLC plates. Before heating, no spot could be observed.

We found that aromatic or sterically hindered azides stain weaker than aliphatic ones. These staining intensities are in accordance with the reaction rates that were typically found for click reactions with these types of compounds.[8] In general, aromatic or sterically hindered azides react slower in click reactions than aliphatic azides, which consequently leads to a lower colorization in the intensity of the occurring spot.

Additionally, the use of other alkynes in the "click-staining-reagent" was investigated to evaluate if they exert their influence on the staining intensity. For example, the staining reagent was prepared with phenyl acetylene instead of propargylic alcohol, because strongly colorized spots as a consequence of the supposed conjugated triazole system were expected to be obtained. These differently composed "click-staining-reagents" were tested with some of the azides of Table 1 and also with the azide-free compounds (Table 2). We observed no staining at all for phenyl acetylene as a dipolarophile. Only after prolonged heating (about one minute), did azide spots for some high energetic polyazides occur (entries 7–10, Table 1) due to their decomposition.

Conclusions

In summary, we have developed and tested a new and selective staining reagent for organic azides based on the Cu(I)-catalyzed Huisgen 1,3-dipolar cycloaddition reaction known from click chemistry. So far, no satisfying method for staining azide-containing organic compounds had been available and some azides that were difficult or impossible to detect with classical staining methods could be selectively stained by the new method. This procedure is convenient, fast to accomplish and the resulting TLC spots have, in general, a sufficient to strong intensity with sharp, well-defined shapes. Additionally, the reagent is storable at room temperature for several months without significant deterioration of quality. Thus, the introduced "click-staining-reagent" is a valuable extension regarding the existing methods for staining azides on TLC plates.

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Notes and references

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