Chem. Anal. (Warsaw), **54**, 807 (2009) **SHORT COMMUNICATION**

Simple and Convenient Protocol for Staining of Organic Azides on TLC Plates by Ninhydrin. A New Application of an Old Reagent

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Operationally simple, general and sensitive method for the visualization of organic azides on TLC plates was developed. The protocol is based on triphenylphosphine-mediated reduction of azides to the corresponding amines which give contrasting color spots with ninhydrin.

Opisano prostą, ogólną i czułą metodę wybarwiania azydków organicznych na płytkach TLC, bazującą na ich redukcji do odpowiednich amin, które w reakcji z ninhydryną dają kontrastowo zabarwione plamy.

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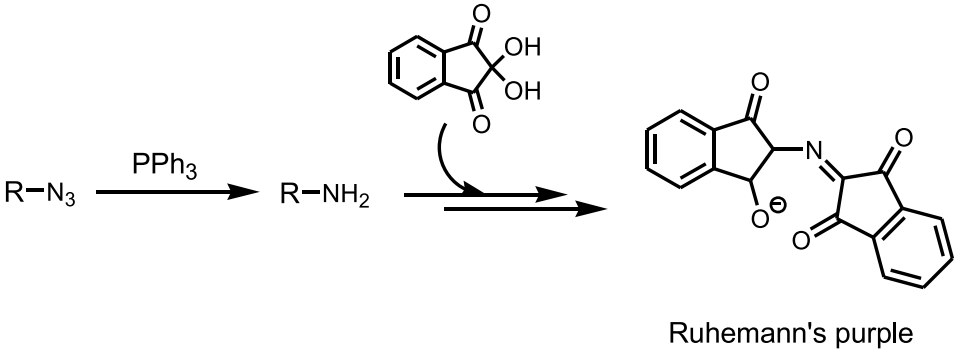
Organic azides are important class of organic compounds widely exploited in organic synthesis as versatile intermediates [1] and recently in so called click chemistry (Huisgen 1,3-cycloaddition) [2, 3].

In the course of our project we synthesized a libraries of azidoacids and azidopeptides *via* diazotransfer from the corresponding amino compounds and needed therefore a reliable and simple test for TLC detection of the progress of these reactions and assessment of purity of the formed azides. Unfortunately, the lack of an aromatic chromophore in many of the azides made the UV-detection impractical. Surprisingly, inspection of the rich literature on TLC staining methods [4] uncovered only a few protocols for the visualization of the azides published so far. Heckel and Seebach used molybdato phosphoric acid [5] which due to a rather strong oxidising characteristics shows lack of selectivity and stains most of the functional groups. Very recently Brase *et al.* published an alternative method based on *in-situ* click reaction between azides (on TLC plate) and the mixture of propargyl alcohol and copper(I) bromide [6]. Unfortunately, this reagent produces white spots on a slightly yellow background which may be not sufficiently contrasting and requires further use of the Seebach reagent for more contrasting staining. For these reasons we turned our attention to the development of a novel staining method free of the abovementioned limitations. Our two-step protocol involves initial reduction of azides with triphenylphosphine (CAUTION: harmful) to the corresponding amines and their further well-known reaction with ninhydrin leading to the Ruhemann's purple (Scheme 1) [7]. This rather obvious sequence was not yet used for staining of azides on TLC plates and a similar colorimetric test was only recently adopted by Punna and Finn for the detection of azides on solid support [8].



Scheme 1

EXPERIMENTAL

Materials and reagents

Silica gel 60 F254 TLC plates (Merck, Fluka) were used. 0.3% solution of ninhydrin (Fluka, analytical grade) in n-butanol/AcOH (100:3, v/v); 10% triphenylphosphine (Fluka, CAUTION: harmful) in CH_2Cl_2. All solvents and reagents were of analytical purity. Azides were prepared according to the published procedures.

General procedure for visualization of azides on TLC plates

A sample of azide was spotted on TLC plate and the plate was developed in chromatographic chamber using an appropriate mobile phase (Fig. 1). The plates were then dried at 80°C for 5 min and dipped into 10% solution of PPh_3 in CH_2Cl_2 for 30 s. Excess of reagent was removed by paper towel. After drying at 80°C for 5 min the plates were dipped into 0.3% solution of ninhydrin in n-butanol/AcOH (100:3, v/v) for 30 s. After removing the excess of reagent, the color was developed by drying the plates in an oven at 80°C for 5 min (or alternatively by using heat gun).

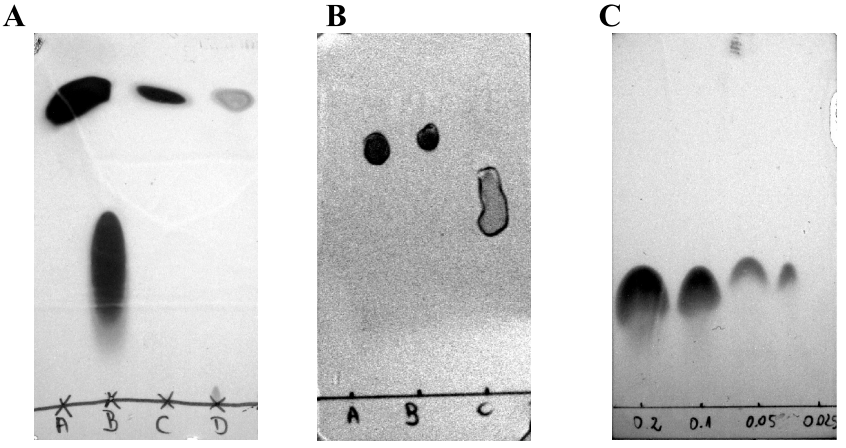


Figure 1. TLC plates staining by PPh_3/ninhydrin protocol. **A**: low-molecular weight azides (from left to right **4**, **2**, **3** and **17**, mobile phase: dichloromethane; **B**: peptide azides **9**, **15**, **10**, mobile phase n-BuOH–AcOH–H_2O (20:5:5 v/v/v); **C**: sensitivity test with 2-azidoethanol (**2**) (0.2, 0.1, 0.05 and 0.025 mg/spot), mobile phase: dichloromethane. The differences of the quality of pictures are due the different photographic conditions

RESULTS AND DISCUSSION

Initially we tried to develop *one pot* protocol for staining of azides by using a mixture of triphenylphosphine (reduction) and ninhydrin (color reaction). This procedure stained the azides, however it produced an intense colored background and

the results were not reproducible enough. Moreover, the stability of the staining mixture was very limited. For these reasons we decided to separate the reduction and staining steps. An optimized protocol thus involves the reduction by dipping the TLC plate into triphenylphosphine solution in dichloromethane and drying at 80°C for 5 min, followed by dipping the plate into a ninhydrin solution and activation for color reaction at 80°C for a few minutes. This protocol was screened against a variety of azides ranging from simple low-molecular aliphatic and benzyl azides (**1–7**) to more complex azido di- and tripeptides (**8–10**) as well as azido deriviatives of diverse natural products (**11–14**) (Fig. 2).

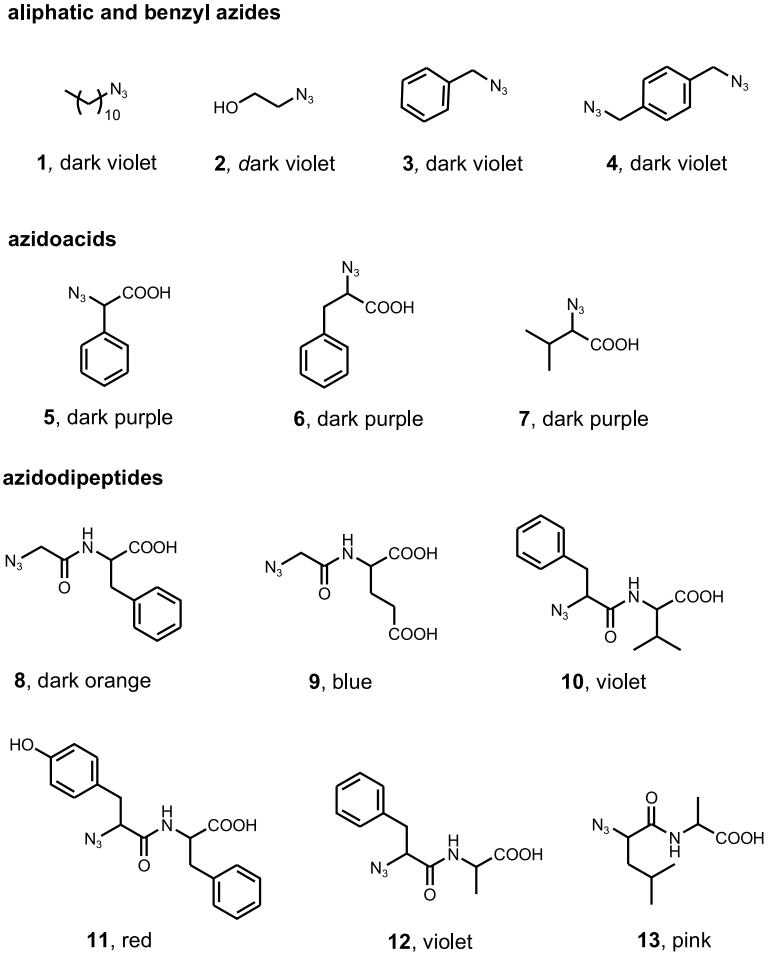


Figure 2. Azides stained using triphenylphosphine-ninhydrin protocol. (Continuation on the next page)

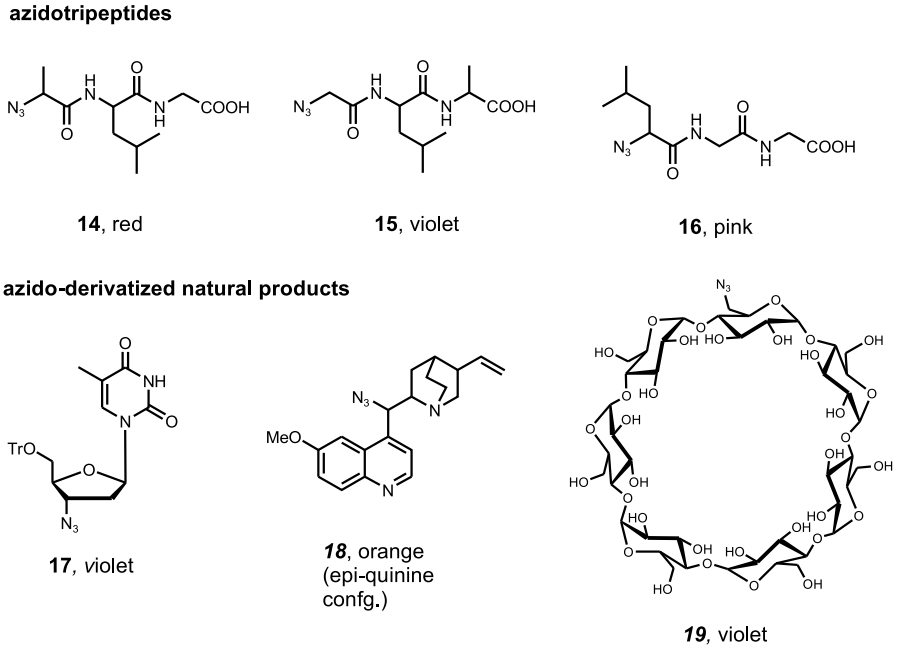


Figure 2. (Continuation)

We were pleased to find a positive reaction of all of the azides tested. Depending on the compound the obtained color varies form yellow to dark violet and is stable for a few days (getting brighter by ageing) (Fig. 1). Especially noteworthy is an easy detection of the azido β-cyclodextrin (**19**) an interesting clickable chiral building block. The desired azide **19** can be unambiguously distinguish form other sugar by-products in the course of preparation and purification. This is not easy by using a standard concentrated H_2SO_4 as an non-selective staining reagent. This method has been also routinely applied for convenient monitoring of diazotransfer reaction of non-aromatic aminoacids and peptides. Using this protocol both amino-compounds and the corresponding azides have been visualized in one step.

In a separate experiment the sensitivity of this protocol was tested using 2-azido-ethanol as a reference (Fig. 1). It has been found that as low amount as 0.025 mg/spot still produces contrasting and visible spot on TLC plate.

In conclusion we have developed a simple, low-cost and general method for the visualization of organic azides on TLC plates. This short method combines the sensitivity and generality of the ninhydrin reaction with amines which are produce *in situ* by the reduction of azides with triphenylphosphine. This new method, we believe, may be helpful for the azido- and click chemistry-related research.

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REFERENCES

1. Brase S., Gil C., Knepper K. and Zimmermann V., *Angew. Chem. Int. Ed*., **44**, 5188 (2005).
2. Tornoe C.W., Christensen C. and Meldal M., *J. Org. Chem*., **67**, 3057 (2002).
3. Rostovtsev V.V., Green L.G., Fokin V.V. and Sharpless K.B., *Angew. Chem. Int. Ed.*, **41**, 2596 (2002).
4. *Thin-Layer Chromatography: Reagents and Detection Methods*, [Jork H., Ed.], VCH, Weinheim, (1994).
5. Heckel A. and Seebach D., *Chem. Eur. J*., **8**, 560 (2002).
6. Schroder T., Gartner M., Grab T. and Brase S., *Org. Biomol. Chem*., **5**, 2767 (2007).
7. Friedman M., *J. Agric. Food Chem*., **52**, 385 (2004).
8. Punna S. and Finn M.G., *Synlett*, 99 (2004).

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