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Metal-Free Click Reaction

Traceless Tosylhydrazone-Based Triazole Formation: A Metal-Free Alternative to Strain-Promoted Azide–Alkyne Cycloaddition**

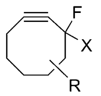
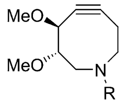
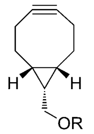
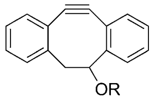
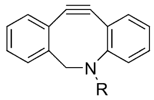
*Sander S. van Berkel, Sebastian Brauch, Lars Gabriel, Michael Henze, Sebastian Stark, Dimitar Vasilev, Ludger A. Wessjohann, Muhammad Abbas, and Bernhard Westermann**

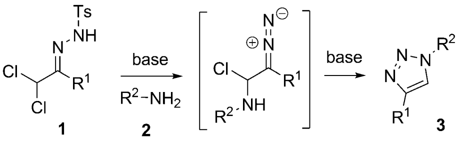
The unprecedented impact of the Cu^{I}-mediated Huisgen cycloaddition reaction is demonstrated by its manifold applications in the life sciences and material sciences.[1,2] The requirement of a metal catalyst, however, has limited the utilization of this cycloaddition reaction in areas such as photophysical chemistry and chemical biology. Consequently, several metal-free processes have been developed to serve as orthogonal ligation methods.[3] At present the foremost method is the strain-promoted azide–alkyne cycloaddition (SPAAC) as is reflected by the fast growing number of cyclooctyne derivatives developed by the groups of Bertozzi,[4] Boons,[5] Rutjes,[6] and van Delft[7] (Table 1). In addition to SPAAC, SPANC,[8] and SPANOC,[9] several other metal-free click methods relying on cycloaddition reactions with alkenes have received considerable attention.[10–13]

Disadvantages of these strategies are: 1) the formation of equimolar quantities of the 1,4 and 1,5 regioisomers; and 2) the requirement of functional handles on both substrates, for example, an activated double or triple bond and the appropriate complementary counterpart. To reduce the synthetic efforts, one of the functionalities should be readily available, that is, inherent to a large variety of starting materials, so that it can be used without further manipulation in a click-type reaction.

In 1986 Sakai et al. described such a reaction for the elegant formation of triazoles and thiadiazoles.[14] The Sakai approach relies on the condensation of a primary amine and an α,α-dichlorotosylhydrazone (**1**) to form regioselectively a 1,4-substituted triazole (**3**) under ambient reaction conditions (Scheme 1). As a result of the highly chemoselective character of this reaction, protecting group strategies, in principal, seem to be unnecessary. Surprisingly, this elegant and mild protocol for the formation of stable 1,2,3-triazoles has found only infrequent applications.[15]

Table 1: Cyclooctyne-based probes for metal-free triazole formation.

Group	Name	Structure
Bertozzi et al.	MOFO, X = H DIFO, X = F	
Bertozzi et al.	DIMAC	
van Delft et al.	BCN	
Boons et al.	DIBO	
Rutjes et al.	DIBAC	



Scheme 1. Proposed mechanism for the Sakai reaction incorporating the Bamford–Stevens intermediate. Ts = tosyl.

Despite the evident potential of this reaction, it has not been fully exploited; that is, neither the mechanism nor the scope and limitations have been explored. Here we report our findings concerning the Sakai triazole formation reaction and demonstrate the suitability of this methodology as a strategy for metal-free triazole conjugation and an alternative to the traditional azide–alkyne cycloaddition.

For the identification of the scope and limitations of the Sakai reaction, two α,α-dichlorotosylhydrazones (**1a**[16] and **1b**)[15a] were prepared and reacted with a variety of primary amines (**2a–j**). The reactions were performed either in a solvent mixture of acetonitrile and ethanol (1:1 v/v%) or in methanol, in the presence of six equivalents of *N,N*-diisopropylethylamine (DiPEA). These simple reaction con-

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[*] Dr. S. S. van Berkel, Dipl.-Chem. S. Brauch, L. Gabriel, Dipl.-Chem. M. Henze, Dipl.-Chem. S. Stark, Dipl.-Chem. D. Vasilev, Prof. Dr. L. A. Wessjohann, Dr. M. Abbas, Prof. Dr. B. Westermann
Department of Bioorganic Chemistry
Leibniz-Institute of Plant Biochemistry
Weinberg 3, 06120 Halle (Germany)
E-mail: bwesterm@ipb-halle.de
Homepage: http://www.ipb-halle.de

Prof. Dr. L. A. Wessjohann, Prof. Dr. B. Westermann
Institute of Chemistry
Martin-Luther-University, Halle-Wittenberg
Kurt-Mothes-Strasse 2, 06120 Halle (Germany)

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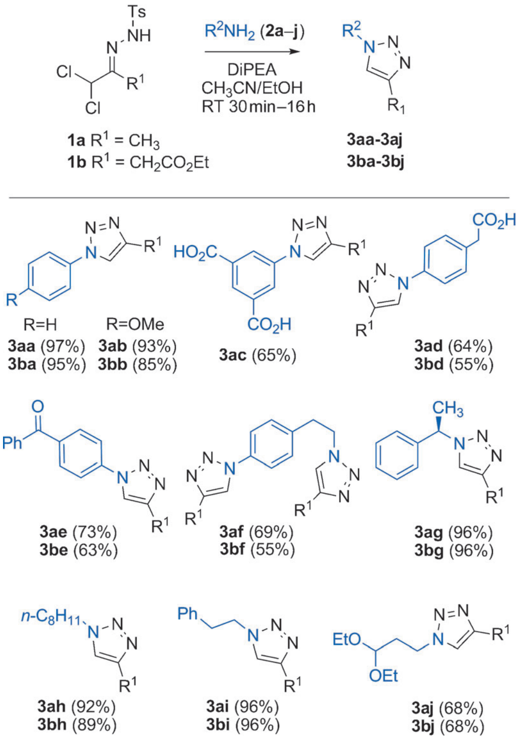
ditions gave triazoles **3aa**–**3aj** and **3ba**–**3bj** (see Scheme 2) in good to excellent yields when the reaction mixtures were stirred for up to 16 h at ambient temperatures.[17]



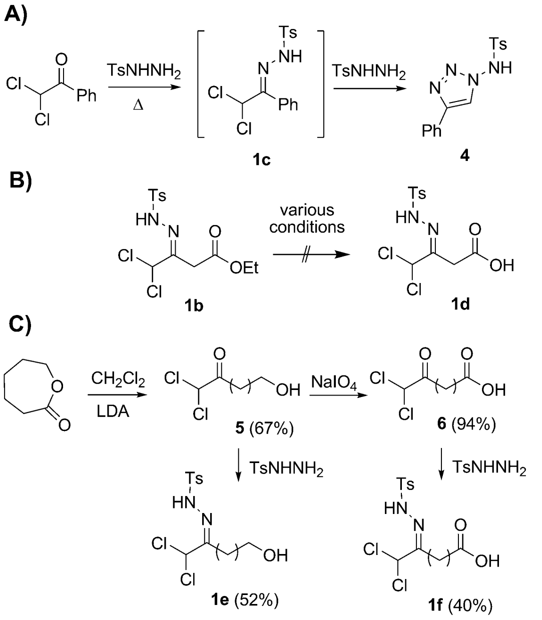
Scheme 2. Primary-amine-derived triazole products (**3**). Segments from the starting amine **2** are depicted in blue.

Focusing on the formation of C_{aryl}–N-containing structures, a frequently reoccurring structural motif in pharmaceutical compounds, often prepared by metal-catalyzed cross-coupling reactions,[18] we tested the suitability of various anilines (**2a**–**e**) in the Sakai reaction. Both electron-rich and electron-poor anilines served as starting material for the production of various phenyl triazoles (**3aa**–**3ae** and **3ba**–**3be**, starting from **1a** and **1b**, respectively). The yields of the isolated products were in most cases good to excellent.[19] Dicarboxylic acid **2c** could be used without protecting groups and triazole **3ac** was obtained smoothly. A fragment frequently used in affinity/activity-based protein profiling is 4-aminobenzophenone (**2e**). Facile conjugation of either tosylhydrazone (**1a** or **1b**) to the amino group was readily achieved, leading to triazoles **3ae** and **3be**, respectively.

Shifting from anilines to benzyl and aliphatic amines (**2f**–**j**), also gave the corresponding triazoles in high yields. To investigate the amine chemoselectivity, tosylhydrazones **1a,b** were reacted with 4-aminophenylethylamine (**2f**). In both cases immediate bis(triazole) formation was observed leading to products **3af** (69%) and **3bf** (55%) in good yields. These results indicate poor chemoselectivity regarding aromatic amines over aliphatic amines; however, more examples are required. No restrictions were observed when aliphatic amines **2h**–**j** were used, including acetal-functionalized amine **2j**, leading to the corresponding triazoles **3ah**–**3aj** and **3bh**–**3bj** in good to excellent yields (up to 96%).

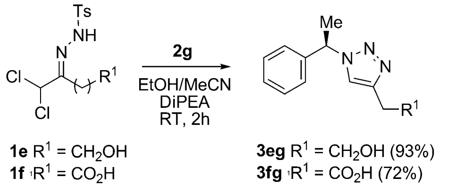
The incorporation of triazoles in peptides and proteins applied in peptidomimetics[20] is often not possible owing to the difficult stereoselective synthesis of azides adjacent to the chiral center of an amino acid. Enantiomerically pure α-azides can be generated from their corresponding alcohols by a modified Mitsunobu reaction[21] or by the kinetic resolution protocol described by Finn and Fokin.[22] The Sakai reaction, however, would allow the use of readily available chiral α-amines. As an example, enantiomerically pure (*S*)-1-phenylethylamine (**2g**) was reacted with tosylhydrazones **1a**,**b** resulting in the formation of triazoles **3ag** and **3bg** in quantitative yield after only 30 min or **1a** and 1 hour for **1b**. HPLC analysis on a chiral stationary phase indicated that no racemization had occurred during the reaction.

In continuation of our efforts to determine the scope and limitations of this procedure, we focused on the use and modification of different α,α-dichloroketones (Scheme 3). As was also demonstrated by Sakai and co-workers,[14] isolation of tosylhydrazone **1c** obtained from α,α-dichloroacetophenone and tosylhydrazine could not be accomplished. Instead, the N-substituted triazole **4** was isolated after crystallization (Scheme 3A). This product is presumably generated after osazone formation of intermediate **1c** with a second equivalent of tosylhydrazine.[23] Saponification of the ester moiety of tosylhydrazone **1b** should result in tosylhydrazone **1d** (Scheme 3B), which could then be utilized in further functional group transformations. Unfortunately, under the employed reaction conditions (mild basic or acidic) the desired product **1d** was not obtained. Most likely, the mild basic conditions favor the formation of the diazo intermediate which undergoes decomposition in a subsequent step following the Bamford–Stevens mechanism.[24]



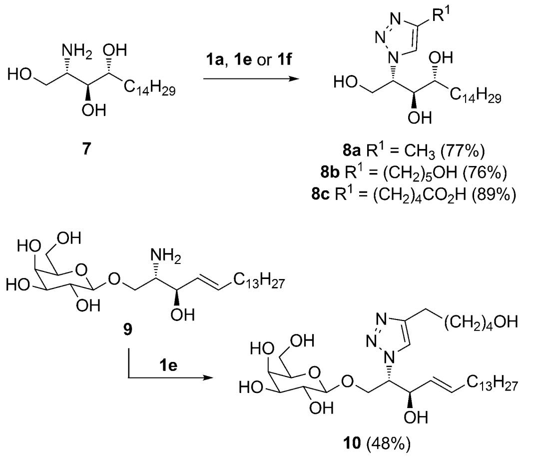
Scheme 3. A) 1-Aminotriazole formation upon treatment of α,α-dichloroacetophenone with tosylhydrazine; B) attempted synthesis of tosylhydrazone **1d**; C) formation of functional tosylhydrazones **1e** and **1f**.

Conversely, mild acidic conditions appeared to give no hydrolysis at all. With the purpose of obtaining functional tosylhydrazones, a concise synthetic strategy was employed (Scheme 3 C). In the first step a cooled solution of ε-lactone in CH_2Cl_2 was treated with lithium diisopropylamide (LDA) resulting in the formation of the desired α,α-dichloroketone **5** (67 %).[25] This compound could then either be transformed into the corresponding tosylhydrazone **1e** (52 %), or treated with periodate and converted into the carboxylic acid derivative **6** (94 %). Finally, α,α-dichloroketone **6** was reacted with tosylhydrazine to generate tosylhydrazone **1f** in 40 % yield (nonoptimized conditions). The obtained tosylhydrazones (**1e** and **1f**) were subsequently subjected to the Sakai reaction following the established protocol (see Scheme 4). In accordance with the observations for **2g**, products **3eg** and **3fg** were obtained in good yields and with retention of configuration.



Scheme 4. Sakai reaction with functional tosylhydrazones **1e** and **1f**.

In work on biologically significant targets we focused on the modification of phytostigmine (**7**) and psychosine (**9**; Scheme 5). The N-acylated phytoceramides, like the analogous *N*-acylated ceramides, show a pronounced cytotoxic effect on different cell types.[26] Moreover, corresponding triazole-modified, metabolically stable ceramides and phytoceramides have previously been synthesized and evaluated successfully,[27] demonstrating the amide-bond mimicry of 1,2,3-triazoles. Elaborating on this topic we employed the Sakai reaction of tosylhydrazone **1a** and phytosphingosine (**7**) to prepare 4-Me-triazolyl-phytoceramide **8a** (77 %) in a single step. In addition to tosylhydrazone **1a**, α,α-dichlorotosylhydrozones **1e** and **1f** were also utilized in the coupling reaction with **7**. This procedure generated the corresponding triazole analogues **8b** and **8c** in high yields (76 % and 89 %, respectively) in a single step, starting from the unprotected phytosphingosine, thus laborious protection and deprotection steps could be sidestepped.



Scheme 5. Application of the Sakai reaction in the synthesis of triazole-modified phytoceramides **8a**–**8c** and modified psychosine **10**.

Psychosine is a glycosphingolipid occurring in the pathology of globoid cell leukodystrophy (GLD), a genetic metabolic disorder that results from the absence of the enzyme galactosyl ceramide.[28] Recently, photoaffinity probes were employed as useful tools for identifying these yet unknown receptors.[29] The derivatization presented in Scheme 5 enables the facile, straightforward synthesis of suitable probes. The triazole-modified psychosine **10** could be synthesized from psychosine (**9**) and tosylhydrazone **1e** in a single step in a moderate 48 % yield. Again, thanks to the superior chemoselectivity of this protocol, previously unknown modifications can be incorporated and a highly functionalized molecule can be prepared without laborious protecting group manipulations.

The examples herein clearly show the versatility of the Sakai reaction as a strategy for metal-free triazole formation. In contrast to the strain-promoted azide–alkyne cycloaddition reaction, this methodology applies readily accessible starting materials such as amines and α,α-dichlorotosylhydrazones. The latter can be prepared from cheap and commercially available starting materials by a two-step procedure. Moreover, highly defined products are obtained as a result of the regioselective formation of exclusively 1,4-substitued triazoles. Currently investigations are underway in our laboratories regarding the use of the Sakai reaction in bioconjugation strategies for the modification of complex biological targets and surfaces.

Experimental Section

General procedure: A cooled solution (0 °C) of amine **2** (0.25 mmol) in ethanol (3 mL) was treated with *N*,*N*-diisopropylethylamine (0.26 mL, 1.50 mmol, 6 equiv). The solution was stirred for 10 min before a solution of hydrazone **1** (0.33 mmol, 1.3 equiv) dissolved in acetonitrile (2 mL) was added dropwise. Stirring was continued at room temperature until the reaction was complete (checked by thin-layer chromatography). Subsequently, all volatiles were removed under reduced pressure and the residue was purified by column chromatography.

Keywords: bioconjugation · click chemistry · nitrogen heterocycles · Sakai reaction

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