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Copper-Free Click Reaction Sequence: A Chemoselective Layer-by-Layer Approach

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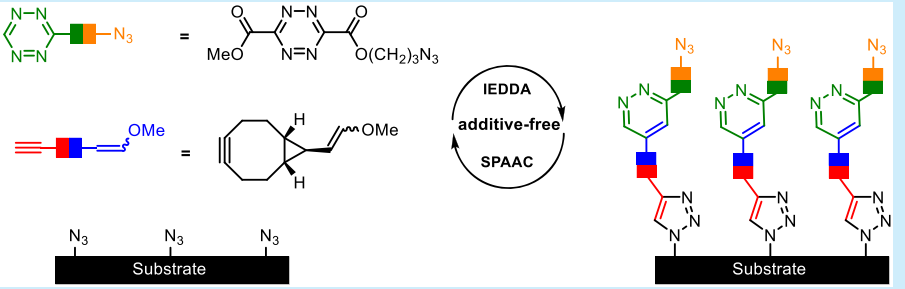
Supporting Information



ABSTRACT: An additive-free chemoselective ligation of dual clickable building blocks is demonstrated. The challenge of balancing reactivity and stability was achieved by employing a small, electron-deficient tetrazine bearing an azido group and an enol ether functionalized cyclooctyne. The chemoselective sequence of strain-promoted azide−alkyne cycloaddition (SPAAC) and inverse-electron-demand Diels−Alder (IEDDA) reaction is demonstrated with a cholic acid derived triazide as a molecular surface model for layer-by-layer synthesis.

"Click" approaches combine rapid conversion and mild reaction conditions with high selectivity and broad functional group tolerance.[1] Due to their modularity, they find wide application in bio-orthogonal chemistry[2] and material science.[3] Bifunctional building blocks expand the scope of click reactions toward more complex systems. Clicking two different monofunctional units selectively to a bifunctional unit requires orthogonal reactivity. Dual clickable organic molecules were employed in material science (synthesis of polymers, dendrimers, nanoparticles, hydrogels, surfactants)[4] and life science (drug development, bio-orthogonal labeling).[5] Orthogonal clicking of two different bifunctional units is a formidable challenge. Toward this end, copper-catalyzed azide−alkyne cycloaddition (CuAAC) was combined with different other click reactions (Figure 1a). Hawker combined the CuAAC with thiol−ene coupling (TEC),[6] Koert with strain-promoted azide−alkyne cycloaddition (SPAAC),[7] Lee with a thiol−Michael reaction,[8] Niu with sulfur(VI) fluoride exchange (SuFEx),[9] and Kakkar with a Diels−Alder reaction (DA).[10] The necessity of copper catalysis in this earlier work displays a major limitation for applications in living systems or surface functionalization via UHV vapor deposition. Similar drawbacks exist for using SuFEx due to additional base and heating to 80 °C. TEC requires a photoinitiator, the DA reaction with furan heating to 50 °C for 3 d, and the thiol−Michael reaction needs reducing agent as additive to inhibit cystin formation. In contrast, SPAAC, as developed by Bertozzi, proceeds metal- and additive-free and can be accomplished at room temperature with high efficiency.[11,1b] Another additive-free click reaction is the inverse-electron-demand Diels−Alder (IEDDA)

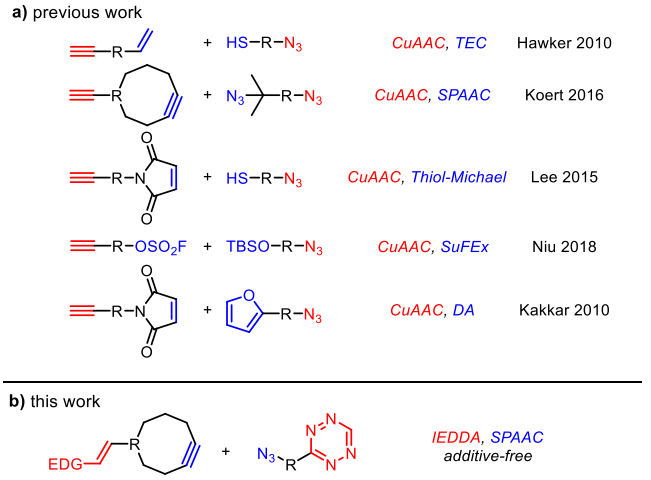


Figure 1. Orthogonal click reaction sequences of bifunctional building blocks in previous work (a) and this work (b).

of a tetrazine with an alkene.[12] In this work, we present an orthogonal solution for a sequential combination of SPAAC and IEDDA using a carefully balanced reactivity pattern of two bifunctional units. While the intramolecular combination of a tetrazine and a cyclooctyne was unfeasible,[13] we intended to investigate the promising additive-free, orthogonal click

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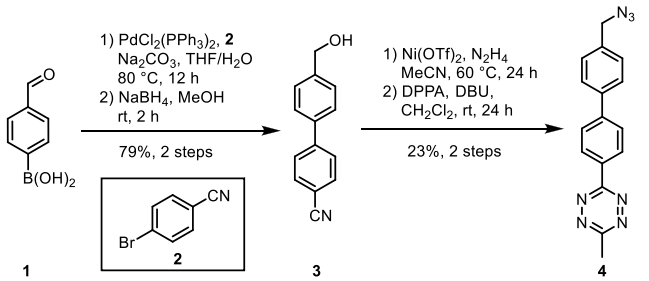
reaction sequence alkene/cyclooctyne and azide/tetrazine (Figure 1b).

The azido tetrazine **4** was chosen as starting point for the bifunctional SPAAC/IEDDA units (Scheme 1). Its synthesis

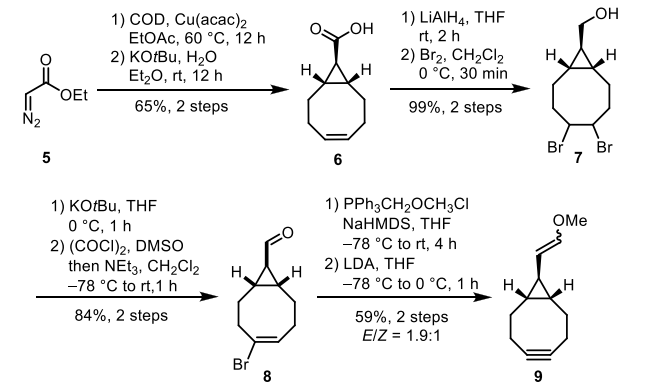
Scheme 1. Synthesis of Tetrazine 4



commenced with boronic acid **1**, which was converted via Suzuki coupling with 4-bromobenzonitrile (**2**) and subsequent reduction to the biaryl **3** (Scheme 1).[14,15] After Lewis acid mediated tetrazine formation adapted from Devaraj's procedure,[16] the azide was installed with DPPA (diphenylphosphoryl azide) to deliver the azido tetrazine **4**.

Having an azide-functionalized tetrazine in hand, the synthesis of the corresponding cyclooctyne enol ether **9** was addressed next (Scheme 2). Starting with ethyl diazoacetate

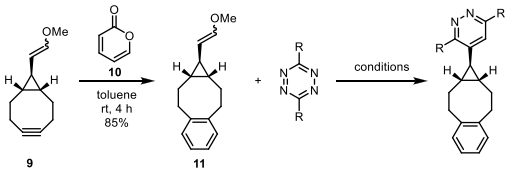
Scheme 2. Synthesis of Cyclooctyne 9

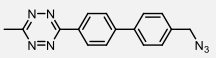
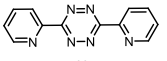
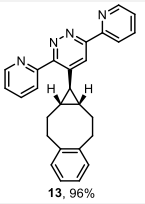
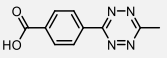
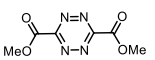
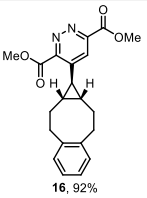


(**5**), a copper-mediated cyclopropanation with COD and subsequent epimerization, accompanied by saponification, furnished *exo* acid **6**.[17,18a] Reduction with $LiAlH_4$ followed by bromination gave dibromo cyclooctane **7**.[18] Stepwise HBr elimination with KO*t*Bu to the corresponding vinyl bromide and Swern oxidation furnished aldehyde **8**. After Wittig reaction to form the enol ether (E/Z = 1.9/1), a second HBr elimination proceeded smoothly with LDA at low temperatures to obtain cyclooctyne enol ether **9** as second dual clickable unit.

In order to examine the IEDDA reactivity of tetrazine **4** and an enol ether, a monofunctional enol ether **11** was prepared first by Diels–Alder reaction of the cyclooctyne enol ether **9** and α-pyrone **10** (Table 1). No IEDDA reaction between **11** and **4** took place at rt. At higher temperatures (>85 °C), only decomposition of enol ether **11** to the respective aldehyde was observed (Table 1, entry 1). Hence, stability of the enol ether was balanced for convenient accessibility and treatment at room temperature, but the reactivity of azide-incorporated tetrazine had to be enhanced. In the past, divergent olefin

Table 1. Reactivity Experiments with Enol Ether 11



tetrazine	conditions[a]	observation
 **4**	CH_2Cl_2, rt then dioxane 30, 55, 70, 85 °C	no reaction until decomp. of **11** at 85 °C
 **12**	dioxane 70 °C, 20 h	 **13**, 96%
 **14**	CH_2Cl_2, rt then dioxane 30, 55, 70, 85 °C	no reaction until decomp. of **11** at 85 °C
 **15**	CH_2Cl_2 rt, 40 sec then aromat.[b]	 **16**, 92%

[a] 3 equiv of enol ether **11**, 1 equiv of tetrazine (0.35 M). [b] Full conversion of starting material; aromatization either in $CHCl_3/CDCl_3$ for 6 h or dioxane, 101 °C, 5 min.

classes were investigated for their reactivity toward tetrazines.[19] Comparisons of tetrazine reactivities were mainly drawn within one particular class (symmetric phenyl substituted,[20a] ether/ester/amide substituted,[20b] and unsymmetric aryl and methyl/proton substituted-*d*[20c]). An estimation attempt for a general trend between different tetrazine classes was made by Slugovc and Knall.[21]

A series of other known more electron-deficient tetrazines **12**,[20a] **14**,[5b] and **15**[12b] were prepared, and their reactivity toward enol ether **11** was examined. The dipyridyl tetrazine **12** furnished pyridazine **13** in excellent yield after heating to 70 °C for 20 h (Table 1, entry 2), whereas acid-functionalized tetrazine **14** gave similar results as initial tetrazine **4** in means of decomposition of the enol ether. The tetrazine diester **15** indicated full conversion after 40 s at room temperature. This was monitored by TLC and vanishing of the strong red color of the respective tetrazine was observed. In order to obtain consistent and clear analytics, the unaromatized dihydropyridazine byproduct was oxidized to the pyridazine **16** by either stirring in $CHCl_3/CDCl_3$ in an open flask or short heating to 101 °C in dioxane.

Due to the promising reactivity of the diester–tetrazine motif, we addressed the introduction of an azido group into this structure. Initial attempts toward saponification, transesterification, reduction, and amidation of tetrazine **15** altogether failed. Reactions on the dihydrotetrazine stage were more promising (Scheme 3). The dihydrotetrazine **17** was prepared from ethyl diazoacetate (**5**).[22] Breaking the symmetry of dihydrotetrazine **17** succeeded via base-promoted

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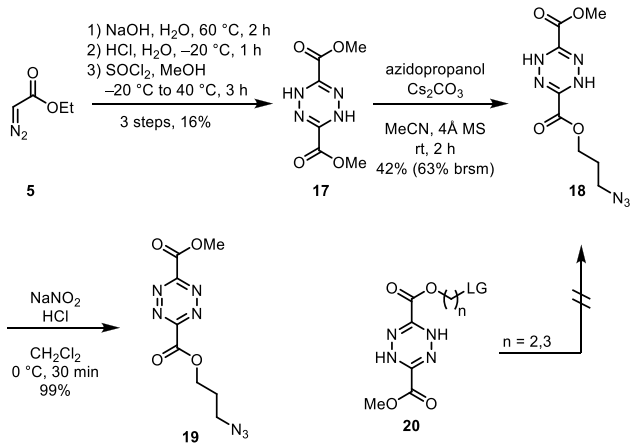
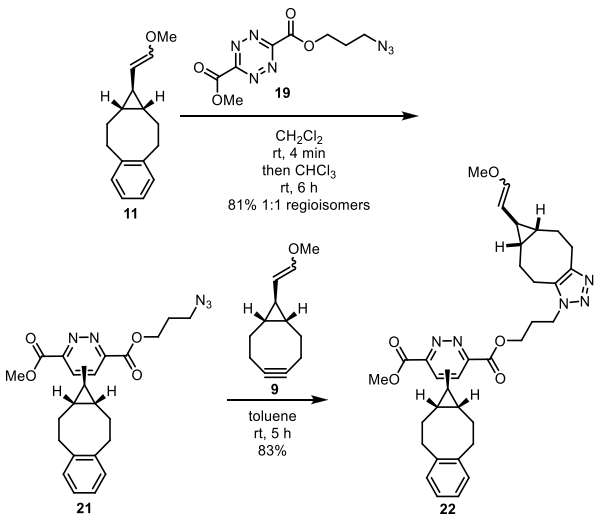
Scheme 3. Synthesis of Azido Tetrazine 19



transesterification using azidopropanol[23] to furnish **18** in good yield. The addition of molecular sieves turned out to be essential in terms of scavenging developing methanol. This way, we circumvented a late-stage nucleophilic substitution with an azide (**20** → **18**), which was found to be difficult due to nucleophilic NH groups of dihydrotetrazine derivatives. Final oxidation of **18** to tetrazine **19** showed the best performance (99% yield) by bubbling nitrous gases through a solution of the substrate in CH_2Cl_2, which was superior to aromatization with MnO_2 (78% yield).

With both dual clickable units **19** and **9** in hand, a sequential combination of IEDDA and SPAAC was examined in order to confirm the orthogonality. The IEDDA between azide bearing tetrazine **19** and enol ether **11** showed complete turnover of the starting materials after 4 min (Scheme 4). This was

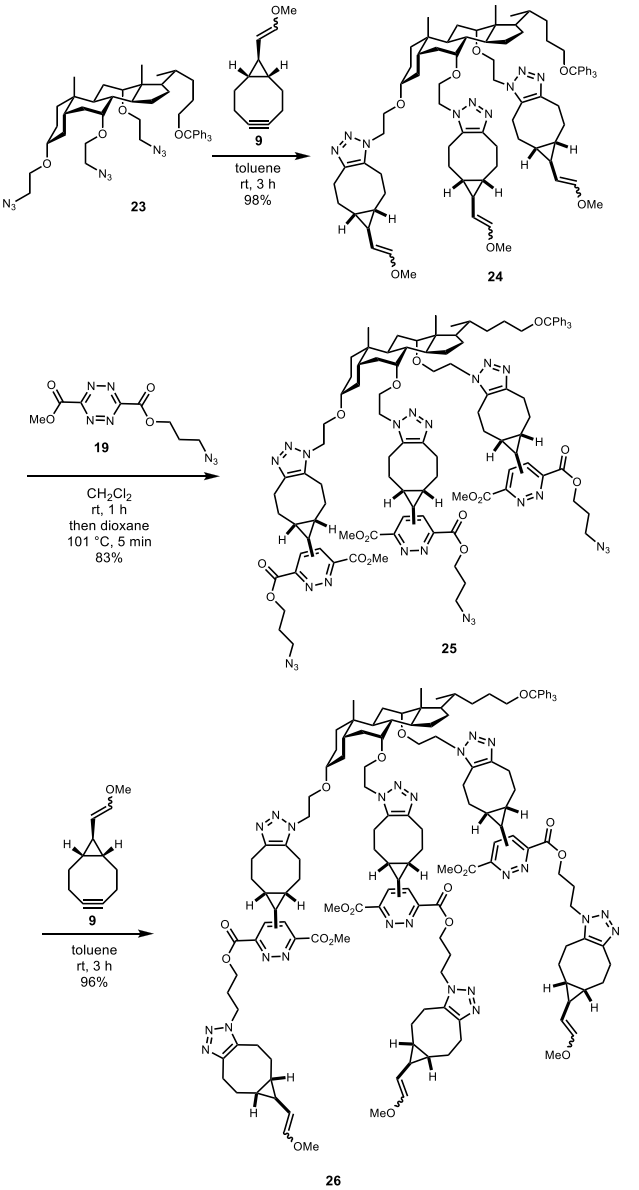
Scheme 4. Orthogonal Click Sequence Using Tetrazine 19 and Cyclooctyne 9



observable by TLC and vanishing of the strong red color of tetrazine **19**. The unaromatized dihydropyridazine byproduct was subsequently oxidized via elimination to obtain the pyridazine **21** as 1:1 regioisomeric mixture. SPAAC of the azido group in **21** with cyclooctyne enol ether **9** led cleanly to the triazole **22**. The click sequence (**19** → **21** → **22**) proves the postulated additive free combination of SPAAC and IEDDA.

With the aim of developing chemoselective surface functionalization, the orthogonal sequence of IEDDA and SPAAC was next examined on a molecular surface model system. Comparable to a former layer-by-layer approach,[7] which entailed a combination of CuAAC and SPAAC and thus the necessity of a copper additive, we employed literature known[24] cholic acid derived triazide **23**. Due to its rigid framework as well as its functionality, it is a suitable target for the following concurrent layer-by-layer approach (Scheme 5).

Scheme 5. Layer-by-Layer Assembly on Molecular Surface



The initial 3-fold SPAAC to tris-triazole **24** proceeded smoothly with nearly quantitative yield. Subsequent IEDDA with azide bearing tetrazine **19** installed three pyridazine units to generate the second layer **25**. The layer-by-layer approach was completed by showing final SPAAC to hexatriazole **26**. Each layer producing step was performed additive-free, at room temperature, and in excellent yields.

In summary, we developed an additive-free click reaction sequence of bifunctional cyclooctyne/alkene and azide/tetrazine building blocks. On the way to a balanced reactivity

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pattern, IEDDA experiments between known tetrazines and a monofunctional dienophile concluded the diester tetrazine as most promising diene. Subsequent repetition of an IEDDA between this motif with an azide present resulted in the desired pyridazine formation. Further conversion with dual clickable cyclooctyne via SPAAC completed the alternating sequence. In contrast to our previous example,[7] the reaction sequence is performed additive-free and at room temperature. Related to the demonstrated layer-by-layer approach on a cholic acid derived triazide in solution, application for solid surface-/semiconductor functionalization via UHV vapor deposition should be adaptable.[25]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.9b02891.

Experimental details and spectroscopic and analytical data of all new compounds (PDF)

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Author Contributions

J.M. accomplished the experimental work.

Notes

The authors declare no competing financial interest.

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