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1,3-Butadiynyl sulfide-based compact trialkyne platform molecule for sequential assembly of three azides†

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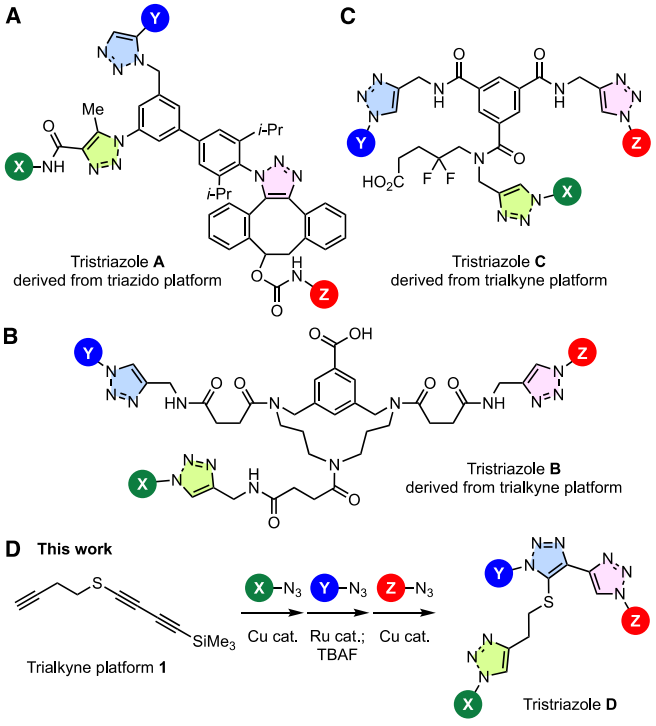
Jumpei Taguchi,[a] Kento Tokunaga,[a] Hitomi Tabuchi,[a] Takashi Nishiyama,[b] Isao Kii[b] and Takamitsu Hosoya*[a]

A compact trialkyne platform with a silyl-protected 1,3-butadiynyl sulfide moiety and a terminal alkyne group has been developed for sequential regioselective transition metal-catalyzed triazole formation reactions with three azides. This method enabled the facile construction of a low-molecular-weight triazole library and the synthesis of middle-molecular-weight trifunctional probes for protein modification.

The sequential assembly of components into a platform molecule with multiple connectable groups is an efficient approach for the synthesis of a wide range of compounds. This strategy enables the concise construction of chemical libraries and the synthesis of multifunctional compounds. Particularly, methods employing sequential click reactions[1] such as azide–alkyne cycloadditions offer a reliable and efficient approach to multicomponent assembly.[2–5] In this context, our research group has developed a series of platform molecules with multiple clickable groups.[3] For instance, we previously reported various platform molecules with three or four distinguishable azido groups.[3a,3d] These platform molecules enabled facile synthesis of multitriazoles via sequential reactions with terminal alkynes, strained cycloalkynes, and β-keto carbonyl compounds and successfully applied to the development of multifunctional probes for protein modification (Scheme 1A). Other groups reported different triclickable platform molecules with three distinguishable alkyne moieties (Scheme 1B and C).[4] These trialkyne platform molecules facilitate the triple click assembly compared with our multiazido and other hetero-triclickable platform molecules[5] because only azides are needed as reaction partners. However, these platform molecules require multistep synthesis procedures and are not suitable for the construction of libraries consisting of low-molecular-weight compounds owing to the large size of their core structures. Herein, we report a novel trialkyne platform molecule **1** with a minimum required structure, which was readily synthesizable enabling the sequential assembly of three azides (Scheme 1D). The electron-rich, strongly coordinating thioalkyne moiety of **1** was expected to react selectively by transition metal-catalyzed azide–thioalkyne cycloaddition before or after the copper-catalyzed azide–alkyne cycloaddition (CuAAC) at the terminal alkyne moiety.[6–8]



Scheme 1 Distinguishable homo-triclickable platform molecules for the synthesis of tristriazoles via sequential click reactions.

[a]*Chemical Bioscience Team, LBB, IIR, Institute of Science Tokyo, 2-3-10 Kanda-Surugadai, Chiyoda-ku, Tokyo 101-0062 (Japan)*
E-mail: thosoya.cb@tmd.ac.jp
[b]*Laboratory for Drug Target Research, Department of Agriculture, Graduate School of Science and Technology, Shinshu University, 8304 Minami-Minowa, Kami-Ina, Nagano 399-4598 (Japan)*
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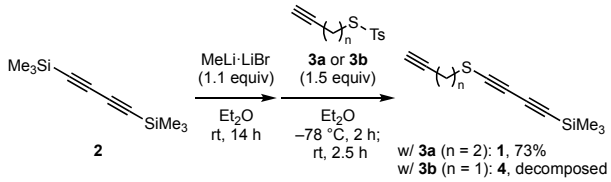
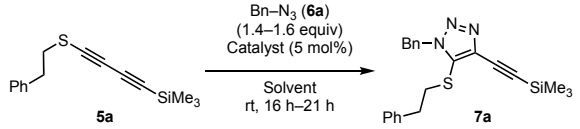
1,3-Butadiynyl homopropargyl sulfide **1** was synthesized via a one-pot procedure from 1,4-bis(trimethylsilyl)-1,3-butadiyne (**2**); monolithiation of **2** with MeLi·LiBr,[9] followed by treatment with thiosulfonate[10] **3a** afforded triyne **1** as a bench-stable oil (Scheme 2). This method was effective for preparing other simple alkyl 1,3-butadiynyl sulfides (Scheme S1) except propargyl analog **4**.[11]



Scheme 2 Synthesis of 1,3-butadiynyl sulfides.

Table 1 Optimization of reaction conditions for triazole formation at the thioalkyne moiety of 1,3-butadiynyl sulfide **5a**.



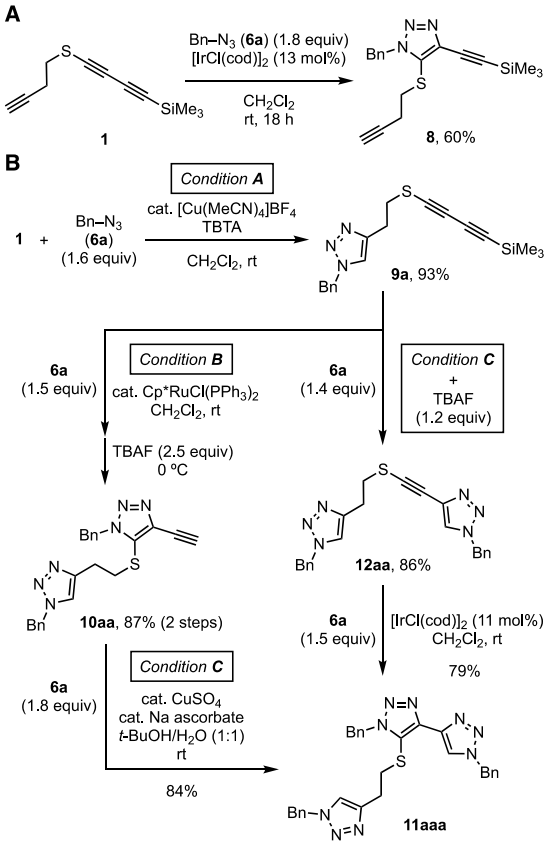
Entry	Catalyst	Solvent	Yield (%)
1	Cp*RuCl(cod)	CH_2Cl_2	69[a]
2	$[IrCl(cod)]_2$	CH_2Cl_2	37
3	$[RhCl(CO)_2]_2$	CH_2Cl_2	30
4	$[Ru(p\text{-cym})Cl_2]_2$	CH_2Cl_2	0
5	$Cp^*RuCl(PPh_3)_2$	CH_2Cl_2	87
6	$Cp^*RuCl(PPh_3)_2$	1,4-dioxane	83
7	$Cp^*RuCl(PPh_3)_2$	toluene	80
8	$Cp^*RuCl(PPh_3)_2$	H_2O	74[a]

[a]A trace amounts of the desilylprotonated derivative of **7a** was detected.

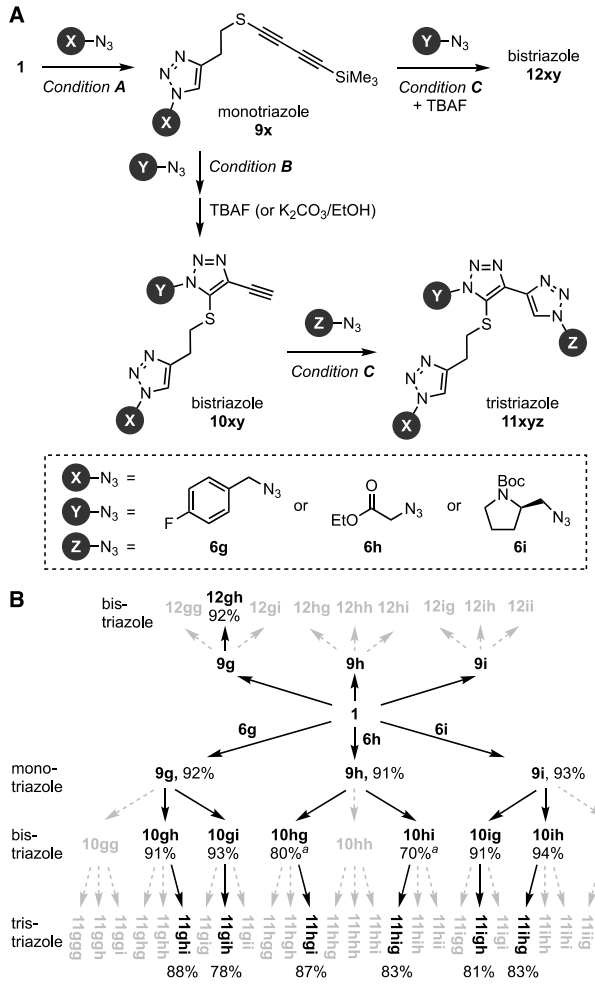
Although reactions of 1,3-butadiyne[12] or thioalkyne[6–8] with azides affording the corresponding triazoles were previously reported, the reaction between 1,3-butadiynyl sulfide and azides remained unexplored. Therefore, before using trialkyne **1**, we investigated the reaction of 1,3-butadiynyl sulfide using **5a** as a model substrate (Table 1). When the reaction of **5a** with benzyl azide (**6a**) was conducted under the reported ruthenium-catalyzed azide–thioalkyne cycloaddition (RuAtAC) conditions,[6b] triazole **7a** was obtained as a sole product in 69% yield, indicating that the reaction occurred chemo- and regioselectively at the thioalkyne moiety (Entry 1). The reaction with an iridium[7] or rhodium[8] complex resulted in lower yield (Entries 2 and 3). Using the ruthenium complex with triphenylphosphine ligands instead of a COD ligand provided the best result, affording **7a** in 87% yield (Entry 5). This reaction could be conducted in various solvents, such as 1,4-dioxane, toluene, and water (Entries 6–8). The optimized conditions was applicable to the reactions with various azides (Scheme S2).

Conducting the reaction of trialkyne platform **1** with azide **6a** under the optimized RuAtAC conditions afforded the desired triazole **8** in 25% (^{1}H NMR yield). However, the formation of another triazole and bistriazole reacted at the terminal alkyne was also observed, which is consistent with the known reactivity of terminal alkynes with azides using a ruthenium catalyst.[6a] Although the reaction using $[IrCl(cod)]_2$ selectively afforded **8**, this approach required to use increased amounts of the catalyst to obtain **8** in a reasonable yield (Scheme 3A). Therefore, no further investigation was performed on this approach.

Alternatively, since internal thioalkynes were reported inert under CuAAC conditions,[6b,8b,8c] we decided to conduct the first triazole formation at the terminal alkyne moiety of trialkyne **1**. The reaction of **1** with azide **6a** using a cationic copper catalyst afforded triazole **9a**, indicating the selective reaction at the terminal alkyne (Scheme 3B). The second triazole formation of diyne **9a** with **6a** using the ruthenium catalyst proceeded chemo- and regioselectively at the thioalkyne moiety, and subsequent deprotection of the silyl group with TBAF afforded bistriazole **10aa**. The remaining terminal alkyne was employed for the third triazole formation with **6a** via CuAAC to afford tristriazole **11aaa**. The second triazole formation of diyne **9a** was also feasible at the silyl-protected alkyne moiety. CuAAC with **6a** in the presence of TBAF resulted in desilylprotonation and subsequent click reaction in one-pot[13] to yield bistriazole **12aa**. For the third triazole formation at the thioalkyne moiety of **12aa**, an iridium catalyst afforded a better result (79% yield of tristriazole **11aaa** using 11 mol% of $[IrCl(cod)]_2$) than a ruthenium catalyst (34% yield using 10 mol% of $Cp^*RuCl(PPh_3)_2$). These results demonstrate that trialkyne **1** serves as an efficient triclickable platform for the facile synthesis of tristriazoles.

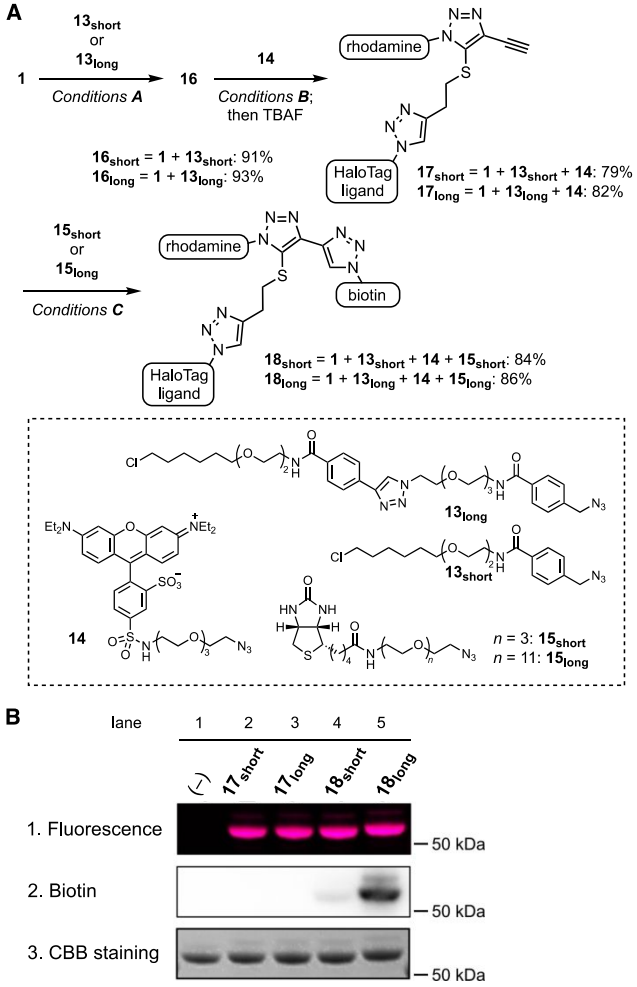


Scheme 3 Sequential assembly of three azides onto trialkyne **1**. (A) First triazole formation via RuAtAC. (B) CuAAC with **1** followed by the second and third triazole formations in two ways.



Scheme 4 Construction of a triazole library using trialkyne **1** and three azides **6g–6i**. [a]Deprotection of the trimethylsilyl group was performed using K_2CO_3 in EtOH to avoid ester hydrolysis. The total yields over two steps are shown.

In principle, various types of triazoles can be synthesized from trialkyne platform **1** simply by changing the azide in each step, enabling the facile construction of a low-molecular-weight compound library (Scheme 4A).[4c] Theoretically, CuAAC/RuAtAC/deprotection/CuAAC and CuAAC/deprotection–CuAAC sequences can provide a chemical library consisting of 48 triazole derivatives that contain three types of monotriazoles **9**, 18 types of bistriazoles **10** and **12**, and 27 types of tristriazoles **11** only from four molecules, i.e., trialkyne **1** and three types of azides (Scheme 4B). To verify this concept, we synthesized 16 of these compounds using three azides **6g–6i** bearing fluoro, ester, and Boc-protected amino groups, respectively, which are substructures commonly found in pharmaceuticals. The first triazole formation via CuAAC of **1** with azides **6g–6i** afforded monotriazoles **9g–9i**, respectively, in 91%–93% yields. Subsequently, the second triazole formation via RuAtAC with **6g**, **6h**, or **6i**, respectively, followed by desilylprotonation gave six bistriazoles **10** in 70%–94% yields. The second triazole formation through the deprotection–CuAAC sequences afforded isomeric bistriazole **12gh** in 92% yield. Finally, the third cycloaddition via CuAAC with azides **6g–6i** resulted in six tristriazoles **11** in 78%–88% yields. The constructed compound library contains 16 triazoles with molecular weights between 335 and 641, which are preferable for drug candidates.[14] Our method outperformed previously reported strategies in terms of the ease of the synthesis of trialkyne platform **1** and the compactness of the core structure of the resulting tristriazoles (Scheme S3). Other advantage of our method is that the size of the compound library can be easily expanded by adopting several simple approaches, including 1) the use of a broader variety of azide compounds, 2) performing RuAAC at the alkyne moiety of bistriazoles to afford regioisomeric 1,5-triazoles, 3) oxidation of sulfide to sulfoxide or sulfone and subsequent transformation such as S_NAr reactions or cross-coupling reactions, and 4) reduction or other transformations at the alkyne moiety of mono- or bistriazoles.



Scheme 5 Protein modification using trifunctional probes prepared from **1**. (A) Synthesis of middle-molecular-weight multifunctional molecules from **1** and functional azide modules. (B) SDS-PAGE analysis of GST-fused HaloTag protein eluted from the resin after modification with multifunctional probes (lanes 2–5).

We further demonstrated the utility of trialkyne platform **1** by preparing a trifunctional probe using azide modules including two HaloTag ligands $\mathbf{13}_{short}$ and $\mathbf{13}_{long}$ and two biotin derivatives $\mathbf{15}_{short}$ and $\mathbf{15}_{long}$ with different linker lengths, as well as tetraethylsulforhodamine derivative **14** (Scheme 5A). The CuAAC of trialkyne **1** with $\mathbf{13}_{short}$ or $\mathbf{13}_{long}$, followed by RuAtAC with **14** and subsequent silyl deprotection, afforded fluorescent HaloTag ligands $\mathbf{17}_{short}$ and $\mathbf{17}_{long}$. The CuAAC of $\mathbf{17}_{short}$ and $\mathbf{17}_{long}$

with biotin–azides **15**$_{short}$ and **15**$_{long}$, respectively, proceeded smoothly to afford trifunctional molecules **18**$_{short}$ and **18**$_{long}$ with molecular weights of 1721 and 2419, respectively.

The synthesized multifunctional molecules were applied for protein modification (Scheme 5B). After adding each probe candidate **17** and **18** to a cell lysate containing a GST-fused HaloTag protein (59 kDa), an SDS-PAGE analysis was performed. The gels were analyzed by fluorescence detection, followed by a Western blot analysis with HRP-conjugated streptavidin or CBB-staining. As a result, the rhodamine-labeled HaloTag proteins were clearly detected in the fluorescence analysis in all experiments, indicating that the covalent formation between HaloTag protein and the ligands proceeded efficiently (lanes 2–5). Meanwhile, the protein labeled with **18**$_{long}$ was successfully detected in the Western blot analysis, whereas that with **18**$_{short}$ was not, indicating that the linker must possess a sufficient length for the streptavidin recognition of the biotinylated protein (lanes 4 and 5).[3a] These results showcase that trialkyne **1** is also a useful platform for the development of effective multifunctional probes because it can expeditiously supply a small library of probe candidates.

In summary, we developed a compact triclickable platform molecule that enabled assembly of three azides via three sequential chemo- and regioselective triazole formation reactions. This method enabled not only the facile construction of a low-molecular-weight tristriazole library that is favorable for drug discovery purposes but also the synthesis of middle-molecular-weight multifunctional molecules that could be used as a dual-detectable probe for protein modification. Further studies on the application of this platform molecule for the synthesis of multifunctional molecules for biological applications are currently underway in our laboratory.

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Data availability

All the data supporting this article have been included in ESI.†

Conflicts of interest

There are no conflicts to declare.

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