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A facile synthesis of multi(triazole)s by iterative click reactions is disclosed. Good functional group tolerance of sequential click assembly by sulfur–fluoride exchange (SuFEx), copper-catalyzed azide–alkyne cycloaddition (CuAAC), and thia-Michael reaction realizes the iterative click reactions. Diverse multi(triazole)-type mid-molecules can be synthesized easily from readily available modules through good chemoselective reactions without functional group transformation steps.

Iterative ligation methods have played significant roles in synthesizing diverse functional molecules including peptides and nucleic acids from simple modules in broad disciplines such as materials chemistry, pharmaceutical sciences, and chemical biology. For example, peptide synthesis is realized by iterative methods through the conjugation of protected amino acids and deprotection (Fig. 1A), which are fundamental ways to prepare bioactive mid-molecules.¹ Herein, we disclose an efficient method to synthesize mid-molecules from simple modules by iterative click reactions onto trivalent platform molecules without deprotection steps.

Click reactions are gaining attention from a wide range of researchers for reliable molecular conjugation.² We have developed a variety of trivalent platforms for efficient molecular assembly by sequential click reactions.^{3–5} In particular, we recently succeeded in the synthesis of trivalent platform **1a** that served in the sequential click conjugation by the SuFEx reaction,⁶ copper-catalyzed azide–alkyne cycloaddition (CuAAC),⁷ and thia-Michael reaction,⁸ where the triazole formation remarkably enhanced the Michael reactivity of acrylamides (Fig. 1B).^{4c,4e} It is worth noting that we succeeded in the one-pot assembly of

Iterative click reactions using trivalent platforms for sequential molecular assembly†‡

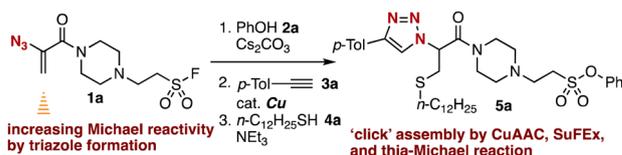
 Gaku Orimoto and Suguru Yoshida *

modules onto trivalent platform **1a**.^{4e} With the orthogonality of the click reactions in mind, we conceived an idea that iterative click reactions can be accomplished by the SuFEx reaction, CuAAC, and thia-Michael reaction using linkers having two clickable groups such as an alkyne and a silyl ether moiety. The robust reliability of click reactions will allow us to synthesize diverse multi(triazole)s from simple starting materials. In this study, we synthesized various trivalent platforms bearing azide, alkene, and fluorosulfonyl groups, and divergent triazoles with a variety of functionalities (Fig. 1C, upper). On the basis of good functional group tolerance in sequential conjugations, we then developed an iterative click reaction which enabled us to synthesize tris(triazole)s from simple modules (Fig. 1C, lower).

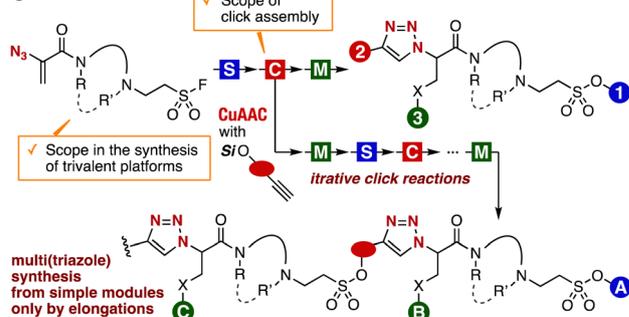
A Iterative reactions for peptide synthesis



B Previous studies



C This work



Department of Biological Science and Technology, Faculty of Advanced Engineering, Tokyo University of Science, 6-3-1 Niijuku, Katsushika-ku, Tokyo 125-8585, Japan.
 E-mail: s-yoshida@rs.tus.ac.jp

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‡ Electronic Supplementary Information (ESI) available: Experimental procedures, characterization for new compounds including NMR spectra. See DOI: <https://doi.org/10.1039/d4cc01177e>

Fig. 1 (A) Peptide synthesis. (B) Our previous studies. (C) Overview of this work.



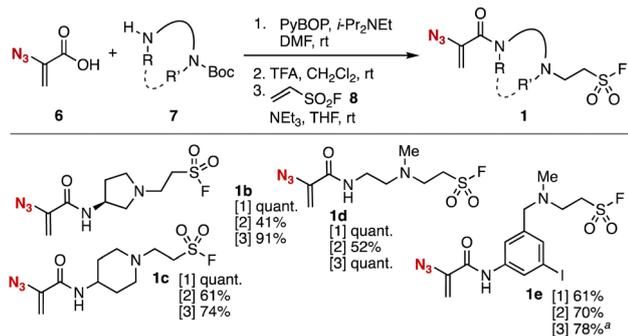


Fig. 2 Syntheses of trivalent platforms **1**. Yields for 1st, 2nd, and 3rd step were shown. See the ESI† for details. ^aCondensation was conducted with chloro-*N,N,N',N'*-tetramethylformamidium hexafluorophosphate and *N*-methylimidazole in MeCN at rt. PyBOP = ((1*H*-Benzo[d][1,2,3]triazol-1-yl)oxy)tri(pyrrrolidin-1-yl)phosphonium hexafluorophosphate.

We first synthesized a range of acrylamides **1b–1e** bearing fluorosulfonyl groups from 2-azidoacrylic acid (Fig. 2). Condensation of 2-azidoacrylic acid (**6**) with amines **7** having an *N*-Boc amide group followed by deprotection of the Boc group and subsequent addition to ethenesulfonyl fluoride (**8**) took place smoothly to afford trivalent platforms **1b–1e** leaving azide, alkene, fluorosulfonyl, secondary or tertiary amide, and iodo moieties untouched. Of note, a broad range of amines such as not only primary and secondary alkyl amines but also aromatic amines participated in the acrylamide synthesis without damaging electrophilic alkene moieties by the undesired Michael addition.

A wide variety of highly functionalized modules **2–4** were conjugated using trivalent platform **1a** by sequential click assembly (Fig. 3). In the SuFEx reaction of **1a** as the first step, various aromatic alcohols reacted selectively at the fluorosulfonyl group to afford sulfonyl esters **9b–9j** in high yields keeping

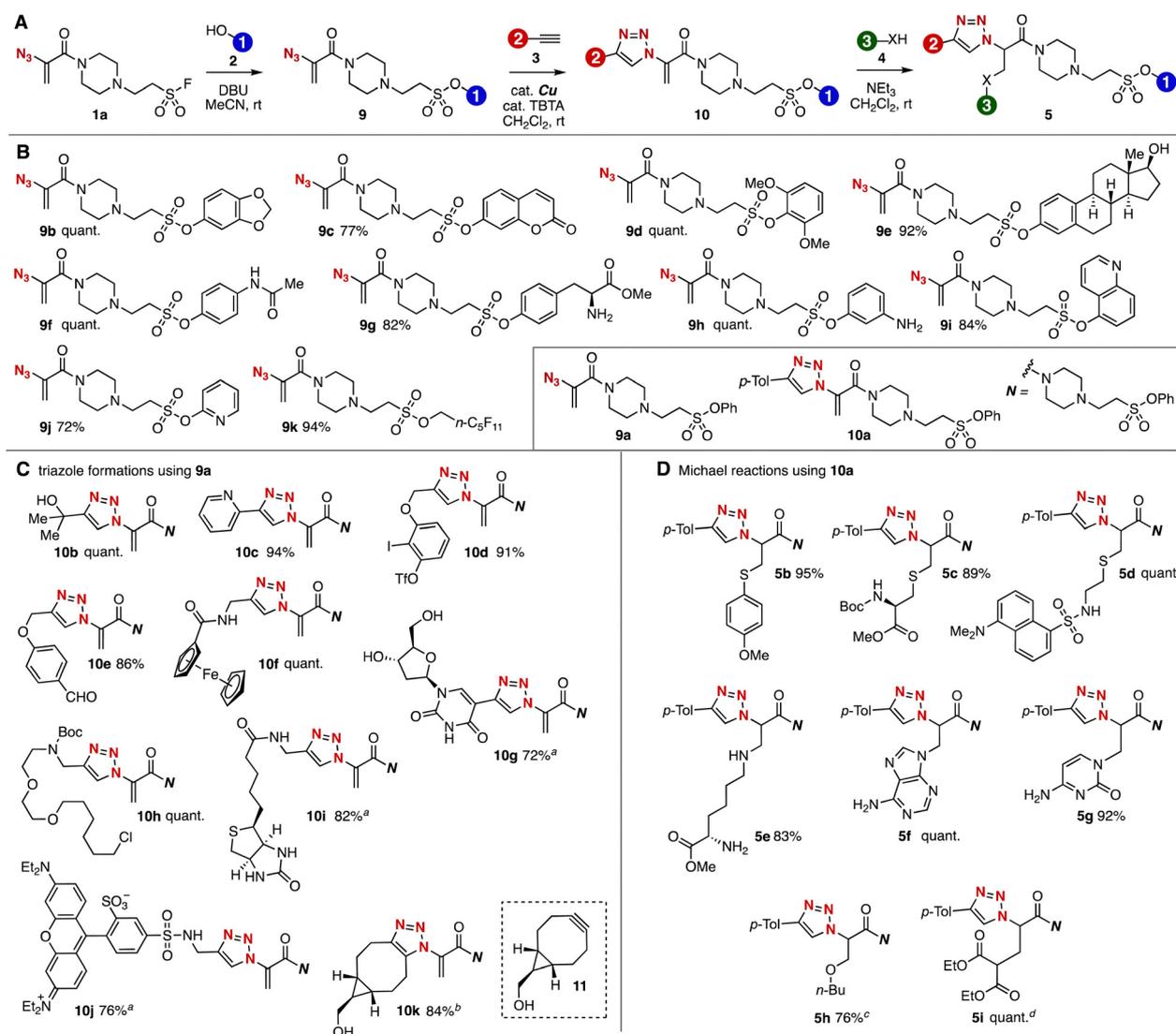


Fig. 3 Sequential click assembly using platform **1a**. See the ESI† for details. (A) General scheme. (B) SuFEx reaction of **1a**. (C) Triazole formation of **9a**. (D) Michael addition to **10a**. ^aDMF was used as a solvent instead of CH₂Cl₂. ^bThe reaction of **9a** with **11** was performed in CH₂Cl₂ at rt. ^cThe reaction of **10a** with *n*-butanol and Cs₂CO₃ in THF was performed at rt. ^dThe reaction of **10a** with diethyl malonate and *t*-BuOK in THF was performed at rt. TBTA = tris(benzyltriazolylmethyl)amine.



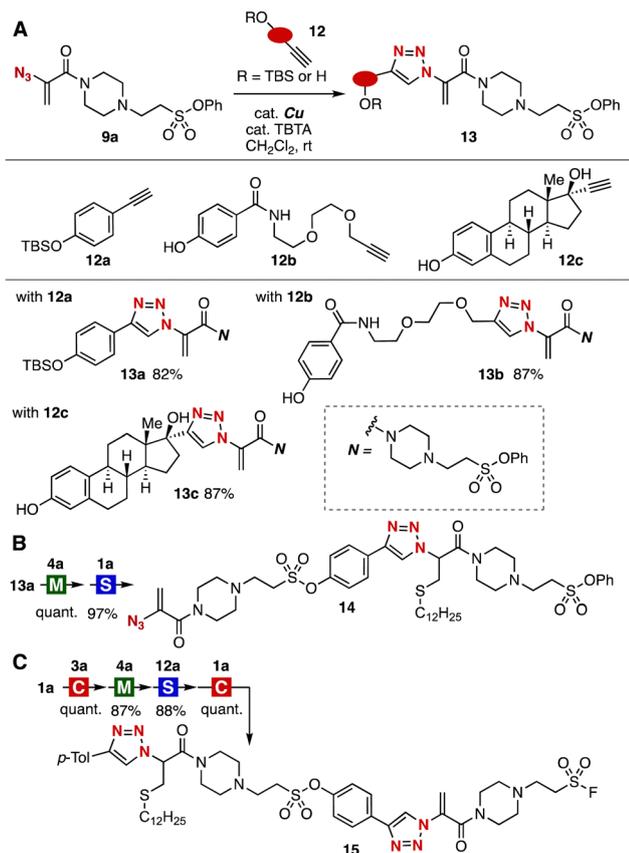


Fig. 4 (A) Synthesis of various triazoles **13**. (B) Synthesis of triazole **14**. (C) Synthesis of bis(triazole) **15**. Reaction conditions; **M**: NEt₃, CH₂Cl₂, rt; **S**: DBU, CH₂Cl₂, rt; **C**: cat. (MeCN)₄CuBF₄, cat. TBTA, CH₂Cl₂, rt; see the ESI† for details.

reactive functionalities (Fig. 3A and B). It is worth noting that the SuFEx reaction proceeded with excellent chemoselectivities at the aromatic hydroxy group in the presence of other nucleophilic groups such as aliphatic alcohol, amide, or amine moieties, in which undesired Michael addition to the remaining acrylamide moiety was not observed. We succeeded in the synthesis of hetero-aromatic sulfonyl esters **9i** and **9j** from 5-quinolinol or 2-pyridone keeping 2-azidoacrylamide moiety intact. In addition, perfluoroalcohol was efficiently conjugated to trivalent platform **1a**, which will serve as a perfluoroalkyl tag moiety.⁹ The synthesis of alkyl sulfonate **9k** also suggests great potential for applications involving ligand-directed labeling,¹⁰ activity-based proteome analyses,¹¹ and covalent drug discovery¹² based on the scission of sulfonate groups.

The second step by the CuAAC reaction using diverse alkynes allowed us to prepare triazoles **10b–10j** bearing various

functionalities in good yields, in which we chose **9a** as a model substrate to simplify the analysis of products (Fig. 3A and C). In particular, 2-(triazolyl)acrylamide moieties were not damaged in the presence of reactive functionalities such as hydroxy, pyridyl, iodo, triflyloxy, formyl, chloro, and amide groups in the products under the Lewis-acidic conditions. It is worthy to note that a wide variety of biochemically significant moieties such as uridine, HaloTag ligand, biotin, and fluorescent sulforhodamine were efficiently introduced by the CuAAC reaction leaving the alkenyl group intact. Strain-promoted azide–alkyne cycloaddition of **9a** with cycloalkyne **11** took place smoothly to provide triazole **10k** in high yield.¹³

A range of nucleophiles **4** were successfully applied to the Michael additions of the resulting 2-(triazolyl)acrylamides **10a**, which was selected as a model substrate to simplify the characterization of products (Fig. 3A and D). For example, aromatic and aliphatic thiols efficiently reacted at the remaining alkene moiety to provide sulfides **5b–5d** by the thia-Michael reaction without damaging amide, ester, and fluorescent dansyl moieties. Not only the primary amino group of lysine methyl ester but also the endocyclic nitrogen atoms of adenine or cytosine served in the efficient Michael addition furnishing amines **5e–5g** in good yields.¹⁴ Additionally, *n*-butanol and dimethyl malonate were also applicable in the Michael reaction under basic conditions, in which 2-(triazolyl)amide and alkoxy sulfonyl groups remained unreacted. These results obviously demonstrate that a broad range of triazoles can be synthesized by assembling various starting materials having diverse reactive functional groups onto trivalent platform **1** in a modular synthetic manner.

The good functional group tolerance of the sequential click assembly realized an iterative conjugation using linkers **12** bearing ethynyl and *tert*-butyl(dimethyl)silyloxy groups by repeating CuAAC and SuFEx reactions (Fig. 4A). Indeed, we accomplished the selective synthesis of triazole **13a** from azide **9a** leaving the remaining acrylamide and silyloxy group untouched. Also, the CuAAC reaction allowed us to synthesize triazoles **13b** and **13c** selectively using linkers **12b** and **12c** with ethynyl and aromatic hydroxy groups. Since the silyloxy group survived in thia-Michael reaction of thiol **4a** to triazole **13a**, we succeeded in the facile preparation of triazole **14** in excellent yield by further SuFEx conjugation with trivalent platform **1a** (Fig. 4B).

We found that the order of the sequential conjugation using trivalent platform **1a** was switchable (Fig. 4C). For instance, after the CuAAC and thia-Michael addition of **1a** with alkyne **3a** and thiol **4a**, respectively, keeping the fluorosulfonyl group unreacted, the SuFEx reaction with linker **12a** took place

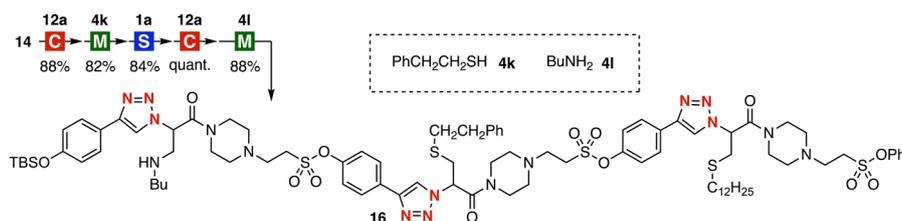


Fig. 5 Synthesis of tris(triazole) **16**. Reaction conditions; **M**: NEt₃, CH₂Cl₂, rt; **S**: DBU, CH₂Cl₂, rt; **C**: cat. (MeCN)₄CuBF₄, cat. TBTA, CH₂Cl₂, rt; see the ESI† for details.



smoothly without damaging the ethynyl group. The resulting alkyne reacted efficiently with trivalent platform **1a** to afford bis(triazole) **15** in high yields leaving the exomethylene and fluorosulfonyl group untouched. Due to the great potential of sulfonyl fluorides in the proximity-promoted conjugation, mid-molecules having fluorosulfonyl groups will gain attention from researchers in pharmaceutical sciences.¹⁵

The synthesis of tris(triazole) **16** was achieved by the iterative click reactions resulting in the elongation of the main chain and assembly of modules **4k** and **4l** (Fig. 5). Indeed, the triazole formation using azide **14** with linker **12a** followed by the thia-Michael reaction of thiol **4k** and the SuFEx reaction with trivalent platform **1a** was realized without side reactions such as undesired elimination by the retro-Michael reaction or decomposition of sulfonic acid ester moieties. Then, CuAAC with linker **12a** and Michael addition of amine **4l** proceeded smoothly to provide tris(triazole) **16** in high yields. Thus, we succeeded in the synthesis of tris(triazole) **16** by the iterative click reactions using thiols **4a** and **4k**, amine **4l**, linker **12a**, and trivalent platform **1a** in a modular synthetic manner. This efficient chain elongation, as well as significant functional group tolerance of click reactions clarified in Fig. 3, obviously demonstrates that the iterative click reaction enables us to synthesize diverse multi(triazole)s, which are of great importance as functionalized peptide bioisosteres.¹⁶

In conclusion, we demonstrated the excellent functional group tolerance of the sequential click assembly by SuFEx, CuAAC, and thia-Michael reaction. Based on the chemoselective molecular conjugation methods, facile synthesis of multi(triazole)s has been developed by iterative click reactions without functional group transformation steps. Due to the wide scope of click reactions and good accessibility of modules, a huge chemical library of multi(triazole)-type mid-molecules will be constructed in a modular synthetic manner. Further studies such as one-pot click assembly of functional molecules onto trivalent platforms and applications to bioassay using diverse mid-molecules are ongoing in our laboratory.

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Conflicts of interest

There are no conflicts to declare.

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