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Click assembly through selective azaylide formation†

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An efficient triple-click assembly using a newly designed trivalent platform is disclosed. We achieved the selective azaylide formation of 2,3,5,6-tetrafluorophenyl azides with *o*-ester-substituted triarylphosphines leaving 2,6-dichlorophenyl azides untouched. Further rapid Staudinger reaction of dichlorophenyl azides and subsequent triazole formation allowed us to prepare trifunctionalized molecules in three steps.

Trivalent platforms having three clickable functional groups for assembling modules gain attention from diverse research fields including pharmaceutical sciences and chemical biology due to the great importance of multifunctional molecules.[1–4] Despite recent emerging advances in sequential click reactions, it is not easy to perform click assembly without deprotection due to the limited trivalent platforms. Herein, we disclose an efficient assembly of functional modules using a new trivalent platform through selective robust azaylide formations by Staudinger reaction.

Staudinger reactions have played pivotal roles as efficient conjugation methods for functional molecules.[5] Rapid Staudinger reactions forming robust azaylides are gaining attention as efficient click reactions from broad researchers in pharmaceutical sciences and materials chemistry.[6–8] Indeed, rapid azaylide formations were developed independently by our group[6] and Ramström and Yan's group[7] using 2,6-dichlorophenyl azides and 2,3,5,6-tetrafluorophenyl azides, respectively. These rapid reactions allowed us to achieve not only the efficient synthesis of difunctionalized molecules[8b,8e] but also bioconjugations using living cells due to good biocompatibility.[6–8] We conceived that selective azaylide formations of 2,6-dichlorophenyl azides and 2,3,5,6-tetrafluorophenyl azides with phosphines enable us to realize efficient sequential conjugations. In this study, we thus decided to design new platforms having these azide moieties and an alkynyl group based on the combination of azaylide formations and triazole formations[9] for the sequential assembly of functional molecules after examining the selective azaylide formations of 2,6-dichlorophenyl azides and 2,3,5,6-tetrafluorophenyl azides with phosphines (Fig. 1).

We at first performed a competition reaction of an equimolar mixture of 2,6-dichloro-4-(*n*-butylaminocarbonyl)phenyl azide (**1a**) and 2,3,5,6-tetrafluoro-4-(*n*-butylaminocarbonyl)phenyl azide (**2a**) with triphenylphosphine (**3a**) in THF at room temperature (Fig. 2). As a result, phosphine **3a** was smoothly consumed to afford a mixture of azaylides **4a** and **5a** quantitatively, where the formation of azaylide **5a** from tetrafluorophenyl azide **2a** was favored in moderate selectivity. This result obviously demonstrated that tetrafluorophenyl azide **2a** showed higher reactivity with triphenylphosphine (**3a**) than dichlorophenyl azide **1a**.

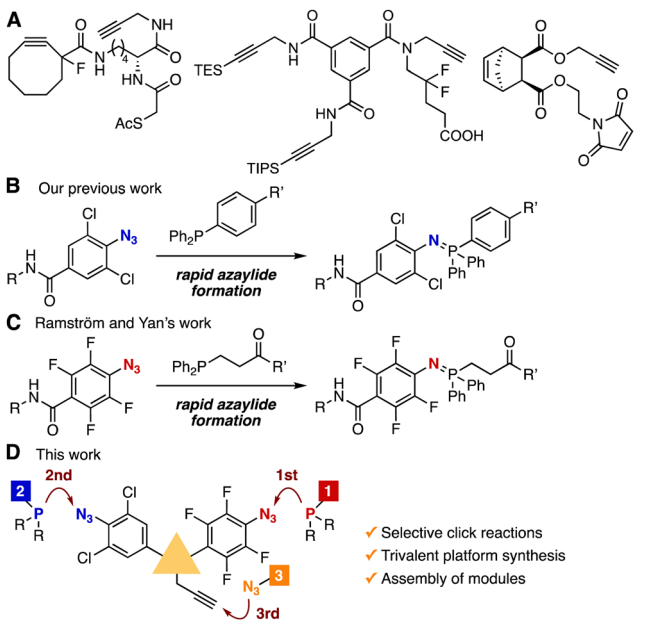

Fig. 1 (A) Examples of trivalent platforms. (B) Our previous study. (C) Ramström and Yan's work. (D) This work.

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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/d4cc02723j

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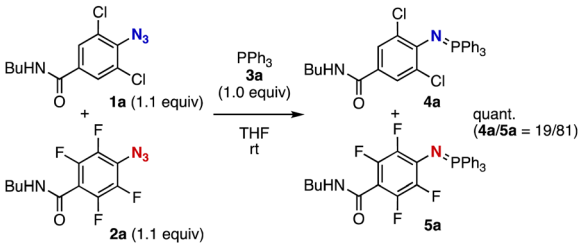
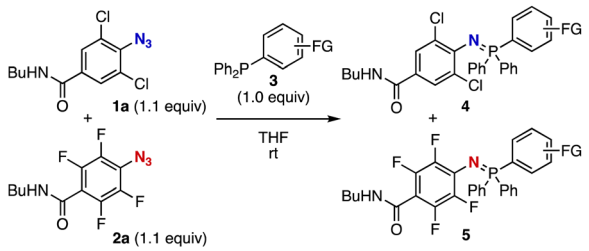


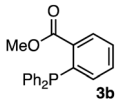
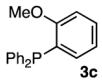
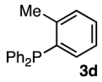
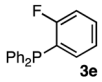
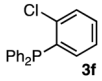
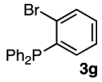
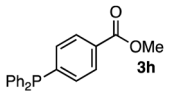
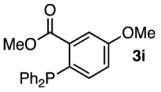
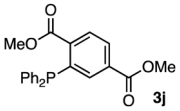
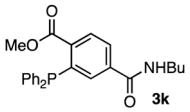
Fig. 2 Competition reaction of azides **1a** and **2a** with phosphine **3a**.

Screening of triarylphosphines **3** resulted in selective azaylide formation using an equimolar mixture of azides **1a** and **2a** (Table 1). Indeed, we found that treatment of an equimolar mixture of azides **1a** and **2a** with diphenyl(2-(methoxycarbonyl)phenyl)phosphine (**3b**) selectively provided azaylide **5** quantitatively from tetrafluorophenyl azide **2a** without the reaction of dichlorophenyl azide **1a** (entry 1). While electron-donating substituents on the benzene ring of phosphines **3b** and **3c** decreased the selectivity (entries 2 and 3), electron-withdrawing and slightly bulky substituents such as chloro and bromo group led to the perfect selectivity as well as ester moiety (entries 4–6). When diphenyl(4-(methoxycarbonyl)phenyl)phosphine (**3h**) was used in the competition reaction, the selectivity was significantly decreased (entry 7). These results clearly showed that the electronic and steric effects of the ester moiety at the *ortho* position of **3b** were key to the success of the selective azaylide formation. Additional substituents such as electron-donating methoxy group and electron-withdrawing ester and amide moieties at the meta position of *o*-ester-substituted phosphines **3i-3g** did not decrease the selectivity (entries 8–10). These results indicated that functions can be installed to the *o*-(methoxycarbonyl)phenyl group of triarylphosphines. Owing to the rapid Staudinger reaction of 2,3,5,6-tetrafluorophenyl azide with **3b** (k_{obs} = 2.7 M^{-1} s^{-1}),[7] this selective azaylide formation will realize broad applications involving bioconjugation.

With an idea for assembling modules through selective azaylide formation, we then examined the stability of azaylides under various conditions (Table 2). Treatment of azaylides **4a**, **4b**, **5a**, and **5b** with an aqueous NaOH showed their good stability under basic conditions (entries 1–4), in which a small amount of phosphine oxide **6b** was observed when using azaylide **5b** prepared from tetrafluorophenyl azide **2a** and diphenyl(2-(methoxycarbonylphenyl))phosphine (**3b**) (entry 4). In the case of hydrolysis of azaylides under acidic conditions with hydrochloric acid, azaylides **4a** and **4b** prepared from dichlorophenyl azide **1a** with triphenylphosphine (**3a**) and phosphine **3b** bearing *ortho* ester moiety were more stable than azaylides **5a** and **5b** from tetrafluorophenyl azide **2a** (entries 5 and 6 *vs.* entries 7 and 8). In particular, we found that quantitative cleavage of azaylide **5a** took place with aqueous hydrochloric acid (entry 7), and the stability was significantly improved by the presence of ester moiety (entry 8). In addition, no reaction proceeded when azaylides **4a**, **4b**, **5a**, and **5b** were treated with $(MeCN)_4CuBF_4$ and tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine (TBTA),[9] which were frequently used for the copper-catalyzed azide–alkyne cycloaddition (CuAAC)

Table 1 Screening of phosphines for the selective azaylide formation



Entry	3	Yield/% (4 + 5)	4/5
1	 **3b**	Quant.	0/100
2	 **3c**	93	17/83
3	 **3d**	92	7/93
4	 **3e**	99%	8/92
5	 **3f**	Quant.	0/100
6	 **3g**	Quant.	0/100
7	 **3h**	Quant.	25/75
8	 **3i**	Quant.	0/100
9	 **3j**	Quant.	0/100
10	 **3k**	97	0/100

(entries 9–12). These results suggested that azaylides will be stable under the CuAAC conditions.

Selective azaylide formation enabled us to accomplish sequential Staudinger reactions of diazide **9** with phosphines **3a** and **3b** (Fig. 2). Diazide **9** having dichlorophenyl and tetrafluorophenyl azide moiety was successfully synthesized from mono-Boc-protected diamine **7** (Fig. 3A). Indeed, after preparation of the corresponding acid chloride from carboxylic acid **1b**, amide formation took place smoothly to afford 2,6-dichlorophenyl azide **8**. Then, deprotection of Boc group with trifluoroacetic acid and acylation with acid chloride **2b** resulted in the efficient synthesis of diazide **9** without damaging two azido groups. Treatment of diazide **9** with *o*-ester-substituted

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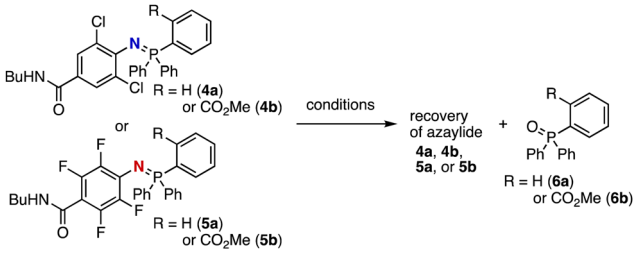
Table 2 Stability of azaylides under various conditions



Entry	Azaylide	Conditions	Recovery of **4a**, **4b**, **5a**, or **5b**/%	**6**, Yield/%
1	**4a**	1 M NaOH aq., THF, rt	Quant.	**6a**, 0
2	**4b**	1 M NaOH aq., THF, rt	Quant.	**6b**, 0
3	**5a**	1 M NaOH aq., THF, rt	Quant.	**6a**, 0
4	**5b**	1 M NaOH aq., THF, rt	94	**6b**, 6
5	**4a**	1 M HCl aq., THF, rt	88	**6a**, 12
6	**4b**	1 M HCl aq., THF, rt	Quant.	**6b**, 0
7	**5a**	1 M HCl aq., THF, rt	0	**6a**, Quant.
8	**5b**	1 M HCl aq., THF, rt	77	**6b**, 23
9	**4a**	$(MeCN)_4CuBF_4$ (5 mol%) TBTA (5 mol%) CH_2Cl_2, rt	Quant.	**6a**, 0
10	**4b**	$(MeCN)_4CuBF_4$ (5 mol%) TBTA (5 mol%) CH_2Cl_2, rt	Quant.	**6b**, 0
11	**5a**	$(MeCN)_4CuBF_4$ (5 mol%) TBTA (5 mol%) CH_2Cl_2, rt	Quant.	**6a**, 0
12	**5b**	$(MeCN)_4CuBF_4$ (5 mol%) TBTA (5 mol%) CH_2Cl_2, rt	Quant.	**6b**, 0

triarylphosphine **3b** furnished azaylide **10** quantitatively leaving 2,6-dichlorophenyl azide moiety untouched (Fig. 3B). The second rapid azaylide formation proceeded smoothly to provide bis(iminophosphorane) **11** in high yield. Due to the fast azaylide formation of 2,6-dichlorophenyl azide with triphenylphosphine (k_{obs} = 0.63 M^{-1} s^{-1}) and good biocompatibility,[6] this

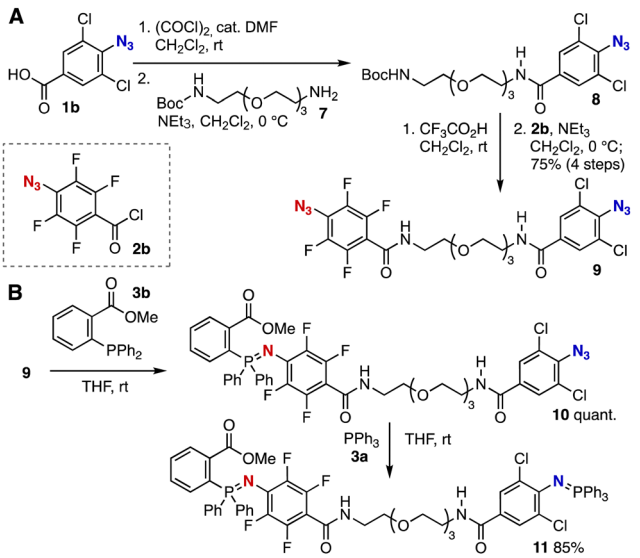


Fig. 3 (A) Synthesis of diazide **9**. (B) Sequential Staudinger reactions.

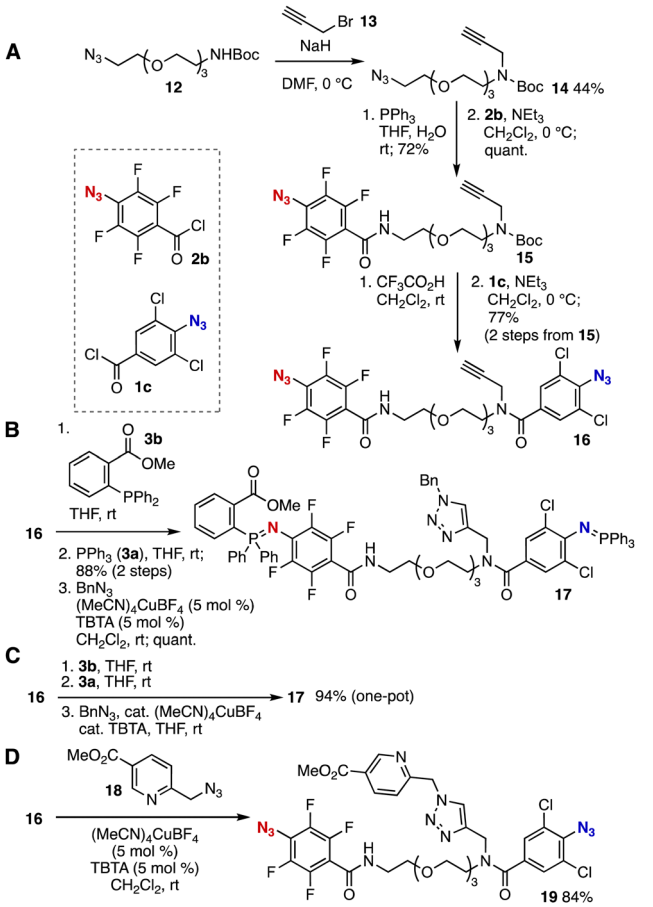


Fig. 4 (A) Synthesis of **16**. (B) Triple-click assembly. (C) One-pot triple click assembly. (D) Alkyne selective reaction.

sequential conjugation would serve in the synthesis of functional molecules and chemical modification of biomolecules.

To showcase the selective azaylide formation, we succeeded in the efficient assembly of modules using newly developed trivalent platform **16** (Fig. 4). Preparation of azide **15** was achieved from azide **12** by *N*-propargylation, Staudinger reduction, and *N*-acylation with acid chloride **2b** in good yields (Fig. 4A). Then, deprotection of Boc group followed by acylation with acid chloride **1c** took place efficiently to afford trivalent platform **16** without damaging two azide moieties and propargyl group. Of note, **16** was easily constructed in modular synthetic manner through the Staudinger reduction of alkyne **14**. We achieved selective azaylide formation by treatment of trivalent platform **16** with ester-substituted phosphine **3b** followed by Staudinger reaction with triphenylphosphine and subsequent CuAAC reaction resulting in the efficient synthesis of bis(iminophosphorane) **17** in good yields leaving terminal alkyne moiety intact (Fig. 4B). The simple protocol realized a one-pot triple-click assembly, which would enable us to synthesize multifunctional molecules such as probe compounds from easily available modules (Fig. 4C). Moreover, alkyne selective reaction was accomplished by treatment of trivalent platform **16** with picolyl azide **18**[10] furnishing triazole **19** with tetrafluorophenyl and dichlorophenyl azide moieties (Fig. 4D). Thus, the

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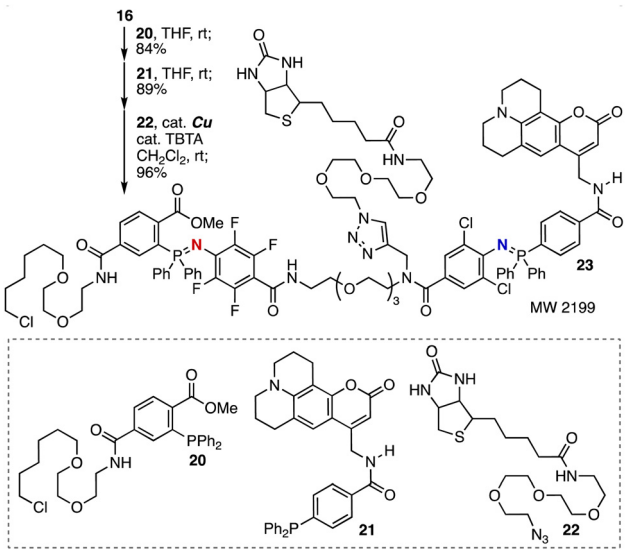

Fig. 5 Synthesis of **23** by triple-click assembly.

order of module assembly could be switched by changing reaction partners. Since rapid azaylide formations served in the efficient chemical labeling using living cells,[6,7] functionalized azides preparable from **16** would be of great significance in various research fields such as chemical biology.

Assembly of HaloTag ligand, fluorescent coumarin 102, and biotin moieties was realized by efficient click reactions with trivalent platform **16** (Fig. 5). Indeed, selective azaylide formation took place smoothly without decomposing primary alkyl chloride. Then, we accomplished the second azaylide formation with fluorescent phosphine **21** and following CuAAC reaction with biotinyl azide **22** providing adduct **23** leaving reactive functional groups untouched. Therefore, highly functionalized molecular probes will be synthesized in short steps in a modular synthetic method onto trivalent platform **16**.

In summary, we have succeeded in the selective azaylide formation of tetrafluorophenyl azide moiety leaving dichlorophenyl azide side intact. Since rapid azaylide formation of dichlorophenyl azide can be performed using the resulting dichlorophenyl azide and these reactions proceed without damaging an alkyne moiety, the newly designed trivalent platform **16** enabled us to achieve efficient triple click assembly of functional modules. Moreover, since we preliminary found that selective azaylide formations of the tetrafluorophenyl azide moiety can be achieved even in the presence of alkyl azide and dichlorophenyl azide moieties,[11] novel trivalent and tetravalent platforms will be developed in future. Further studies including applications to develop novel molecular probes and sequential bioconjugation methods using trivalent platforms are ongoing.

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Data availability

The data supporting this article have been included as part of the ESI.†

Conflicts of interest

There are no conflicts to declare.

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11 See the ESI† for details.