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Sequential conjugation methods based on triazole formation and related reactions using azides

Suguru Yoshida

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The recent remarkable progress in azide chemistry has realized sequential conjugation methods with selective 1,2,3-triazole formation. On the basis of the diverse reactivities of azides and azidophiles, including terminal alkynes and cyclooctynes, various selective reactions to furnish triazoles and a wide range of platform molecules, such as diynes, diazides, triynes, and triazides, have been developed so far for bis- and tris(triazole) syntheses. This review highlights recent transformations involving selective triazole formation, allowing the efficient preparation of unsymmetric bis- and tris(triazole)s using diverse platform molecules.

Introduction

Azides are recognized as reliable compounds to conjugate with several types of azidophiles, such as terminal alkynes and cycloalkynes.[1] In particular, copper-catalyzed azide–alkyne cycloaddition (CuAAC) and strain-promoted azide–alkyne cycloaddition (SPAAC) have been utilized as "click reactions" for connecting two molecules in broad disciplines, including the pharmaceutical sciences, chemical biology, and materials science (Fig. 1).[2–6] These reliable methods for forming a 1,2,3-triazole ring have enabled the preparation of a wide range of compounds involving functionalized proteins.

In 2002, Sharpless's group and Meldal's group independently reported that a catalytic amount of copper(I) salt efficiently facilitated the [3 + 2] cycloaddition reaction between azides and terminal alkynes.[4a,b] This catalytic reaction realizes the selective synthesis of a wide range of 1,4-triazoles, leaving diverse functional groups unreacted. When cyclooctynes were treated with azides, the triazole formation took place smoothly without copper catalysis.[5] The SPAAC reaction reported by Bertozzi and coworkers in 2004 served in the chemical modification of proteins.[6a] Various cycloalkynes have so far been developed for efficient conjugation with azides.[5,6]

The diversity of synthesizable triazoles has been expanded by the development of sequential triazole formation and related reactions. Sequential reactions using platform molecules, such as diynes, triynes, diazides, and triazides, have allowed for the modular synthesis of bis- and tris(triazole)s from simple modules.

This review summarizes recent sequential conjugation methods based on triazole formation and related chemistry using azides. In particular, various platform compounds bearing two or more clickable moieties are highlighted in terms of their azido- or alkyne-type selectivities.

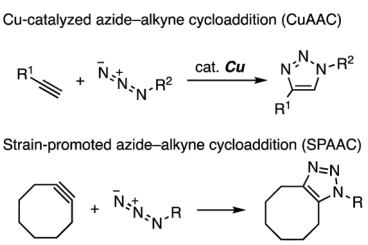

Fig. 1 Triazole formation by cycloaddition of azides with terminal alkynes or cyclooctyne.

Laboratory of Chemical Bioscience, Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University (TMDU), 2-3-10 Kanda-Surugadai, Chiyoda-ku, Tokyo 101-0062, Japan. E-mail: s-yoshida.cb@tmd.ac.jp

Suguru Yoshida

Suguru Yoshida received his Ph.D. in 2009 from Kyoto University under the supervision of Prof. Koichiro Oshima. After a postdoctoral fellowship in the group of Prof. Katsuhiko Tomooka at Kyushu University and the group of Prof. Marcus Tius at the University of Hawaii in Manoa, he became Assistant Professor at Tokyo Medical and Dental University (TMDU), Japan, working with Prof. Takamitsu Hosoya in 2010 and was promoted to Associate Professor in 2015. His research interests include new methods in synthetic organic chemistry. He received the Chemical Society of Japan Award for Young Chemists (2017) and the Thieme Chemistry Journal Award (2019).

Multi(triazole) syntheses *via* selective reactions of diynes or related compounds

One-pot bis(triazole) synthesis using a peptide platform with two types of alkynyl groups, *i.e.* an ethynyl group and a trimethylsilyl-protected alkyne moiety, was developed by Aucagne and Leigh in 2006 (Fig. 2A).[7] Indeed, the copper-catalyzed reaction of diyne **1** with azide **2** proceeded selectively at the terminal alkyne moiety. The subsequent Ag(I)-mediated desilylprotonation and chemoselective triazole formation catalyzed by copper catalysis with azide **3** provided bis(triazole) **4** in high yield. The unsymmetrical bis(triazole) synthesis through selective triazole formation using 1-trimethylsilyl-1,3-butadiyne (**5**) was reported by Fiandanese and coworkers in 2009 (Fig. 2B).[8] The Cu(II) catalyzed azide–alkyne cycloaddition between diyne **5** and benzyl azide (**6**) and subsequent Cu(I)-promoted triazole formation with *n*-decyl azide (**7**) in the presence of tetrabutylammonium fluoride yielded bis(triazole) **8** in good yield. In 2010, Aizpurua, Fratila, and coworkers reported the efficient preparation of bis(triazole) **13** by selective desilylprotonation of 1,4-bis(trimethylsilyl)-1,3-butadiyne (**9**) with methyllithium followed by a first CuAAC reaction with azide **10**, desilylprotonation with cesium fluoride, and a second CuAAC reaction with azide **12** (Fig. 2C).[9] Simpson *et al.* also developed a bis(triazole) synthesis from TIPS-protected 1,3-diyne **14** in a one-pot manner (Fig. 2D).[10]

Sequential triple-triazole-formation methods have been accomplished through selective desilylprotonation (Fig. 3). For example, the synthesis of tris(triazole) **17** using triazacyclophane-scaffold **16** was achieved by Liskamp *et al.* in 2014 (Fig. 3A).[11] Indeed, a first CuAAC reaction with an azide followed by Ag(I)-mediated selective deprotection of the TES group proceeded efficiently. Then, a second CuAAC, desilylprotonation of the TIPS group with TBAF, and a third CuAAC resulted in the convergent synthesis of tris(triazole) **17** bearing three cyclic peptide moieties. In 2015, Jiráček and coworkers developed a versatile trifunctional scaffold **18** with three alkynyl groups; an ethynyl group and TES- and TIPS-protected alkynyl moieties, which enabled a solid-phase triple-click synthesis (Fig. 3B).[12] Stepwise triple-click functionalization of a peptide-type triyne platform **19** was also accomplished by Vrabel *et al.* in 2018.[13]

Bis(triazole) syntheses using diynes without silyl protective groups have also been achieved through selective triazole for-

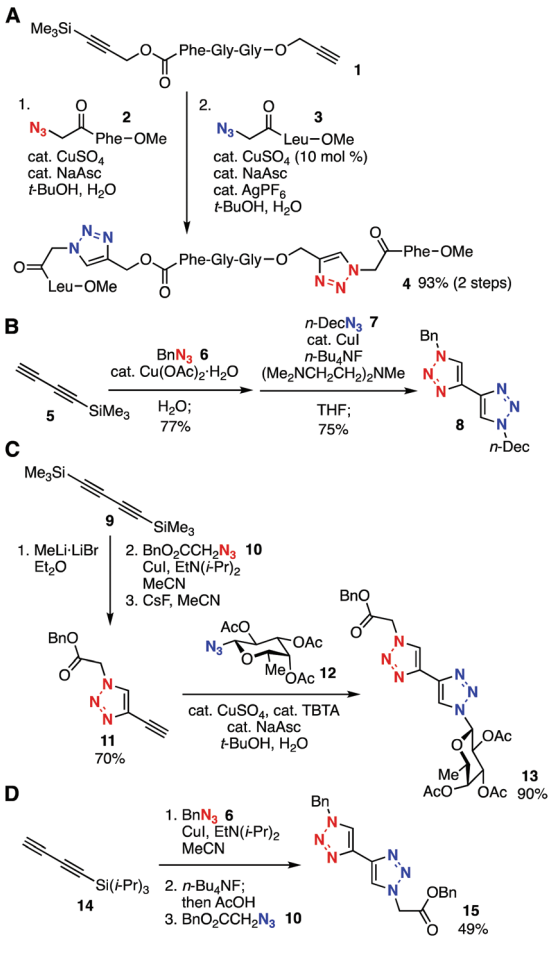

Fig. 2 Bis(triazole) syntheses by two CuAAC reactions of diynes. (A) Aucagne and Leigh's work. (B) Fiandanese's work. (C) Aizpurua and Fratila's work. (D) Simpson's work. NaAsc = sodium ascorbate; TBTA = tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine.

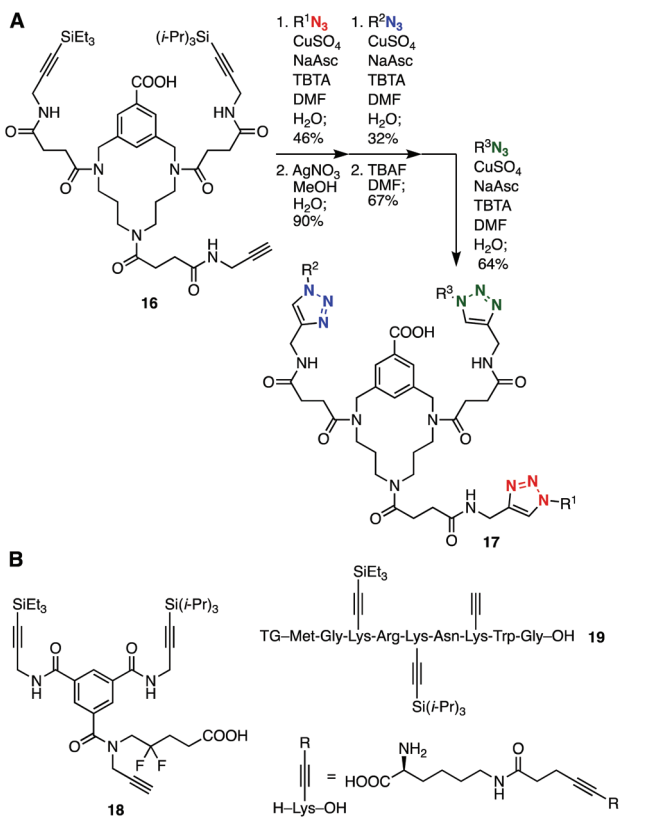

Fig. 3 Tris(triazole) syntheses using triynes. (A) Liskamp's work. (B) Other triyne platforms for the tris(triazole) syntheses. TG = TentaGel NH_2 resin.

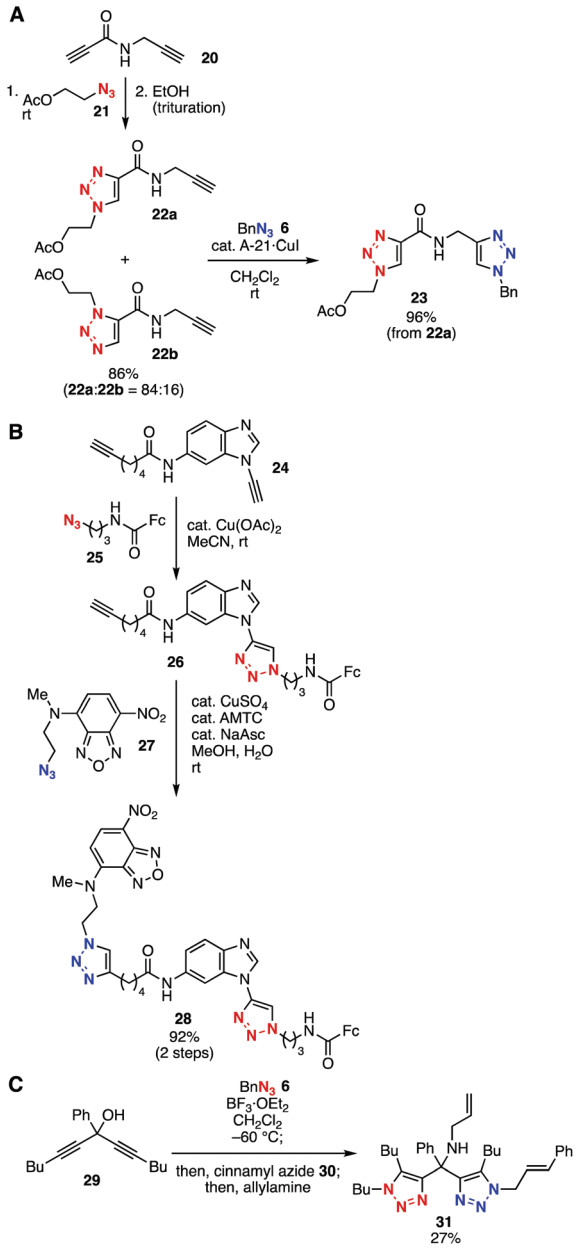

Fig. 4 Bis(triazole) syntheses using diynes. (A) Girard's work. (B) Watson and Burley's work. (C) Tanimoto's work. A-21 = Amberlyst A-21. AMTC = 2-(4-(dimethylamino)methyl-1,2,3-triazol-1-yl)cyclohexan-1-ol.

mation (Fig. 4). For example, Girard and coworkers reported selective triazole formation of *N*-propargyl propiolic amide (**20**) in 2011 (Fig. 4A).[14] The remaining terminal alkyne in **22a** reacted with azide **6** in the presence of a copper catalyst to yield bis(triazole) **23** in high yield. In 2016, Burley, Watson, and coworkers found that aromatic ynamines showed higher reactivity than simple terminal alkynes in triazole formation catalyzed by Cu(II) (Fig. 4B).[15] On the basis of the different reactivities, selective bis(triazole) synthesis was accomplished by selective Cu(II)-catalyzed triazole formation followed by Cu(I)-catalyzed azide–alkyne cycloaddition.

Tanimoto and coworkers developed a unique azide–alkyne cycloaddition between azides and propargyl alcohols through cationic intermediates (Fig. 4C).[16] Since the cationic intermediates generated from diyne **29** and azide **6** can react with azides, bis(triazole) synthesis was accomplished in moderate yield.[16a] Furthermore, an elegant four-component coupling by sequential triazole formation and subsequent amination has been achieved using diyne **29**, azides **6** and **30**, and allylamine (Fig. 4C).[16b]

Since aryne intermediates spontaneously react with azides without catalysis to efficiently provide benzotriazoles,[17] we developed an efficient bis(triazole) synthesis using aryne precursors bearing a terminal alkyne moiety (Fig. 5A).[18] Indeed, treatment of *o*-iodoaryl triflate **32** with a silylmethyl Grignard reagent in the presence of azides followed by a CuAAC reaction furnished bis(triazole)s **33a** and **33b** in good yields. The aryne–azide cycloaddition and following azide–alkyne cycloaddition catalyzed by ruthenium afforded bis(triazole) **34a** with a 1,5-triazole moiety.[19] Furthermore, the synthesis of tris(triazole) **39** was achieved from *o*-iodoaryl triflate **35** and azides **36**–**38** by aryne–azide cycloaddition, a first CuAAC, Ag(I)-mediated desilylprotonation, and a second CuAAC (Fig. 5B).

Bis(triazole) synthesis using a platform with an ethynyl group and a cyclooctyne moiety was accomplished (Fig. 6). Dual labeling of biomolecules using diyne **40** through SPAAC and CuAAC was reported by Kele, Wolfbeis, and coworkers in 2009 (Fig. 6A).[20] The SPAAC reaction between cyclooctyne **40**

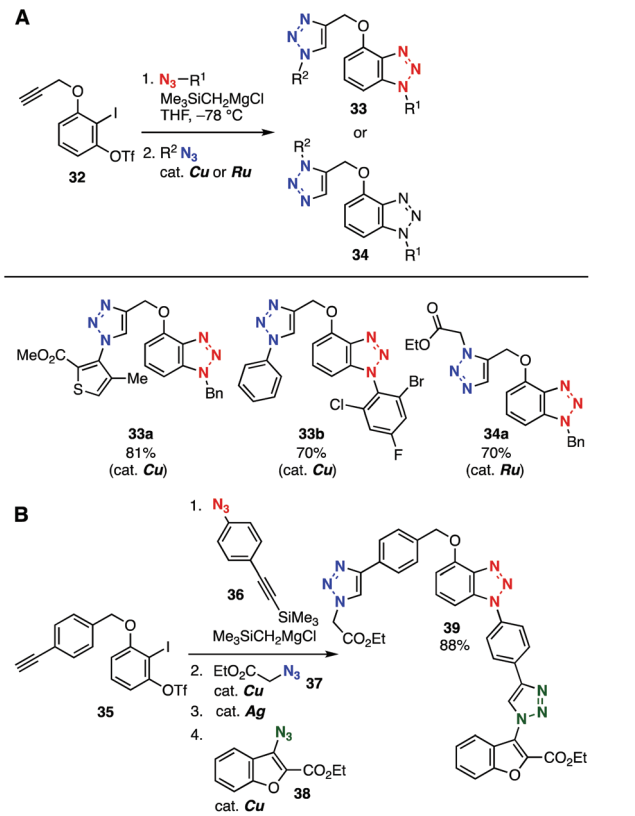

Fig. 5 Bis- and tris(triazole) syntheses through aryne intermediates. (A) Bis(triazole) synthesis. (B) Tris(triazole) synthesis.

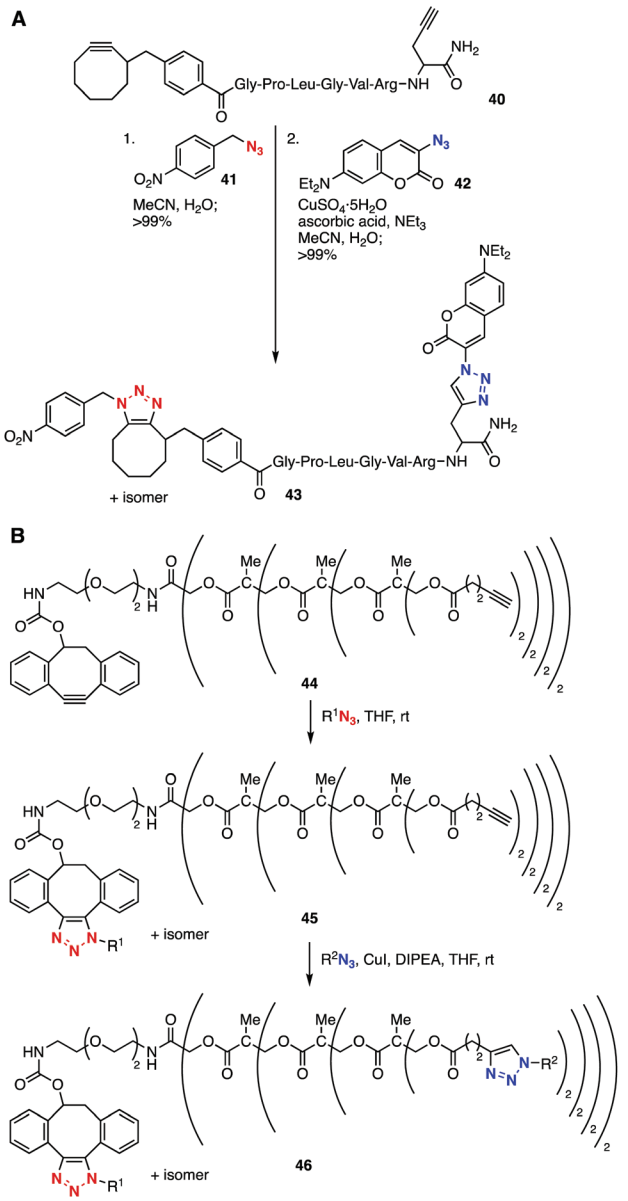

Fig. 6 Multi(triazole) syntheses by SPAAC followed by CuAAC. (A) Kele and Wolfbeis's work. (B) Boons's work. DIPEA = *N*,*N*-diisopropylethylamine.

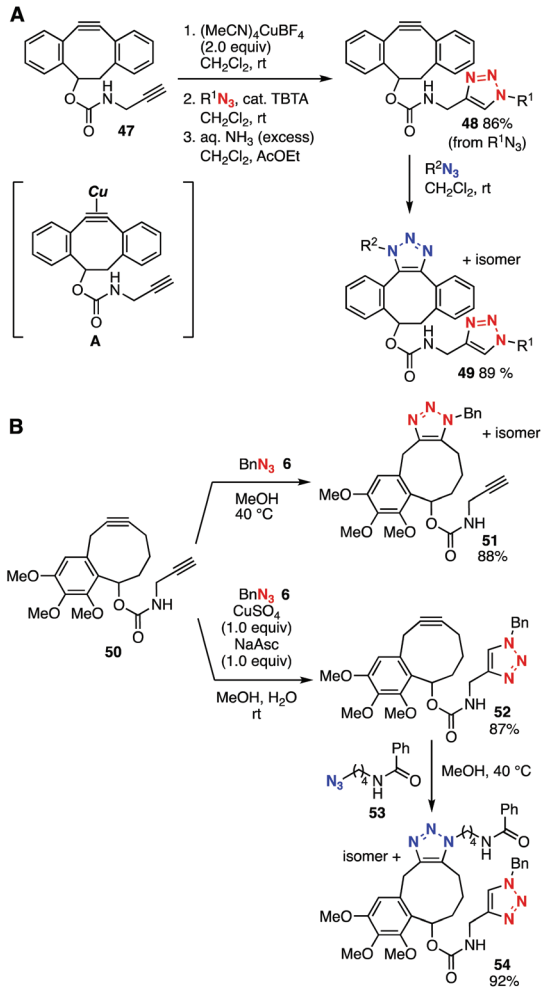

Fig. 7 Bis(triazole) syntheses through transient protection of cycloalkynes. (A) Our work. (B) Dudley's work.

and azide **41** took place smoothly without catalysis, and subsequent CuAAC reaction at the remaining ethynyl group efficiently furnished a regioisomeric mixture of bis(triazole)s **43**. In 2011, Boons *et al.* reported that the selective bis(triazole) synthesis allowed for a dendrimer-type multi(triazole) synthesis using platform **44** with ethynyl groups and a cycloalkyne moiety by a SPAAC reaction and following CuAAC reaction efficiently providing multi(triazole) **46** (Fig. 6B).[21]

In 2014, we reported a novel method to prepare bis(triazole) **49** using diyne **47** through transient protection of the cyclooctyne moiety toward the click reaction with azides (Fig. 7A).[22a] Indeed, terminal alkyne-selective triazole formation took place smoothly *via* a copper–cyclooctyne complex **A**[23] with $(MeCN)_4CuBF_4$ and a following CuAAC reaction with azide and removal of the copper salt with aqueous ammonia solution. Since the terminal alkyne-selective click reaction *via* the transient protection of diyne **47** with $(MeCN)_4CuBF_4$ was realized to retain a more reactive cycloalkyne moiety, the azide-to-cycloalkyne switching approach served in the preparation of a wide range of cyclooctynes with diverse functional groups.[24] This approach achieved the chemical modification of azido-incorporated proteins with functional azides.[22b] In 2016, Dudley and coworker developed a novel platform **50** bearing a cyclononyne and terminal alkyne moieties (Fig. 7B).[25] The SPAAC reaction of the cyclononyne moiety of **50** with azide **6** took place efficiently with gentle heating. Transient protection of the cycloalkyne moiety by complexation with copper enabled the selective CuAAC reaction of platform **50** at the ethynyl group followed by SPAAC with azide **53** to afford bis(triazole) **54** in good yield.

Bis(triazole) synthesis using platform **56** with a dicobalt-protected cycloheptyne moiety and an ethynyl group was also achieved by Fouquet, Hermange, and coworker in 2019 (Fig. 8A).[26] Platform **56** was prepared through the Nicholas reac-

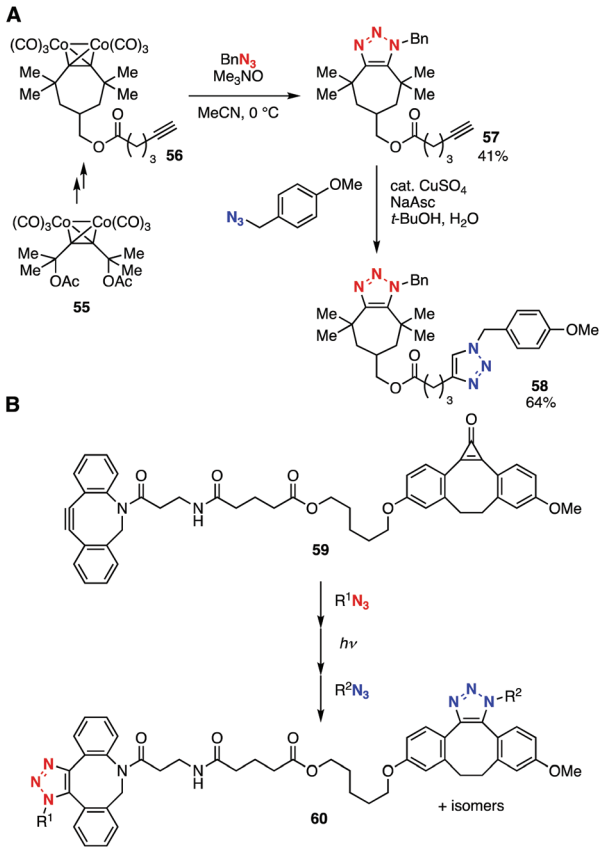

Fig. 8 Bis(triazole) syntheses using platforms **56** and **59**. (A) Fouquet and Hermange's work. (B) Popik's work.

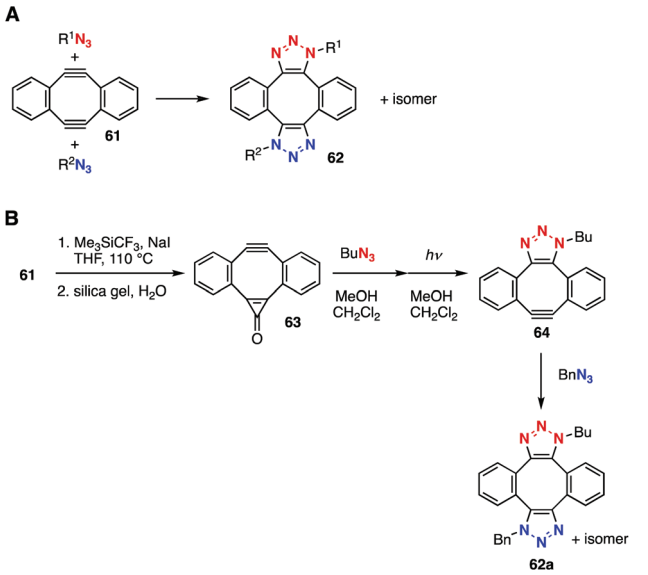

Fig. 9 Double-click reactions using diyne **61**. (A) Hosoya and Kii's work. (B) Popik's work.

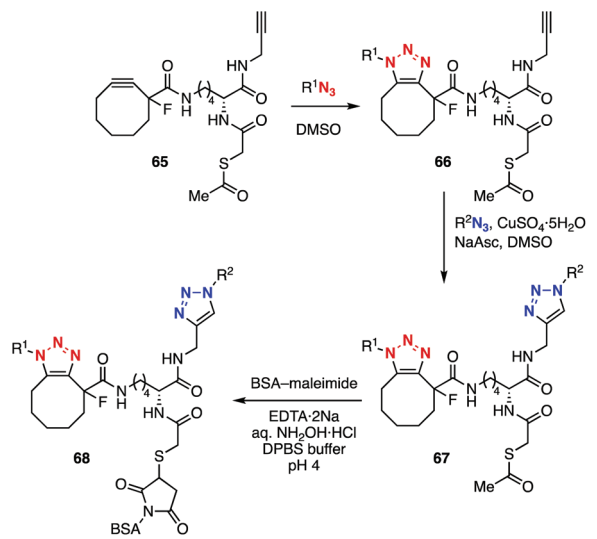

Fig. 10 Three-component coupling using platform **65**.

tion[27] of dicobalt-protected alkyne **55**. Then, the generation of cycloheptyne by deprotection with trimethylamine *N*-oxide and SPAAC reaction with benzyl azide took place to afford triazole **57** in moderate yield without damaging the ethynyl group. Bis(triazole) **58** was synthesized by the CuAAC reaction of **57**.

Based on the photo-triggered click chemistry developed by Popik, Boons, and coworkers,[28a] bis(triazole) synthesis by sequential SPAAC reactions was accomplished using platform **59** by Popik *et al.* in 2014 (Fig. 8B).[28b] Indeed, the first SPAAC reaction of diyne **59** followed by photoirradiated removal of carbon monoxide to generate dibenzo-fused cyclooctyne and a subsequent SPAAC reaction efficiently provided bis(triazole) **60**.

In 2010, the double-click reaction of Sondheimer–Wang diyne **61** [29] was accomplished by Hosoya, Kii, and coworkers (Fig. 9A).[30] The double-click reaction served in the chemical modification of azido proteins with diyne **61** and azides, but selective bis(triazole) synthesis was not easy.[31] In 2016, Popik *et al.* succeeded in a selective bis(triazole) synthesis *via* the mono-cyclopropene formation of diyne **61** followed by SPAAC reaction with butyl azide and a further SPAAC reaction with benzyl azide through the generation of a cycloalkyne moiety (Fig. 9B).[28c]

Bis(triazole) synthesis and a thiol–ene reaction using platform **65** enabled the assembly of three modules by reliable conjugation methods (Fig. 10).[32] In 2012, Beal and coworkers developed platform **65** with cyclooctyne and terminal alkyne moieties and an acetylthio group. Sequential SPAAC and CuAAC reactions efficiently yielded bis(triazole) **67**. Then, removal of the acetyl group followed by a thiol–ene reaction using a maleimide conjugated with bovine serum albumin (BSA) resulted in the dual-modification of the BSA protein.

Multi(triazole) syntheses using platforms with both azido and alkyne moieties

Platform compounds bearing both azido and alkyne moieties have also served in the preparation of bis(triazole)s and tris(tri-

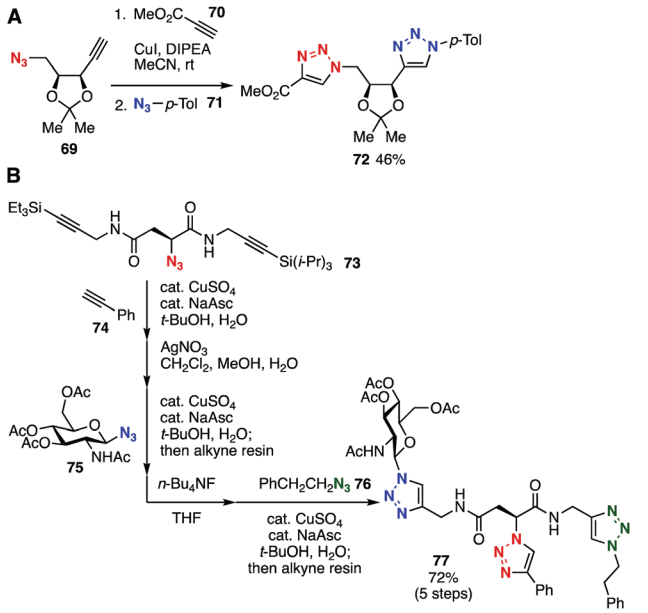

Fig. 11 Bis(triazole) syntheses using platforms **69** and **73**. (A) Kalippan's work. (B) Aucagne's work.

azole)s (Fig. 11). For example, Kaliappan *et al.* reported that the CuAAC reaction of platform **69** with methyl propiolate (**70**) and a following CuAAC reaction with *p*-tolyl azide (**71**) furnished bis(triazole) **72** in moderate yield by virtue of the higher clickability of methyl propiolate than that of the ethynyl group of platform **69** (Fig. 11A).[33] Platform **73**, with both an azido group and TES- and TIPS-protected alkyne moieties, was developed by Aucagne and coworkers in 2009 (Fig. 11B).[34] Indeed, an elegant 5-step transformation was achieved through a first CuAAC with alkyne **74**, TES-selective desilylprotonation with silver nitrate, a second CuAAC with azide **75**, TIPS-selective desilylprotonation by TBAF, and a third CuAAC with azide **76**.

In 2015, Workentin, Gilroy, and coworkers developed platform **80** bearing an azide and dicobalt-protected cyclooctyne moieties (Fig. 12A).[35] The protection of bicyclononyne **78** with dicobalt octacarbonyl successfully proceeded to afford quantitative amounts of **79**, enabling the formation of carbamate **80** using 2-azidoethylamine without an SPAAC reaction. Subsequent a CuAAC reaction using the azido group followed by removal of the dicobalt moiety and a further SPAAC reaction furnished bis(triazole) **83**.

Platform compound **84** with an azido group and alkyne precursor moiety has been developed by Wright, Couty, and coworkers (Fig. 12B).[36] Indeed, the CuAAC reaction of platform **84** with alkyne **74** followed by alkyne formation by DIC took place efficiently to provide **86**, which reacted with azide **87** catalyzed by copper to afford bis(triazole) **88** in excellent yield.

Recently, we reported bis(triazole) syntheses using platform compounds with an azido group and an aryne precursor moiety (Fig. 13). In 2015, platform compound **89** bearing both an azido group and an *o*-silylaryl triflate moiety for aryne generation was prepared through Ir-catalyzed C–H borylation[37,38] of

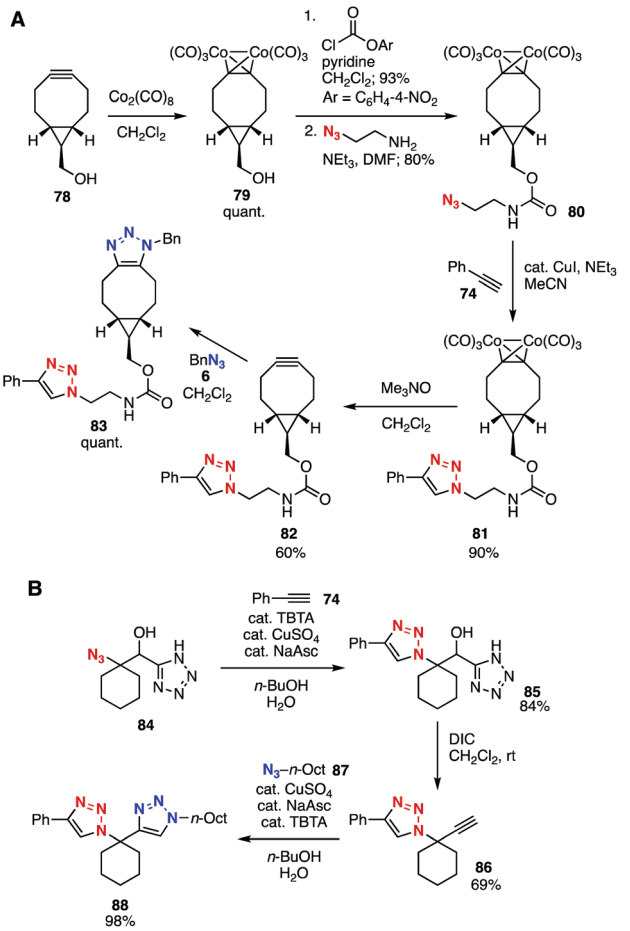

Fig. 12 Bis(triazole) syntheses using platforms **80** and **84**. (A) Gilroy and Workentin's work. (B) Wright and Couty's work. DIC = diisopropylcarbodiimide.

o-silylaryl triflate and following deborylazidation (Fig. 13A).[39a] Since aliphatic azide **6** showed higher reactivity than azide **89** in cycloaddition with aryne intermediate **B**, bis(triazole) **91** was efficiently prepared by azide–aryne cycloaddition and subsequent CuAAC with alkyne **90**. Platform **92** with both an azido group and an *o*-iodoaryl triflate moiety was also developed in 2016 (Fig. 13B).[39b] For instance, a CuAAC reaction with terminal alkynes and aryne–azide cycloaddition with a silylmethyl Grignard reagent as an activator enabled the facile synthesis of bis(triazole)s **93a–93c**, leaving various functional groups untouched.

Multi(triazole) syntheses using multi-azido platforms

Azido-type selective reactions have served in multi(triazole) syntheses. In 2012, Zhu *et al.* found efficient reactions between 2-picolyl azides and terminal alkynes in the presence of a catalytic amount of copper(II) acetate by virtue of significant enhancement in the clickability of the picolyl azido group

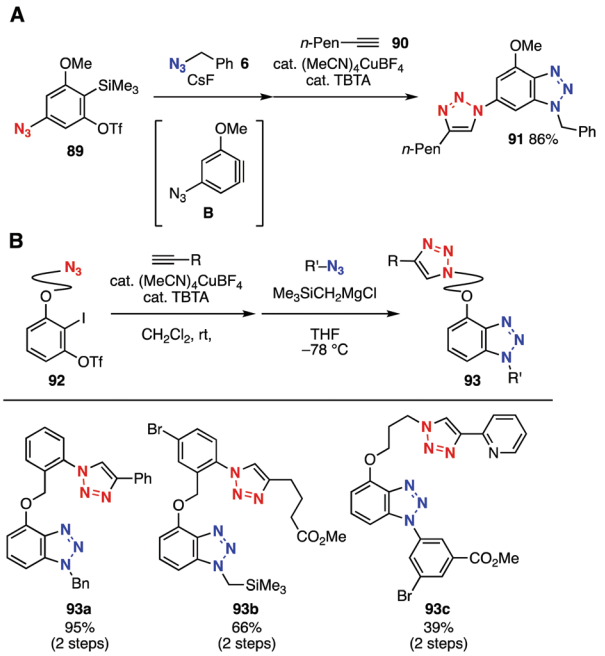

Fig. 13 Bis(triazole) syntheses using platforms **89** and **92**. (A) Platform **89** with both an azido group and an *o*-silylaryl triflate moiety. (B) Platform **92** with both an azido group and an *o*-iodoaryl triflate moiety.

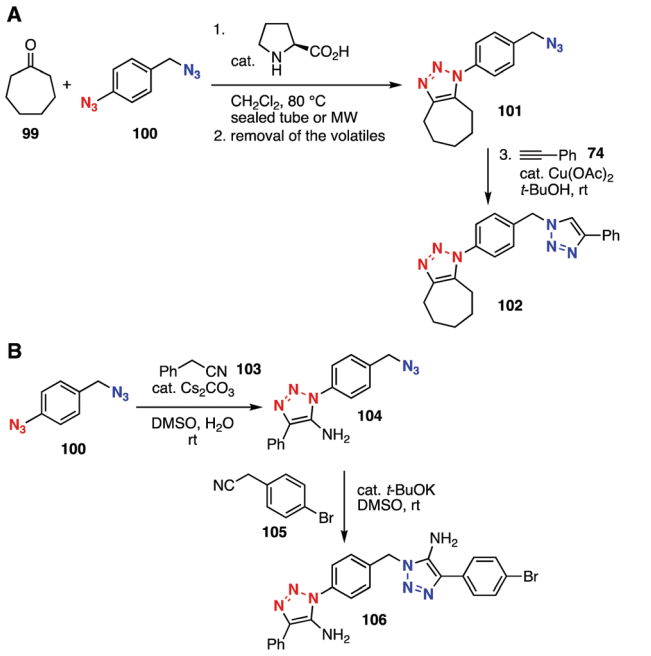

Fig. 15 Bis(triazole) syntheses using diazide **100**. (A) Belkheira, Pons, and Bressy's work. (B) Ramachary's work.

facilitated by chelation (Fig. 14).[40] On the basis of this remarkable clickability of the 2-picolyl azido group, bis(triazole) **98** was efficiently prepared from diazide **94** by Cu(II)-catalyzed azide–alkyne cycloaddition of 2-picolyl azido group with alkyne **95** followed by Cu(I)-catalyzed cycloaddition of the remaining azido group with alkyne **97**. This method allowed for the stepwise click functionalization of alkyne-installed DNA.[41]

Efficient bis(triazole) syntheses using platform compound **100** with an aromatic and an aliphatic azido group were achieved through triazole formation with nucleophilic species (Fig. 15).[42–44] In 2011, Belkheira, Pons, Bressy, and coworker succeeded in efficient bis(triazole) synthesis by an organocatalytic azide–ketone [3 + 2]-cycloaddition reaction. Indeed, triazole **102** was obtained selectively by the reaction between ketone **99** and diazide **100** in the presence of a catalytic amount of proline *via* an enamine intermediate followed by CuAAC reaction of the remaining benzylic azido group (Fig. 15A).[43] In 2015, Ramachary *et al.* reported that benzyl cyanide also reacted with azides to afford triazoles (Fig. 15B). In the case of using diazide **100**, triazole formation took place selectively at the aromatic azido group in the presence of cesium carbonate, and bis(triazole) **106** was successfully prepared in good yield by further cycloaddition with benzyl cyanide **105** using potassium *tert*-butoxide.[44]

In 2014, Delft, Bickelhaupt, and coworkers reported that electron-deficient aromatic azides showed significantly higher reactivity than aliphatic azides in the SPAAC reaction with bicyclo[6.1.0]non-4-yne (BCN).[45] On the other hand, the clickability of aliphatic azides was higher than that of aromatic azides in the SPAAC reaction with dibenzo-fused azacyclooctyne. These SPAAC reactions enabled selective bis(triazole) formation using diazide **108** with two-types of cyclooctynes **107** and **109**, showcasing the labeling of BCN-installed protein **107** with fluorescent cyclooctyne **109** (Fig. 16).

Bulky tertiary azides also served in selective SPAAC and CuAAC reactions (Fig. 17). In 2016, Koert and coworkers reported that the CuAAC reaction of diazide **113** took place selectively at the primary azido group (Fig. 17A).[46] On the basis of this selective CuAAC reaction and cyclooctyne-selective triazole formation using diyne **111**, a novel layer-by-layer method was developed. Bis(triazole) synthesis using diazide **116** through two SPAAC reactions was realized by Bickelhaupt, Mikula, and coworkers (Fig. 17B).[47] Indeed, the selective SPAAC reaction of diazide **116** at the primary azido group using dibenzo-fused cyclooctyne **117** and a subsequent SPAAC reaction at the remaining bulky azido group with BCN **118** provided bis(triazole) **119** in a quantitative amount.

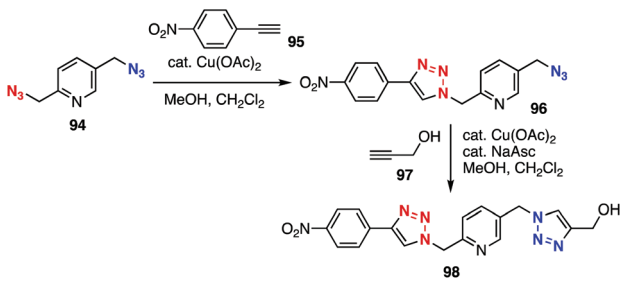

Fig. 14 Bis(triazole) synthesis using diazide **94**.

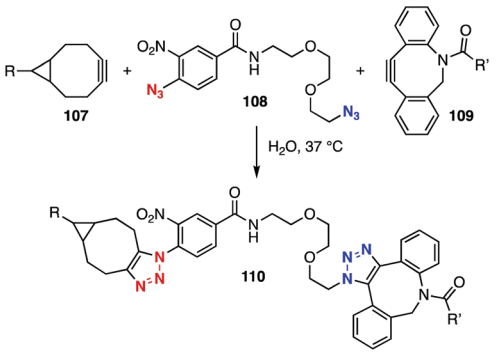

Fig. 16 Labeling of BCN-installed protein **107** using diazide **108** and fluorescent cyclooctyne **109**.

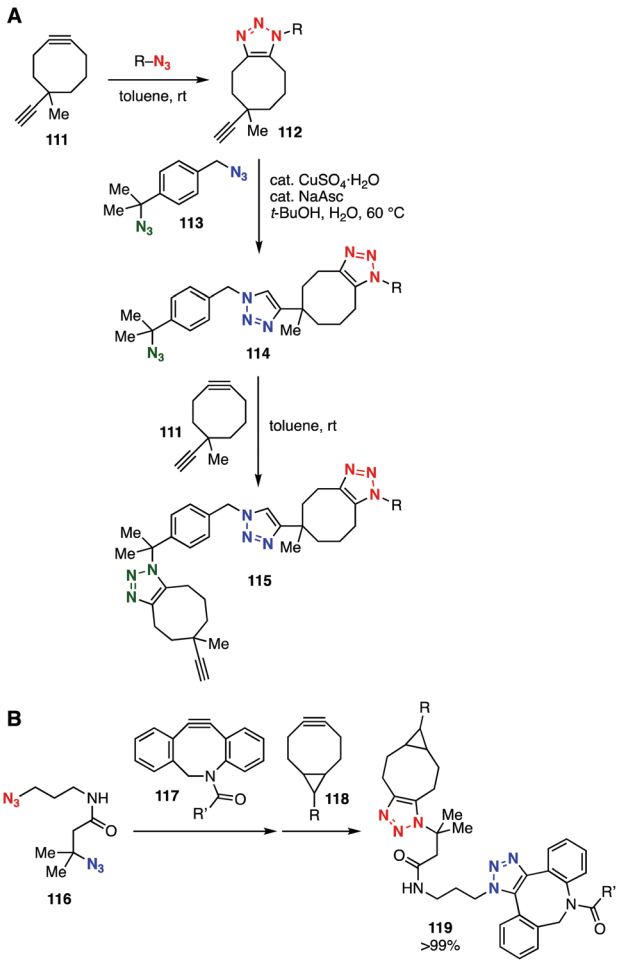

Fig. 17 Bis(triazole) syntheses using diazides **113** and **116**. (A) Koert's work. (B) Bickelhaupt and Mikula's work.

Remarkable clickability of doubly sterically-hindered aromatic azides also realized selective bis(triazole) formation (Fig. 18).[48] In 2011, we found that 2,6-diisopropylphenyl azide showed 76 times higher reactivity than phenyl azide in the SPAAC reaction with dibenzo-fused cyclooctynes due to the steric inhibition of resonance. Thus, a selective SPAAC reaction

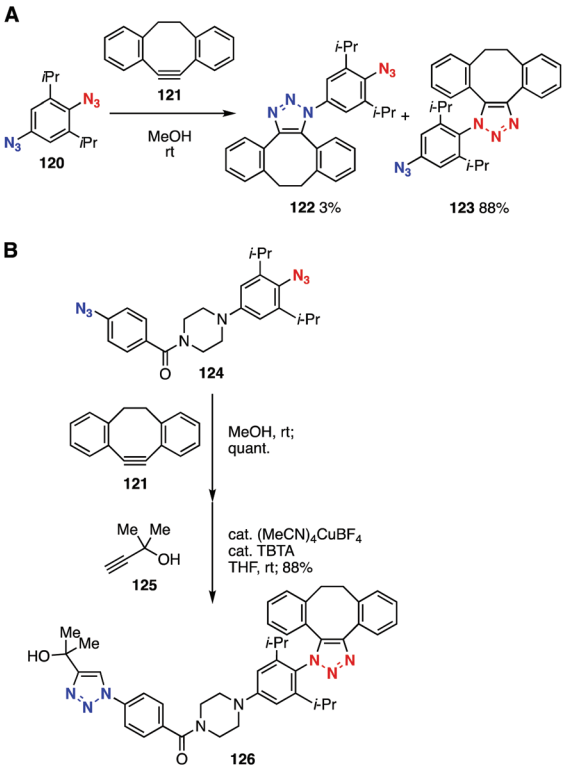

Fig. 18 Selective triazole formation of diazides **120** and **124**. (A) SPAAC reaction between diazide **120** and cyclooctyne **121**. (B) Bis(triazole) synthesis using diazide **124**.

with cyclooctyne **121** took place efficiently at the doubly sterically hindered aromatic azido group of diazide **120** (Fig. 18A).[48a] In 2018, a further enhancement in clickability was achieved using 4-amino-2,6-diisopropylphenyl azides, allowing the selective SPAAC reaction of diazide **124** with dibenzo-fused cyclooctyne **121** (Fig. 18B).[48b] Thus, a following CuAAC reaction with alkyne **125** furnished bis(triazole) **126** in excellent yield.

In 2018, we found three types of selectivities in triazole formation, namely SPAAC, Ru-catalyzed azide–alkyne cycloaddition (RuAAC),[19] and base-catalyzed triazole formation with 1,3-dicarbonyl compounds,[42c] by competitive experiments using an equimolar mixture of 2,6-diisopropylphenyl azide, phenyl azide, and benzyl azide.[49] On the basis of these findings, we succeeded in a consecutive tris(triazole) synthesis using triazide platform **127** (Fig. 19). Indeed, selective base-catalyzed triazole formation with 1,3-diketone **128** at the unhindered aromatic azido group followed by selective RuAAC with alkyne **125** at the benzylic azido group and SPAAC reaction with cyclooctyne **121** at the remaining 2,6-diisopropylphenyl azido group efficiently provided tris(triazole) **129a** (Fig. 19A). Triple-triazole formation was also achieved by SPAAC reaction with **121** at the doubly sterically-hindered aromatic azido group, aromatic azido-selective base-catalyzed cycloaddition with diketone **128**, and CuAAC reaction with alkyne **74**. A trifunctional chemical probe **129c** as a dual-label-

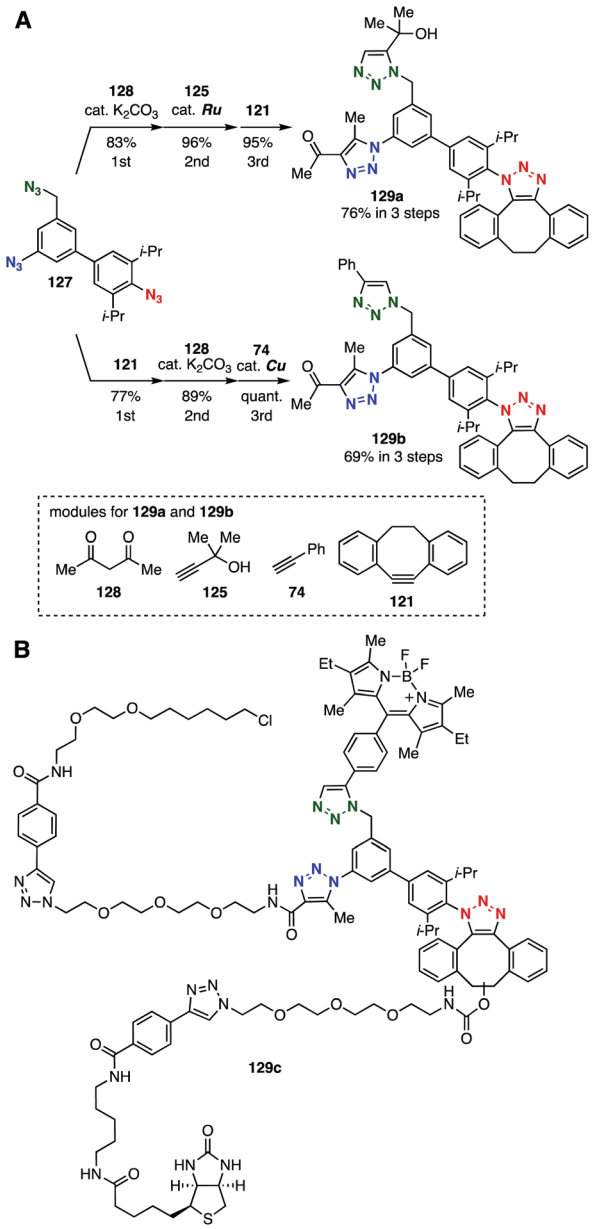

Fig. 19 Tris(triazole) synthesis using triazide **127**. (A) Synthesis of **129a** and **129b**. (B) Dual labeling ligand **129c**.

ing ligand was efficiently developed by assembling three modules onto triazide platform **127** in three steps (Fig. 19B).

A transient protection method realized selective triazole formation using diazide platform **130** (Fig. 20).[50] In 2018, we found that azides were efficiently protected toward SPAAC and CuAAC reactions by phosphazide formation with Amphos (**131**). Since the formation of phosphazide **132** from diazide **130** selectively proceeded at the aromatic azido group by the addition of an equimolar amount of Amphos (**131**),[51] we accomplished selective SPAAC and CuAAC reactions of diazide **130** with cyclooctyne **78** and alkyne **134**, respectively, at the aliphatic azido group through deprotective removal of Amphos by elemental sulfur.

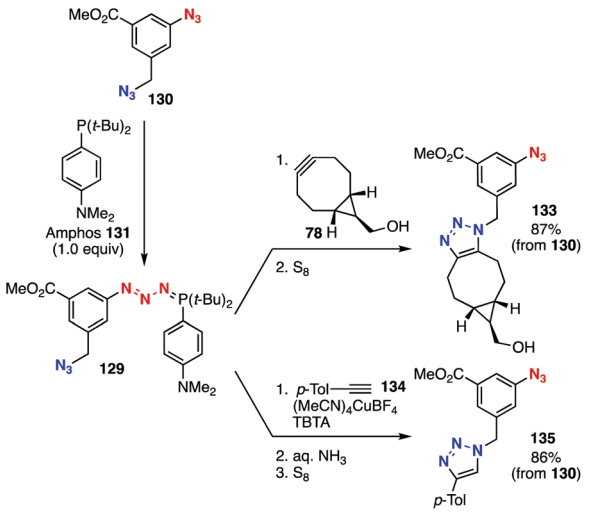

Fig. 20 Selective triazole formations through phosphazide formation.

Related sequential conjugation methods based on transformations using azides

Azides show diverse reactivities in conjugation reactions with various azidophiles. In 2015, platform compound **136** with a masked-phosphonite and terminal alkyne moieties was developed by Hackenberger and coworkers (Fig. 21).[52,53] Indeed, sequential conjugation was achieved by CuAAC reaction, removal of borane, and reaction with an azide to form a P–N bond.

Recently, Yan and Ramström's group,[54] Yi and Xi's group,[55] and our group[56] independently reported the Staudinger reaction affording robust aza-ylides. In 2018, we found that the Staudinger reaction between 2,6-dichlorophenyl azide (**141**) and triphenylphosphine (**140**) took place smoothly to provide stable aza-ylide **142** even in the presence of cyclooctyne **121**, while the SPAAC reaction of benzyl azide (**6**) with cyclooctyne **121** proceeded faster than that with phosphine **140** (Fig. 22A). We also demonstrated that aza-ylides formed by the Staudinger reaction between 2,6-dichloroaryl azides and triphenylphosphine derivatives showed significant stability in the presence of biomolecules, enabling the chemical modification of azido-incorporated proteins. We also reported a novel sequential conjugation method using diazide **146** with tri-

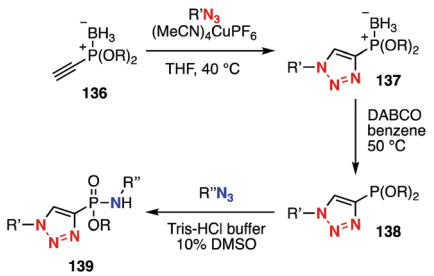

Fig. 21 Sequential conjugation using platform **136**.

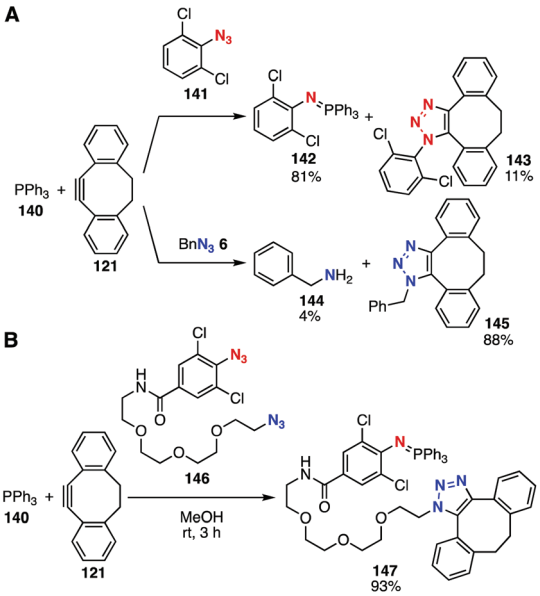

Fig. 22 Selective reactions of azides with phosphine **140** and cyclooctyne **121**. (A) Competitive experiments using an equimolar mixture of **140** and **121**. (B) Three-component reaction between diazide **146**, phosphine **140** and cyclooctyne **121**.

phenylphosphine (**140**) and cyclooctyne **121**, providing triazole **147** in high yield by three-component coupling (Fig. 22B).

In 2019, Workentin *et al.* developed a sequential conjugation method using platform **148** (Fig. 23A).[57] Indeed, the generation of cyclooctyne with an *ortho*-alkoxycarbonyl-substituted triarylphosphine moiety from **148** enabled the SPAAC reaction with azide **6** followed by a rapid Staudinger reaction with tetrafluorophenyl azide **149**, yielding stable aza-ylide **150**. In 2019, Yi, Xi, and coworkers developed diazide platform **151** (Fig. 23B).[58] Staudinger reaction of diazide **151** with triphenylphosphine derivative **152** proceeded smoothly to afford a stable aza-ylide followed by an SPAAC reaction with cyclooctyne **118**, resulting in efficient sequential conjugation.

Elegant sequential conjugations using triazide **154** have been accomplished by Tanimoto and coworkers (Fig. 23). Indeed, selective transformations of azidomethyl groups into

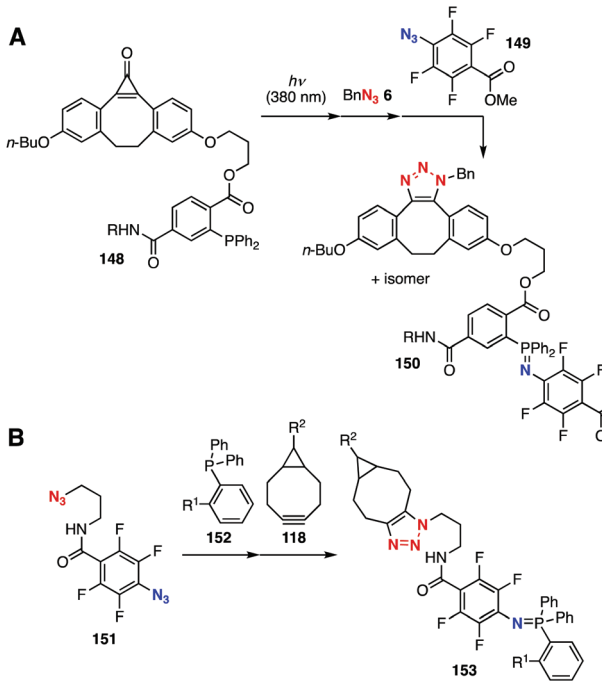

Fig. 23 Sequential conjugations through stable aza-ylide formation. (A) Workentin's work. (B) Yi and Xi's work.

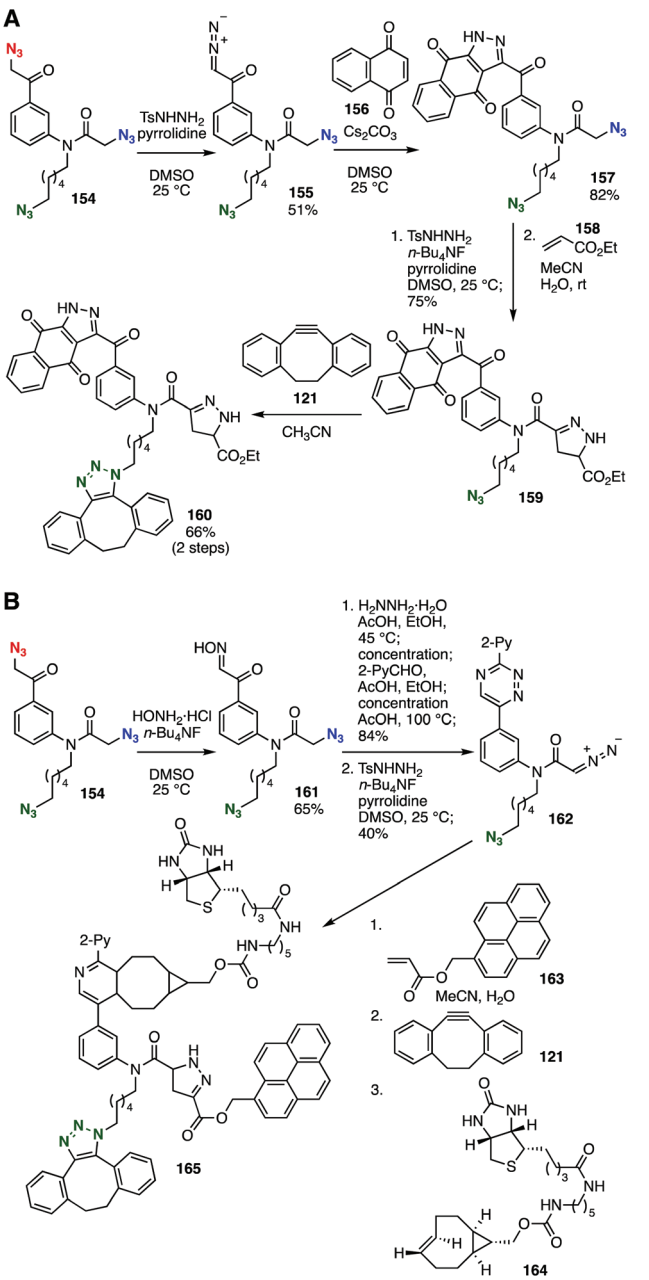

Fig. 24 Sequential three-component assembly using triazide **154**. (A) Transformations *via* two generations of a diazo group. (B) Transformations *via* oxime and diazo intermediates.

diazomethyl groups enabled sequential cycloadditions to efficiently provide **160** (Fig. 24A).[59a] Furthermore, the selective preparation of oxime was achieved by the transformation of α-azidoacetophenone, leaving two azido groups untouched (Fig. 24B).[59b] Following the synthesis of triazine **162** with 2-pyridyl aldehyde, diazomethane formation, cycloaddition with acrylic acid ester **163**, SPAAC reaction with cyclooctyne **121**, and conjugation with *trans*-cyclooctene **164** at the triazine ring realized efficient three-component assembly.

Conclusions

Complicated multi(triazole)s have been prepared by the consecutive syntheses of simple modules onto platform molecules, such as multiazides, through selective triazole formation. However, three (or more) component coupling still remains challenging due to the limited methods available to control triazole formation. Various methods to synthesize multi(triazole)s would be useful in broad disciplines, including materials science, the pharmaceutical sciences, and chemical biology.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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