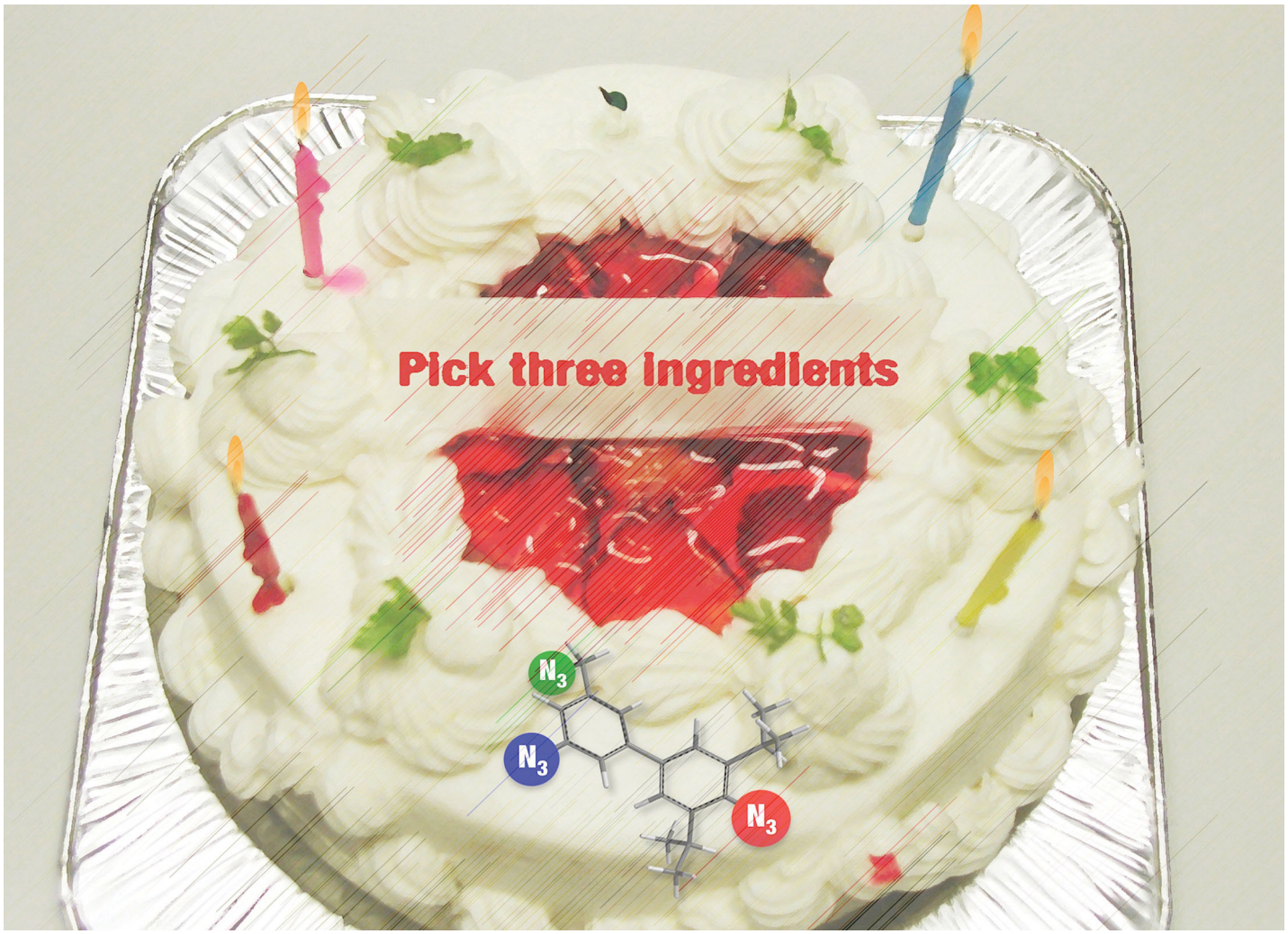

Showcasing research from the group of Professor Takamitsu Hosoya at the Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University (TMDU), Japan.

Convergent synthesis of trifunctional molecules by three sequential azido-type-selective cycloadditions

Like making an original cake by choosing your favorite 3 ingredients, trifunctional molecules are easily prepared by 3 sequential mechanistically different azido-type-selective triazole-forming reactions using a triazido platform molecule and 3 functional modules.

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Convergent synthesis of trifunctional molecules by three sequential azido-type-selective cycloadditions†

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A facile strategy for the synthesis of trifunctional molecules involving three sequential selective triazole-forming reactions is proposed. This method exploits three kinds of mechanistically different azido-type-selective cycloadditions. Three different azidophiles could be efficiently connected to a triazido platform molecule with three types of azido groups in a consecutive manner, which rendered a practical trifunctional molecule readily available.

In recent years, multifunctional molecules that are capable of playing multiple roles have received great attention in a broad range of disciplines.[1] However, these well-designed molecules are usually prepared by time-consuming, linear, and multi-step synthetic routes that include many cumbersome protection/deprotection procedures and functional group transformations (Fig. 1A). This incurs low overall yields for the desired molecules and makes it difficult to prepare libraries of related candidates for certain purposes, being a bottleneck in the development of optimal molecules. Therefore, novel strategies that make multifunctional molecules more easily accessible are increasingly sought-after.

In principle, the easiest way to prepare a multifunctional molecule is to assemble multiple monofunctional components into a single molecule. Over the last decade, click reactions,[2] such as copper(I)-catalyzed azide–alkyne cycloaddition (CuAAC)[3] and the copper-free variant, strain-promoted azide–alkyne cycloaddition (SPAAC),[4,5] have become some of the most reliable methods for connecting two molecules. Although several efficient click-like reactions other than azide–alkyne combination have been developed by many other groups and have been successfully used for conjugating two molecules,[6] synthesis of multifunctional molecules with three or more functional moieties in short steps is not easy using these methods, and only limited examples have been reported.[7] To address this issue, we conceived the idea of performing a sequential triple-click reaction for the synthesis of trifunctional molecules by advancing our double-click strategies mediated by diynes[8] (Fig. 1B).

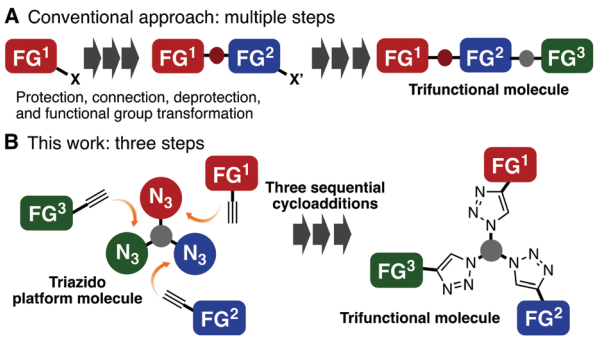

Fig. 1 Synthetic approaches to trifunctional molecules. (A) General scheme of a conventional synthesis by a linear multi-step route. (B) The proposed convergent method based on three sequential triazole formations using a triazido platform molecule. FG = functional group.

Based on azido chemistry,[9] including our previous findings on two-step photoaffinity labeling using a diazido probe and the dual reactivity of 2,6-disubstituted phenyl azides,[10] we inferred that using a suitably designed triazido platform compound, three sequential selective cycloadditions would be possible if we could exploit three different types of cycloadditions that are orthogonal to each other. This convergent triple-click assembly approach shows significant advantages over the conventional linear conjugation approaches because it proceeds *via* reliable cycloaddition reactions of azides with a broad substrate scope. Furthermore, a variety of functional azidophiles, the reaction partners of azides, are readily available. However, due to the high reactivity of azides, discrimination between three or more azido

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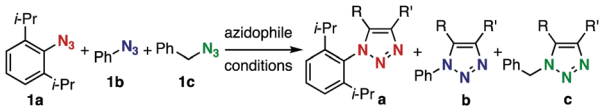
groups is a challenging issue. Herein, we demonstrate three good combinations of azido groups and azidophiles applicable to the synthesis of trifunctional molecules by three sequential azido-type-selective cycloadditions.

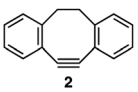
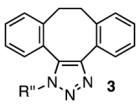
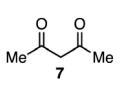
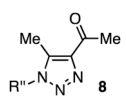
We chose three simple azides, 2,6-diisopropylphenyl azide (**1a**), phenyl azide (**1b**), and benzyl azide (**1c**), as the representatives for the three types of azides, *i.e.*, doubly sterically-hindered aromatic azides, standard aromatic azides, and aliphatic azides, respectively, to explore their orthogonal reactivity under different conditions. Using an equimolar mixture of azides **1a–c**, we performed competitive experiments for various triazole-forming reactions to find a reaction that consumed one of the three azides selectively. After extensive screening, we identified three kinds of mechanistically different cycloadditions that preferred a specific type of azide (Table 1).

In the reaction with cyclooctyne **2**, which proceeds in a concerted manner, sterically-hindered aromatic azide **1a** was preferentially converted to the corresponding triazole **3a** (entry 1). While a small amount of the benzyl azide-derived product **3c** was also obtained, only a trace amount of triazole **3b** was formed from the phenyl azide, demonstrating the high azido-type selectivity. This result is in good agreement with our previous observations, where the order of clickability for azides in reactions with a strained alkyne was strongly dominated by the distortability of the azido groups.[10f] In transition-metal-catalyzed cycloadditions with terminal alkyne **4a**, benzyl azide (**1c**) showed the highest reactivity (entries 2–4). Although the selectivity was moderate under the copper-catalyzed conditions[11] (entries 2 and 3), higher selectivity was achieved using a ruthenium catalyst,[12] affording 1,5-substituted 1,2,3-triazoles **6** (entry 4). These results indicate that the selectivity depends on the steric environment of the azido groups, with the most sterically unhindered azide **1c** reacting more favorably than the others. Furthermore, base-catalyzed cycloadditions of azides with anionic azidophiles showed high selectivity toward aromatic azides, particularly toward the more unhindered **1b** (entries 5 and 6). The anionic intermediates of these reactions, which are generated by the nucleophilic attack of the anions on the azides, are likely to be more stabilized by the aryl group, resulting in high selectivity. While the cycloaddition with the terminal alkyne **4a** under ammonium hydroxide-catalyzed conditions[13] proceeded preferentially with aromatic azide **1b** in high selectivity, complete decomposition of benzyl azide (**1c**) was also observed (entry 5), which was unsuitable for our purpose. To our delight, cycloaddition with 1,3-diketone **7**,[14] employing a weaker base such as potassium carbonate, proceeded smoothly with high **1b**-selectivity, leaving **1c** intact (entry 6).

We designed and synthesized triazide **11** bearing three types of azido groups, and examined the feasibility of this molecule as a platform molecule for three sequential selective cycloadditions (Fig. 2). Triazide **11** was easily prepared in a convergent

Table 1 Screening of conditions for azido-type-selective cycloadditions



Entry	Azidophile	Conditions	Product	Yield[a] (%)		
1	 2	**1a–c** (1.2 equiv. each) **2** (1.0 equiv.) MeOH, r.t., 1 h	 3	**3a** 85	**3b** <1	**3c** 14
2	Ph—≡ **4a**	**1a–c** (1.2 equiv. each) **4a** (1.0 equiv.) $(MeCN)_4CuBF_4$ (5 mol%) TBTA (5 mol%) DMSO, r.t., 24 h	 5	**5a** 26	**5b** 13	**5c** 57
3	**4a**	**1a–c** (1.2 equiv. each) **4a** (1.0 equiv.) IMesCuBr (5 mol%) *t*-BuOH–H_2O, r.t., 24 h	**5**	**5a** 19	**5b** 14	**5c** 65
4	**4a**	**1a–c** (1.0 equiv. each) **4a** (4.0 equiv.) $Cp^*Ru(PPh_3)_2Cl$ (8 mol%) Benzene, r.t., 24 h	 6	**6a** 0	**6b** 17	**6c** 83
5	**4a**	**1a–c** (1.2 equiv. each) **4a** (1.0 equiv.) Me_4NOH (10 mol%) DMSO, r.t., 48 h	**6**	**6a** 7	**6b** 72	**6c**[b] 0
6	 7	**1a–c** (1.0 equiv. each) **7** (1.0 equiv.) K_2CO_3 (18 mol%) DMF, r.t., 24 h	 8	**8a** 7	**8b** 84	**8c** 0

[a] Yields were determined by ^1H NMR analysis. [b] Benzyl azide (**1c**) was completely consumed.

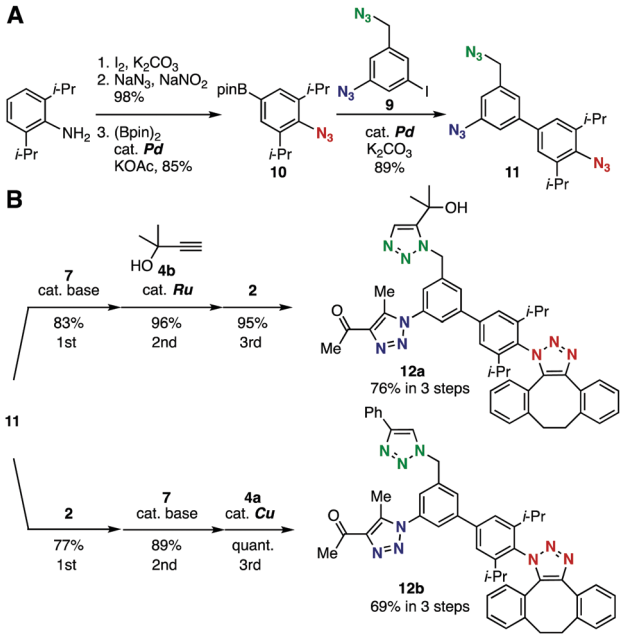


Fig. 2 Three sequential azido-type-selective cycloadditions. (A) Synthesis of triazido platform molecule **11**. (B) Examples of the three-step synthesis of tristriazoles in different orders. See the ESI† for details.

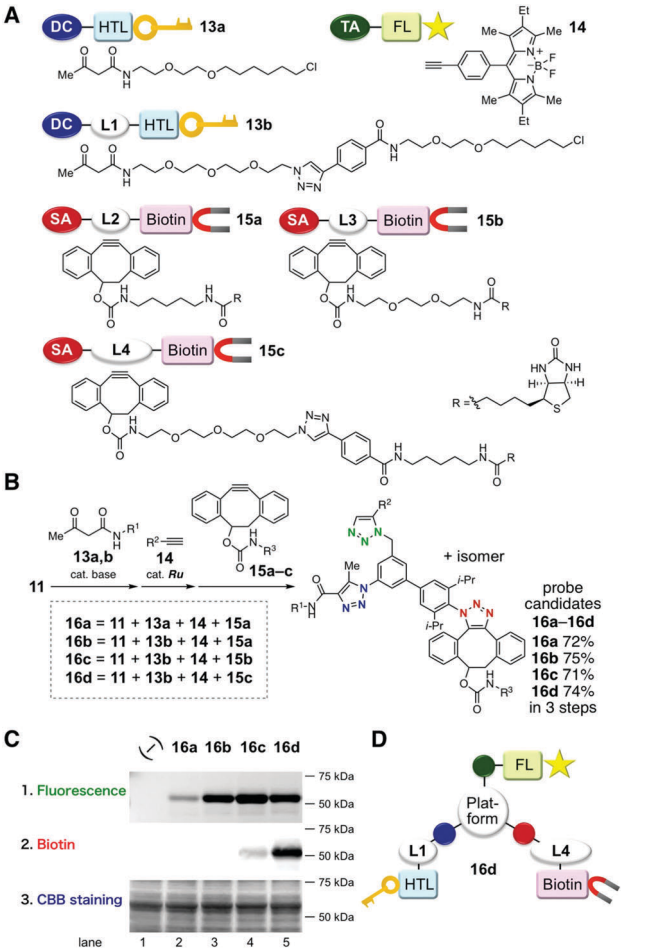


Fig. 3 Synthesis of trifunctional probe candidates and functional evaluations. (A) Structures and schematic diagrams of azidophilic modules. (B) Synthesis of probe candidates **16a–d**. (C) SDS-PAGE analysis of the GST-HaloTag proteins labeled with trifunctional probe candidates **16a–d**. The gels were (1) scanned with a fluorescence image analyzer, and then (2) analyzed by western blot, or (3) stained with CBB. (D) The schematic diagram of trifunctional probe **16d**. DC, HTL, TA, FL, SA, and L1–L4 indicate 1,3-dicarbonyl, HaloTag ligand, terminal alkyne, fluorescent, strained alkyne, and linker moieties, respectively.

manner by the Suzuki–Miyaura cross-coupling reaction between diazide **9** bearing an iodo group[10g] and readily synthesized arylboronic acid pinacol ester **10** (Fig. 2A). The three sequential cycloadditions of triazide **11** with three kinds of azidophiles proceeded smoothly to afford the desired tristriazole compound in high yield (Fig. 2B). For example, cycloaddition of triazide **11** with 1,3-diketone **7** catalyzed by potassium carbonate proceeded predominantly at the aromatic azido group to provide the monotriazole product in high yield. Subsequent ruthenium-catalyzed cycloaddition of the monotriazole bearing two unreacted azido groups with terminal alkyne **4b** proceeded selectively at the aliphatic azido moiety to afford the bistriazole in excellent yield. Finally, the remaining sterically-hindered azido group reacted efficiently with strained alkyne **2**, affording tristriazole **12a**. In this scheme, the three sequential cycloadditions were achieved in 76% overall yield. Furthermore, the order of the cycloadditions was exchangeable; tristriazole **12b** was prepared in 69% overall yield from triazide **11** by the three sequential reactions, *i.e.*, the strain-promoted click reaction with **2**, base-catalyzed cycloaddition with **7**, and copper-catalyzed cycloaddition with **4a**.

Three sequential azido-type-selective cycloadditions of triazide **11** with three functional modules enabled to develop a practical trifunctional probe in a short period of time. We demonstrated the utility of the strategy by preparing HaloTag ligands bearing a fluorescent BODIPY moiety and a biotinyl group (Fig. 3).[15] Initially, we prepared simple azidophilic modules that contained the respective functional groups with different kinds of linkers. These included the two β-ketoamide-type HaloTag ligands **13a** and **13b**, terminal alkyne-type BODIPY derivative **14**, and three kinds of biotinylated cyclooctyne derivatives **15a–c** (Fig. 3A). Using these monofunctional modules, we synthesized four kinds of trifunctional tristriazoles **16a–d** from triazide **11** by (1) base-catalyzed cycloaddition with **13**, (2) ruthenium-catalyzed cycloaddition with **14**, and (3) strain-promoted click reaction with **15** (Fig. 3B). In all of these cases, the three sequential cycloadditions proceeded efficiently without affecting the functional groups to afford the trifunctional tristriazoles **16a–d** in 71–75% overall yields. The performances of the synthesized probe candidates **16a–d** were evaluated. Each of them was added to a cell lysate that contained a GST-fused HaloTag protein (59 kDa) to ligate the probe candidates, followed by SDS-PAGE analysis. The gels were analyzed by fluorescence detection, and then analyzed by western blot with horseradish peroxidase (HRP)-conjugated streptavidin or Coomassie brilliant blue (CBB)-staining (Fig. 3C; ESI,† Fig. S1 and S2). The intensity of the fluorescent bands reflected the efficiency of the covalent binding of the probe candidates to the HaloTag protein. The weak fluorescence signal observed when using **16a** without a linker in its HaloTag ligand moiety indicates that the covalent bond formation between **16a** and the HaloTag protein was prevented, possibly due to the bulkiness of the platform core

structure (Fig. 3C, lane 2). This is supported by the remarkable enhancement of the fluorescence intensity observed when **16b–d** bearing a triethyleneoxy linker were used (Fig. 3C, lanes 3–5). Western blot analysis using HRP-streptavidin also indicates that a linker between the biotinyl group and the platform core with a sufficient length is critical for efficient recognition of the biotinylated protein by streptavidin (Fig. 3C, lanes 3–5). Resultantly, among the four probe candidates examined, **16d** showed the best performance for dual modification of the HaloTag protein with BODIPY and biotin (Fig. 3D).[16]

In summary, we have demonstrated that a triazido platform molecule bearing three types of sterically and electronically different azido groups is useful for the synthesis of trifunctional molecules by three sequential azido-type-selective cycloadditions. Although ruthenium- or base-catalyzed cycloadditions do not precisely meet the criteria of click chemistry, the broad scope of the cycloaddition reactions would allow for the facile synthesis of diverse trifunctional molecules. Further studies to enhance the selectivity of each triazole-forming reaction and applications to develop a variety of practical multifunctional molecules are currently underway in our laboratory.

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Conflicts of interest

The authors declare no conflicts of interest.

Notes and references

1 (*a*) A. Louie, *Chem. Rev.*, 2010, **110**, 3146–3195; (*b*) M. Rudin and R. Weissleder, *Nat. Rev. Drug Discovery*, 2003, **2**, 123–131; (*c*) R. H. Kimura, Z. Miao, Z. Cheng, S. S. Gambhir and J. R. Cochran, *Bioconjugate Chem.*, 2010, **21**, 436–444; (*d*) G. Bottari, Od. Trukhina, M. Ince and T. Torres, *Coord. Chem. Rev.*, 2012, **256**, 2453–2477.

2 (*a*) H. C. Kolb, M. G. Finn and K. B. Sharpless, *Angew. Chem., Int. Ed.*, 2001, **40**, 2004–2021; (*b*) C. S. McKay and M. G. Finn, *Chem. Biol.*, 2014, **21**, 1075–1101; (*c*) J. Lahann, *Click Chemistry for Biotechnology and Materials Science*, John Wiley & Sons, West Sussex, 2009.

3 (*a*) C. W. Tornøe, C. Christensen and M. Meldal, *J. Org. Chem.*, 2002, **67**, 3057–3064; (*b*) V. V. Rostovtsev, L. G. Green, V. V. Fokin and K. B. Sharpless, *Angew. Chem., Int. Ed.*, 2002, **41**, 2596–2599; (*c*) M. Meldal and C. W. Tornøe, *Chem. Rev.*, 2008, **108**, 2952–3015.

4 (*a*) E. M. Sletten and C. R. Bertozzi, *Angew. Chem., Int. Ed.*, 2009, **48**, 6974–6998; (*b*) M. F. Debets, C. W. J. van der Doelen, F. P. J. T. Rutjes and F. L. van Delft, *ChemBioChem*, 2010, **11**, 1168–1184; (*c*) J. C. Jewett and C. R. Bertozzi, *Chem. Soc. Rev.*, 2010, **39**, 1272–1279; (*d*) J. Dommerholt, F. P. J. T. Rutjes and F. L. van Delft, *Top. Curr. Chem.*, 2016, **374**, 16.

5 (*a*) G. Wittig and A. Krebs, *Chem. Ber.*, 1961, **94**, 3260–3275; (*b*) N. J. Agard, J. A. Prescher and C. R. Bertozzi, *J. Am. Chem. Soc.*, 2004, **126**, 15046–15047; (*c*) S. T. Laughlin, J. M. Baskin, S. L. Amacher and C. R. Bertozzi, *Science*, 2008, **320**, 664–667; (*d*) J. A. Codelli, J. M. Baskin, N. J. Agard and C. R. Bertozzi, *J. Am. Chem. Soc.*, 2008, **130**, 11486–11493; (*e*) X. Ning, J. Guo, M. A. Wolfert and G.-J. Boons, *Angew. Chem., Int. Ed.*, 2008, **47**, 2253–2255; (*f*) A. A. Poloukhtine, N. E. Mbua, M. A. Wolfert, G.-J. Boons and V. V. Popik, *J. Am. Chem. Soc.*, 2009, **131**, 15769–15776; (*g*) J. C. Jewett, E. M. Sletten and C. R. Bertozzi, *J. Am. Chem. Soc.*, 2010, **132**, 3688–3690; (*h*) J. Dommerholt, S. Schmidt, R. Temming, L. J. A. Hendriks, F. P. J. T. Rutjes, J. C. M. van Hest, D. J. Lefeber, P. Friedl and F. L. van Delft, *Angew. Chem., Int. Ed.*, 2010, **49**, 9422–9425; (*i*) J. Dommerholt, O. van Rooijen, A. Borrmann, C. F. Guerra, F. M. Bickelhaupt and F. L. van Delft, *Nat. Commun.*, 2014, **5**, 5378.

6 (*a*) A.-C. Knall and C. Slugovc, *Chem. Soc. Rev.*, 2013, **42**, 5131–5142; (*b*) C. E. Hoyle and C. N. Bowman, *Angew. Chem., Int. Ed.*, 2010, **49**, 1540–1573; (*c*) M. R. J. Vallée, L. M. Artner, J. Dernedde and C. P. R. Hackenberger, *Angew. Chem., Int. Ed.*, 2013, **52**, 9504–9508; (*d*) H. Liu, D. Audisio, L. Plougastel, E. Decuypere, D.-A. Buisson, O. Koniev, S. Kolodych, A. Wagner, M. Elhabiri, A. Krzyczmonik, S. Forsback, O. Solin, V. Gouverneur and F. Taran, *Angew. Chem., Int. Ed.*, 2016, **55**, 12073–12077.

7 (*a*) D. M. Beal and L. H. Jones, *Angew. Chem., Int. Ed.*, 2012, **51**, 6320–6326; (*b*) L. I. Willems, N. Li, B. I. Florea, M. Ruben, G. A. van der Marel and H. S. Overkleeft, *Angew. Chem., Int. Ed.*, 2012, **51**, 4431–4434; (*c*) D. M. Beal, V. E. Albrow, G. Burslem, L. Hitchen, C. Fernandes, C. Lapthorn, L. R. Roberts, M. D. Selby and L. H. Jones, *Org. Biomol. Chem.*, 2012, **10**, 548–554; (*d*) Y. Sun, X. Ma, K. Cheng, B. Wu, J. Duan, H. Chen, L. Bu, R. Zhang, X. Hu, Z. Deng, L. Xing, X. Hong and Z. Cheng, *Angew. Chem., Int. Ed.*, 2015, **54**, 5981–59842; (*e*) V. Vaněk, J. Pícha, B. Fabre, M. Buděšínský, M. Lepšík and J. Jiráček, *Eur. J. Org. Chem.*, 2015, 3689–3701; (*f*) A.-C. Knall, M. Hollauf, R. Saf and C. Slugovc, *Org. Biomol. Chem.*, 2016, **14**, 10576–10580.

8 (*a*) I. Kii, A. Shiraishi, T. Hiramatsu, T. Matsushita, H. Uekusa, S. Yoshida, M. Yamamoto, A. Kudo, M. Hagiwara and T. Hosoya, *Org. Biomol. Chem.*, 2010, **8**, 4051–4055; (*b*) S. Yoshida, Y. Hatakeyama, K. Johmoto, H. Uekusa and T. Hosoya, *J. Am. Chem. Soc.*, 2014, **136**, 13590–13593.

9 (*a*) S. Bräse, C. Gil, K. Knepper and V. Zimmermann, *Angew. Chem., Int. Ed.*, 2005, **44**, 5188–5240; (*b*) S. Bräse and K. Banert, *Organic Azides: Syntheses and Applications*, John Wiley & Sons, West Sussex, 2010.

10 (*a*) T. Hosoya, T. Hiramatsu, T. Ikemoto, M. Nakanishi, H. Aoyama, A. Hosoya, T. Iwata, K. Maruyama, M. Endo and M. Suzuki, *Org. Biomol. Chem.*, 2004, **2**, 637–641; (*b*) T. Hosoya, T. Hiramatsu, T. Ikemoto, H. Aoyama, T. Ohmae, M. Endo and M. Suzuki, *Bioorg. Med. Chem. Lett.*, 2005, **15**, 1289–1294; (*c*) T. Ikemoto, T. Hosoya, K. Takata, H. Aoyama, T. Hiramatsu, H. Onoe, M. Suzuki and M. Endo, *Diabetes*, 2009, **58**, 2802–2812; (*d*) T. Hosoya, A. Inoue, T. Hiramatsu, H. Aoyama, T. Ikemoto and M. Suzuki, *Bioorg. Med. Chem.*, 2009, **17**, 2490–2496; (*e*) R. Kohta, Y. Kotake, T. Hosoya, T. Hiramatsu, Y. Otsubo, H. Koyama, Y. Hirokane, Y. Yokoyama, H. Ikeshoji, K. Oofusa, M. Suzuki and S. Ohta, *J. Neurochem.*, 2010, **114**, 1291–1301; (*f*) S. Yoshida, A. Shiraishi, K. Kanno, T. Matsushita, K. Johmoto, H. Uekusa and T. Hosoya, *Sci. Rep.*, 2011, **1**, 82; (*g*) S. Yoshida, Y. Misawa and T. Hosoya, *Eur. J. Org. Chem.*, 2014, 3991–3995.

11 (*a*) T. R. Chan, R. Hilgraf, K. B. Sharpless and V. V. Fokin, *Org. Lett.*, 2004, **6**, 2853–2855; (*b*) S. Díez-González, E. C. Escudero-Adán, J. Benet-Buchholz, E. D. Stevens, A. M. Z. Slawin and S. P. Nolan, *Dalton Trans.*, 2010, **39**, 7595–7606.

12 (*a*) L. Zhang, X. Chen, P. Xue, H. H. Y. Sun, I. D. Williams, K. B. Sharpless, V. V. Fokin and G. Jia, *J. Am. Chem. Soc.*, 2005, **127**, 15998–15999; (*b*) B. C. Boren, S. Narayan, L. K. Rasmussen, L. Zhang, H. Zhao, Z. Lin, G. Jia and V. V. Fokin, *J. Am. Chem. Soc.*, 2008, **130**, 8923–8930.

13 S. W. Kwok, J. R. Fotsing, R. J. Fraser, V. O. Rodionov and V. V. Fokin, *Org. Lett.*, 2010, **12**, 4217–4219.

14 E. P. J. Ng, Y.-F. Wang, B. W.-Q. Hui, G. Lapointe and S. Chiba, *Tetrahedron*, 2011, **67**, 7728–7737.

15 C. G. England, H. Luo and W. Cai, *Bioconjugate Chem.*, 2015, **26**, 975–986.

16 Similarly, we prepared three bifunctional tristriazoles, in which each functional group of **16d** is replaced with a functionless dummy group, clearly demonstrating that each functional group of **16d** worked practically. See the ESI† for the details.

