Selective strain-promoted azide–alkyne cycloadditions through transient protection of bicyclo[6.1.0]nonynes with silver or gold†

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Complexation of bicyclo[6.1.0]nonynes with a cationic silver or gold salt results in protection from a click reaction with azides. The cycloalkyne protection using the silver or gold salt enables selective strain-promoted azide–alkyne cycloadditions of diynes keeping the bicyclo[6.1.0]nonyne moiety unreacted.

Click reactions, such as strain-promoted azide–alkyne cycloaddition (SPAAC) using cycloalkynes, have been used for reliable molecular conjugation in a broad range of research fields including materials chemistry, pharmaceutical sciences, and chemical biology. 1–7 In particular, remarkable reactivities of bicyclo[6.1.0]nonynes (BCNs) realized catalyst-free functionalizations by reaction with a number of ynophiles such as azides, nitrones, nitrile oxides, tetrazines, triazines, syndiones, thiophene S,S-dioxides, and so on. 5 In the course of our studies on click chemistry, 6 we recently developed a transient protection method for cycloalkynes involving BCNs from the SPAAC reaction by complexation with (MeCN)4CuBF4, which was easily deprotected by treatment with chelators (Fig. 1A). 7 A wide variety of functionalized cycloalkynes were synthesized using an azide-to-cycloalkyne switching approach by the protection of a cycloalkyne having a terminal alkyn moiety with (MeCN)4CuBF4 followed by copper-catalyzed azide–alkyne cycloaddition (CuAAC) with functionalized azides and subsequent deprotection with an aqueous solution of disodium ethylenediaminetetraacetate (EDTA·2Na) (Fig. 1B). 8 We herein disclose a selective protection method for BCNs from other cycloalkynes by silver or gold complexation, realizing facile synthesis of functionalized BCNs by selective SPAAC reactions using diyne platforms leaving the BCN moiety intact (Fig. 1C).

With previous reports of cyclooctyne–metal complexes in mind, 9,10 we envisioned that silver and gold salts can protect cycloalkynes from the SPAAC reaction by complexation. Thus, we screened silver and gold salts in the complexation with BCN 1a in CDCl3 followed by the addition of azide 2a (Table 1). As a result, a variety of silver salts decreased the yield of triazole 3a along with the recovery of azide 2a and precipitate formation of 1a–metal complexes (entries 2–7), while azide 2a was completely consumed when the reaction was performed without any metal salt (entry 1). In particular, the examination using AgBF4 resulted in no triazole formation and almost complete recovery of azide 2a (entry 7), clearly showing that the cationic silver salt completely prevented the SPAAC reaction. Furthermore, AuBF4 prepared from AuCl and AgBF4 also realized the protection of cycloalkyne 1a from the SPAAC reaction (entry 8).

Deprotection of BCN–metal complexes 1a–AgBF4 and 1a–AuBF4 was achieved by proper choice of the silver and gold salts, and chelators (Table 2). For example, the treatment of 1a–AgBF4 with

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Fig. 1 Protection methods of cycloalkynes. (A) Protection with copper. (B) The azide-to-cycloalkyne switching approach. (C) This work. FG = functional group.
an aqueous solution of ammonia or EDTA-2Na resulted in the recovery of 1a in low to moderate yields along with the decomposition of cycloalkyne 1a (entries 1 and 2). In sharp contrast, the deprotection of 1a–AgBF₄ with a solid-phase metal scavenger SiliaMetS Thiourea proceeded efficiently (entry 3). In particular, increasing the amount of SiliaMetS Thiourea improved the recovery yield (entry 4). The deprotection of 1a–AgBF₄ with a polystyrene-conjugated phosphine also took place smoothly (entry 5). Among a variety of conditions screened for the deprotection of 1a–AuBF₄ (entries 6–10), we succeeded in the efficient deprotection with SiliaMetS Thiourea in THF (entry 9).

Attempts to protect dibenzo-fused cyclooctyne (DIBO) 1b, 1c and 4,8-diazacyclononyne (DACN) 1c with silver or gold revealed their decreasing coordination strength compared to the complex between BCN 1a and silver or gold (Table 3). When (MeCN)₄CuBF₄ was used, cycloalkynes 1b and 1c were successfully protected resulting in the recovery of azide 2a in excellent yields (entries 1 and 4). On the other hand, the treatment of cycloalkynes 1b and 1c with AgBF₄ or AuBF₄ in CDCl₃ followed by the addition of azide 2a furnished triazoles 3b and 3c in low to high yields (entries 2, 3, 5, and 6). Of note, the coordination strength between DACN 1c and silver or gold was significantly weak, leading to the recovery of azide 2a in low yields. These results clearly show the contrasting differences in the complexation of cycloalkynes with copper, silver, and gold depending on the electronic nature and ring strain of cycloalkynes.

Complexation of BCN 1a with AgBF₄ served as protection from [2+3] cycloaddition with nitrone 4 (Table 4). While BCN 1a smoothly reacted with nitrone 4 without the protection providing dihydroisoxazole 5 in high yield (entry 1), complexation of BCN 1a with transition metals prevented the cycloalkyne–nitrone cycloaddition (entries 2–4). However, nitrone 4 was consumed completely by decomposition when using (MeCN)₄CuBF₄ or AuBF₄ (entries 2 and 4). On the other hand, the complexation of 1a with AgBF₄ realized the protection of BCN 1a along with the recovery of nitrone 4 in good yield (entry 3).

Table 1 Screening of metal salts for the protection of cycloalkyne 1a from the formation of 3a

| Entry | Metal salt | 3a (%) | 2a (%) |
|-------|------------|--------|--------|
| 1     | None       | 97     | 0      |
| 2     | AgCl       | 92     | 7      |
| 3     | AgF        | 80     | 16     |
| 4     | AgOAc      | 91     | 9      |
| 5     | AgSCN      | 72     | 28     |
| 6     | AgNO₃      | 36     | 72     |
| 7     | AgBF₄      | 0      | 97     |
| 8     | AuBF₄      | 0      | 94     |

Table 2 Screening of chelators for the deprotection of BCN–metal salts

| Entry | Metal salt | Chelator | 1a (%) |
|-------|------------|----------|--------|
| 1     | AgBF₄      | 15 M aq. NH₃ | 55     |
| 2     | AgBF₄      | 0.1 M aq. EDTA-2Na | 15     |
| 3     | AgBF₄      | SiliaMetS Thiourea | 69     |
| 4     | AgBF₄      | (PS-TPP) | 82     |
| 5     | AgBF₄      | SiliaMetS Thiourea | 0      |
| 6     | AuBF₄      | 0.1 M aq. EDTA-2Na | 24     |
| 7     | AuBF₄      | SiliaMetS Thiourea | 58     |
| 8     | AuBF₄      | (PS-TPP) | 83     |
| 9     | AuBF₄      | (PS-TPP) | 0      |

Table 3 Screening of metal salts for the protection of cycloalkynes 1b and 1c

| Entry | Metal salt | 3b (%) | 2a (%) |
|-------|------------|--------|--------|
| 1     | (MeCN)₄CuBF₄ | 3b     | 0      |
| 2     | AgBF₄      | 3b, 66 | 33     |
| 3     | AuBF₄      | 3b, 22 | 54     |
| 4     | (MeCN)₄CuBF₄ | 3c     | 0      |
| 5     | AgBF₄      | 3c, 85 | 15     |
| 6     | AuBF₄      | 3c, 67 | 19     |

Table 4 Screening of metal salts for the protection of cycloalkyne 1a from formation of 5

| Entry | Metal salt | 5 (%) | 4 (%) |
|-------|------------|-------|-------|
| 1     | None       | 93    | 0     |
| 2     | (MeCN)₄CuBF₄ | 0     | Decomposed |
| 3     | AgBF₄      | 0     | 93    |
| 4     | AuBF₄      | 0     | Decomposed |

a Yields were determined using ¹H NMR analysis. b AuBF₄ was prepared from AuCl and AgBF₄.
Table 5  Screening of metal salts for the selective protection of cycloalkynes

| Entry | 1b or 1c | Metal salt | 3a (a) (%) | 3b or 3c (a) (%) | 2a (a) (%) |
|-------|----------|------------|------------|-----------------|------------|
| 1     | 1b       | None       | 43         | 3b, 53          | 0          |
| 2     | 1b       | (MeCN)4CuBF4 | 5         | 3b, 58          | 36         |
| 3     | 1b       | AgBF4      | 17         | 3b, 74          | 0          |
| 4     | 1b       | AuBF4      | 0          | 3b, 92          | 1          |
| 5     | 1c       | None       | 60         | 3c, 37          | 0          |
| 6     | 1c       | (MeCN)4CuBF4 | 0         | 3c, 68          | 28         |
| 7     | 1c       | AgBF4      | 0          | 3c, 99          | 0          |
| 8     | 1c       | AuBF4      | 0          | 3c, 74          | 18         |

(a) Yields were determined using 3H NMR analysis. AuBF4 was prepared from AuCl and AgBF4.

Complementation with silver or gold realized the selective protection of BCN in the presence of other cycloalkynes (Table 5). An equimolar mixture between BCN 1a and DIBO 1b (1.0 equiv. each) smoothly reacted with benzyl azide (2a) (1.0 equiv.) without protection to furnish a ca. 1:1.2 mixture of triazoles 3a and 3b (entry 1). The pretreatment of cycloalkynes 1a and 1b (1.0 equiv. each) with 1.0 equiv. of metal salts drastically changed the ratio of 3a to 3b (entries 2–4). In particular, we succeeded in the selective SPAAC reaction of DIBO 1b affording triazole 3b in high yield when using AuBF4 by virtue of the BCN-selective protection (entry 4). The SPAAC reaction of an equimolar mixture of BCN 1a and DBCN 1c with azide 2a also proceeded efficiently to provide a ca. 1.6:1 mixture of triazoles 3a and 3c (entry 5). The pretreatment of cycloalkynes 1a and 1c with metal salts prevented the formation of triazole 3a (entries 6–8). In particular, treatment of cycloalkynes 1a and 1c (1.0 equiv. each) with AgBF4 (1.0 equiv.) followed by the addition of azide 2a exclusively furnished triazole 3c in excellent yield (entry 7).

The synthetic utility of the DIBO- and DBCN-selective SPAAC reactions in the presence of a BCN moiety through the complementation was showcased by the selective triazole formation of diyne 6 and 8 keeping the BCN moiety unreacted (Fig. 2). Indeed, the pretreatment of diyne 6 with AuBF4 followed by the addition of azide 2b and subsequent removal of the gold salt with SiliaMetS Thiourea provided triazole 7 in good yield by the SPAAC reaction at the DIBO moiety without reacting the BCN moiety (Fig. 2A). Furthermore, we also achieved the DBCN-selective triazole formation of diyne 8 by complexation with AgBF4, addition of azide 2b, and the removal of the silver salt with SiliaMetS Thiourea (Fig. 2B). Since the remaining BCN moiety contributes significantly to the catalyst-free click conjugation with various ynophiles in materials chemistry and chemical biology, the DIBO- and DBCN-selective triazole formation of diyne allowed for sequential conjugations of a broad range of functional molecules.

In conclusion, we have developed an efficient method for the transient protection of BCNs by complementation with silver or gold, enabling DIBO- or DACN-selective triazole formation. The selective SPAAC reactions realized the preparation of functionalized BCNs by the selective click conjugation of diyne. Further studies of cycloalkyne–metal complexes involving detailed solvent effects, protection from various ynophiles, and applications of sequential triazole formations of diyne are now in progress.

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Conflicts of interest

There are no conflicts to declare.

Notes and references

1 (a) H. C. Kolb, M. G. Finn and K. B. Sharpless, *Angew. Chem., Int. Ed.*, 2001, 40, 2004; (b) C. S. McKay and M. G. Finn, *Chem. Biol.*, 2014, 21, 1075; (c) J. Lahann, *Click Chemistry for Biotechnology and Materials Science*, John Wiley & Sons, West Sussex, 2009.
2 M. Meldal and C. W. Tornøe, *Chem. Rev.*, 2008, 108, 2952.
3 (a) M. F. Debets, C. W. J. van der Doelen, F. P. J. T. Rutjes and F. L. van Delft, *ChemBioChem*, 2010, 11, 1168; (b) J. C. Jewett and C. R. Bertozzi, *Chem. Soc. Rev.*, 2010, 39, 1272; (c) E. M. Sletten and C. R. Bertozzi, *Acc. Chem. Res.*, 2011, 44, 666; (d) S. Arumugam, S. V. Orski, N. E. Mbua, C. McNitt, G.-J. Boons, J. Locklin and V. V. Popik, *Pure Appl. Chem.*, 2013, 85, 1499; (e) J. Dommerholt, F. P. J. T. Rutjes and F. L. van Delft, *Top. Curr. Chem.*, 2016, 374, 16.
4. A.-C. Knall and C. Slugovec, Chem. Soc. Rev., 2013, 42, 5131.
5. Z.-J. Zheng, D. Wang, Z. Xu and L.-W. Xu, Beilstein J. Org. Chem., 2015, 11, 2557; (c) S. Yoshida, Bull. Chem. Soc. Jpn., 2018, 91, 1293; (d) S. Yoshida, Org. Biomol. Chem., 2020, 18, 1550.
6. (a) J. Dommerholt, S. Schmidt, R. Temming, L. A. Hendriks, F. P. J. T. Rutjes, J. C. M. van Hest, D. J. Lefebre, P. Friedl and F. L. van Delft, Angew. Chem., Int. Ed., 2010, 49, 9422; (b) A. M. Jawalearak, E. Reubsaeit, F. P. J. T. Rutjes and F. L. van Delft, Chem. Commun., 2011, 47, 3198; W. Chen, D. Wang, C. Dai, D. Hamelberg and B. Wang, Chem. Commun., 2012, 48, 1736; (c) T. Cruchter, K. Harms and E. Meggers, Chem. – Eur. J., 2013, 19, 16682; (d) D. Wang, W. Chen, Y. Zheng, C. Dai, K. Wang, B. Ke and B. Wang, Org. Biomol. Chem., 2014, 12, 3950; (e) S. Wallace and J. W. Chin, Chem. Sci., 2014, 5, 1742; (f) L. Plougastel, O. Koniev, S. Specklin, E. Decuypere, C. Creminon, D.-A. Buiossis, A. Wagner, S. Kolodych and F. Taran, Chem. Commun., 2014, 50, 9376; (g) D. Wang, E. Viennois, K. Jj, K. Dammera, A. Dragano, Y. Zheng, C. Dai, D. Merlin and B. Wang, Chem. Commun., 2014, 50, 15890; (h) J. Dommerholt, O. van Rooijen, A. Bormann, C. F. Guerra, F. M. Bickelhaupt and F. L. van Delft, Nat. Commun., 2014, 5, 5378; (i) T. H. Poole, J. A. Reisz, W. Zhao, L. B. Poole, C. M. Purdifu, S. M. Deks, M. A. Wolfert, G.-J. Boon and F. L. van Delft, Angew. Chem., Int. Ed., 2017, 56, 4130; (j) S. Bernard, D. Audisio, M. Riomet, S. Ursuegui, M. Recher, W. Kreßel and A. Wagner, J. Am. Chem. Soc., 2018, 140, 5322.

Although further detailed studies are required, the selectivity would be concerned with the following two factors; (1) the coordination of metal ions, and (2) the electronic effect of the ligands. Strengthen the coordination.

7. (a) M. A. Bennett and H. P. Schwemlein, Chem. – Eur. J., 2018, 24, 14064; (b) A. Herrmann, L. Kaufmann, P. Dey, R. Haag and U. Schöder, ACS Appl. Mater. Interfaces, 2010, 18, 11382; (c) F. Friscourt and E. Friscourt, Angew. Chem., 2018, 26, 1503; (d) M. Shelbourne, X. Chen, T. Brown and A. H. El-Sagheer, Chem. Commun., 2011, 47, 6257; (e) A. Das, C. Dash, M. Youssufuddin, M. A. Celik, G. Frenking and H. V. R. Dias, Angew. Chem., Int. Ed., 2012, 51, 3940; (f) A. Das, C. Dash, M. A. Celik, M. Youssufuddin, G. Frenking and H. V. R. Dias, Organometalics, 2013, 32, 3135; (g) P. Gobbo, T. Romagnoli, S. M. Barbon, J. T. Price, J. Keir, J. B. Gilroy and M. S. Workentin, Chem. Commun., 2015, 51, 6647.

For an alternative approach, see; R. S. Ramsubhag and B. D. Dudley, Org. Biomol. Chem., 2016, 14, 5028.

8. (a) L. Hui, Z. Li, B. Wang, C. Dai, D. Diao and B. Wang, Chem. Commun., 2017, 53, 1370; (b) P. Werther, J. S. Möhler and R. Wombacher, Chem. – Eur. J., 2017, 23, 18216; (c) M. Bjerknes, H. Cheng, C. D. McNitt and V. V. Popik, Bioconjugate Chem., 2017, 28, 1560; (d) L. C.-C. Lee, H. M.-H. Cheung, H.-W. Liu and K. K.-W. Lo, Chem. – Eur. J., 2018, 24, 14064; (e) J. K. Harms and E. Meggers, Org. Biomol. Chem., 2017, 15, 15898; (f) K. A. Horn, D. N. M. Valette and M. E. Webb, Chem. – Eur. J., 2015, 21, 14376; (g) T. Hoogenboom, H. Zuilhof and T. Wennekes, Org. Lett., 2015, 17, 15550; (h) A. Bormann, O. Fatunsin, J. Dommerholt, A. M. Jonker, D. W. P. M. Lówik, J. C. M. van Hest and F. L. van Delft, Bioconjugate Chem., 2015, 26, 257; (i) E. Galardon and D. Padovani, Bioconjugate Chem., 2015, 26, 1013; (j) F. P. J. T. Rutjes, J. C. M. van Hest, D. J. Lefeber, P. Friedl and F. L. van Delft, Angew. Chem., Int. Ed., 2015, 54, 5981; (k) D. Wang, W. Chen, Y. Zheng, C. Dai, D. Merlin and B. Wang, Chem. Commun., 2014, 50, 15890; (l) T. H. Poole, J. A. Reisz, W. Zhao, L. B. Poole, C. M. Purdifu, S. M. Deks, M. A. Wolfert, G.-J. Boon and F. L. van Delft, Angew. Chem., Int. Ed., 2017, 56, 4130; (m) S. Bernard, D. Audisio, M. Riomet, S. B. King, V. V. Popik, Bioconjugate Chem., 2014, 1370; (n) Z. Deng, L. Xing, X. Hong and Z. Cheng, Chem. Lett., 2015, 44, 7440; (o) W. Chen, X. J., Z. Du and B. Wang, Chem. Commun., 2015, 53, 1370; (p) W. Chen, Y. J. S. Möhler and R. Wombacher, Chem. – Eur. J., 2017, 23, 18216; (q) S. Bernard, D. Audisio, M. Riomet, S. Bennett and P. Schwemlein, Angew. Chem., Int. Ed. Engl., 1999, 28, 1296.

9. T. H. Poole, J. A. Reisz, W. Zhao, L. B. Poole, C. M. Purdifu, S. M. Deks, M. A. Wolfert, G.-J. Boon and F. L. van Delft, Angew. Chem., Int. Ed., 2010, 49, 3065.

10. R. N. M. N. Matsuda, T. Kashivagi, K. Igawa and K. Tonomura, Angew. Chem., Int. Ed., 2015, 54, 1190; (b) Y. Kawasaki, Y. Yamanaka, K. Iii and T. Hosoya, Chem. Commun., 2019, 55, 3536.

11. R. N. M. N. Matsuda, T. Kashivagi, K. Igawa and K. Tonomura, Angew. Chem., Int. Ed., 2015, 54, 1190; (b) Y. Kawasaki, Y. Yamanaka, K. Iii and T. Hosoya, Chem. Commun., 2019, 55, 3536.