Chinese Chemical Letters 34 (2023) 107587

Contents lists available at ScienceDirect

Chinese Chemical Letters

journal homepage: www.elsevier.com/locate/cclet

N-Acetylenethio phthalimides: Sequential linkage for compositional click reaction

Wen-Chao Gao[a,b,*], Kai Feng[a], Jun Tian[a], Juan Zhang[a], Hong-Hong Chang[a], Xuefeng Jiang[b,*]

[a] *College of Biomedical Engineering, Taiyuan University of Technology, Taiyuan 030024, China*
[b] *School of Chemistry and Molecular Engineering, East China Normal University, Shanghai 200062, China*

ARTICLE INFO

Article history:
Received 6 April 2022
Revised 31 May 2022
Accepted 5 June 2022
Available online 10 June 2022

Keywords:
Diversity-oriented synthesis
Compositional click
Reversible linkage
Iridium catalysis
Hydrophobic tagging

ABSTRACT

Click chemistry has become a useful tool for diverse molecular linkage and modification, and the development of new click strategy that enable reversibility and multifunctionality is of high demand for the multifunction and drug release. Herein, compositionally clicking combined regioselective iridium-catalyzed azide-alkynthio cycloaddition (Ir-AAC) and disulfuration has been developed for the sequential linkage from *N*-acetylenethio phthalimides, naturally occurring thiols and readily available azides. This method has been successfully applied to the construction of drug hybrids, peptide modification and glycosylation. Furthermore, by the design of diacetylenethio phthalimide as a platform molecule, trifunctional conjugants were sequentially linked through independent Ir-AAC, disulfuration and Cu-AAC reaction for hydrophobic tagging ternary PROTACs.

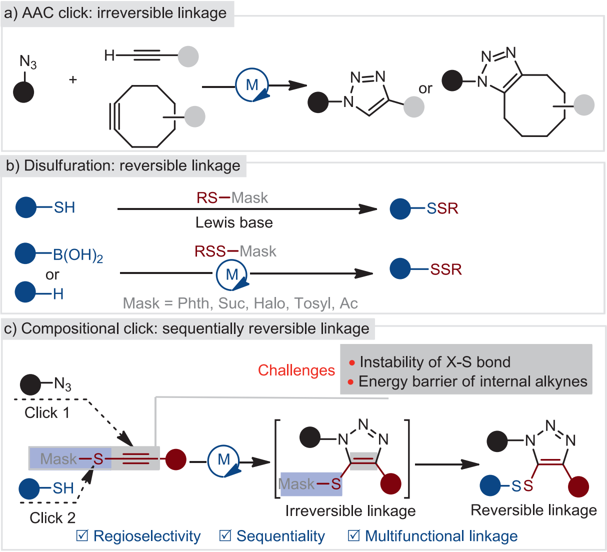
Over the past 20 years, chemists have strived to develop mutually selective pairs of chemical handles those could react together efficiently for either reversible or irreversible covalent linkages to meet different functionalities [1,2]. As an irreversibly clicking reaction, the alkyne-azide cycloaddition (AAC) has been widely applied for molecular functionalization and linkage in chemical biology, drug discovery, and material science [3–5]. Generally, the widespread AAC-click proceeded through the triazole annulation by Cu catalysis [6] or with the aid of strained alkynyl-rings under metal free conditions (Scheme 1a) [7]. Moreover, in the click toolbox, other irreversible linkages such as thiol-ene, thiol-yne and more recently thiol-alkylation have been also well developed for molecular conjugation modification rely on the availability and nucleophility of cysteine and related peptides [8–10]. On the other hand, to facilitate the drug release and biomolecular function, the design and construction of reversible linkage is in high demand and the disulfide bond has been an ideal and prevalent choice [11]. Despite the formation of disulfide bond through oxidation of cysteine residue in nature [12], the access to the disulfide by the use of masked sulfurating reagents (RS–LG or RSS-LG) has become more convenient and practicable through either nucleophilic disulfuration or metal-catalyzed C-S bond formation (Scheme 1b) [13–18].

Previously, some masked sulfurating reagents have been designed by our group and applied to access un-symmetrical disulfides, steric chiral disulfides, and bi-lateral disulfide linkage [13–15]. With the demands of diversity-oriented synthesis (DOS) in chemical biology, the compositional click [19–21] and multicomponent reactions [22–26] have aroused much attention for modular and sequential linkage. We envisioned that the masked acetylenethio reagents can serve as both alkynyl handle and sulfuryl handle to construct compositionally irreversible triazole and reversible disulfide linkage, which connect the cysteine or thiol-bearing compounds with azides sequentially (Scheme 1c). However, because of the lower S-X bond energy, masked sulfurating reagents were not compatible with most metal catalysts, such as palladium, copper, silver, gold [27–30], and the oxidative metal-insertion would easily occur *via* the cleavage of S-X bond. Furthermore, the cycloaddition of internal alkyne with azides is also challenging since the inherent higher energy barrier than terminal alkynes are needed for orbital match. Given the weak and reversible chelation of sulfur to platinum-group metals [31–34], the reaction of alkynylthio phthalimides with azides would possibly proceed *via* cycloaddition rather than S-N insertion in the presence of platinum-group-catalysis, and the resulted *N*-triazolylthio phthalimides would gain extraordinary regioselectivity and reactivity, continuing to form disulfides from various naturally

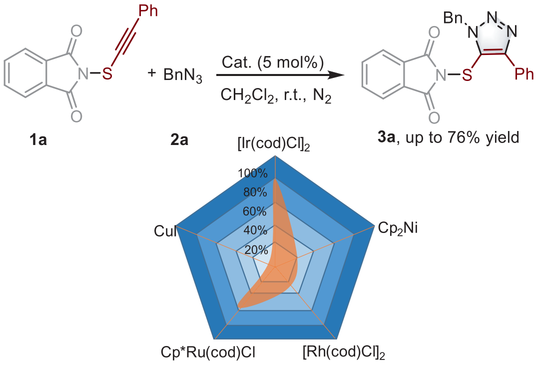
---

* Corresponding authors.
E-mail addresses: gaowenchao@tyut.edu.cn (W.-C. Gao), xfjiang@chem.ecnu.edu.cn (X. Jiang).

https://doi.org/10.1016/j.cclet.2022.06.010
1001-8417/



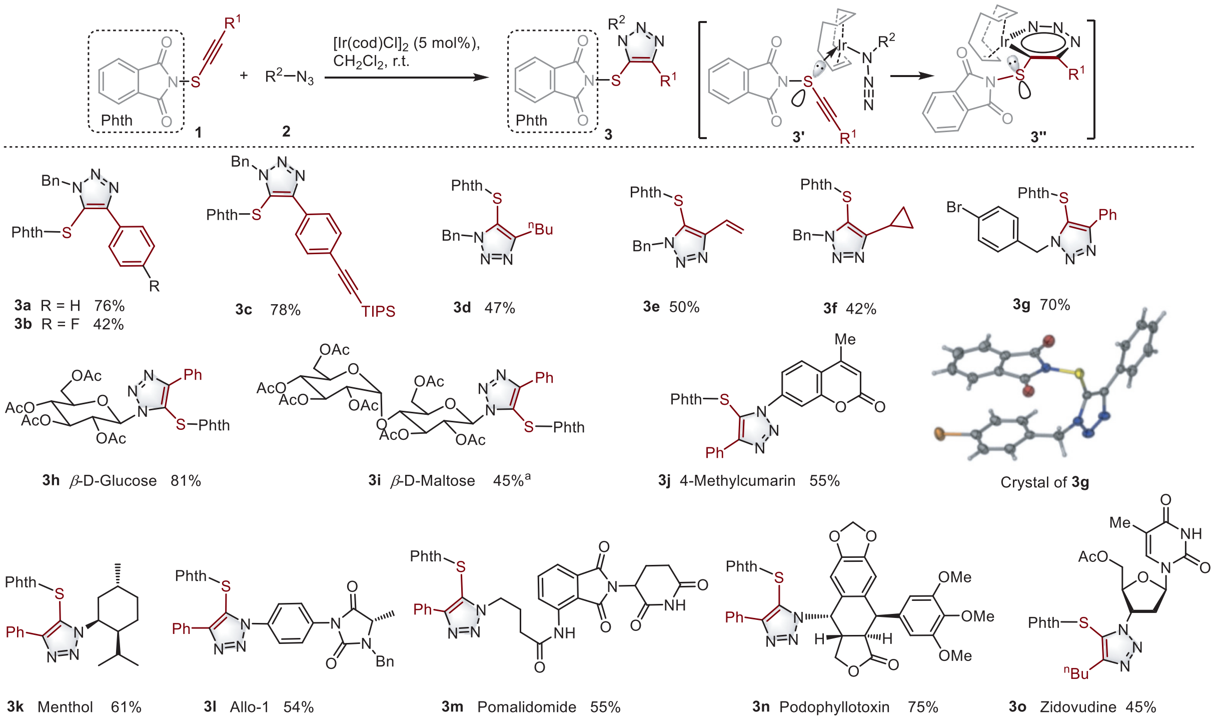
Scheme 1. Alkyne-azide cycloaddition and disulfuration.



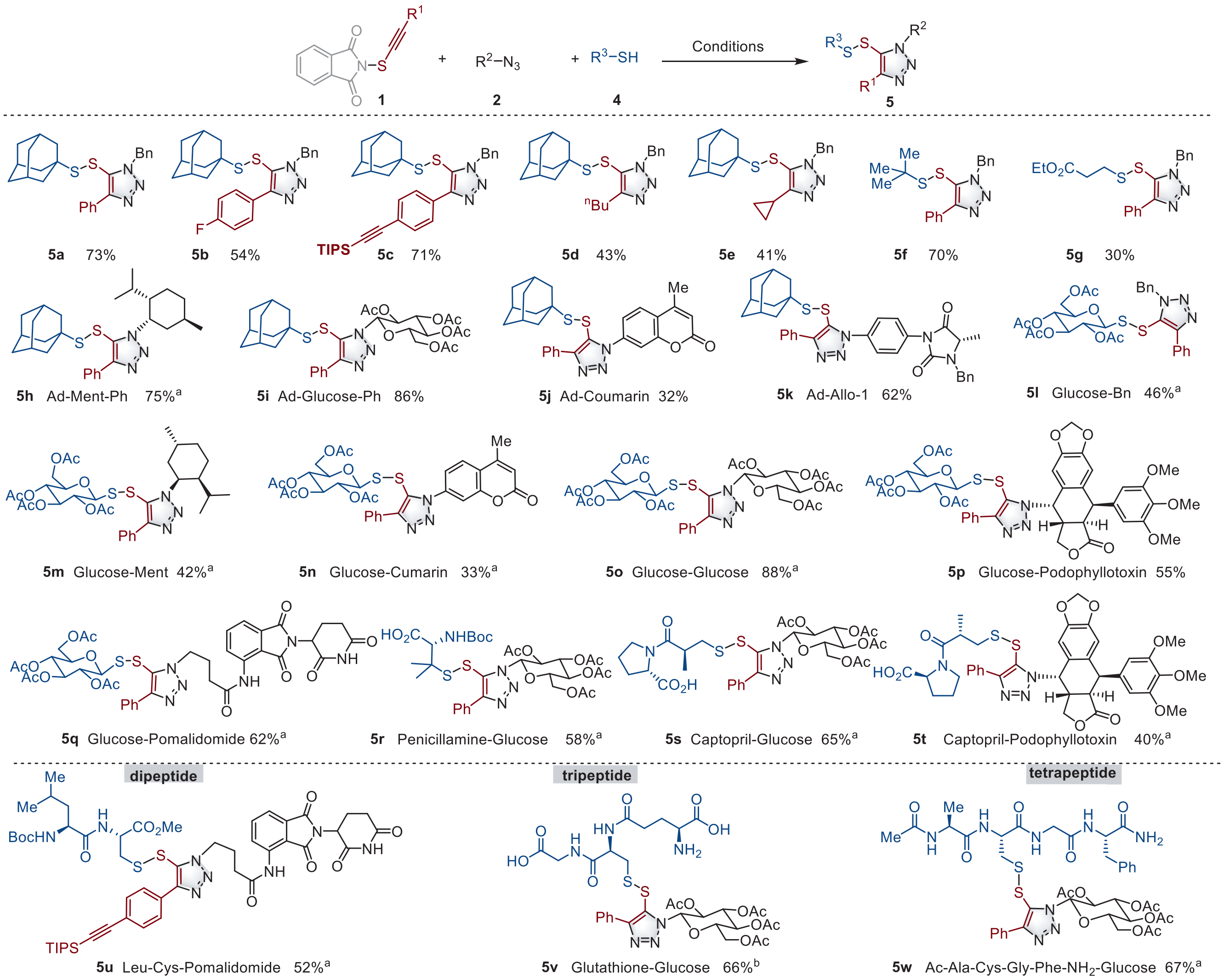
Scheme 2. Conditions of AAC Click. Conditions: **1a** (0.15 mmol), **2a** (0.165 mmol) and catalyst (5 mol%) in CH_2Cl_2 (1.5 mL) under N_2 at r.t. for overnight.

occurring thiols. Herein, we report an iridium-catalyzed compositional click for sequential bi- or tri-conjugation from *N*-acetylenethio phthalimides, azides and thiols. Moreover, with a di-acetylenethio phthalimide as the platform, a series of adamantane tagged trifunctional conjugates have been sequentially constructed through Ir-AAC, disulfuration as well as Cu-AAC reaction.

To explore the azide-alkynylthio cycloaddition, the library of reactive sulfurating reagents bearing phthalimide mask and internal alkynylthio group was firstly built following our previous report (**1a**-**1j**, Supporting information) [35]. Notably, besides the generally aryl and alkyl substituents on the alkynyl termini, the novel di-acetylene precursor (**1c**), which contains two C-C triple bonds for further derivation, could also be accessed in 77% yield. With the library of masked alkynylthio reagents in hand, azide-alkynylthio cycloaddition were firstly attempted with *N*-acetylenethio phthalimide **1a** and benzyl azide **2a** as substrates (Table S1 in Supporting information). In contrast to the well-established Cu-catalyzed azide-alkyne cycloaddition, most of *N*-acetylenethio phthalimide **1a** was decomposed under CuI catalysis, and only trace amount of triazole product **3a** was detected (Table S1, entry 1). Other metal catalysts for internal alkynes-azide cycloaddition such as Cp_2Ni, $[Rh(cod)Cl]_2$ and Cp*Ru(cod)Cl could be able to catalyze the triazole annulation, while the desired product **3a** was produced only in moderate to low efficiency (Scheme 2 and Table S1, entries 2-4). To our delight, when $[Ir(cod)Cl]_2$ was used as the catalyst, the cycloaddition could proceed to afford **3a** in 76% yield with high regioselectivity, and the N-S bond was well tolerated under this condition. The increase of reaction temperature could accelerate the reaction, while both the *N*-acetylenethio phthalimide **1a** and product **3a** could not stand long time at higher temperature (Table S1, entry 6). Other solvents were also tested for this reaction, while



Scheme 3. Ir-AAC Click reaction. Conditions: **1** (0.15 mmol), **2** (0.225 mmol) and $[Ir(cod)Cl]_2$ (5 mol%) in CH_2Cl_2 (1.5 mL) were stirred under N_2 at room temperature for 24 h. ^{a} Cp*Ru(cod)Cl (5 mol%) was used as the catalyst.

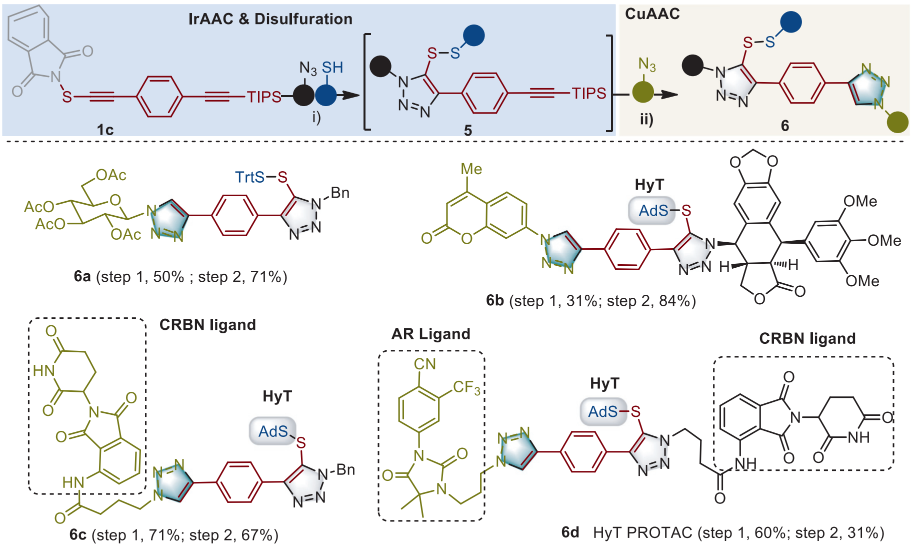


Scheme 4. Compositional click *via* Ir-AAC & disulfuration. Conditions A: **1** (0.165 mmol), **2** (0.15 mmol), **4** (0.165 mmol) and [Ir(cod)Cl]$_2$ (5 mol%) in CH_2Cl_2 (1.5 mL) were stirred under N_2 at room temperature for 12-48 h, isolated yields. ^{a} Conditions B: **1** (0.165 mmol), **2** (0.165 mmol) and Ir(cod)Cl]$_2$ (5 mol%) in CH_2Cl_2 (1.5 mL) were stirred under N_2 at room temperature for 12-48 h, then **2** (0.15 mmol) was added, and the mixture was stirred for another 30 s. ^{b} Conditions C: **1** (0.165 mmol), **2** (0.165 mmol) and [Ir(cod)Cl]$_2$ (5 mol%) in CH_2Cl_2 (1.5 mL) were stirred under N_2 at room temperature for overnight, glutathione (0.15 mmol) and TFA (0.15 mmol) dissolved in methanol (1 mL) was added to the reaction mixture for another 2 h.

none of them could give a better result than that of CH_2Cl_2, which indicated that the reaction was sensitive to the type of solvents (Table S1, entries 8-13).

With the standard conditions for Ir-AAC Click (Table S1, entry 5), the generality of masked triazolylthio reagents was next evaluated (Scheme 3). Various terminal functional groups including butyl, cyclopropyl, aryl, C-C double bond or C-C triple bond could be all well tolerated in this transformation, affording the desired *N*-triazolylthio phthalimides **3a**-**3f** in moderate to good yields. Other azides were also carefully evaluated, and the structure of product **3g** derived from 4-bromobenzyl azide was further confirmed by the X-ray diffraction analysis, which highlighted the regiochemistry of present annulation. Glycosylation of *N*-alkynylthio phthalimides could be realized smoothly with glucosyl azide and maltosyl azide in moderate yields (**3h** and **3i**). The fluorogenic coumarin derivative, the natural product menthol derivative, and the allosteric ligand Allo-1 [36] could be successfully linked to *N*-alkynylthio phthalimides as well (**3j**-**3l**). Furthermore, a subset of drugs containing the azide group such as pomalidomide, podophyllotoxin, and zidovudine were also successfully incorporated, furnishing *N*-triazolylthio phthalimides **3m**-**3o** in moderate to good yields. Notably, due to the relatively weak η^2-Ir-S interaction [37] and strong η^1-N-S coordination (intermediate **3′**) [38], the *N*-thio phthalimide was regioselectively located at 5-position of 1,2,3-triazole ring *via* the irido-heterocycle **3″**. This dominantly regioselective Ir-AAC Click provided diverse masked *N*-triazolylthio linkers for next disulfuration.

Taking the developed Ir-AAC click reaction and the reactivity of *N*-thio phthalimides, a compositional click progress was next considered for the dual and sequential cross-linkage of different functional molecules directly from thiols, azides and *N*-alkynylthio phthalimides (Scheme 4). Although thiols had been known to poison metal-catalysts [39], the present Ir-AAC reaction was not affected by the sulfur-metal interaction. As shown in Scheme 4, these compositional click reactions could successfully proceed to produce a series of triazolyl disulfide-linked conjugants: the tertiary thiols such as adamantanethiol and *tert*-butylthiol could be connected with benzyl azide *via* either 4-aryl or -aliphatic triazolyl disulfide (**5a**-**5f**), while the conjugation with primary thiols, only afforded the de-sired product in low yields (**5g**), and disulfides derived from homocoupling of thiols were isolated as major products; functional azides derived from natural products



Scheme 5. Trifunctional molecules linked with diacetylenethio phthalimide. Conditions: (i) **1c** (0.165 mmol), azide-1 (0.165 mmol) and $[\mathrm{Ir(cod)Cl}]_2$ (5 mol%) in CH_2Cl_2 (1.5 mL) were stirred under N_2 at room temperature for 6 h, then thiol (0.15 mmol) was added, and the mixture was stirred for 30 s; (ii) Disulfide **5** (0.15 mmol), TBAF (1 mol/L in THF), azide-2 (0.165 mmol), DIPEA (4 mol%), HOAc (4 mol%) and CuI (2 mol%) was stirred for 4 h.

(menthol, **5h**), saccharides (**5i**), fluorophores (**5j**), and allosteric ligands (**5k**) could be well conjugated with 1-adamantanethiol. Furthermore, various disulfuryl glycosides such as glucose-linked menthol (**5l**), glucose-linked coumarin (**5m**), dual glucose linkage (**5n**), glucose-linked pomalidomide (**5o**), glucose-linked podophyllotoxin (**5p**), and glucose-linked penicillamine (**5q**), glucose-linked penicillamine (**5r**), and glucose-linked captopril (**5s**) could be all successfully accessed, providing alternative way to diverse dithioglycosylation [40]. The modification and glycosylation of peptides *via* disulfide bond has proven to be an effective approach to overcome the intrinsic disadvantages of natural peptide drugs such as inadequate absorption and rapid degradation by proteolytic enzymes. Using the present method, the dipeptide (Leu-Cys), tripeptide glutathione (Gly-Cys-Glu), and the tetrapeptide (Ac-Ala-Cys-Gly-Phe-NH$_2$) could be successfully modified by anti-cancer drug pomalidomide or glucose in moderate to good yields (**5u**-**5w**), and the cell permeability and stability of peptides would be potentially improved by this modification [41,42].

Due to the stability and lipophility of hindered disulfides, the tertiary disulfides have been widely designed as linkers in ADCs and bridged polypeptides for drug release or improving the lipidation of biomolecules [43,44]. After the hindered disulfide bond could be singly introduced onto bifunctional molecules, we turned our intention to the development of trifunctional molecules [45] that contained the tertiary disulfide bond to enhance the functionality and improve the stability and pharmacokinetics of drugs. A common challenge for the trifunctional linkage is the requirement of three independent sites, attendant orthogonal, and mutually compatible reactivity. With our platform molecule diacetylenethio phthalimide **1c**, the Ir-catalyzed azide-alkynylthio click combined with the thiol-triazolylthio click reactions could proceed in sequence to afford the tertiary bifunctional disulfides **5**. Subsequently addition of fluoride and the second azide, would then undergo another Cu-AAC click reaction to furnish the tertiary trifunctional disulfides **6** (Scheme 5). This sequential linkage could connect galactopyranosyl azide and benzyl azide with triphenylmethanethiol to form conjugant **6a**, and realize the modification of anti-cancer drug podophyllotoxin with fluorescent coumarin and adamantanethiol to form hybrid **6b**. Synthetic hydrophobic tag (HyT), like adamantane, could attach to a protein's surface and mimic the partially unfolded state, leading proteasomal degradation of target protein [46,47]. The present disulfide linkage could efficiently build tagged molecule hybrids for protein-targeted degradation by incorporate the admantane tag to protein-of-interest (POI) recruiters. For example, the hydrophobic tag adamantane cage could not only combined with a cereblon (CRBN) ligand pomalidomide *via* the hindered disulfide bond (**6c**) [48], but also conjugated with a proteolysis-targeting chimeras (PROTAC), in which the CRBN ligand linked to an androgen receptor (AR) (**6d**) [49].

In summary, we have developed a compositional click strategy for sequential and reversible linkage *via* iridium-catalyzed alkyne-azide cycloaddition and disulfuration. This compositional click could regioselectively construct bi- or tri-functional molecules from diverse natural thiols and readily available azides. Moreover, with a diacetylenethio phthalimide as the platform, the click combination of Ir-AAC, disulfuration and Cu-AAC could be applied for the modular synthesis of trifunctional conjugants including hydrophobic tagging PROTACs. Given the reversibility, regioselectivity, and sequentiality of current compositional click, the resulted linkage breakthrough would provide high diversity and molecular complexity in the field of chemical biology and pharmacological therapy.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (Nos. 21901179 and 22125103), the Scientific Activities of Selected Returned Overseas Professionals of Shanxi Province (No. 20200002), the Natural Science Foundation of Shanxi Province (No. 202103021224067), and the Research Project of Shanxi Scholarship Council (No. HGKY2019029).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cclet.2022.06.010.

References

[1] M.G.Finn H.C.Kolb, K.B. Sharpless, Angew. Chem. In. Ed. 40 (2001) 2004–2021.
[2] V. Rigolot, C. Biot, C. Lion, Angew. Chem. In. Ed. 60 (2021) 23084–23105.
[3] W. Xi, T.F. Scott, C.J. Kloxin, C.N. Bowman, Adv. Funct. Mater. 24 (2014) 2572–2590.
[4] P. Thirumurugan, D. Matosiuk, K. Jozwiak, Chem. Rev. 113 (2013) 4905–4979.
[5] A.D. Moorhouse, J.E. Moses, ChemMedChem 3 (2008) 715–723.
[6] V.V. Rostovtsev, L.G. Green, V.V. Fokin, K.B. Sharpless, Angew. Chem. In. Ed. 41 (2002) 2596–2599.
[7] J.C. Jewetta, C.R. Bertozzi, Chem. Soc. Rev. 39 (2010) 1272–1279.
[8] C.E. Hoyle, C.N. Bowman, Angew. Chem. In. Ed. 49 (2010) 1540–1573.
[9] R. Hoogenboom, Angew. Chem. In. Ed. 49 (2010) 3415–3417.
[10] E.M.D. Allouche, E. Grinhagena, J. Waser, Angew. Chem. In. Ed. 60 (2021) e202112287.
[11] M. Góngora-Benítez, J. Tulla-Puche, F. Albericio, Chem. Rev. 114 (2014) 901–926.
[12] S.B. Gunnoo, A. Madder, ChemBioChem 17 (2016) 529–553.
[13] X. Xiao, J. Xue, X. Jiang, Nat. Commun. 9 (2018) 2191–2200.
[14] W.C. Gao, J. Tian, Y. Shang, X. Jiang, Chem. Sci. 11 (2020) 3903–3908.
[15] J. Xue, X. Jiang, Nat. Commun. 11 (2020) 4170.
[16] C.M. Park, B.A. Johnson, J. Duan, et al., Org. Lett. 18 (2016) 904–907.
[17] W. Wang, Y. Lin, Y. Ma, C.H. Tung, Z. Xu, Org. Lett. 20 (2018) 3829–3832.
[18] Z. Wu, D.A. Pratt, Angew. Chem. In. Ed. 60 (2021) 15598–15605.
[19] C.J. Smedley, G. Li, A.S. Barrow, et al., Angew. Chem. In. Ed. 59 (2020) 12460–12469.
[20] R. Tessier, J. Ceballos, N. Guidotti, R. Simonet-Davin, B. Fierz, J. Waser Chem. 5 (2019) 2243–2263.
[21] W.R. Galloway, A. Isidro-Llobet, D.R. Spring, Nat. Commun. 1 (2010) 80.
[22] Q.W. Gui, F. Teng, S.N. Ying, et al., Chin. Chem. Lett. 31 (2020) 3241–3244.
[23] N. Meng, Y. Lv, Q. Liu, et al., Chin. Chem. Lett. 32 (2021) 258–262.
[24] J. Jiang, F. Xiao, W.M. He, L. Wang, Chin. Chem. Lett. 32 (2021) 1637–1644.
[25] Q.W. Gui, F. Teng, Z.C. Li, et al., Chin. Chem. Lett. 32 (2021) 1907–1910.
[26] Y. Wu, J.Y. Chen, J. Ning, et al., Green Chem. 23 (2021) 3950–3954.
[27] P. Saravanan, P. Anbarasan, Org. Lett. 16 (2014) 848–851.
[28] D. Zhu, X. Shao, X. Hong, L. Lu, Q. Shen, Angew. Chem. In. Ed. 55 (2016) 15807–15811.
[29] H. Li, Z. Cheng, C.H. Tung, Z. Xu, ACS Catal. 8 (2018) 8237–8243.
[30] J. Zou, J. Chen, T. Shi, Y. Hou, et al., ACS Catal. 9 (2019) 11426–11430.
[31] S. Ding, G. Jia, J. Sun, Angew. Chem. In. Ed. 53 (2014) 1877–1880.
[32] Y. Liao, Q. Lu, G. Chen, Y. Yu, et al., ACS Catal. 7 (2017) 7529–7534.
[33] P. Destito, J.R. Couceiro, H. Faustino, et al., Angew. Chem. In. Ed. 56 (2017) 10766–10770.
[34] A. Gutiérrez-González, P. Destito, J.R. Couceiro, et al., Angew. Chem. In. Ed. 60 (2021) 16059–16066.
[35] W.C. Gao, Y.Z. Shang, H.H. Chang, et al., Org. Lett. 21 (2019) 6021–6024.
[36] F. Zhou, K. Ding, Y. Zhou, Y. Liu, et al., J. Med. Chem. 62 (2019) 9983–9989.
[37] A.F. Dalebrook, L.J. Wright, Organometallics 28 (2009) 5536–5540.
[38] G. Albertin, S. Antoniutti, D. Baldan, et al., Inorg. Chem. 47 (2008) 742–748.
[39] D.A. Evans, S.J. Miller, T. Lectka, P. von Matt, J. Am. Chem. Soc. 121 (1999) 7559–7573.
[40] S.V. Moradi, W.M. Hussein, P. Varamini, P. Simerska, I. Toth, Chem. Sci. 7 (2016) 2492–2500.
[41] A.K. Mishra, R. Tessier, D.P. Hari, J. Waser, Angew. Chem. In. Ed. 60 (2021) 17963–17968.
[42] B.M. Cooper, J. Iegre, D.H. O' Donovan, M.Ö. Halvarsson, D.R. Spring, Chem. Soc. Rev. 50 (2021) 1480–1494.
[43] G. Saito, J.A. Swanson, K.D. Lee, Adv. Drug Delivery Rev. 55 (2003) 199–215.
[44] K.C. Nicolaou, S. Rigol, Angew. Chem. In. Ed. 58 (2019) 11206–11241.
[45] S. Imaide, K.M. Riching, N. Makukhin, et al., Nat. Chem. Biol. 17 (2021) 1157–1167.
[46] J.L. Gustafson, T.K. Neklesa, C.S. Cox, et al., Angew. Chem. In. Ed. 54 (2015) 9659–9662.
[47] T.K. Neklesa, H.S. Tae, A.R. Schneekloth, et al., Nat. Chem. Biol. 7 (2011) 538–543.
[48] C. Steinebach, I. Sosič, S. Lindner, et al., Med. Chem. Commun. 10 (2019) 1037–1041.
[49] H. Xie, J.J. Liang, Y.L. Wang, et al., Eur. J. Med. Chem. 204 (2020) 112512.