Ruthenium-Catalyzed Azide–Thioalkyne Cycloadditions in Aqueous Media: A Mild, Orthogonal, and Biocompatible Chemical Ligation

Paolo Destito, José R. Couceiro, Hélio Faustino, Fernando López,* and José L. Mascareñas*

Abstract: The development of efficient metal-promoted bio-orthogonal ligations remains as a major scientific challenge. Demonstrated herein is that azides undergo efficient and regioselective room-temperature annulations with thioalkynes in aqueous milieu when treated with catalytic amounts of a suitable ruthenium complex. The reaction is compatible with different biomolecules, and can be carried out in complex aqueous mixtures such as phosphate buffered saline, cell lysates, fetal bovine serum, and even living bacteria (E. coli). Importantly, the reaction is mutually compatible with the classical CuAAC.

The copper-catalyzed azide–alkyne cycloaddition (CuAAC), paradigm of “click” chemistry,[1] can be considered among the most relevant chemical transformations discovered in the last decades, with countless applications in many areas of science.[2] The biological relevance of this reaction stems from its robustness and compatibility with aqueous media, as well as from its good bioorthogonality.[3] However, the transformation still presents important limitations. Thus, in addition to being fairly incompatible with thiols, the reaction is essentially restricted to terminal alkynes, a consequence of a mechanism which requires the formation of copper acetylide intermediates (Scheme 1a). An important additional drawback has to do with the side reactivity and toxicity of copper ions in biological contexts.[4] Furthermore, to reach efficient conversions in typically diluted biological settings, the reactive copper(I) species need to be generated in situ using excess amounts of a copper(II) source and sodium ascorbate, a reductant which is not innocent in biological contexts.[5] These issues have been partially addressed by using copper-stabilizing ligands which enhance the biocompatibility and kinetic of the reactions.[6,7] Copper-free, strain-promoted annulations have been shown to be an efficient alternative,[8] however, these reactions also present limitations associated to the side-reactivity of the reactants. Therefore, the development of new bioorthogonal and biocompatible reactions which address some of the above limitations remains as a major challenge.[9] In particular, the discovery of robust and aqueous-compatible metal-catalyzed annihilations, as alternatives to the CuAAC, represents a highly appealing goal.[10]

Several azide–alkyne cycloadditions using metals other than copper have been described in recent years,[11] but only the ruthenium variant (RuAAC)[12] has shown a meaningful scope (Scheme 1b).[13] In contrast to the CuAAC, which encompasses dinuclear copper intermediates such as I and II,[14] the ruthenium-promoted reaction involves intermediate species like III, which evolve into IV by oxidative cyclometalation, and eventually to the triazole product.[15] In keeping with this scenario, the RuAAC, essentially developed in organic solvents, tolerates disubstituted alkynes but can produce mixtures of regioisomers. Probably, the notion that it is not compatible with water and air atmospheres has precluded more biofocused investigations.[16,13] Recent data suggest that some ruthenium complexes can promote the process in water, but the reactions require thermal activation and present a limited scope.[17]

Herein, we demonstrate that certain ruthenium(II) complexes can indeed catalyze the cycloaddition between azides and alkynes in water, and at room temperature. Importantly,
the reaction is especially efficient when thioalkynes are used as reaction partners (Scheme 1c). Moreover, the process is mutually compatible with the CuAAC, tolerant to different types of biomolecules, including thiols, and can be carried out in either phosphate buffered saline, cell lysates, or cell culture media, even in the presence of living bacteria (E. coli).

At the outset, we were inspired by a report of Jia, Sun, and co-workers on an iridium-promoted azide–thioalkyne cycloaddition.[19] Although the method was developed in anhydrous CH₂Cl₂, an isolated example in water using benzyl azide caught our attention. Unfortunately, when we tested the reaction of the thioalkyne 2a with the fluorogenic anthracenyl-azide probe 1a,[19][20] the yield of the corresponding adducts (3aa/3aa‘) was modest (Table 1, entry 1).[20] Remarkably, when using Cp*Ru(cod)Cl as a catalyst,[15a] we observed, improved by using 2 equivalents of thioalkyne, (entry 5). And other ruthenium(II) catalysts, such as Cp*Ru(PPh₃)₂Cl,[12] RuH₂(CO)(PPh₃)₄[17b] were not efficient (entries 6 and 7). In contrast, the tetramer [Cp*RuCl₄][23] was quite effective (entry 8). The reaction between 1a and 2a, could also be carried out in CH₂Cl₂. However, obtaining good yields required the use of anhydrous solvent and inert atmospheres (entries 9 vs. 10), which is not necessary in water. It looks like the aqueous solvent is somewhat precluding the ruthenium species from being rapidly deactivated.[24]

The cycloaddition is also feasible using a typical internal alkyne such as 2b, albeit somewhat slower (10% less conversion after 30 min), and it led to a 5:1 mixture of regioisomers (Table 1, entry 11). Other alkynes such as 2c were unreactive under identical reaction conditions (entry 12). The higher reactivity of the thioalkyne partner was clearly visible in a cross-competition experiment: when the azide 1a was reacted with a 1:1 mixture of 2a and 2b (equiv each), the triazole 3aa, arising from the cycloaddition with the thioalkyne was exclusively observed in 98% yield (entry 13). Interestingly, NMR analysis of the interaction between Cp*Ru(cod)Cl and the alkynes (in CD₂Cl₂) demonstrated that while 2a displaces the cod ligand at room temperature, 2b does not induce any change (see Pages S6–S9 of the Supporting Information). Analogous experiments using [Cp*RuCl₂], and 2a, revealed rapid formation of a new complex identified as [Cp*Ru(2a)Cl], whereas with alkyn 2b no new ruthenium species could be detected, even after 3 hours.[25] Thus, the good performance of thioalkynes might be in part related to their ability to strongly coordinate the Cp*RuCl moiety at room temperature. Additionally, the presence of the sulfur atom should also favor the formation of the required ruthenocyclic intermediate of type IV (Scheme 1b).

With these reaction conditions in hand, we analyzed the scope of the method (Ru α1,4AC). Despite the relatively poor water solubility of many of the azides and thioalkynes, the reactions proved to be general at room temperature, and the corresponding triazoles were obtained in good yields and with excellent regioselectivities (Table 2). Thus, aliphatic substituents either attached to the sulfur atom or to the terminal position of the azide were tolerated (e.g. 3aa–af). Terminal or trimethylsilyl-substituted thioalkynes (2g, 2h) provided the corresponding adducts 3ag and 3ah in good yields. Importantly, not only the anthracenyl and benzyl azide (1a, 1b) participated in the process, but aliphatic azides such as (2-azidoethyl)benzene (1ce) or 2-azidoethan-1-ol (1df) also reacted cleanly to provide the corresponding triazoles (3ca, 3ce, 3de). p-Tolyl azide reacted with 2a to provide 3ea with a moderate 40% yield, a value that could be improved up to 61% by using [Cp*RuCl₄], as a catalyst. Interestingly, different types of fluorophore-equipped trisubstituted triazoles could be generated by either using a dansyl-based azide (such as in 3fa) or by incorporating a coumarin moiety, either as part of the thioalkyne (e.g. 3aj) or of the organic azide (3ga and 3ha). Additionally, following work developed by Waser and co-workers,[26] 2-thioglucose and a cysteine-containing dipetide were selectively thio-alkynylated with EBX reagents. Gratifyingly, although the low water solubility of the thio-

---

**Table 1: Identification of reaction conditions in water.**

| Entry | Cat. (X mol%) | 1a/2 Solv. | t [h] | Conv [%] | 3/3’ [％] | Yield [%] |
|-------|---------------|------------|------|----------|----------|----------|
| 1     | [Ir(cod)Cl]₂ (2.5) | 1:1 H₂O | 24 42 | 1.0 | 29 |
| 2     | Cp*Ru(cod)Cl (S) | 1:1 H₂O | 24 65 | 19:1 | 58 |
| 3     | Cp*Ru(cod)Cl (S) | 1:2 H₂O | 9 99 | 19:1 | 99 |
| 4     | Cp*Ru(cod)Cl (S) | 1:2 H₂O | 0.80 | 19:1 | 78 |
| 5     | [Ir(cod)Cl]₂ (2.5) | 1:2 H₂O | 24 36 | 10 | 20 |
| 6     | Cp*Ru(PPh₃)₂Cl (S) | 1:2 H₂O | 24 47 | 23:1 | 17 |
| 7     | RuH₂(CO)(PPh₃)₄ (S) | 1:2 H₂O | 24 0 | – | 0 |
| 8     | [Cp*Ru(cod)Cl]₂ (1.25) | 1:2 H₂O | 24 99 | 14:1 | 99 |
| 9     | Cp*Ru(cod)Cl (S) | 1:2 H₂O | 2 99 | 19:1 | 99 |
| 10    | Cp*Ru(cod)Cl (S) | 1:2 H₂O | 2 99 | 18:1 | 37 |
| 11    | Cp*Ru(cod)Cl (S) | 1:2 H₂O | 9 99 | 5:1 | 95 |
| 12    | Cp*Ru(cod)Cl (S) | 1:2 H₂O | 10 | – | <5 |
| 13    | Cp*Ru(cod)Cl (S) | 1:4 H₂O | 4 99 | 19:1 | 98 |

[a] Unless otherwise noted, 2a (1–2 equiv), water, and 1a (1 equiv, 75 mm) were sequentially added under air to a vial containing the catalyst (that had been kept under N₂), and the mixture was stirred at RT.

[b] Determined by ¹H NMR spectroscopy of the crude reaction mixture with an internal standard. [c] Combined yield of 3/3’. [d] Carried out under an inert atmosphere in anhydrous solvent. [e] Carried out with 2b.

---

**Communications**

**Angew. Chem. Int. Ed. 2017, 56, 10766–10770 © 2017 The Authors. Published by Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim www.angewandte.org 10767**
alkynylated protected dipeptide 2k demanded the use of small amounts of a cosolvent (i.e. CHCl₃, 5–10% vol.), its reaction with 1a and 1f proceeded efficiently, thus affording the desired products 3ak and 3fk in good yields. The reaction between an alkynylated thioglucose and 1a took place smoothly in water to give the triazole 3al in 64% yield.

We next explored the bioorthogonality of the chemistry by performing the reaction in either the presence of different biomolecular additives or under biologically relevant conditions (Table 3). Gratifyingly, the RuAtAC could be efficiently carried out in the presence of glutathione (20-fold excess respect to the [Ru]; entry 1), different aminoacids (entry 2), and even in the presence of a random miniprotein (entry 3). The desired triazole was obtained in moderate to excellent yields and similar regioselectivities compared to those obtained in pure water (>15:1). The reaction could also be carried out in phosphate-buffered saline (PBS; entry 4). Additionally, frequently used culture cell media, such as DMEM, fetal bovine serum (FBS), and Hela cell lysates are also excellent reaction media, so the triazole 3aa was obtained in yields varying from 77 to 91% (entries 5–7).

Analogue reactions in these media between (2-azidoethyl)benzene (1c) and the thioalkyne 2e gave good yields of 3ce (see Table S3).

At this point, it was of interest to contrast the performances of the RuAtAC and CuAAC in water to establish strengths and weaknesses of each method. Thus, we carried out parallel experiments using (2-azidoethyl)benzene (1c; 75 mm), 5 mol% of each catalyst, and either 2e (for Ru) or phenylacetylene (for Cu). While the ruthenium-promoted reaction was significantly faster than the copper counterpart, when using CuSO₄ and sodium ascorbate for the latter (60% vs. 22% yield, after 2 h), the CuAAC became faster by including additives such as BTTAA (75% yield after 2 h; see Table S4). Importantly, the CuAAC failed with internal alkynes, including thioalkynes like 2a and, not surprisingly, it is essentially inhibited in the presence of thiols like glutathione (0% yield after 24 h). In contrast, the RuAtAC works effectively even in the presence of a 20-fold excess of glutathione (75% yield), and provides the products with both internal and terminal thioalkynes (as shown in Table 2). On the weak side, the efficiency of the RuAtAC decreases upon dilution (16% yield at 250 μM), while the ligand-accelerated CuAAC provided a 57% yield under similar micromolar conditions (see Pages S11–S13).

The lack of reactivity of internal thioalkynes in the presence of copper catalysts suggested that the CuAAC and the RuAtAC could be mutually orthogonal. Gratifyingly, the engineered diyne 4 was quantitatively converted into the bis(triazole) 5, without cross-reactivity, by performing a CuAAC with the dansyl azide 1f, and subsequent in situ addition of the ruthenium catalyst and the anthracenyl azide 1a (1 equiv with respect to thiokynle; Scheme 2). Considering the scarcity of mutually compatible bioorthogonal reac-

---

**Table 2:** Scope of the RuAtAC in water at room temperature.

| Entry | Conditions | Conv. [%] | 3/3' [%] | Yield [%] |
|-------|------------|-----------|-----------|-----------|
| 1     | H₂O/glutathione | 80        | 19:1      | 60        |
| 2     | H₂O/His + Fmoc-ala | 93       | 18:1      | 82        |
| 3     | H₂O/Peptide 39 aa (0.5 mm) | 99     | 23:1      | 98        |
| 4     | PBS         | 99        | 18:1      | 97        |
| 5     | cell cultured media (DMEM) | 99      | 15:1      | 84        |
| 6     | fetal bovine serum (FBS) | 88     | 17:1      | 77        |
| 7     | cell lysates (Hela) | 99      | 16:1      | 91        |

[a] Reaction conditions: 2 (2 equiv), water, and 1a (1 equiv, 75 mm) were sequentially added under air to a vial containing Cp*Ru(cod)Cl (5 mol%) which had been kept under N₂. The vial was closed, and the mixture stirred at RT for 15–24 h. Regioselectivities (3/3') were >18:1 unless otherwise noted (determined by ¹H NMR analysis of the crude reaction mixtures with an internal standard). Yield of isolated pure 3a determined by ¹H NMR analysis of the crude reaction mixture with an internal standard. [b] Corresponds to a 18:1 mixture of 3ca/3ca'. [c] Combined yield of Cp*RuCl₂. (1.25 mol%).
tions, the possibility of using both annihilations in tandem in one pot is certainly promising. [27]

Finally, and importantly, we found that the RuAtAC can also be carried in the presence of bacteria (*E. coli*) without compromising their viability. Therefore, incubation of PBS containing *E. coli* with 1a (1 mM), 2a (2 mM), and CuCl2 (100 μM) led to a rapid increase in the fluorescence. After 24 hours, centrifugation and analysis in a plate reader of both the extracellular supernatant and the methanol/water (8:2) extracts of the resulting bacteria pellet showed a combined increase in fluorescence of eight times with respect to controls (see Figure S29). Importantly, the fluorescence was mainly concentrated inside the bacteria, and the product 3aa was also detected by HPLC-ESI. Analysis of the optical density of the bacterial cultures revealed that neither the catalyst nor the reactants are meaningfully toxic (see Table S5).

In summary, we have discovered a new methodology to achieve catalytic, orthogonal chemical annihilations in water, at room temperature. The reaction is promoted by specific ruthenium(II) catalysts, works efficiently with a variety of azides and thioalkynes, and can be carried out in presence of biomolecules (glutathione, amino acids, peptides). The reaction is also efficient in phosphate buffered saline, and in complex biological media such as cell lysates and fetal bovine serum, and even in presence of living bacteria. Importantly, the reaction is mutually compatible with the classical CuAAC, thus providing the option of tandem biorthogonal processes.

Acknowledgments

This work has received financial support from Spanish grants (SAF2016-76689-R and SAF2013-41943-R), the Xunta de Galicia (2015-CP082 and Centro Singular de Investigación de Galicia accreditation 2016-2019 ED431G-09), the European Union (European Regional Development Fund - ERDF), and the ERC (Adv. Grant 340055). We also thank the Orfeo-Cinca network CTQ2016-81797-REDC, and T. Seedat and V. Fraga for preliminary experiments.

Conflict of interest

The authors declare no conflict of interest.

Keywords: alkynes - azides - chemical ligations - click chemistry - ruthenium

How to cite: Angew. Chem. Int. Ed. 2017, 56, 10766–10770
Angew. Chem. 2017, 129, 10906–10910

[1] H. C. Kolb, M. G. Finn, K. B. Sharpless, Angew. Chem. Int. Ed. 2001, 40, 2004; Angew. Chem. 2001, 113, 2056.

[2] For instance, see: a) J. E. Hein, V. V. Fokin, Chem. Soc. Rev. 2010, 39, 1302; b) F. Musumeci, S. Schenone, A. Desogus, E. Nieddu, D. Deodato, L. Botta, Curr. Med. Chem. 2015, 22, 2022; c) T. Zhang, Z. Zheng, X. Cheng, X. Ding, Y. Peng, Prog. Chem. 2008, 20, 1090; d) J. Matysákovský, P. Perliková, V. Malmut, R. Pohl, M. Hocek, Angew. Chem. Int. Ed. 2016, 55, 15886; Angew. Chem. 2016, 128, 16808.

[3] a) L. Li, Z. Zhang, Molecules 2016, 21, 1393; b) V. K. Tiwari, B. B. Mishra, K. B. Mishra, N. A. Singh, X. Chen, Chem. Rev. 2016, 116, 3086; c) D. Schulz, A. Rentmeister, ChemBioChem 2014, 15, 2342.

[4] D. C. Kennedy, C. S. McKay, M. C. Legault, D. C. Danielson, J. A. Blake, A. F. Pegoraro, A. Stolow, Z. Mester, J. P. Pezacki, J. Am. Chem. Soc. 2011, 133, 17993.

[5] V. Hong, N. F. Steinmetz, M. Manchester, M. G. Finn, Bioconjugate Chem. 2010, 21, 1912.

[6] For selected examples, see: a) V. O. Rodionov, S. I. Presolski, D. Díaz Díaz, V. V. Fokin, M. G. Finn, J. Am. Chem. Soc. 2007, 129, 12705; b) S. I. Presolski, V. Hong, S.-H. Cho, M. G. Finn, J. Am. Chem. Soc. 2010, 132, 14570; c) B. T. Worrell, J. A. Malik, V. V. Fokin, P. M. Guha, R. J. Clark, T. Zheng, L. Feng, D. Soriano del Amo, W. Wang, L. M. Kivlinsky, F. L. Marlow, Y. Liu, P. Wu, Angew. Chem. Int. Ed. 2011, 50, 8051; Angew. Chem. 2011, 123, 8201; d) C. Uittampiantant, A. Tangerechak, S. Grecian, S. Clarke, U. Singh, P. Slade, K. R. Gee, A. Y. Ting, Angew. Chem. Int. Ed. 2012, 51, 5852; Angew. Chem. 2012, 124, 5954; e) S. Li, L. Wang, F. Yu, Z. Zhu, D. Shobaki, H. Chen, M. Wang, J. Wang, G. Qin, U. J. Erasquier, L. Ren, Y. Wang, C. Cai, Chem. Sci. 2017, 8, 2107.

[7] For the use of nanoparticle-based catalysts, see: a) J. Clavadescher, S. Hoffmann, A. Lilienkampf, L. Mackay, R. M. Yusop, S. A. Rider, J. J. Mullins, M. Bradley, Angew. Chem. Int. Ed. 2016, 55, 15662; Angew. Chem. 2016, 128, 15891; b) Y. Bai, X. Feng, H. Xing, Y. Xu, B. K. Kim, N. Baig, T. Zhou, A. A. Gewirth, Y. Lu, E. Oldfield, S. C. Zimmerman, J. Am. Chem. Soc. 2016, 138, 11077.

[8] a) J. C. Jewett, C. R. Bertozzi, Chem. Soc. Rev. 2009, 39, 1272; b) N. K. Devaraj, R. Weissleder, Acc. Chem. Res. 2011, 44, 816. For photoinitable annihilations, see: a) R. K. V. Lim, Q. Lin, Acc. Chem. Res. 2011, 44, 828.

[9] a) M. Vrabl, T. Carell, Cycloadditions in Bioorthogonal Chemistry, Springer, Heidelberg, 2016; b) P. Shieh, C. R. Bertozzi, Org. Biomol. Chem. 2014, 12, 9307; c) C. S. McKay, M. G. Finn, Chem. Biol. 2014, 21, 1075; d) M. King, A. Wagner, Bioconjugate Chem. 2014, 25, 825; e) D. M. Patterson, L. A. Nazarova, J. A. Prescher, ACS Chem. Biol. 2014, 9, 592; f) C. P. Ramil, Q. Lin, Chem. Commun. 2013, 49, 11007.

[10] a) M. Yang, Y. Yang, P. R. Chen, Top. Curr. Chem. 2016, 374, 2; b) M. Yang, J. Li, P. R. Chen, Chem. Soc. Rev. 2014, 43, 6511.

[11] C. Wang, D. Ihklef, S. Kahal, J.-Y. Saillard, D. Astruc, Coord. Chem. Rev. 2016, 316, 1.

[12] L. Zhang, X. Chen, P. Xue, H. H. Y. Sun, I. D. Williams, K. B. Sharpless, V. V. Fokin, G. Jia, J. Am. Chem. Soc. 2005, 127, 15998.

[13] J. R. Johanssen, T. Beke-Somfai, A. Said Stalsmeden, N. Kann, Chem. Rev. 2016, 116, 14726.

[14] For a recent review, see: a) L. Zhu, C. J. Brassard, X. Zhang, P. M. Guha, R. J. Clark, Chem. Rev. 2016, 16, 1501. See also: b) B. T. Worrell, J. A. Malik, V. V. Fokin, Science 2013, 340, 457.

[15] a) B. C. Boren, S. Narayan, L. K. Rasmussen, L. Zhang, H. Zhao, Z. Lin, G. Jia, V. V. Fokin, J. Am. Chem. Soc. 2008, 130, 8923; b) E. Boz, N. Ş. Tüzün, J. Organomet. Chem. 2013, 724, 167.

[16] J. S. Oakdale, V. V. Fokin, S. Umezaki, T. Fukuyama, Org. Synth. 2013, 90, 96.
In anhydrous CH$_2$Cl$_2$ under argon, 3aa was obtained in 78% yield (see Table S1).

Increasing the amount of the catalyst doesn’t lead to substantial changes in the rate (see the Supporting Information).