

## Heterocycle Synthesis

## Relayed Regioselective Alkynylation/Olefination of Unsymmetrical Cyclic Diaryliodonium Species Catalyzed by Cu and Pd: Affording Fluorescent Cytotoxic Benzoxazoles

Daqian Zhu,<sup>[a, b]</sup> Panpan Liu,<sup>[a]</sup> Wenhua Lu,<sup>[a]</sup> Haiwen Wang,<sup>[b]</sup> Bingling Luo,<sup>[a, b]</sup> Yumin Hu,<sup>[a]</sup> Peng Huang,<sup>\*[a]</sup> and Shijun Wen<sup>\*[a, b]</sup>

Abstract: Although cyclic diaryliodonium species have the potential to act as valuable synthons for cascade transformations, they still remain largely unexplored. The regioselectivity associated with unsymmetrical cyclic diaryliodonium species has previously been known to pose a challenge. A regioselective relayed alkynylation and olefination of unsymmetrical cyclic diaryliodonium species has been achieved by installation of a directing amido group. These relayed transformations were delayed until an oxazole ring had formed, delivering a series of unique fluorescent benzoxazoles. Moreover, some of these synthetic benzoxazoles showed apparent inhibitory activity against malignant cancer cells. Further confocal visualization revealed that benzoxazoles targeted cell nuclei. These findings might provide a novel structural scaffold to develop desirable anticancer agents.

Transition metal-catalyzed regio- and site-selective cross-coupling reactions are very powerful tools to form C–C and C–X bonds and remain a long-standing challenge in organic chemistry. In recent years, remarkable progress has been achieved in site-selective transformations by directed C–H activation (Scheme 1 a).<sup>[1]</sup> Generally, it is believed that functionalization of aryl *ortho* C–H bonds is possible through  $\sigma$ -chelationassisted metalation with directing groups (DGs) such as pyridines,<sup>[2]</sup> amides,<sup>[3]</sup> ketones,<sup>[4]</sup> and others.<sup>[5]</sup> With the assistance of DGs, thermodynamically favorable six- or seven-membered metal complex intermediates are formed and then subjected to subsequent functionalization.

 [a] D. Zhu,<sup>+</sup> P. Liu,<sup>+</sup> W. Lu, B. Luo, Dr. Y. Hu, Prof. P. Huang, Dr. S. Wen Sun Yat-sen University Cancer Center, State Key Laboratory of Oncology in South China, Collaborative Innovation Center for Cancer Medicine Sun Yat-sen University 651 Dongfeng East Road, Guangzhou 510060 (China)
 E-mail: huangpeng@sysucc.org.cn wenshj@sysucc.org.cn

- [b] D. Zhu,<sup>+</sup> H. Wang, B. Luo, Dr. S. Wen School of Pharmaceutical Sciences, Sun Yat-sen University 132 Waihuan East Road, Guangzhou 510006 (China)
- [<sup>+</sup>] Both authors contributed equally to this work.
- Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.201503791.

Linear diaryliodonium species have been extensively exploited as arylating agents in the formations of C-X bonds.<sup>[6]</sup> Recently, significant achievements have been made in regioselective arylation with unsymmetrical linear diaryiodonium species.<sup>[7]</sup> Compared to their linear analogues, cyclic diaryliodonium species have not found broad applications in synthetic organic chemistry.<sup>[8]</sup> Our group is interested in the development of novel transformations with cyclic diaryliodonium species to construct important structural scaffolds. However, regioselectivity associated with unsymmetrical cyclic diaryliodonium species remained an unsolved problem to us in our previous study.<sup>[9]</sup> Inspired by the directing group coordination effect (Scheme 1 a), we were eager to explore whether unsymmetrical cyclic diaryliodonium species containing common DGs, for example, an amido group, will exhibit the expected regioselectivity (Scheme 1 b). Herein, we report a relayed regioselective alkynylation and olefination of unsymmetrical cyclic diaryliodonium species by installing a directing amido group, leading to simultaneous benzoxazole formation. In these reactions, diverse alkynes and olefins could be selectively introduced into unsymmetrical cyclic diaryliodonium species and the directing amide groups were converted into oxazole motifs through intramolecular rearrangement. C-C and C-O bonds were formed under dual relaying catalysis by Cul and Pd(OAc)<sub>2</sub>. Furthermore, the fluorescent properties and anticancer activities of the obtained products were also investigated.

To assess our aforementioned hypothesis, a benzamido group was installed *ortho* to the iodine center in cyclic diaryliodonium **1a**. Our initial efforts for the *ortho*-selective alkynylation of unsymmetrical cyclic diaryliodonium species were guided by the reaction of **1a** with *p*-tolylacetylene **2a** catalyzed by copper and palladium, a similar reaction system to that developed in our previous study.<sup>[9]</sup> To our surprise, the expected product **3a**' was not obtained, although **2a** was hypothesized to be selectively introduced *ortho* to the directing amido group. Instead, a new benzoxazole ring was formed and a subsequent relayed alkynylation occurred to provide the novel product **3a**. The absolute configuration of **3a** was then determined by single-crystal X-ray diffraction (Figure 1).<sup>[10]</sup>

Benzoxazole frameworks are often applied in pharmaceuticals, dyes, and functional materials.<sup>[11]</sup> Many endeavors have been made to construct this type of aromatic heterocycle.<sup>[12]</sup> However, to our knowledge, the synthesis of benzoxazoles from diaryliodonium species has not been reported to date. Thus, it was of interest to us to further investigate these find-

Wiley Online Library



Scheme 1. Strategies for site-selective functionalization enabled by directing groups. a) General strategy with ortho metalation; b) this work.



Figure 1. Single-crystal X-ray structure of 3a.<sup>[10]</sup>

ings, whereby a regioselective alkynylation took place and a directing amide group was converted into an oxazole motif by intramolecular rearrangement.

Firstly, we undertook screenings of catalysts, ligands, and bases to optimize the reaction conditions for the formation of **3a** (see the Supporting Information, Table S1). The optimal reaction conditions were identified as Cul/Pd(OAc)<sub>2</sub> as catalysts, PPh<sub>3</sub> and Na<sub>2</sub>CO<sub>3</sub> as ligand and base, respectively, at 100 °C under Ar (Table S1, entry 1). It is worth noting that reactions with single copper or palladium catalysts gave extremely poor yield or no product (Table S1, entries 12 and 13), implying that the Cu/Pd dual transition metal system is critical to the generation of **3a**.

Then, the scope of both alkynes and cyclic diaryliodonium species were investigated (Scheme 2). Aryl alkynes bearing electron-donating groups (Me, OMe) or electron-withdrawing groups (CO<sub>2</sub>Me, F, Cl) were found to perform well (3a-g). Gratifyingly, alkyl alkynes also gave the desired products in modest yields (3h, 3i). Meanwhile, similar results were obtained for cyclic diaryliodonium substrates in which the phenyl ring carried various substituents (3j-m). To our delight, alkyl amide group (pivalamide) also provided the desired benzoxazole 3n. A substrate with the electron-rich amido group 3,4,5-trimethoxybenzamide also underwent smooth conversion (3o), where-

as the presence of a highly electron-poor amido group (pentafluorobenzamide) led to no conversion (data not shown).

To deliver structures with greater synthetic value and make this methodology more general, we speculated that various olefins could also be amenable to this dual metal relay catalysis strategy (Scheme 3). To our satisfaction, in a screening of olefination conditions (see the Supporting Information, Table S2), methyl acrylate (4a) was effectively introduced with 1,2-bis(diphenylphosphino)ethane (dppe) as a ligand. In terms of olefin compatibility, the reactions were tolerant of methyl acrylate, tert-butyl acrylate, ethyl vinyl ketone, affording 5a, 5b, and 5c, respectively (Scheme 3). Aryl olefin substrates with a variety of substituents including 2-nitro (5d), 3-nitro (5e), 4nitro (5 f), 4-cyano (5 g), and 4-methoxycarbonyl (5 h) were readily coupled with 1a. Moreover, iodonium species bearing diverse groups including trifluoromethyl (5 i), methoxycarbonyl (5j), phenyl (5k), and methoxy (5l, 5m) were well tolerated. More importantly, other amido groups, including alkyl amides, were also tolerated to some degree (5 n, 5 o).

For better insight into the reaction mechanism, we performed some control experiments (Scheme 4). In the absence of alkyne 2a, iodonium 1a was subjected to the standard conditions for the formation of 3a with various catalysts. Intermediate 6 was obtained in similarly good yields with Cul only or with a combination of Cul and  $Pd(OAc)_2$ . However, 6 was not formed in the presence of only the palladium catalyst. Next, 3a was readily generated in high yield through the reaction of 6 with 2a under standard conditions. Based on these observations, a potential mechanism of these transformations was proposed, consisting of two reaction cycles (see the Supporting Information, Scheme S1). The first intramolecular reaction to form intermediate 6A was mediated by copper catalysts. Then, a relayed intermolecular reaction of intermediate 6A with alkynes to form the final product 3 was catalyzed by Pd or Pd/Cu. In these transformations, a radical pathway cannot be excluded, as neither 6 nor 3a was formed in the reaction of 1 a and 2 a under the standard conditions in the presence of the radical scavenger TEMPO. Further investigation to elucidate the mechanism is still underway.





Scheme 2. Relayed alkynylation of unsymmetrical cyclic diaryliodonium species. Reaction conditions: 1 (0.27 mmol), 2 (1.5 equiv), Pd(OAc)<sub>2</sub> (10 mol%), Cul (10 mol%), PPh<sub>3</sub> (20 mol%), Na<sub>2</sub>CO<sub>3</sub> (3.0 equiv), DMF (1.4 mL), 100 °C, 15 h, Ar.

Benzoxazole is an important structural motif that is present in anticancer reagents.<sup>[13]</sup> In light of this, a preliminary anticancer screening was performed on these novel benzoxazoles (Figure 2). To our delight, some of the products, including **5 c**, **5 i**, **5 m**, and **5 o**, demonstrated obvious inhibition activity on colon cancer HCT-116 cells at 10  $\mu$ M concentration. In addition, malignant lung cancer A549 cells were also susceptible to these benzoxazoles (Figure S1, Supporting Information). Our findings suggest that these new obtained compounds may provide a useful scaffold to develop anticancer agents.

These synthetic benzoxazoles were also found to be fluorescent under irradiation at  $\lambda = 365$  nm. Compounds **5h**, **5j**, **5m**, and **5o** were selected for further study of their photophysical properties by UV/Vis and fluorescence spectroscopy (Figure 3). The UV/Vis absorption peak band of **5j** was narrow compared to those of the other compounds, whereas **5h** and **5o** showed broader emission bands than **5j** and **5m**. The fluorescence quantum yields were measured for **5h** (0.16), **5j** (0.234), **5m** (0.029) and **5o** (0.094) using quinine sulfate as a standard (see the Supporting Information, Table S3). Small biologically active molecules with fluorescence are highly valuable, due to a combination of potential diagnostic and therapeutic abilities in one molecule.<sup>[14]</sup>

Given the combined fluorescence and anticancer activity, confocal experiments were carried out to determine a cellular target of **5o** (Figure 4). HCT-116 cells treated with **5o** clearly showed blue fluorescence, implying their potential target might be the cell nuclei. Thus, a commercially available fluorescent selective cell nucleus marker, SYTO green, was employed to validate our observation. Indeed, the blue subcellular fluorescence of **5o** was well overlaid with the green subcellular fluorescence. These results imply that **5o** might target cell nuclei to cause its cytotoxic effects, although the exact mechanism remains to be elucidated.

In summary, relayed regioselective alkynylation and olefination of unsymmetrical cyclic diaryliodonium species were successfully realized under Cu/Pd dual-metal relay catalysis. These transformations were delayed until the oxazole ring had





Scheme 3. Relayed olefination of unsymmetrical cyclic diaryliodonium species. Reaction conditions: 1 (0.27 mmol), 4 (2.0 equiv), Pd(OAc)<sub>2</sub> (10 mol%), Cul (10 mol%), dppe (20 mol%), Na<sub>2</sub>CO<sub>3</sub> (3.0 equiv), DMF (1.4 mL), 100 °C, 24 h, Ar.



Scheme 4. Control experiments. Reaction conditions: Pd(OAc)<sub>2</sub> or/and Cul (10 mol% if used), PPh<sub>3</sub> (20 mol%), Na<sub>2</sub>CO<sub>3</sub> (3.0 equiv), 100 °C, DMF.

formed, providing a concise synthetic method to access a series of novel fluorescent benzoxazoles. In subsequent biological evaluation, some compounds showed obvious antiproliferative activity against malignant cancer cells. Moreover, the compounds appeared to target the cell nuclei, according to confocal experiments. These findings may provide a novel structural scaffold to develop anticancer agents with unique fluorescent properties. Further work on employing the current transformation to develop benzoxazoles with long absorption wavelengths and better cytotoxicities is currently underway in our laboratory.



Figure 2. Preliminary anticancer screening of synthetic benzoxazoles



**Figure 4.** Subcellular localization of **5 o** in HCT-116 cells. a) Fluorescent image of cells treated with **5 o** ( $\lambda_{\text{Ex}} = 405 \text{ nm}$ ;  $\lambda_{\text{Em}} = 430-470 \text{ nm}$ ); b) fluorescent image of cells treated with SYTO Green ( $\lambda_{\text{Ex}} = 500 \text{ nm}$ ;  $\lambda_{\text{Em}} = 530 \text{ nm}$ ); c) overlay of images (a) and (b). All images share the same scale bar (10 µm).



**Figure 3.** Photophysical properties of **5 h**, **5 j**, **5 m**, and **5 o** (0.5  $\mu$ m in CHCl<sub>3</sub>, 25 °C). Left: UV/Vis absorption spectra; middle: emission spectra (measured after their respective max excitation wavelength); right: luminescent colors of **5 h** (green), **5 j** (brown), **5 m** (blue), **5 o** (light blue) on irradiation at  $\lambda$ =365 nm.

## **Experimental Section**

General procedure for synthesis of 3, exemplified by 3a: To iodonium 1a (150 mg, 267.2 µmol) in a flask was added Na<sub>2</sub>CO<sub>3</sub> (85 mg, 802 µmol), Pd(OAc)<sub>2</sub> (6.0 mg, 26.72 µmol), PPh<sub>3</sub> (14 mg, 53.5 µmol), and Cul (5.1 mg, 26.72 µmol). The reaction flask was evacuated and backfilled with argon three times, and a solution of 1-ethynyl-4-methylbenzene 2a (50.1 µL, 400.85 µmol) in DMF (1.4 mL) was added via syringe. The reaction mixture was stirred at 100 °C under Ar atmosphere for 15 h. The reaction mixture was then diluted with EtOAc (3×15 mL), washed with H<sub>2</sub>O (2×5 mL) and brine (2×5 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (petroleum ether (60–90)/EtOAc = 100:1–20:1) to give 3a (76 mg, 71% yield) as a white solid.

General procedure for synthesis of 5, exemplified by 5 a: To 1 a (150 mg, 267.2 µmol) in a flask was added  $Na_2CO_3$  (85 mg, 802 µmol), Pd(OAc)<sub>2</sub> (6.0 mg, 26.72 µmol), dppe (21.29 mg, 53.45 µmol), and Cul (5.1 mg, 26.72 µmol). The reaction flask was evacuated and backfilled with argon three times, and a solution of methyl acrylate **4a** (48.13 µL, 534.46 µmol) in DMF (1.4 mL) was added via syringe. The reaction mixture was sealed in a tube and stirred at 100 °C heated by an oil bath under Ar atmosphere for 24 h. The reaction mixture was then diluted with EtOAc (3×15 mL), washed with H<sub>2</sub>O (2×5 mL) and brine (2×5 mL), dried over anhydrous  $Na_2SO_4$ , and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (petroleum ether (60–90)/EtOAc=50:1–20:1) to give **5a** (72 mg, 73% yield) as a white solid.

## Acknowledgements

This work was supported by Guangdong Department of Science and Technology (2013B051000034, 2014A030313196), Guangzhou Innovation Research Program (LCY201317), and National Basic Research Program of China (2012CB967004).

**Keywords:** alkynylation · fluorescence · iodonium · olefination · regioselectivity

- a) C. Yeung, V. Dong, Chem. Rev. 2011, 111, 1215–1292; b) D. Colby, R. Bergman, J. Ellman, Chem. Rev. 2010, 110, 624–655; c) O. Daugulis, H. Do, D. Shabashov, Acc. Chem. Res. 2009, 42, 1074–1086; d) T. Lyons, M. Sanford, Chem. Rev. 2010, 110, 1147–1169; e) K. Engle, T. Mei, M. Wasa, J.-Q. Yu, Acc. Chem. Res. 2012, 45, 788–802.
- [2] A. Tsai, M. Tauchert, R. Bergman, J. Ellman, J. Am. Chem. Soc. 2011, 133, 1248–1250.
- [3] S. Rakshit, C. Grohmann, T. Besset, F. Glorius, J. Am. Chem. Soc. 2011, 133, 2350-2353.
- [4] N. Schröder, J. Wencel-Delord, F. Glorius, J. Am. Chem. Soc. 2012, 134, 8298-8301.
- [5] a) G. Li, D. Leow, L. Wan, j.-Q. Yu, Angew. Chem. Int. Ed. 2013, 52, 1245–1247; Angew. Chem. 2013, 125, 1283–1285; b) C. Wang, H. Chen, Z. Wang, J. Chen, Y. Huang, Angew. Chem. Int. Ed. 2012, 51, 7242–7245; Angew. Chem. 2012, 124, 7354–7357; c) C. Tang, N. Jiao, J. Am. Chem. Soc. 2012, 134, 18924–18927; d) B. Xiao, Z. Liu, L. Liu, Y. Fu, J. Am. Chem. Soc. 2013, 135, 616–619.
- [6] a) R. J. Phipps, N. P. Grimster, M. J. Gaunt, J. Am. Chem. Soc. 2008, 130, 8172–8174; b) E. Skucas, D. W. MacMillan, J. Am. Chem. Soc. 2012, 134, 9090–9093; c) E. Cahard, N. Bremeyer, M. J. Gaunt, Angew. Chem. Int.

Chem. Eur. J. 2015, 21, 18915 - 18920

www.chemeurj.org

18919

Ed. 2013, 52, 9284–9288; Angew. Chem. 2013, 125, 9454–9458; d) M. G. Suero, E. D. Bayle, B. S. L. Collins, M. J. Gaunt, J. Am. Chem. Soc. 2013, 135, 5332–5335; e) D.-T. Tang, K. D. Collins, J. B. Ernst, F. Glorius, Angew. Chem. Int. Ed. 2014, 53, 1809–1813; Angew. Chem. 2014, 126, 1840–1844; f) S. Schäfer, T. Wirth, Angew. Chem. Int. Ed. 2010, 49, 2786–2789; Angew. Chem. 2010, 122, 2846–2850; g) N. Jalalian, E. E. Ishikawa, L. F. Silva, B. Olofsson, Org. Lett. 2011, 13, 1552–1555.

ChemPubSoc Europe

- [7] a) R. J. Phipps, M. J. Gaunt, *Science* 2009, *323*, 1593–1597; b) B. Chary, S. Kim, Y. Park, J. Kim, P. Lee, *Org. Lett.* 2013, *15*, 2692–2695; c) M. Iyanaga, Y. Aihara, N. Chatani, *J. Org. Chem.* 2014, *79*, 11933–11939; d) B. Xiao, Y. Fu, J. Xu, T. Gong, J. Dai, J. Yi, L. Liu, *J. Am. Chem. Soc.* 2010, *132*, 468–469.
- [8] a) Z. Liu, D. Zhu, B. Luo, N. Zhang, Q. Liu, Y. Hu, R. Pi, P. Huang, S. Wen, Org. Lett. 2014, 16, 5600-5603; b) D. Zhu, Q. Liu, B. Luo, M. Chen, R. Pi, P. Huang, S. Wen, Adv. Synth. Catal. 2013, 355, 2172-2178; c) Y. Wu, X. Peng, B. Luo, F. Wu, B. Liu, F. Song, P. Huang, S. Wen, Org. Biomol. Chem. 2014, 12, 9777-9780; d) D. Zhu, M. Chen, M. Li, B. Luo, Y. Zhao, P. Huang, F. Xue, A. Rapposelli, R. Pi, S. Wen, Eur. J. Med. Chem. 2013, 68, 81-88.
- [9] D. Zhu, Y. Wu, B. Wu, B. Luo, A. Ganesan, F.-H. Wu, R. Pi, P. Huang, S. Wen, Org. Lett. 2014, 16, 2350–2353.
- [10] CCDC 1408091 (3 a) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre.
- [11] a) J. S. Carey, D. Laffan, C. Thomson, M. T. Williams, Org. Biomol. Chem.
  2006, 4, 2337–2347; b) A. Kraft, A. C. Grimsdale, A. B. Holmes, Angew. Chem. Int. Ed. 1998, 37, 402–428; Angew. Chem. 1998, 110, 416–443;
   c) J. E. Moses, A. D. Moorhouse, Chem. Soc. Rev. 2007, 36, 1249–1262;

d) M. Meldal, C. W. Tornoe, *Chem. Rev.* **2008**, *108*, 2952–3015; e) J.-F. Lutz, *Angew. Chem. Int. Ed.* **2007**, *46*, 1018–1025; *Angew. Chem.* **2007**, *119*, 1036–1043; f) S. Gorla, M. Kavitha, M. Zhang, L. Hedstrom, G. Cuny, *J. Med. Chem.* **2013**, *56*, 4028–4043; g) M. Cui, M. Ono, H. Kimura, M. Ueda, H. Saji, *J. Med. Chem.* **2012**, *55*, 9136–9145; h) D. Chancellor, K. Davies, O. Moor, D. Wren, G. Wynne, *J. Med. Chem.* **2011**, *54*, 3241–3250.

- [12] a) N. Aljaar, C. Malakar, J. Conrad, W. Frey, U. Beifuss, J. Org. Chem. 2013, 78, 154–166; b) P. Boissarie, Z. Hamilton, S. Lang, J. Murphy, C. Suckling, Org. Lett. 2011, 13, 6256–6259; c) J. Bonnamour, C. Bolm, Org. Lett. 2008, 10, 2665–2667; d) J. Peng, C. Zong, M. Ye, T. Chen, D. Gao, Y. Wang, C. Chen, Org. Biomol. Chem. 2011, 9, 1225–1230; e) G. Evindar, R. Batey, J. Org. Chem. 2006, 71, 1802–1808.
- [13] a) F. Lin, A. Damu, T. Wu, J. Nat. Prod. 2006, 69, 93–96; b) M. Don, C. Shen, Y. Lin, W. Syu, Y. Ding, C. Sun, J. Nat. Prod. 2005, 68, 1066–1070; c) M. McKee, S. Kerwin, Bio. Med. Chem. 2008, 16, 1775–1783; d) D. Kumar, M. Jacob, M. Reynoldsa, S. Kerwin, Bio. Med. Chem. 2002, 10, 3997–4004; e) S. Rida, F. Ashour, S. El-Hawash, M. ElSemary, M. Badr, M. E. Shalaby, Eur. J. Med. Chem. 2005, 40, 949–959.
- [14] a) J. Jokerst, S. Gambhir, Acc. Chem. Res. 2011, 44, 1050-1060; b) Y.
  Yuan, C. Zhang, M. Gao, R. Zhang, B. Tang, B. Liu, Angew. Chem. Int. Ed.
  2015, 54, 1780-1786; Angew. Chem. 2015, 127, 1800-1806; c) Y. Yuan,
  R. Kwok, R. B. Tang, B. Liu, J. Am. Chem. Soc. 2014, 136, 2546-2554.

Received: September 21, 2015 Published online on November 24, 2015