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Overcoming Synthetic Challenges of Oridonin A-Ring Structural Diversification: Regio- and Stereoselective Installation of Azides and 1,2,3-Triazoles at the C-1, C-2, or C-3 Position

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Abstract

Efficient and concise synthetic approaches have been developed for the rapid and diverse installation of azide functionalities at the C-1, C-2 or C-3 of oridonin (1) with highly controlled regio- and stereoselectivity, while keeping key reactive pharmacophores intact by utilizing unique preactivation strategies based on the common synthon 4. Further functionalization of these azides through click chemistry yielding triazole derivatives successfully provides access to an expanded natural scaffold-based compound library for potential anticancer agents.

Oridonin (**1**), a highly oxygenated 7,20-epoxy-*ent*-kaurane-type diterpenoid isolated from the traditional Chinese herb *isodon rubescens*, is characterized by its densely functionalized and stereochemistry-rich frameworks including an *exo*-methylene cyclopentanone moiety in the D-ring and a 6-hydroxyl-7-hemiketal group in the B-ring (Fig. 1).[1] It has been attracting considerable attention in recent years due to its remarkably unique and safe anticancer pharmacological profile.[1e–f] In China, oridonin injection is used alone or in combination with other drugs to treat liver cancer and carcinoma of gastric cardia.[2] Nevertheless, more extensive clinical applications of oridonin for cancer therapy have been restricted, to a large degree, due to its relatively moderate potency, limited aqueous solubility and bioavailability.[3] Consequently, modifications on the scaffold of **1** have become an attractive strategy to create better natural product-like compound libraries. Most previous approaches were attempted by coupling ester appendages to hydroxyl groups of **1**.[1b,4] To date, little

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Supporting Information Available. Experimental procedures, characterization data, copies of NMR spectra for new compounds, and X-ray CIF files for compounds **6**, **14** and **21**. This material is available free of charge via the Internet at http://pubs.acs.org.

chemical effort has been directed toward modifications of the oridonin Aring to generate diversity, likely owing to the synthetic challenges arising from its structural complexity with multiple reactive functionalities. Recently, we reported a concise synthesis of thiazole-fused oridonin derivatives with enhanced activity and solubility,[5] indicating that the rational modifications on oridonin A-ring may have great potential to generate better anticancer agents.

Click chemistry, a concept initiated by Sharpless,[6] has provided a number of nearly perfect "spring-loaded" chemical reactions to elaborate an elegant and selective modification on complex natural products. Particularly, the Cu(I)-catalyzed azide–alkyne cycloaddition (CuAAC)[7] can efficiently afford 1,2,3-triazole scaffolds under mild conditions even in the presence of chemically reactive functionalities. Importantly, the resulting triazole derivatives are fairly stable to metabolic degradation and capable of actively participating in hydrogen bonding and dipole-dipole interactions, providing potential advantages including target binding and cell permeability improvement.[8] Herein, we, for the first time, disclose our effort for the efficient synthesis of novel nitrogen-enriched oridonin derivatives with azide and 1,2,3-triazole functionalizations at the C-1, C-2 or C-3 position in a highly regio- and stereo-specific manner.

As illustrated in Fig. 1, our general synthetic strategy to achieve these molecules involves a key and challenging step of azide installation at each of three sites of the Aring with controlled regio- and stereoselectivity, while not destroying other featured functionalities. It is well documented that the presence of the α,β-unsaturated ketone (enone) system in the D-ring of **1** is the main structural determinant for its anticancer activity and destruction of this enone system could counteract its bioactivity.[1a–b,d] On the other hand, this bioactive enone system is also a chemical electrophilic center susceptible to nucleophilic attack (Michael addition) by various nucleophiles including azide reagents under certain reaction conditions, leading to adducts at the β-position as evidenced in literature.[9] Accordingly, a regioselective installation of the azide functionality to the desired sites of the A-ring, but not the enone moiety in the D-ring, is synthetically challenging and essentially required, in developing efficient synthetic protocols. With this goal in mind, we attempted to achieve a differential reactivity of these two sites by activating the functional group of the Aring for a successfully controlled selectivity.

Our synthesis commenced with **1**, which is naturally abundant and commercially available (Scheme 1). Protection of 7,14-dihydroxyl of **1** with 2,2-dimethoxypropane[10] followed by selective activation of the 1-hydroxyl group with MsCl solely provided mesylate **2** in 74% yield over two steps, which was subsequently subjected to NaN_3 in DMF. However, the anticipated substitution reaction at 60 °C failed to give 1-azide **3** but resulted in high recovery of **2**. Increasing the reaction temperature to 120 °C produced a complex mixture, presumably owing to the Michael addition to the enone system in the D-ring by azide anions. Therefore, a more reactive electrophilic center deemed necessary to be created at the A-ring. To introduce an allylic alcohol functional moiety to the A-ring, mesylate **2** was chosen as a substrate to undergo an elimination reaction in the presence of Li_2CO_3 at 110 °C to provide 1-ene **4** in 84% yield, followed by an allylic oxidation with selenium dioxide in refluxing 1,4-dioxane, stereoselectively leading to the 3β-allylic hydroxyl **6** in excellent yield. In this step, the 1-allylic seleninic acid intermediate **5** was only formed from the β-face of the A-ring because of a steric effect of the 7,20-expoxy ring, eventually leading to an installation of the hydroxyl group at the 3β-position in a stereoselective manner that was unambiguously determined through X-ray crystallographic analysis. Initially, allylic alcohol **6** without any preactivation was directly treated with diphenylphosphoryl azide (DPPA)[11] and DBU in THF at 0 °C followed by warming up to 60 °C to install an azide group at C-3 for the purpose of atom-economy. Unfortunately, only a diphenyl phosphate intermediate **7**, instead of anticipated 3-allylic azide **8**, was obtained in 80% yield. Mechanistically, the reactive 3-allylic phosphate of intermediate **7** could not undergo further substitution reaction with the azide anion from the *α*-face due to the surrounding steric hindrance of C-3*α*. Accordingly, the 3-allylic alcohol of **6** has to be preactivated in the form of a better leaving group to install the 3-azide with high regioselectivity.

In pursuance of this goal, we intended to convert the 3-allylic alcohol into the 3-allylic chloride for installation of the 3-azide group (Scheme 2). Interestingly, chlorination of **6** with $SOCl_2$ in dry ether at rt exclusively led to 1*β*-allylic chloride **10** (73%),[12] the regio- and stereostructure of which was determined by HMBC and NOESY experiments (Fig. S1 in Supporting Information). In this step, owing to the intrinsic structural feature of the substrate, the in situ generated 3-allylic mesylate **9** underwent an S_Ni' reaction with the internal attack of chloride on C-1, instead of the allylic site C-3, leading to the shift of the double bond, in which a stable hexacyclic ring transition state was likely formed from the *β*-face.[13] Subsequent nucleophilic substitution reaction of **10** with NaN_3 in dry DMF at rt neatly afforded 1-allylic azide **11** (71%) with inversion of configuration despite steric crowding.[14] The high propensity of allylic azides to undergo [3,3]-sigmatropic rearrangement[15] suggested a facile route potentially available to 3*α*-allylic azide **8**. Gratifyingly, performing this substitution reaction at 70 °C smoothly redirected the reaction to 3*α*-azide **8** (74%) with a total control of regio- and stereoselectivity as determined by HMBC and NOESY experiments (Fig. S2 in Supporting Information). These results implied that 1*α*-azide **11** would be initially produced and then undergo [3,3]-sigmatropic shift to provide 3*α*-azide **8**. Further evidence for this isomerization was also achieved by heating 1*α*-azide **11** in refluxing CH_3CN to solely give 3*α*-azide **8**. This surprisingly high 3*α*-allylic azide preference is probably ascribed to its less crowded steric environment. As depicted in Scheme 2, the 3-azide group is far away from the bridgehead 20-methylene group, and possesses a more favorable steric surrounding relative to the 1-azide group, leading to this selective thermal isomerization of 1- to 3-type allylic azide.

With key intermediates **11** and **8** in hand, the subsequent click reaction with phenylacetylene catalyzed by CuI in dry CH_3CN at rt followed by removal of the acetonide groups with 5% HCl (aq) achieved the corresponding 1,2,3-triazole derivatives **14** and **15** in excellent yields, the structures of which were also confirmed by 1D and 2D NMR experiments (Figs. S3 and S4 in Supporting Information). The stereochemistry of **14** was further unambiguously secured by X-ray crystallographic analysis. As additional proof for selective isomerization of the 1- to 3-type allylic azide, the click reaction of 1-azide **11** in refluxing CH_3CN solely provided 3-triazole **13** (70%) instead of 1-triazole **12**. To further explore their versatility, triazole derivatives **18** and **19** have also been synthesized in a same fashion with good yields.

Regioselective installation of an azide group at the C-2 of the A-ring also utilized the preactivation strategy to achieve differential reactivity with respect to other reactive functionalities (Scheme 3). After considering various possibilities, the key synthon **4** prepared in Scheme 1 was subjected to epoxidation though *m*-CPBA-mediated Prilezhaev reaction.[16] To our delight, this reaction not only occurred preferentially at the 1-ene rather than the 16-*exo*-methylene, but also formed 1,2-epoxide ring stereoselectively from the less sterically-hindered *β*-face to exclusively give 1*S*,2*R*-epoxide **20** (86%). Ring-openings of unsymmetrical epoxides by nucleophiles usually lead to non-regioselctive products.[17] In our case, azidolysis of epoxide **20** with NaN_3 occurred preferentially with attack of azide anion on C-2 due to its less sterically-crowded surrounding relative to C-1. Conducting the reaction in $EtOH/H_2O$ (1:1) at 85 °C using NH_4Cl as a coordinating salt for 24 h solely afforded 2-azide **21** (51%),[18] the structure of which was also unequivocally determined by X-ray crystallographic analysis. In this step, different solvents such as $MeOH/H_2O$, $CH_3CN/$

H_2O, acetone/H_2O or DMF resulted in lower yields than that in aqueous ethanol; extending reaction time decreased the yield; after screening other coordinating salts, $LiClO_4$ proved to be optimal leading to a better yield (60%) than NH_4Cl (Table S1 in Supporting Information). The subsequent click reactions of **21** with phenyacetylene or 4-*tert*-butylphenylacetylene followed by treatment with 5% HCl (aq) successfully generated 2-triazole compounds **24** and **25** in 58% and 80% yields (two steps), respectively.

The antiproliferative effects of representative 1,2,3-triazole-substituted analogues against breast cancer cell lines including MCF-7 (ER-positive) and MDA-MB-231 (triple-negative) are summarized in Table 1, indicating that these new compounds with 1,2,3-triazole installed in the A-ring exhibited significantly improved anti-breast cancer activity in comparison with oridonin.

In summary, efficient and concise synthetic approaches have been developed for the rapid and diverse installation of azide functionalities at the C-1, C-2 or C-3 position of the oridonin A-ring, while keeping key reactive pharmacophores intact by utilizing unique preactivation strategies based on the common synthon **4**. Selective chlorination of 3-allylic alcohol **6** followed by substitution reaction with NaN_3 provided 1-azide **11**, which further underwent a selective [3,3]-sigmatropic rearrangement to solely afford 3-azide **8**; while selective Prilezhaev epoxidation of 1-ene **4** followed by epoxide ring-opening with azide exclusively led to 2-azide **21**. By harnessing the intrinsic structural features of the oridonin scaffold, a high degree of regio- and stereoselective control was achieved throughout the whole sequence. Further functionalizations of these azides through click chemistry yielding 1,2,3-triazole derivatives readily provides access to an expanded natural scaffold-based compound library. Intriguingly, these new molecules have demonstrated superior antiproliferative effects against breast cancer cells to oridonin. Our success in the efficient construction of oridonin-based azides or triazoles opens new synthetic avenues to the development of novel potential natural product-like anticancer agents.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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12. Formation of mesylate followed by treatment with LiCl was also attempted, but failed to give regio- and stereoselective product **10**, while resulting in a mixture of several isomers instead.
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14. Indeed, **11** was not very stable and a minor amount of **8** was identified when purified with silica gel column.
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18. This ring opening reaction required harsh condition and was still incomplete probably due to 1,3-diaxial interactions in the resulting product **21**.

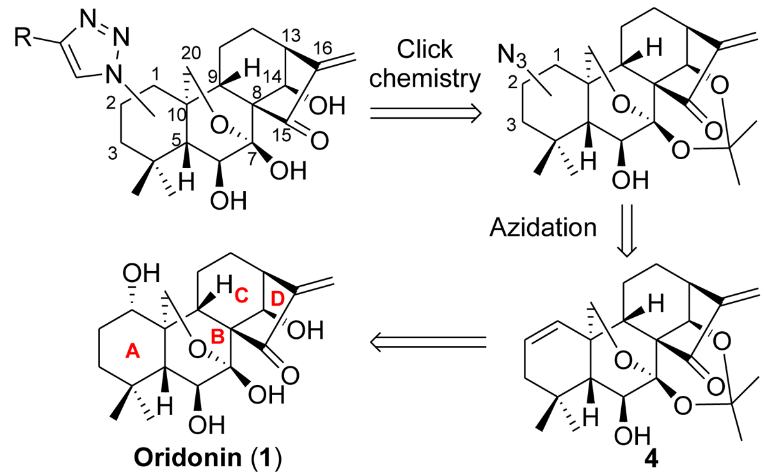
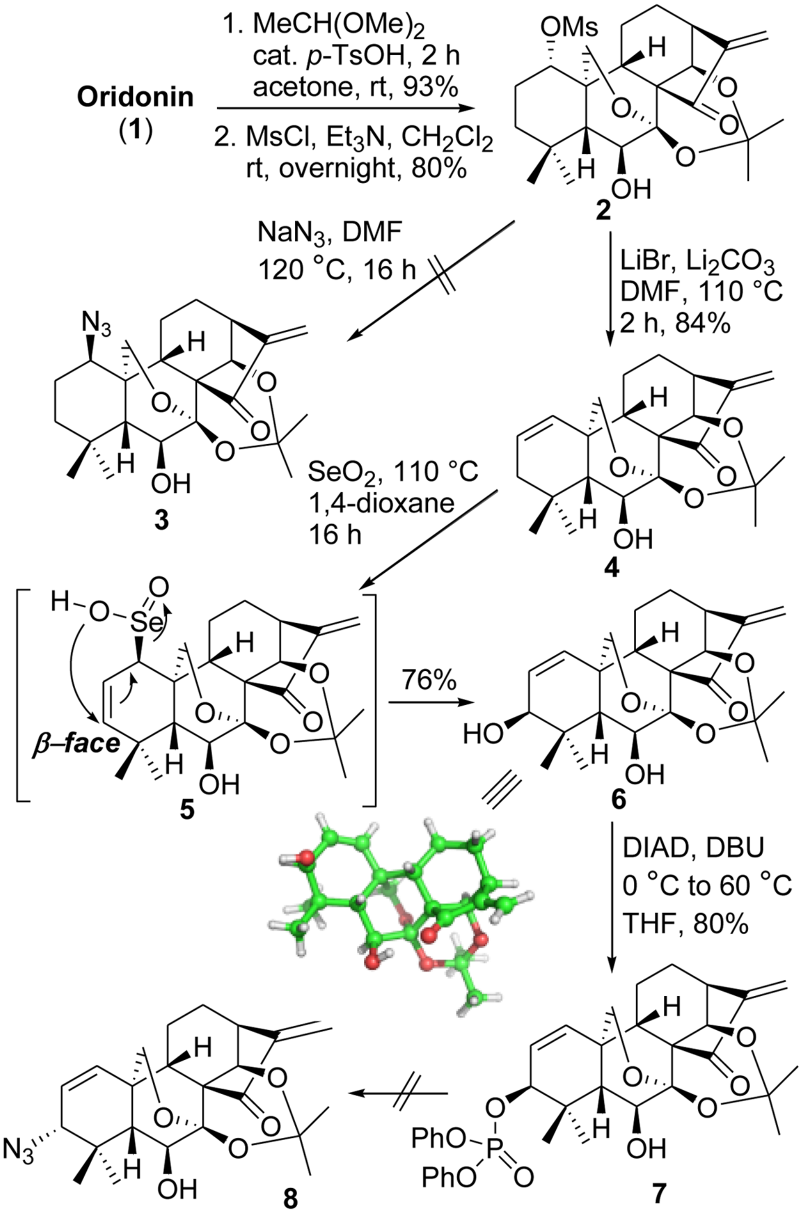


Figure 1.
Retrosynthetic analysis of azide- and 1,2,3-triazole-substituted oridonin derivatives.



Scheme 1.

Attempts to synthesize 1-azide and 3-azide derivatives **3** and **8**

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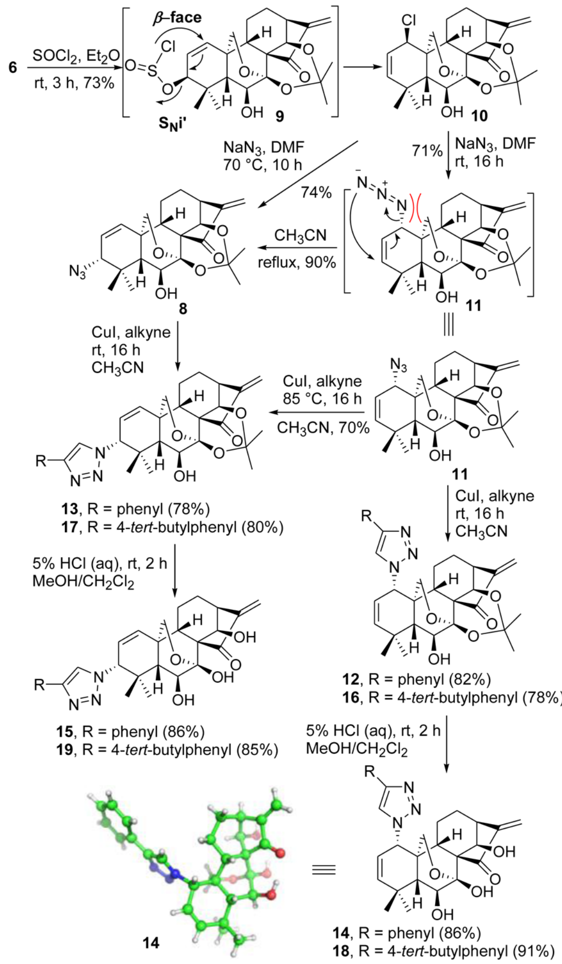
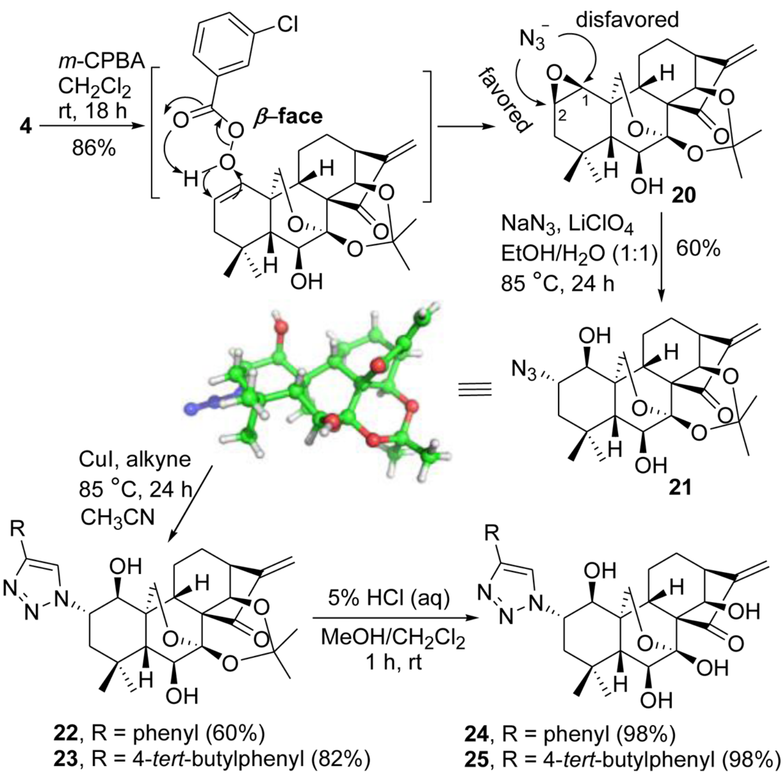


Scheme 2.

Synthesis of 3-azide-, 1-azide-, 1,2,3-triazole-substituted oridonin derivatives **8**, **11**, **14**, **15**, **18** and **19**



Scheme 3.

Synthesis of 2-azide-, 1,2,3-triazole-substituted oridonin derivatives **21**, **24** and **25**

Table 1

Antiproliferative effects of representative triazole-substituted analogues against human breast cancer cell lines

compounds	**IC_{50} (μM)[a]**	
	MCF-7	**MDA-MB-231**
Oridonin (**1**)	6.6	29.4
12	0.38	0.48
14	2.1	1.8
15	2.3	1.8
23	2.0	1.1
25	1.9	3.5

[a]Breast cancer cell lines: MCF-7 and MDA-MB-231. Software: MasterPlex ReaderFit 2010, MiraiBio, Inc. Values are mean ± SE of three independent experiments.