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Linking of Alcohols with Vinyl Azides

Yi-Feng Wang, Ming Hu, Hirohito Hayashi, Bengang Xing,* and Shunsuke Chiba*

Division of Chemistry and Biological Chemistry, School of Physical and Mathematical Sciences, Nanyang Technological University, Singapore 637371, Singapore

Supporting Information Placeholder

ABSTRACT: A protocol to link alcohols with vinyl azides has been established through fluoro- or bromo-alkoxylation of vinyl azides to provide α -alkoxy- β -haloalkylazides. A series of primary and secondary alcohols including natural products and their derivatives such as sugars and steroids were successfully anchored with vinyl azides. The as-prepared cyanine dye-linked testosterone derivatives were capable of rapid cell membrane imaging in real-time.

Owing to their rich and diverse chemical reactivity, organic azides are recognized as versatile synthons for the preparation of nitrogen-containing compounds, in particular, nitrogen-heterocycles.¹ The 1,3-dipole character of organic azides is most commonly used to construct heterocyclic frameworks through [3+2]-cycloaddition reactions with a range of unsaturated bonds such as alkynes, alkenes, and nitriles. Among them, the azide-alkyne cycloaddition² to form 1,2,3-triazoles is actively applied for various areas such as medicinal chemistry,³ chemical biology,⁴ and material sciences.⁵ For example, the azide-alkyne [3+2]-cycloaddition can be used to label natural products, drugs, and biomolecules *in vitro* and/or *in vivo* for bioimaging, drug delivery, or bioconjugation purposes.⁶ For these opportunities, installation of the azido unit at the appropriate position of the targeted materials is necessarily indispensable. Hydroxyl groups (alcohols) are prevalent in various molecules of biologically and medically importance.⁷ Chemical conversion of alcohols into azides is thus commonly implemented by substitution of the activated hydroxyl group with the azido ion.^{8,9} Use of 2-azido-1,3-dimethylimidazolium salts^{9a} or diarylphosphorylazides with DBU as a base^{9b-9d} enables direct azidation of alcohols (Scheme 1A).¹⁰ On the other hand, the protocol to install the azide unit onto the alcohols would be an alternative strategy to use alcohols for further applications.¹¹ We conceived that such a linking method could be achieved through electrophilic halogenation of vinyl azides **1** in the presence of alcohols **2**, in which the initially formed α -haloiminodiazonium ions **A**¹² could be trapped by external alcohols **2** (Scheme 1B). Neighboring group participation of the installed halogen atom (**X**) to the C=N bond might result in stabilization of iminodiazonium ions **A** through halonium ions **B**, preventing formation of nitrilium ions by migration of substituent R.^{12a,b}

Scheme 1. Installation of Azido Unit onto Alcohols

(a) Direct Conversion of Aliphatic Alcohols to Azides

(b) Linking Alcohols with Vinyl Azides through Haloalkoxylation (This Work: X = F or Br)

Herein, we demonstrate this concept with the fluoro- or bromo-alkoxylation of vinyl azides. Alcohols are uniquely connected to haloalkylazide moieties in the resulting unprecedented aminal-type structure. Concise transformation of the products, α -alkoxy- β -haloalkylazides to halomethyl nitrogen-heterocycles was demonstrated using thermal intramolecular [3+2]-azide-alkene/alkyne cycloaddition. Moreover, tagging of different types of fluorescent probes to testosterone-linked azide was implemented by the click reactions. The as-prepared fluorescent testosterone derivatives having cyanine probes exhibited unique properties for rapid imaging of cell membranes in real time.

Screening of a variety of halonium ion sources in the reactions of vinyl azide **1a** with cinnamyl alcohol (**2a**) revealed that 1-chloromethyl-4-fluoro-1,4-diazoniabicyclo[2.2.2]octane

bistetrafluoroborate (Selectfluor)^{13,14} and 2,4,4,6-tetrabromocyclohexa-2,5-dienone (TBCO)¹⁵ worked well to give desired α -alkoxy- β -haloalkylazides **3aa** (for fluoride) and **4aa** (for bromide) in 80% and 82% yields, respectively (Scheme 2, conditions A and B. See the Supporting Information for optimization of the reaction conditions). Having these protocols, the generality of alcohols **2** to link with vinyl azide **1a** was next examined (Scheme 2. See the Supporting Information for the substituent compatibility on vinyl azides **1b-1i** for these linking protocols). Both alkenyl (**2b** with aryl-substituted alkene and **2c** with terminal alkene) and alkynyl (**2d-2f** with both terminal and internal alkynes) alcohols reacted smoothly, providing the corresponding β -haloalkylazides **3** and **4** in good yields. Other functional groups such as *N*-tosylindolyl (for **2g**), phenylsulfonyl (for **2h**), diethyl phosphoryl (for **2i**), and pinacolboryl (for **2j**) moieties were all compatible under the present reaction conditions. In the reactions of diol **2k** having primary and secondary hydroxyl groups, the primary one was preferentially linked (for **3ak** and **4ak**).¹⁶ For linking of 3,5-dimethylphenol (**2l**), the bromination protocol with TBCO only worked to give **4al**, while the reaction was sluggish due to over-bromination. We next investigated to tag natural products and their derivatives having hydroxyl group(s) in their structure.¹⁷ Glucose derivative **2m**¹⁸ and rac-menthol (**2n**) were readily employed for the present linking protocol. It was found that nopol (**2o**) and nerol (**2p**) having reactive alkenes (strained and/or electron-rich) are

not applicable for the linking protocol with Selectfluor, while that with TBCO delivered the corresponding azides **4ao** and **4ap** in good yields. Steroids such as androsterone (**2q**) and testosterone (**2r**) could be anchored well with both protocols, while the bromination method was only amenable for linking of cholesterol (**2s**) and betulin (**2t**) having an electron-rich alkene. In the reaction of betulin (**2t**), its primary hydroxyl group was selectively linked, while the secondary one next to the quaternary carbon was kept intact due to the steric hindrance.

Intramolecular 1,3-dipolar azide-alkene cycloaddition¹ of alkenyl alcohol-linked azides under thermal reaction conditions afforded unique nitrogen heterocycles, 2-halomethyl-2,5-dihydrooxazoles (for **5aa** and **6aa**) and 2-fluoromethyl-5,6-dihydro-2*H*-1,3-oxazine **7ab** in good yields (Scheme 3). Similarly, intramolecular 1,3-dipolar azide-alkyne cycloaddition reactions of **3ae** and **3af** were also performed well to give bicyclic triazoles **8ae** and **9af**, respectively, in good yields.

We next explored the potential applicability of the present linking protocol in bioimaging. As indispensable important cell components, steroids have been found to maintain cell membrane's integrity and fluidity as well as to secure the membrane-associated biological activities in living cells. It has been reported that several dye-labeled steroid analogues can be used for specific membrane staining.^{19,20}

Scheme 2. Substrate Scope on Alcohols^{a,b}

^a Unless otherwise noted, the reactions were carried out by treatment of alcohols **2** and vinyl azide **1a** (1.2-2.2 equiv) with Selectfluor (1.2-2.2 equiv) in CH₃CN (conditions A) or TBCO (1.2-2.2 equiv) in CH₂Cl₂ (conditions B) at 0 °C to room temperature under a N₂ atmosphere.

^b Isolated yields and diastereoselectivity (dr) determined by ¹H and ¹⁹F NMR measurements were recorded above. ^c Isolated yields of the linking product of the secondary hydroxyl group. ^d The reaction was conducted in CH₃CN-CH₂Cl₂ (5:1). ^e The reaction was conducted in CH₂Cl₂-THF (1:1).

Scheme 3. Intramolecular Azide-Alkene/Alkyne Cycloaddition. ^a 2 step yield from vinyl azide **1a** (see Supporting Information).

Thus, testosterone-linked azide **3ar** was chosen, and the click reactions of **3ar** and alkynes tethered to fluorescent probes such as *N*-propargyl dansylamide and azadibenzocyclooctyne-cyanine dyes (DBCO-Cy3 and DBCO-Cy5) were implemented to prepare **10-12** (Figure 1). It is noteworthy that the resulting triazole-linked aminal-type moiety was fairly stable under physiologically relevant serum conditions (pH 7.4) and in aqueous phosphate buffer solution (pH 7.6–3.0) (see the Supporting Information). Moreover, MTT assay showed that there was little cell viability impaired during the process of cell staining, indicating less toxicity and suitable biocompatibility of the triazole-linked aminal-type moiety in living cell applications (see the Supporting Information).

Human embryonic kidney 293 (HEK293) cells were then incubated with **10-12** (2.0 μ M). The capability of these dye-linked testosterone probes as a membrane staining probe was examined by confocal microscopy. As shown in Figure 1 and Figure S2, while fluorescence of dansyl probe **10** was found inside the cells rather than on the membrane surface probably due to the rapid internalization of dansyl probe **10** into the cells (Figure 1a), the strong fluorescence signals of **11** and **12** were found on the cellular membrane (Figures 1c and 1d). These observations are most likely attributed to the difference of the charge properties between dansyl-linked testosterone **10** and cyanines-linked ones **11** and **12**. The fluorescence signals overlap of dansyl probe **10** and organelle specific dyes (Figure 1b with BODIPY TR ceramide, one commonly used Golgi apparatus tracker) indicated more prominent localization in Golgi apparatus than other major organelles (e.g. mitochondria and lysosomes) (Figure S4). Further investigation is undergoing to account for such interesting difference between dansyl probe **10** and cyanines-linked probes **11** and **12**.

Further studies to develop modular and selective linking protocols of other essential functional groups, such as amines and thiols with vinyl azides, are currently underway in our laboratory. We envision that such a unique protocol would enable promising applicability for medicinal and biological studies.

Figure 1. Synthesis of dye-linked testosterone **10-12** and fluorescence imaging of live HEK293 cells by incubation of **10-12**, at 37 °C, respectively. Cells incubation with a) dansyl **10** (2 μ M) for 5 min, λ_{ex} = 405 nm; b) fluorescence signals overlap of dansyl **10** with BODIPY TR ceramide for the Golgi apparatus staining; c) Cy-3 **11** (2.0 μ M) for 2 min, λ_{ex} = 514 nm; and d) Cy-5 **12** (2.0 μ M) for 2 min, λ_{ex} = 633 nm. The cell nucleus staining (c and d) by Hoechst 33258 (λ_{ex} = 405 nm) was used as control. Scale bar in all images = 20 μ m.

ASSOCIATED CONTENT

Supporting Information

Experimental procedures, characterization of new compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>.

AUTHOR INFORMATION

Corresponding Author

*E-mail: shunsuke@ntu.edu.sg, bengang@ntu.edu.sg

Notes

The authors declare no competing financial interest.

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