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Linking of Alcohols with Vinyl Azides

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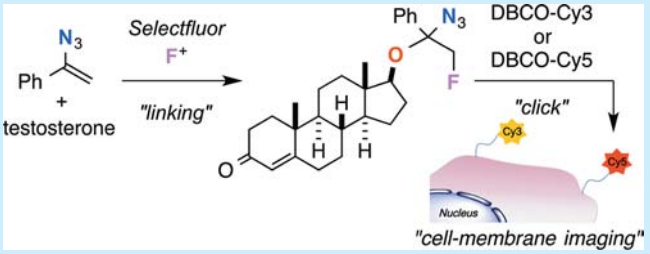
Supporting Information

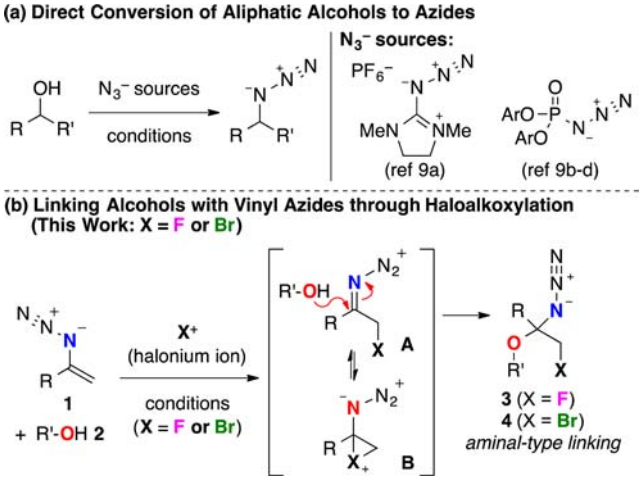
ABSTRACT: A protocol to link alcohols with vinyl azides has been established through fluoro- or bromo-alkoxylation of vinyl azides to provide α-alkoxy-β-haloalkyl azides. A series of primary and secondary alcohols including natural products and their derivatives such as sugars and steroids were successfully anchored with vinyl azides. The as-prepared cyanine dye linked testosterones 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 cycloaddition[2] 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 medicinally 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-dimethylimidazolinium salts[9a] or diarylphosphoryl azides with DBU as a base[9b−d] 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]

Herein, we demonstrate this concept with the fluoro- or bromo-alkoxylation of vinyl azides. Alcohols are uniquely connected to haloalkyl azide moieties in the resulting unprecedented aminal-type structure. Concise transformation of the products, α-alkoxy-β-haloalkyl azides to halomethyl

Scheme 1. Installation of Azido Unit onto Alcohols



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 the desired α-alkoxy-β-haloalkyl azides **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

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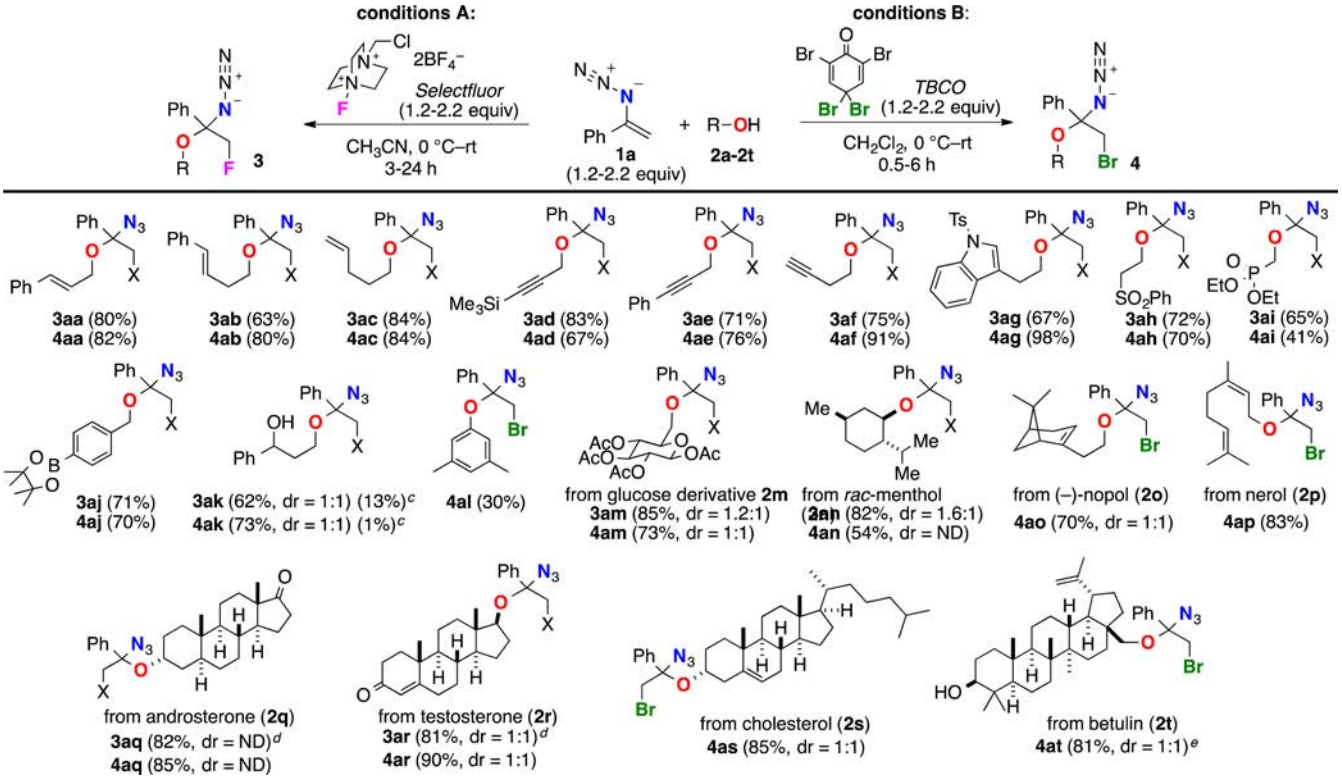
Scheme 2. Substrate Scope on Alcohols[a,b]

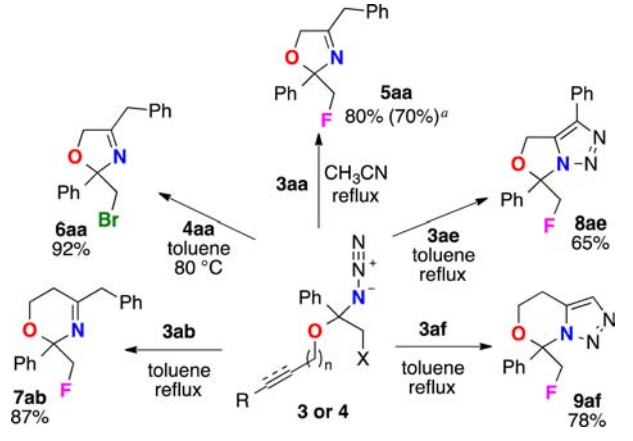


[a]Unless otherwise noted, the reactions were carried out by treatment of alcohols **2** and vinyl azide **1a** (1.2−2.2 equiv) with Selectfluor (1.2−2.2 equiv) in CH_3CN (conditions A) or TBCO (1.2−2.2 equiv) in CH_2Cl_2 (conditions B) at 0 °C to room temperature under a N_2 atmosphere. [b]Isolated yields and diastereoselectivity (dr) determined by ^1H and ^{19}F NMR measurements were recorded above. [c]Isolated yields of the linking product of the secondary hydroxyl group. [d]The reaction was conducted in CH_3CN−CH_2Cl_2 (5:1). [e]The reaction was conducted in CH_2Cl_2−THF (1:1).

protocols in hand, 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−i** for these linking protocols). Both alkenyl (**2b** with aryl-substituted alkene and **2c** with terminal alkene) and alkynyl (**2d−f** with both terminal and internal alkynes) alcohols reacted smoothly, providing the corresponding *β*-haloalkyl azides **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 overbromination. We next investigated tagging 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 steric hindrance.

Intramolecular 1,3-dipolar azide−alkene cycloaddition[1] 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

Scheme 3. Intramolecular Azide−Alkene/Alkyne Cycloaddition



[a]Two-step yield from vinyl azide **1a** (see the Supporting Information).

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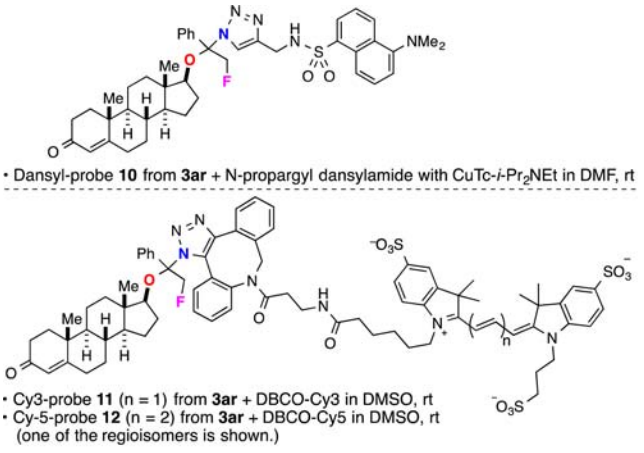
reactions of **3ae** and **3af** were also performed to give biyclic triazoles **8ae** and **9af**, respectively, in good yields.

We next explored the potential applicability of the present linking protocol in bioimaging. As indispensable important cell components, steroids have been found to maintain the 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]

Thus, testosterone-linked azide **3ar** was chosen, and the click reactions of **3ar** and alkynes were tethered to fluorescent probes such as *N*-propargyldansylamide, and azadibenzocyclooctyne−cyanine dyes (DBCO−Cy3 and DBCO−Cy5) were implemented to prepare **10**−**12** (Figure 1). It is noteworthy



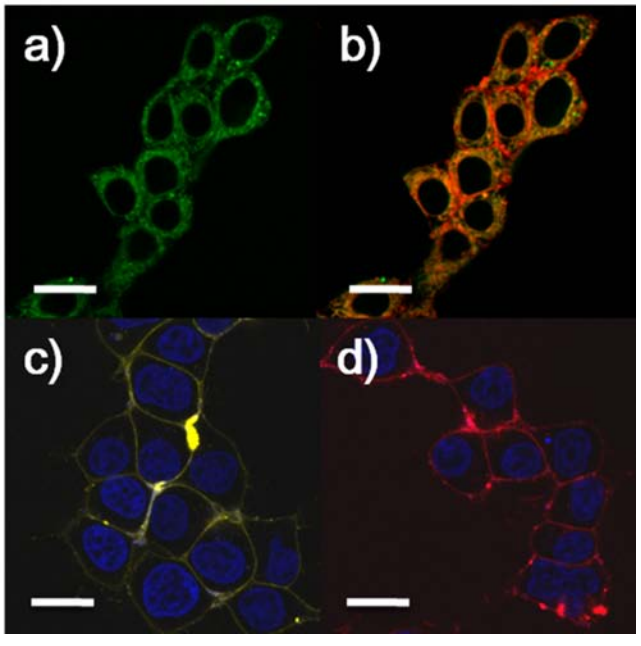


Figure 1. Synthesis of dye-linked testosterones **10**−**12** and fluorescence imaging of live HEK293 cells by incubation of **10**−**12** at 37 °C, respectively. Cells incubation with (a) dansyl **10** (2 μM) for 5 min, λ_{ex} = 405 nm; (b) fluorescence signals overlap of dansyl **10** with BODIPY TR ceramide for the Golgi apparatus staining; (c) Cy-3 **11** (2.0 μM) for 2 min, λ_{ex} = 514 nm; and (d) Cy-5 **12** (2.0 μM) 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 μm.

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 6.6−3.0) (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 μM). The capability of these dye-linked testosterones **10**−**12** 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,d). These observations are most likely attributed to the difference of the charge properties between dansyl-linked testosterone **10** and cyanine-linked ones **11** and **12**. The fluorescence signal 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 the Golgi apparatus than other major organelles (e.g., mitochondria and lysosomes) (Figure S4). Further investigation is ongoing to account for such interesting differences between dansyl probe **10** and cyanine-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.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b00116.

Experimental procedures and characterization of new compounds (PDF)

X-ray crystallographic data for compound **5aa** (CIF)

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Notes

The authors declare no competing financial interest.

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