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N-Sulfonyl-β-lactam hapten as an effective labeling reagent for aldolase mAb

Tsubasa Inokuma *, Roberta P. Fuller, Carlos F. Barbas III †

The Skaggs Institute for Chemical Biology and the Departments of Chemistry and Molecular Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, United States

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ABSTRACT

Utilization of chemically programmed antibodies (cpAbs) is regarded to be one of the most efficient methods for the development of therapeutic systems. cpAbs can extend the half-life of programming reagents, activate immune systems via the Fc region of antibodies and achieve universal vaccination by attaching varieties of small, programmed molecules. In the current study, we aimed to develop a novel labeling reagent for the preparation of cpAbs and found that *N*-sulfonyl-β-lactams (NSBLs) were optimal. NSBL can be synthesized from readily available 4-(bromomethyl)benzenesulfonyl chloride via few simple manipulations and can label the aldolase monoclonal antibody (mAb) 84G3, which could not be labeled effectively by the conventional labeling reagent, *N*-acyl-β-lactam (NABL). We also demonstrated that the conjugate, which consists of mAb 84G3 and an NSBL bearing a biotin moiety, maintained strong binding activity to streptavidin. In addition, the stability assay of NSBL revealed that NSBLs can tolerate aqueous media without significant decomposition over 24 h.

Bioconjugation is an essential tool to attach valuable functional units onto proteins, such as antibodies,[1,2] nucleic acids[3,4] and carrier proteins,[5] by assembling proteins and biologically active small molecules.[6,7] Among them, conjugates of antibodies and small molecules, which are called 'chemically programmed antibodies (cpAbs),' have proved to be one of the most useful devices for the development of therapeutic systems (Fig. 1).[8,9] If the antibody could be labeled properly by certain small-molecule compounds, the resulting conjugate would possess an extended serum half-life relative to that of the parent small molecule due to a significant increase in molecular weight. In addition, the Fc domain of the antibody would activate effector function systems such as T-cell or complement systems, and show several preferable activities such as antibody-dependent cell cytotoxic activity (ADCC) or complement-dependent cell cytotoxic activity (CDC).[10] By using various kinds of therapeutic agents such as anti-HIV,[11] anti-cancer[12] or anti-flu[13] compounds as the programming molecules, one could potentially target a wide range of diseases via universal vaccination. For the preparation of cpAbs, several procedures for labeling antibodies by small molecules have been reported.[14–16] We reported an effective labeling system of the aldolase monoclonal antibody (mAb) 38C2,[17–21] which was generated by conjugating 1,3-diketone **1** (see Fig. 2 for the structure of **1**) and carrier protein KLH via *N*-acyl β-lactam (NABL)-mediated amidation (Scheme 1).[13,22–26] In this case, the NABLs react selectively and irreversibly with the two amino groups of the lysine residues to form stable covalent bonds with mAb 38C2.

For effective generation of catalytic antibodies by immunization with small molecules, it is essential to use a small molecule that has a structure, that is, close to the transition state of the target reaction. For this purpose, we also reported that hapten **2**, which mimics the tetrahedral transition state of the C–C bond-forming step of an aldol reaction, induced seven out of seventeen antibodies showing aldolase activity, while only two out of twenty possessed catalytic activity in the case of the immunization by hapten **1**.[27,28] Although the availability of the catalytic antibody series generated by the sulfonyldiketone hapten **2** is superior to those generated by the diketone hapten **1**, labeling studies using these compounds have yet to be accomplished. Therefore, we surmised that if we could develop the methodology of effectively labeling the aldolase antibody series that are derived from the immunization by **2**, it would be valuable for the preparation of cpAbs due to the availability of the catalytic antibodies. Herein, we report the labeling study of 84G3, which is one of the aldolase

* Corresponding author at present address: Department of Bioorganic Synthetic Chemistry, Institute of Health Biosciences and Graduate School of Pharmaceutical Sciences, The University of Tokushima, Shomachi, Tokushima 770-8505, Japan. Tel.: +81 88 633 9534; fax: +81 88 633 9505.

E-mail address: tinokuma@tokushima-u.ac.jp (T. Inokuma).

† C.F. Barbas III passed away on June 24th, 2014, during the preparation of the manuscript.

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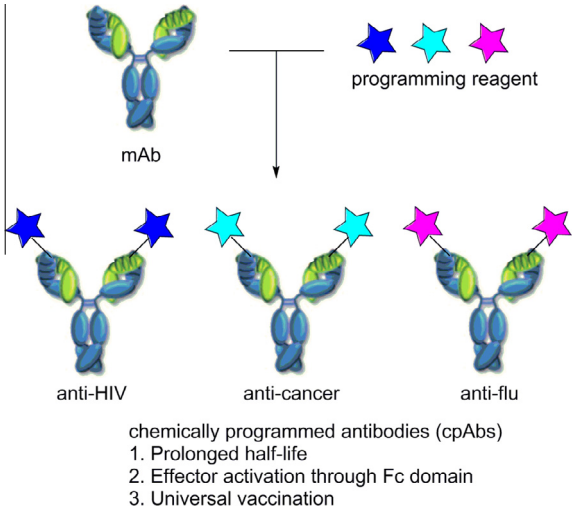


Figure 1. Concept and expected benefits of cpAbs.

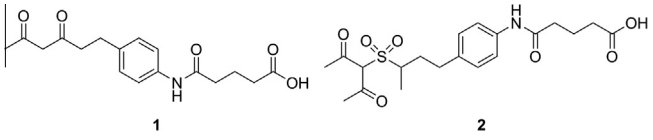
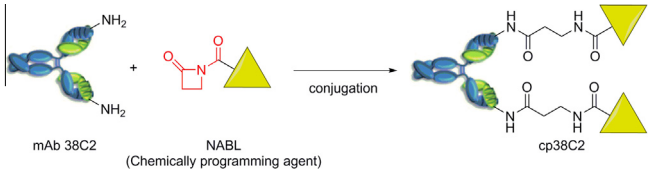
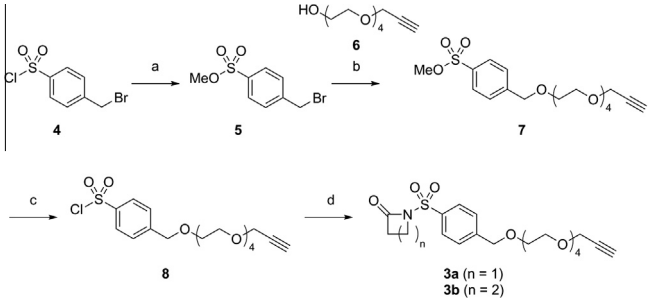


Figure 2. Small-molecule haptens that induce aldolase antibodies.



Scheme 1. Labeling of mAb 38C2 by NABL.

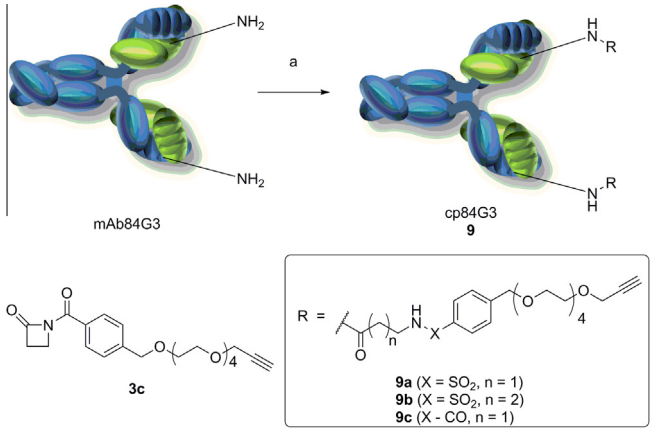


Scheme 2. Synthesis of the *N*-sulfonyl lactam haptens **3a** and **3b**. Reagents and conditions: (a) Et_3N, MeOH, rt, 30 min (52%). (b) NaH, **6**, THF, 0 °C to rt, 3 h (33%). (c) LiCl, acetone, 55 °C, 2 h then phosgene, DMF, CH_2Cl_2, 0 °C to rt, 4 h (74%). (d) NaHMDS, 2-azetidinone, THF, −78 °C, 2 h or *n*-BuLi, 2-pyrrolidinone, −78 °C, 1 h (16% for **3a**, 12% for **3b**).

antibodies induced by the immunization with hapten **2**, by using a newly designed labeling reagent.

Inspired by the structure of hapten **2**, which has an additional sulfonyl group at the carbon between the two carbonyl groups of the 1,3-diketone moiety, we speculated that the *N*-sulfonyl β-lactam (NSBL) would label 84G3 more effectively than the NABL hapten that was reported previously.[13,22–24] Therefore, we synthesized *N*-sulfonyl-β-lactam **3a** (Scheme 2). At first, the commercially available 4-(bromomethyl)benzene-1-sulfonyl chloride (**4**) was converted to the corresponding methylsulfonate **5**[29] by methanolysis in the presence of Et_3N. Methylsulfonate **5** was etherified with alcohol **6**,[30] which is derived from tetraethyleneglycol, to produce **7**. In an effort to convert the methylsulfonate moiety of **7** to a sulfonyl chloride, we found that it can be realized by nucleophilic demethylation with LiCl followed by chlorination with phosgene in one-pot. The resulting sulfonyl chloride **8** was coupled with 2-azetidinone to afford the desired NSBL **3a**. We also prepared the corresponding *N*-sulfonyl γ-lactam (NSGL) **3b** in an analogous manner to evaluate the influence of the size of the lactam moiety.

With the novel *N*-sulfonyl lactam haptens in hand, we evaluated the efficacies of the labeling of mAb 84G3 by these haptens (Scheme 3). The labeling was performed by incubating the mixture of 84G3 and 2.2 equiv of each hapten in neutral PBS solution at room temperature for 60 min. NABL **3c**, which is the effective labeling reagent for mAb 38C2, was also tested for labeling of 84G3 to compare the labeling efficacy with those of *N*-sulfonyl lactams **3a** and **3b**. We evaluated the efficacy of this conjugation process by monitoring the catalytic activity of the *retro*-aldol reaction of methodol **10** to form the fluorescent compound **11** as reported previously (Fig. 3).[20] As we expected, NSBL **3a** reacted smoothly and the catalytic activity of the resulting antibody **9a** completely disappeared within 1 h. In contrast, NSGL **3b** did not affect the aldolase activity of the antibody presumably due to its stable and less reactive five-membered ring structure. We also found that the existing NABL hapten **3c** did not label the catalytic site of 84G3 as well. These results imply that both the highly strained, less hindered four-membered lactam moiety and *N*-sulfonyl functional group are necessary for effective labeling of 84G3 selectively at the catalytic lysine site. The ESI-MS analysis of the conjugate of **9a** and 84G3 indicated that the two molecules of **3a** labeled 84G3 (Figs. S1 and S2).



Scheme 3. Labling of 84G3 with lactam haptens. Reagents and conditions: (a) **3a** or **3b** or **3c**, PBS (pH 7.4), rt, 60 min.

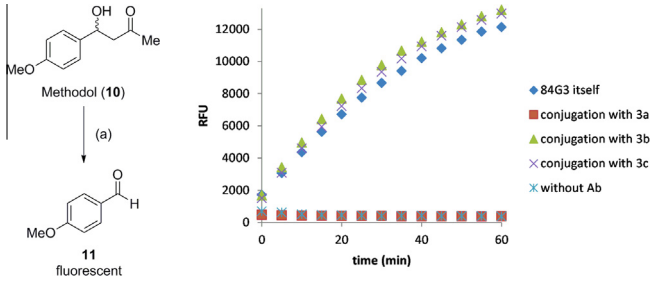


Figure 3. Labeling study of mAb 84G3 with lactam haptens **3a**–**3c**. Reagents and conditions: (a) cpAb (0.3 mol %), PBS (pH 7.4), rt.