Reactive Immunization: Antibody Selection Through Glycoside Bond Formation

Matthew R. Dintzner[*,†], John A. Neves[‡], Roberta Fuller[‡], Tom Lynch[†], Elena Lucchetta[†], Debbie Coligado[†], Andrea Toth[†], Nick Richert[†], M.G. Finn[*,‡]

Contribution from Department of Chemistry, DePaul University, 1036 W. Belden Ave., Chicago, IL 60660, and Department of Chemistry and The Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 N. Torrey Pines Rd., La Jolla, CA 92037

The work described herein represents ongoing efforts in our laboratories aimed at expanding technologies for the development of new catalytic antibodies through the process of reactive immunization. The preparation and use of protein-conjugates **3**–**5** to recruit antibodies via glicoside bond formation is described.

Lerner and Schultz introduced the concept of reactive immunization as a method for developing antibodies with well-defined catalytic properties but without lock-and-key constraints on reaction scope.[1] Reactive immunization works by presenting the immune system with immunogens capable of eliciting antibodies via formation of a covalent bond. The evolution of antibodies through reactive immunization is, then, a function of binding energy, and results in the generation of antibodies that have not shaped themselves around a particular substrate. The process of reactive immunization can result in the development of catalytic antibodies if the selectable chemistry is part of a catalytic mechanism, as was demonstrated for the catalytic antibody aldolases.[2]

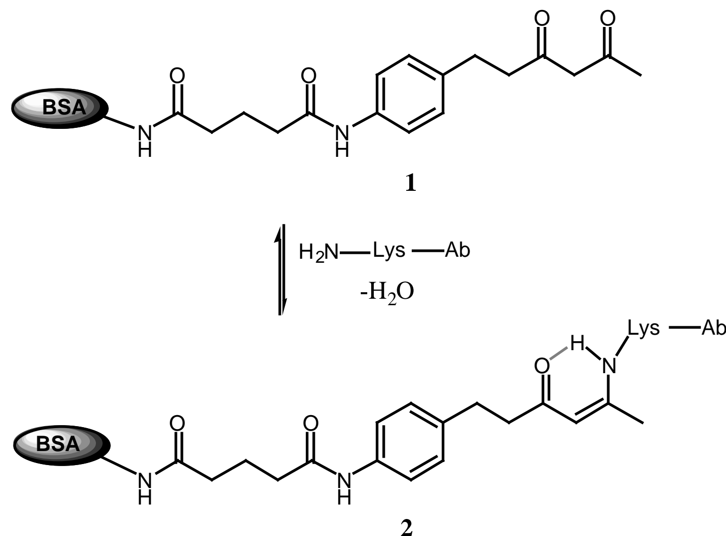


Figure 1. Recruitment of catalytic antibody aldolases via reactive immunization.

The catalytic antibody aldolases generated by reactive immunization were observed to be extraordinarily similar in mechanism and efficiency to the natural class I aldolase enzymes, and demonstrated an even broader substrate scope.[3] The aldolase antibodies 38C2, 33F12, 40F12 and 42F1 were recruited by -diketone hapten **1** through formation of a covalent antibody-hapten complex (enamine **2**, figure 1) with the -amino group of a lysine residue in the binding site of the antibody.[2] The antibodies function as aldol catalysts through formation of enamines from ketone substrates, which subsequently react with aldehydes to give aldol products upon hydrolysis.

In our laboratories, the concept of reactive immunization was extended with haptens **3**–**5**, which were designed to elicit nucleophilic antibodies via formation of stable glycoside antibody-hapten complex **6** *in vivo* (figure 2). Preliminary investigation of the affinity for aldolase antibody 38C2 for **3**–**5** was encouraging. 38C2 demonstrated similar affinities for **3**–**5** as for **1** (the hapten by which it was originally recruited), as evidenced by several enzyme-linked immunosorbent assay experiments (ELISA). Binding of 38C2 with **3**–**5** was inhibited by pre-incubation of the antibody with 2,4-pentanedione (1.0 M/PBS). Binding of 38C2 with **3**–**5** was unaffected, however, by treatment with 2,4-pentanedione (1.0 M/PBS) *after* the antibody was incubated with the hapten. These data suggest 38C2 binds haptens **3**–**5** at the active site lysine amine and that binding is irreversible.

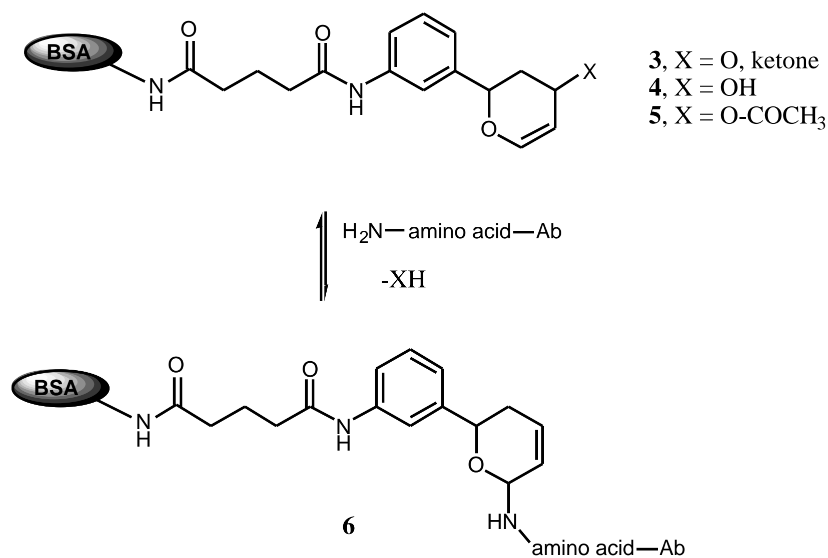
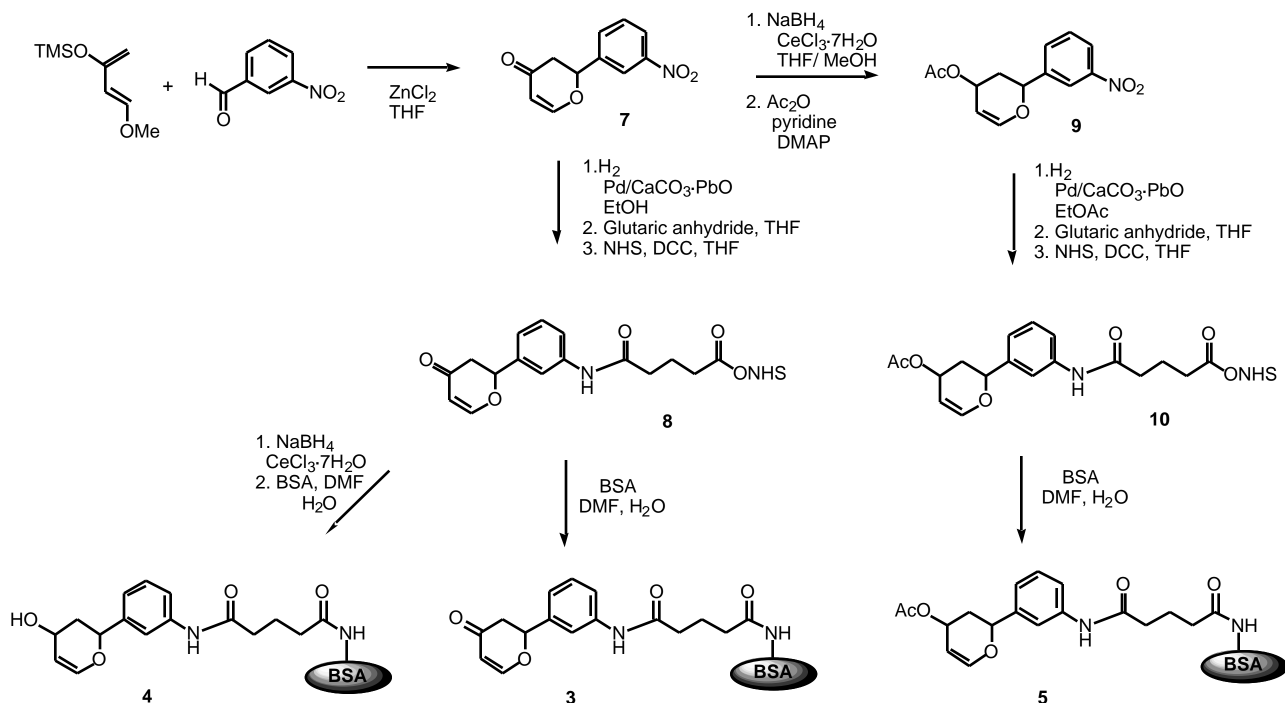


Figure 2. Recruitment of novel antibodies via glycosidation.

Immunization of mice with **3** is currently being investigated. Immunization of mice with **4** resulted in generation of four antibodies (2D1, 6A11, 9D11 and 12G3), while immunization of mice with **5** resulted in generation of five antibodies (1A1, 26C, 3C1, 7B12 and 10D2). The catalytic abilities of these antibodies are currently being investigated in our laboratories.

Protein conjugates **3**–**5** were prepared according to the synthetic sequence shown in scheme 1. Lewis acid-catalyzed hetero-Diels Alder condensation between 3-nitrobenzaldehyde and Danishefsky's diene[4] gave dihydropyrone **7** in good yield (82 %). Intermediate **7** was employed in two synthetic pathways en route to BSA conjugates **3** and **4**, and **5**, respectively. Reduction of the aromatic nitro group of **7**, followed by successive treatment with glutaric anhydride in tetrhydrofuran (THF) and esterification of the resulting carboxylic acid with N-hydroxysuccinimide (NHS) in the presence of 1,3-dicyclohexylcarbodiimide (DCC) afforded NHS-ester **8** in excellent yield (85 % for three steps). Treatment of NHS-ester **8** with bovine serum albumin (BSA) in DMF and aqueous buffer gave protein conjugate **3**. Luche[6] reduction of enone **8** with $NaBH_4$ in the presence of $CeCl_3 \cdot 7H_2O$, followed by conjugation with BSA gave, hapten

Scheme 1.



4. Hapten **5** was synthesized from intermediate **7** by Luche[5] reduction of the enone followed by acetylation to give acetate **9** (82 % for two steps). Reduction of the aromatic nitro group of **9**, followed by successive treatment with glutaric anhydride and NHS, gave activated ester **10** in good yield (80 % for three steps). Treatment of NHS-ester **10** with BSA in DMF and aqueous buffer gave protein conjugate **5**.

---

*To whom correspondence should be addressed.
†Department of Chemistry, DePaul University.
‡Department of Chemistry and The Skaggs Institute for Chemical Biology,
The Scripps Research Institute.

References and Notes:

1. Barbas, C. F., III, et al. *Science* **1997**, *278*, 2085; Lin, C. H. , et al. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 11773; Bjornestedt, R.; Ahong, G. F.; Lermer. R. A.; Barbas, C. F., III *J. Am. Chem. Soc.* **1996**, *118*, 11720.
2. Wagner, J.; Lerner, R. A.; Barbas, C. F., III *Science*, **1995**, *270*, 1797.
3. Karlstrom, A.; Zhong, G.; Rader, C., Larsen, A. H.; Fuller, R.; List, B.; Tanaka, F.; Wilson, I. A.; Barbas, C. F., III; Lerner, R. A. *Proc. Natl. Acad. Sci. U.S.A.*, **2000**, *97*, 3878.
4. Larson, E. R.; Danishefsky, S. *J. Am. Chem. Soc.* **1982**, *104*, 6458.
5. Luche, J.-L.; Gemal, A. L. *J. Am. Chem. Soc.* **1979**, *101*, 5848.