Dibromomaleimide as a facile, versatile linker for delivery applications

“The ease and versatility of dibromomaleimide chemistry has allowed the reversible attachment of biomolecules in a wide variety of applications such as polymer synthesis, cancer, and gene delivery.”

Keywords: degradation • dibromomaleimide • functionalization • linker

With the advances in material design and synthesis, more complex delivery systems with active targeting and multiple functionalities are being developed for a wide variety of applications. New chemistries that utilize thiols, amines, and carboxylic acid moieties allow for facile, efficient functionalization and bioconjugation of macromolecules such as peptides, proteins, and polymers. To improve drug efficacy or lower cytotoxicity, reversible linkers have been developed for the degradation of delivery vehicles in certain conditions. One molecule that has been gaining attention in the field of delivery for its versatile conjugation properties is dibromomaleimide (DBM).

The DBM structure consists of a maleimide with a bromine attached to each of the alkene carbons. Unlike typical maleimides, DBM allows for two points of attachment since each bromine can be substituted with a thiol to form two thioether bonds. The mild nature and selectivity of the DBM reaction allows for the use of sensitive biomolecular reagents while the high efficiency of the reaction requires only equimolar amounts of expensive biologics. Since its first utility in protein modification and bioconjugation as demonstrated by James R Baker and Stephen Caddick [1,2], a wide variety of thiolated molecules have been substituted onto DBM such as peptides [2–4], proteins [2,5,6] and polymers [7,8]. DBM's utility has been further expanded as a fluorescent tag; when small alkyl chains are substituted to the DBM molecule, the resulting conjugate exhibits bright fluorescence in a range of solvents [9,10]. DBM also features a unique ability to exchange

with an excess of thiols. Under reducing conditions, such as the cell cytoplasm, the DBM linkages break apart allowing for controlled degradation and the release of the original, thiolated molecule as monitored by fluorescence resonance energy transfer (FRET) in HeLa cells [11]. Another advantage is that the DBM linker has another point of attachment off the imide nitrogen, from which researchers have been able to attach fluorophores [2,8] and polymers [4–7]. The ease and versatility of DBM chemistry has allowed the reversible attachment of biomolecules in a wide variety of applications such as polymer synthesis, cancer, and gene delivery. The ability of DBM to improve delivery in these applications will be discussed further.

Polymer synthesis applications

A wide variety of polymer architectures such as linear, branched, star, and dendrimer shapes are being developed with their own distinct routes of efficient synthesis [12]. As a small linker with three points of attachment, DBM has the ability to reversibly connect polymers together and provide site-specific functionalization by relatively simple chemical reactions. Cui *et al.* utilized DBM as the central connector of three polymer chains [7]. The DBM bromines were substituted with either poly(ethylene oxide) (PEG), poly(*N*-(2-acryloyloxyethyl) pyrrolidone) or poly(*N,N*-dimethyl acrylamide) while the imide of DBM had a bromobutylene that was changed into an azido unit for the attachment of another PEG polymer by copper-catalyzed azide-alkyne cycloaddition. In another example, polymers were synthesized

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in the presence of unprotected DBM. Robin *et al.* synthesized a DBM and reversible addition chain-transfer macroinitiator and polymerized butyl acrylate, methyl methacrylate, and triethylene glycol methyl ether acrylate with controlled size and polydispersity [13]. While materials design and synthesis continue to create advanced architectures with multiple functionalities by complex chemistries, DBM can provide simpler methods of polymer attachment, chemical functionalization, and degradation to create structures of great complexity for a variety of delivery applications.

Imaging applications

In all delivery applications, the ability to track and monitor the cargo is important in understanding the delivery location, mechanism and distribution. Since DBM is a versatile, small molecule linker that fluoresces upon conjugation with a variety of thiol-containing ligands [10,14,15], it can be used in imaging and vehicle tracking applications. By substituting DBM with different thiols, Robin *et al.* prepared different dithiomaleimide (DTM) small molecules that have bright emissive fluorescence properties at about 500 nm [15]. In addition, they have shown that these DTM fluorophores can be readily incorporated to polymers and proteins. Similarly, they have developed DTM-based fluorescent polymers that can self-assemble into nanoparticles and be used as self-reporting probes for delivery applications. Most recently, Wang *et al.* prepared a DTM-based camptothecin nanoparticle used as an anticancer drug delivery system [16]. This nanoparticle showed high drug loading and was FRET responsive by taking advantage of the intracellular glutathione concentration. The inherent fluorescence of DBM upon conjugation allows for the design and synthesis of a new class of materials for the imaging and tracking of particles, including small molecule drug release. These materials are capable of facile assembly and disassembly, a desirable feature for monitoring therapeutic agent delivery.

Cancer treatment applications

Anticancer drugs are known to cause severe indiscriminate side effects when administered systemically. Thus, cancer treatments focus on delivering drugs specifically to cancer cells by incorporating cancer-homing moieties, such as small molecules, peptides and proteins into their delivery systems. One construct of growing interest is the antibody–drug conjugate, which involves the specific attachment of a cytotoxic drug to an antibody (Ab). With the appropriate conjugation technique, these ‘magic bullets’ offer distinct advantages of homogeneity, high yield, and long stability without undesirable toxic side effects [17]. There-

fore, the choice of conjugation method in antibody–drug conjugate synthesis is of crucial importance to its cancer treatment application.

“DBM is an adaptable linker that can be ... used to bridge peptides, fluorophores, antibodies, and polymers.”

DBM offers a facile and promising conjugation strategy that allows the use of the Ab’s native disulfide bonds in the attachment and delivery of chemotherapeutic drugs. For example, the Fab fragment and the functional bridging disulfide interchain of trastuzumab (Herceptin®) was utilized and conjugated to an alkyne-functionalized DBM that can be readily reacted with azide-containing drugs such as porphyrins and doxorubicin [18,19]. The chemical reactions were mild enough to retain Ab binding, an important attribute for Ab-based cancer therapy. One distinguishing advantage of the use of DBM chemistry is the abolishment of the need for Ab functionalization by protein engineering. Utilizing the native Ab disulfide bonds removes any additional complicated steps for Ab production, thus making it simpler and more economical. While some issues may arise, such as formation of a half Ab due to intrachain disulfide bridging [19] and changes in protein function upon insertion of DBM, optimizing the reaction conditions and utilizing the appropriate DBM linker may potentially address these challenges. Nevertheless, this technology presents a novel way to conjugate a native Ab to a drug while keeping the Ab’s structural features intact. In addition, the degradation of DBM in the reducing condition of the cell cytoplasm can allow for the release of the anticancer drug for a more potent effect, a feat not possible for nonlabile linkers.

Nonviral gene delivery applications

In nonviral gene delivery, typically cationic polymers are complexed with negatively charged nucleic acids to form a gene transfer vehicle. Polyethylenimine (PEI) is one of the most common and effective cationic polymers utilized for gene delivery; however, its high density of positive charge and nondegradable nature limit its application *in vivo* [20]. To surmount this shortcoming, degradable PEI and other cationic polymers have been prepared to decrease cytotoxicity *in vitro* by dissociating into smaller, less toxic pieces.

The chemical versatility of DBM allows for the development of an ideal nonviral vector capable of addressing the barriers of gene delivery such as high toxicity and the release of nucleic acids after internalization into cells. The ability of DTM conjugates for thiol exchange by intracellular glutathione allows for these nucleic acid-polymer complexes to be cleaved *in vivo*; thus allowing for the dissociation of a large density of

cationic charge in the cell cytoplasm and nucleic acid release. Furthermore, DBM allows for the development of multifunctional polymers by further functionalization at the *N*-maleimide moiety with small molecules, such as fluorophores, and macromolecules, such as peptide and proteins. To showcase these features, Tan *et al.* synthesized a panel of cleavable cationic polymers incorporating DBM as a reversible linker to bridge statistical copolymers of (2-dimethyl-amino)ethyl methacrylate and oligo(ethylene glycol)methyl ether methacrylate [8]. These polymers exhibited better cell viability than the nonreducible analogs, while maintaining gene transfer capabilities. In addition, a fluorophore was incorporated and cellular uptake was monitored by flow cytometry. The versatility and degradation of DBM allow for an apropos application in gene delivery.

Overall, DBM is an adaptable linker that can be applied to a wide variety of molecules and applications.

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Financial & competing interests disclosure

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No writing assistance was utilized in the production of this manuscript.