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A versatile acid-labile linker for antibody–drug conjugates†

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Antibody drug conjugates (ADC) couple therapeutic monoclonal antibodies (mAb) with potent toxins through a linker that is stable within systemic circulation, but cleaves within the target cells. In this report, silyl ether chemistry was used to couple the mAb trastuzumab with the chemotherapeutic, gemcitabine, to demonstrate the use of silyl ethers as a potential linker for ADCs.

Antibody drug conjugates (ADCs) aim to combine the cellular selectivity of a monoclonal antibody (mAb) with a highly toxic agent into a singular molecular entity. The principle underpinning ADCs is based on the mAb guiding the toxic agent specifically to diseased tissue, while minimizing the peripheral damage to healthy tissue.[1] ADCs augment the toxicity of the mAb, while simultaneously reducing the systemic side effects of the toxic agent.

Many different pieces must work in concert for an ADC to be successful at increasing antitumor efficacy while reducing toxicity. Choosing an adequately potent toxin, defining the proper molecular target and determining the optimal drug to antibody ratio have all proven to be important factors in developing an efficacious ADC.[2,3] Arguably, the chemical linker between the toxin and antibody is one of most important features in ADC development. The nature of the chemical linker is responsible for shaping the release profile of the toxin from the ADC. Ideally, the ADC would be stable in systemic circulation but upon cellular internalization and in response to a trigger, would release its toxic payload. The majority of ADCs in clinical development use a few different chemical linkers, hydrazones, disulfides, peptides or thioether bonds.[4–7] These linkers exploit differences in intracellular pH, reduction potential and enzyme concentration, respectively, to trigger toxin release. The two ADCs currently approved by the FDA, Adcetris® and Kadcyla® use a peptide linker cleaved by cathepsin B or a non-cleavable thioether linker that releases the drug after the mAb is degraded. Synthesizing ADCs that exploit disulfide or thioether linkers requires a toxin with a free thiol for conjugation. However, few clinically relevant toxins contain free thiols, therefore many potential drug targets would require synthetic modification to install a thiol group for conjugation. Such synthetic modifications may not be possible for all potential toxic agents and it is unknown how the required synthetic modifications would impact the toxicity profile of the therapeutic. With this limitation in mind, we developed a novel acid-labile linker as an additional option to the already established hydrazone, disulfide, thioether and peptide linkers for the future development ADCs.

We have previously explored the use of silyl ethers as: (1) acid labile crosslinkers in degradable chemotherapeutic loaded PRINT® nanoparticles (2) as pH triggered tethers in chemotherapeutic loaded PRINT® nanoparticles and (3) as acid labile moieties to reversibly tune the hydrophobicity of small molecule chemotherapeutics loaded into PRINT® nanoparticles.[8–10] In each instance, we were able to demonstrate that precise control of the steric bulk surrounding the silicon atom resulted in predictable transformations in the degradation profile of the silyl ether group. Consequently, by altering the steric bulk surrounding the silicon atom of our silyl ether systems we were able to fine-tune the properties of the PRINT® nanoparticle delivery system.[8] Given our previous success using silyl ether groups to develop finely tuned pH triggered therapeutic release systems, we hypothesized that we could successfully develop a silyl ether linker for ADCs that would be stable in systemic

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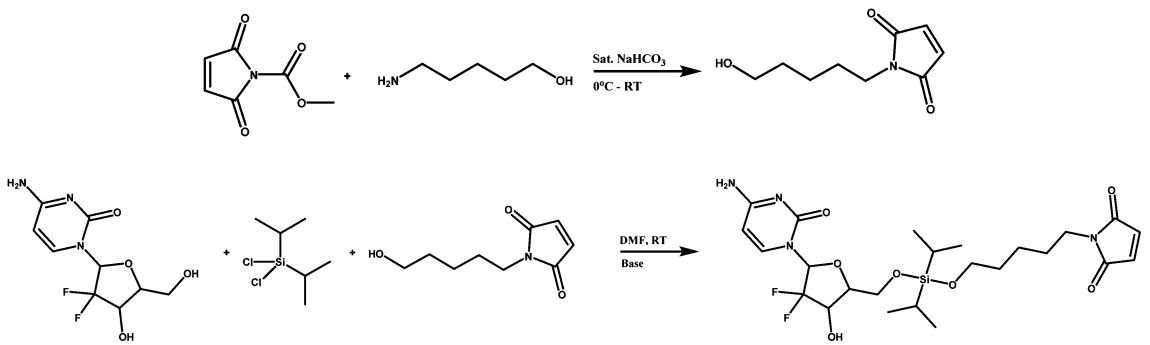
circulation (pH = 7.2) but selectively release chemotherapeutic payloads at endosomal pH (pH = 5.0).[11]

We designed our silyl ether linker system to be a facile one-pot synthesis from its constituent molecular components, so that each component, spacer, linker and drug, could be easily varied for screening purposes. Our spacer, (*N*-(5-hydroxypentyl)-maleimide), was synthesized from previously reported methods by combining *N*-methoxycarbonylmaleimide and 5-amino-1-pentol in saturated sodium bicarbonate.[12] Next the (*N*-(5-hydroxypentyl)maleimide) was reacted with dichlorodiisopropyl silane at low temperature over several hours. The mixture was then added to a solution of gemcitabine hydrochloride to form the desired gemcitabine silyl ether maleimide complex (Scheme 1). Gemcitabine was chosen because it is a clinically relevant drug with a short half-life, however, we have shown previously that silyl ether linkers are amenable with a multitude of drugs containing hydroxyl functional groups.[9]

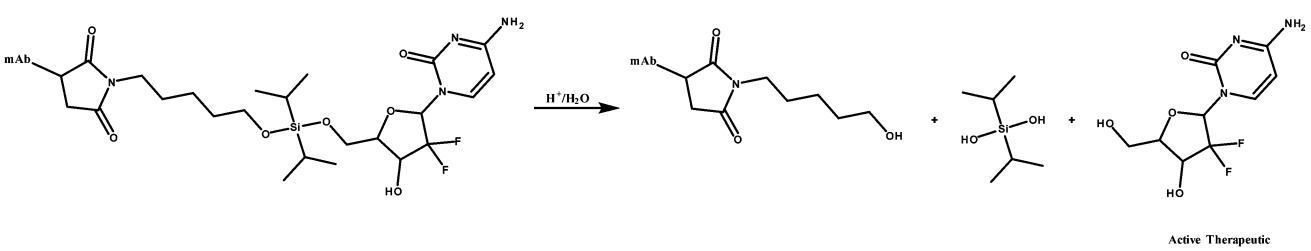
After the target cell internalizes an ADC, it is trafficked to the lysosome where the linker is cleaved to release the toxic agent.[13] The silyl ether linkage degrades under specific acid catalyzed hydrolysis within the acidic pH of the lysosome as shown in Scheme 2. To demonstrate the use of silyl ether as a potential ADC linker, the gemcitabine silyl ether maleimide was conjugated to trastuzumab through reduced disulfide bonds, a method that is widely accepted in the literature.[14] Trastuzumab has been successfully used to develop a clinically approved ADC.[7] The synthesized trastuzumab–gemcitabine ADC was then evaluated for stability at pH = 7.0 in phosphate buffer, under acidic conditions in pH = 5.0 buffer and at physiological conditions in 50% mouse plasma (Fig. 1A–C). Minimal gemcitabine was released over 72 hours when the ADC was incubated in pH 7 buffer, but at pH 5, ~80% of the gemcitabine was released at 72 hours. The linker also had high stability in plasma as minimal drug was released. Thus, a silyl ether linker may minimize release of the toxic agent from the ADC while in circulation, but selectively release the toxic agent after internalization and endosomal compartmentalization in the target cell. Given that mAbs have circulation half lives of multiple days, a linker with stability at systemically relevant pHs for multiple days is preferable.

Additionally, conjugation of the linker and toxin should not adversely affect the stability of the mAb. To improve ADC formulation stability, hydrophilic spacers have been developed to reduce ADC aggregation at higher drug-to-antibody ratios (DAR).[15] Using previously published methods, ADC aggregation as measured by size-exclusion chromatography was evaluated for ADCs with variable drug-to-antibody ratios.[16] ADCs were prepared at three different DARs by adjusting the molar equivalents of the gemcitabine silyl ether maleimide linker added during the conjugation step. At all DAR levels, minimal aggregation (<5%) was detected (Fig. 2). At a DAR of 5.8, only ~2% aggregation was detected as shown by the small peak that eluted prior to trastuzumab, which is comparable to other ADCs in clinical development.[17,18]

As we have demonstrated, silyl ether linkers provide the option to modify a different functional handle of the parent toxin compared to the hydrazone, disulfide, thioether and peptide bonds commonly used in ADCs. Silyl ether linkers also provide an alternative mechanism of release compared to peptide and disulfide linkers and have more stability than hydrazone linkers. Both of these factors contribute to making silyl ethers a flexible tool for ADC development.



Scheme 1 Synthetic scheme of *N*-(5-hydroxypentyl)maleimide) and gemcitabine silyl ether maleimide.



Scheme 2 Degradation of linker and release of active therapeutic.

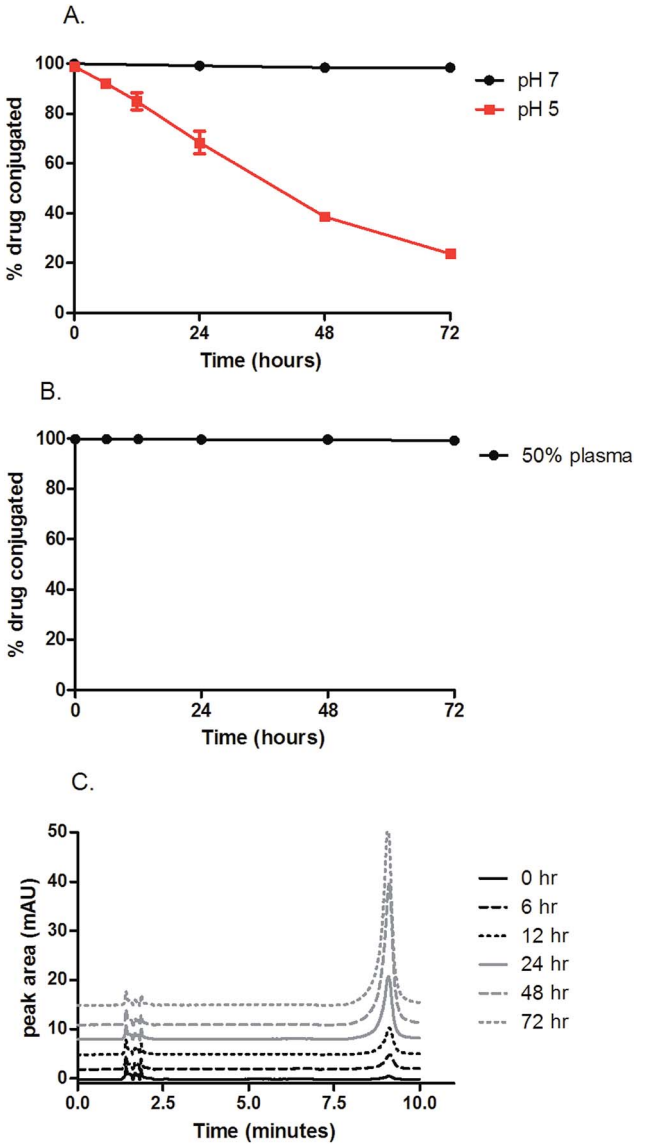


Fig. 1 (A) Stability of ADC in pH = 7.0 and pH = 5.0 at 37 °C ($N = 3$) (B) stability of ADC in 50% plasma at 37 °C ($N = 3$). (C) HPLC chromatogram of increasing gemcitabine peak at retention time of 9 minutes.

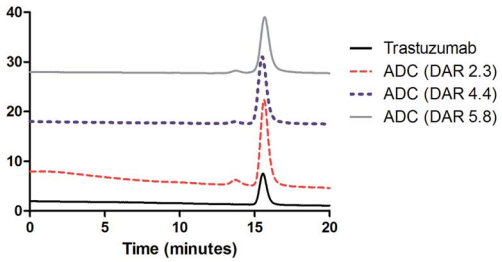


Fig. 2 Aggregation of ADC compared to trastuzumab measured by size-exclusion chromatography.

Author contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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