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Site-Specific Antibody Drug Conjugates Using Streamlined Expressed Protein Ligation

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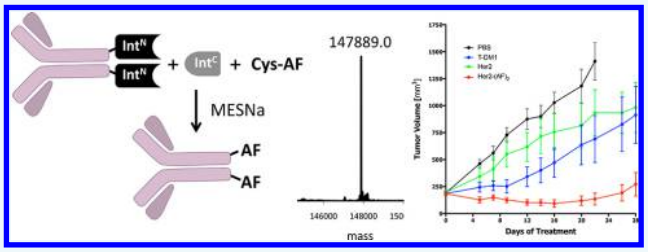
Supporting Information

ABSTRACT: Antibody-Drug Conjugates (ADCs) have been shown to produce clinical benefit in cancer patient thanks to their ability to target highly cytotoxic small molecules to tumor cells. However, the development of these complex molecules faces significant challenges due to the need to combine a large biologic drug with a small molecule drug to generate the desired bioconjugate. We describe here the use of a protein ligation methodology, based on the native chemical ligation reaction to generate site-specific Antibody-Drug Conjugates, which does not require the incorporation of unnatural modifications into the antibody. Fully native antibodies, with only the desired cytotoxic molecules attached, can be generated, thus minimizing the risk that additional modifications required for the site-specific conjugation pose a risk to the antibody activity. We demonstrate that our approach can be used to generate site-specifically modified ADCs, with potent *in vitro* and *in vivo* antitumor activity in a breast cancer tumor model.



INTRODUCTION

One of the major goals of anticancer drug development is to achieve appropriate selectivity to specifically kill tumor cells without affecting healthy ones. Antibody based biologic drugs have been instrumental toward this objective, and among them Antibody Drug Conjugates (ADCs) have been proposed over the past decade to hold great promise for the selective elimination of cancer cells. ADCs combine the highly specific targeting properties of antibodies with the high toxicity of small molecule cytotoxic agents to specifically kill cancer cells over healthy ones.[1,2] ADC development faces significant challenges, stemming from their molecular complexity, which requires the combination of large protein based biologic drugs with small molecule synthetic cytotoxics. Despite these challenges there are currently four ADCs approved for clinical use, and hundreds more are being investigated in ongoing clinical trials. Approved ADCs include brentuximab vedotin (Adcetris), for treatment of Hodgkin lymphoma; trastuzumab emtansine (Kadcyla), for breast cancer; gemtuzumab ozogamicin (Mylotarg) for leukemia; and inotuzumab ozogamicin (Besponsa), for lymphoblastic leukemia.

First and second generation ADCs were developed using traditional conjugation methods to link the cytotoxic payload to the antibody-targeting moiety.[1,3] These approaches are based on the reactivity of lysine and cysteine residues on antibodies to conjugate the desired cargo, and result in a large heterogeneity in the number of drugs attached per antibody molecule (drug antibody ratio (DAR) between 0 and 8), as well as on the site of attachment.[3] This heterogeneity negatively affects therapeutic index, and poses challenges to the identification of optimal antibody-payload combinations and CMC development, all of which greatly impact the chances of success for ADC development. To address these limitations, reduce ADC development risks and improve their therapeutic properties[1,4,5] several methods for the site-specific conjugation of cytotoxic payloads to antibodies have been developed over the last years.

Partially specific conjugation can be achieved by engineering solvent exposed Cys residues for their subsequent chemical modification.[4,6] Truly site-specific conjugation approaches, on the other side, require the incorporation of specific chemical groups on the antibody of interest so they can be used as molecular handles for the specific attachment of any desired cargo. These molecular handles can be introduced through genetic incorporation of unnatural amino acids,[7−9] enzymatic modification of side chains,[10−12] manipulation of the glycans,[13] or transpeptidase based approaches.[14] Although these strategies do provide access to homogeneous ADCs they often result in the incorporation of collateral modifications on the antibody, that is modifications required to install the necessary chemical handles for conjugation, which may affect its properties.

Protein semisynthesis approaches,[15] based on native chemical ligation,[16] are advantageous as they do not cause collateral modifications on the mAb, and have been shown to be extremely versatile regarding the chemical diversity they

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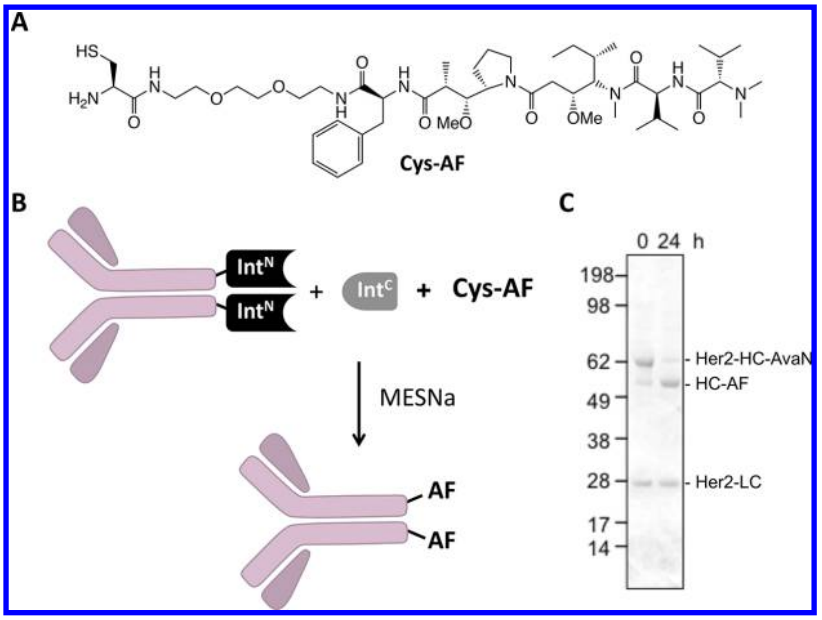

Figure 1. (A) Chemical structure of the drug-linker compound Cys-Auristain F (Cys-AF). (B) Reaction scheme for the synthesis of the anti-Her2-$(AF)_2$ ADC via SEPL. (C) Coomassie stained SDS-PAGE analysis, under reducing conditions, of one-pot thiolysis/ligation reaction for 24 h at r.t. At t = 0 the starting material (Her2-HC-AvaN and Her2-LC) is observed. After 24 h the conjugate product Her2-HC-AF and Her2-LC are detected.

allow to be introduced into proteins.[17] A variation on the widely used Expressed Protein Ligation (EPL) approach, termed Streamlined EPL (SEPL), has recently been shown to be particularly powerful for the modification of antibodies and antibody fragments.[18−20] SEPL is based on the use of ultrafast split-inteins to generate protein α-thioesters, for their subsequent ligation to cargoes containing 1,2-amino thiol groups, or other nucleophiles. We report here a unique and robust strategy using SEPL for efficient generation of ADCs where a native, stable, peptide bond is formed between a full-length therapeutic antibody and a cytotoxic cargo. The ADCs obtained through this strategy fully retain the antibody binding properties and display strong antitumor efficacy, with undetectable premature drug release. Moreover, SEPL allows the incorporation of not only one but several modifications, without the incorporation of unintended collateral modifications. Here we demonstrate that ADCs with DAR 2 and 4 can be prepared with an absolute control on the stoichiometry between cargo and antibody, yielding anti-Her2 ADCs with superior antitumor activity than a benchmark conjugate.

RESULTS AND DISCUSSION

Design of Site-Specific Antibody Drug Conjugate. We developed a method based on SEPL to conjugate Auristatin F (AF), a highly potent tubulin inhibitor,[21] to trastuzumab (anti-Her2, Herceptin), a humanized IgG1 monoclonal antibody targeting the human Her2 receptor, highly expressed in breast, ovarian, and gastric cancers[22] (Figure 1). Herceptin is currently being used as a treatment for breast cancer and also it is the antibody moiety present in the approved ADC trastuzumab emtansine (T-DM1, Kadcyla). We chose the cytotoxic Auristatin because it is well characterized and can be readily modified to incorporate the required chemical warheads for bioconjugation.[22]

SEPL is based on the use of split inteins for the generation of antibody C-terminal α-thioesters,[18] which prevents premature intein cleavage during antibody production observed with full-length inteins. Several ultrafast DnaE N-inteins (IntN) were fused to the C-terminus of anti-Her2's heavy chain (Her2-HC) (Figure S1), to identify those yielding highest titers of the corresponding anti-Her2-IntN fusion. As previously observed for other monoclonal antibodies[18] and antibody fragments,[19] the N-terminal fragment of the intein from *Anabaena variabilis* (AvaN) was the one that yielded the highest expression levels (Figure S2). Anti-Her2-AvaN fusions were transiently transfected and recombinantly expressed in Expi293 cells using standard protocols, and purified over Protein G. Importantly, combined expression and purification yields for the anti-Her2-AvaN fusion were not significantly lower than those obtained for the wild-type *inteinless* anti-Her2 antibody (Figure S2). Antibody production yields are key parameters for any site-specific conjugation technology,[7,23] considering their great impact on CMC development and GMP production, hence the confirmation that fusion of AvaN to the anti-Her2 antibody did not negatively affect them, was a crucial result in determining the viability of the approach.

Auristatin F was modified through its N-terminus[7,24] to introduce an N-terminal Cys residue, required for ligation, and a noncleavable polyethylenglycol linker (Figure 1A; see Supporting Information for synthesis details Figure S3 and S4).

Generation of Site-Specific ADC by SEPL. We decided to perform SEPL one-pot reactions in solution, as previously described,[19] to facilitate monitoring and optimization of reaction conditions in a homogeneous phase, based on our prior successful use of the approach to modify antibody fragments.[19] Thiolysis and ligation reactions were carried out in one-pot upon mixing the purified anti-Her2-AvaN antibody, with the engineered IntC fragment and the modified Auristatin F (AF) analogue (Cys-AF, Figure 1B). The reaction was monitored by SDS-PAGE (Figure 1C), RP-HPLC, and MS, which confirmed the formation of the desired product with ligation yields over 95% (Figure 2A and Figure S5). Excess reagents were removed by dialysis and the anti-Her2-$(AF)_2$ conjugate was purified by size exclusion chromatography under

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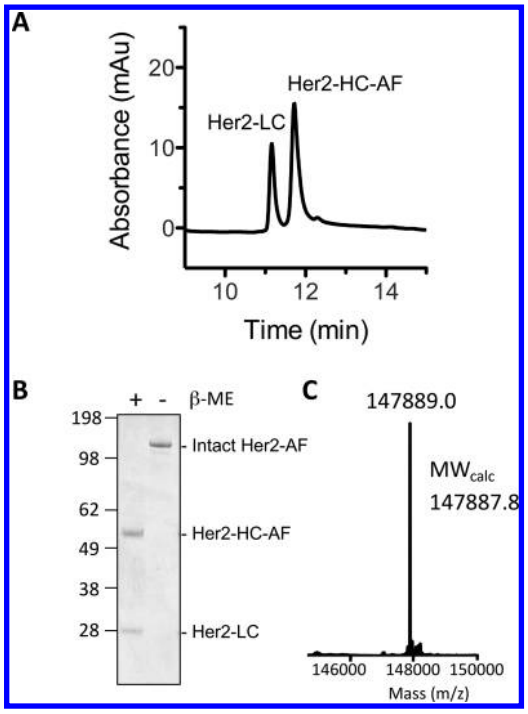

Figure 2. (A) RP-HPLC analysis (detection at 280 nm) of one-pot thiolysis/ligation reaction after 24 h at r.t. Sample was deglycosylated and fully reduced prior to RP-HPLC. (B) Coomassie stained SDS-PAGE analysis of purified Her2-$(AF)_2$ under reducing (+) and nonreducing (−) conditions. (C) ESI-MS analysis of the anti-Her2-$(AF)_2$ ADC obtained by SEPL and analyzed under nonreducing conditions. Sample was deglycosylated prior to the analysis.

native, nonreducing conditions (Figure S6). The anti-Her2-$(AF)_2$ conjugate elution volume was consistent with a monomeric antibody containing two light and two heavy chains, and SDS-PAGE analysis under reducing and non-reducing conditions confirmed the conjugate was a disulfide containing tetramer with the expected MW (Figure 2B). Furthermore, LC-MS analysis of the purified, nonreduced, anti-Her2-$(AF)_2$ conjugate confirmed the expected MW for a monomeric antibody with two molecules of cytotoxic covalently attached to it (DAR = 2) (Figure 2C). Importantly, neither the mass of the unmodified (hydrolyzed) antibody (anti-Her2-OH) nor any conjugate, with only one of the two heavy chains modified, were detected. This is a direct consequence of the high ligation yields of the SEPL reaction, resulting in a very efficient process to prepare ADCs.

Drug-Antibody Ratio (DAR) was determined by Hydrophobic Interaction Chromatography (HIC)[25] and intact Mass Spectrometry (MS). According to HIC analysis, the purified conjugate had a DAR of 1.9 ± 0.1 (Figure S7), which was in good agreement with the DAR observed by MS (DAR = 2, Figure 2C).

Additionally, in order to demonstrate ADCs with more than two payloads could be prepared with an absolute controlled stoichiometry using SEPL, we set on to generate a conjugate with DAR 4. For this purpose, a bidentate linker was designed and synthesized (Figure S8), containing an N-terminal Cys for ligation, and two anchoring points on which to attach the cytotoxics. Two molecules of Auristatin F were coupled to the linker (Figure S8), and the resulting cargo conjugated to the antibody via SEPL The SEPL reaction was carried out as described above and the reaction monitored by SDSPAGE. RP-HPLC and MS analysis of the fully reduced and desglycosilated conjugate confirmed formation of the desired product, and labeling yields over 95% (Figure S9).

Physicochemical Characterization of ADC. Conjugation of hydrophobic cytotoxic cargoes to antibodies typically

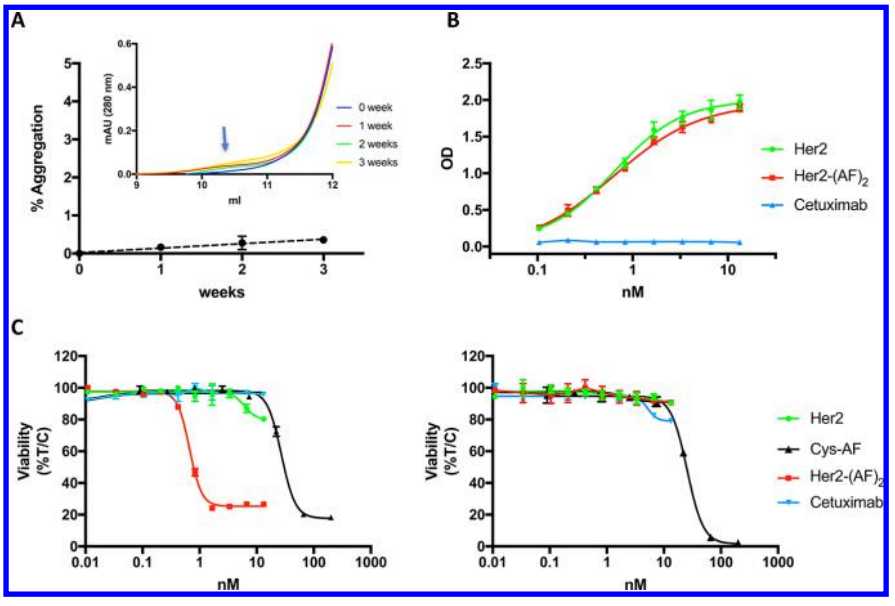

Figure 3. (A) Aggregation of anti-Her2-$(AF)_2$ site-specifically conjugated ADC. The percentage of aggregate formation of Her2-$(AF)_2$ over time was determined by SEC using a Superdex 200. Inset shows the SEC chromatograms of the anti-Her2-$(AF)_2$ conjugate incubated for the indicated times; the arrow indicates the peak at 10.25 mL elution volume assigned to aggregated conjugate. (B) Direct-binding ELISA assay to determine the affinity of the naked antibody and the ADC for their Her2 antigen. The unconjugated and the conjugated anti-Her2 antibodies had similar binding affinities. As a negative control anti-Her1 Cetuximab was used. (C) *In vitro* cytotoxicity against Her2-overexpressing cell lines SKBR-3 (left), and Her2 negative control cell line A-431 (right). The cytotoxic activity of the anti-Her2-$(AF)_2$ ADC prepared by SEPL, the corresponding free linker-drug, the unconjugated anti-Her2, and anti-Her1 control antibody (Cetuximab) were tested.

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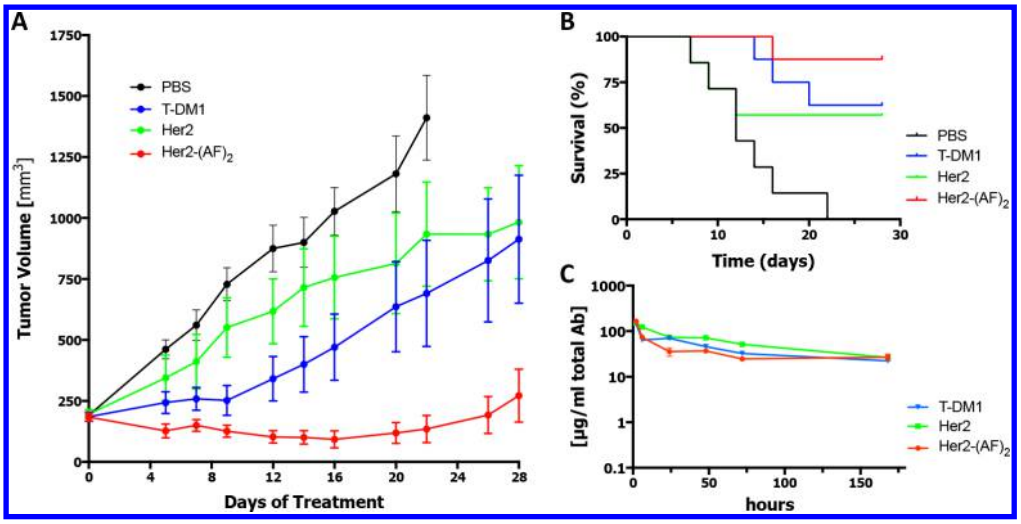

Figure 4. *In vivo* efficacy and stability. (A) *In vivo* efficacy studies were performed with a single injection of 15 mg/kg i.v. dose of ADC anti-Her2 by SEPL (Her2-$(AF)_2$), naked anti-Her2 antibody (Her2), benchmark anti-Her2 ADC (T-DM1) or DPBS in B474/Her2$^+$ tumor bearing mice. (B) Survival curve, animals with tumor volumes over 800 mm^3 were considered dead. (C) In vivo pharmacokinetics study. Concentration of total anti-Her2 in plasma of live mice was monitored over time for animals treated with 10 mg/kg of anti-Her2-$(AF)_2$, T-DM1, and anti-Her2. Compounds was injected intravenously at time 0, blood was collected at regular intervals and anti-Her2 content in plasma determined by ELISA.

results in conjugates with higher hydrophobicity than the unconjugated antibodies. This increased hydrophobicity can affect the physicochemical properties of the conjugate, including aggregation profile, shelf life, and pharmacokinetics.[26,27] Therefore, we set out to investigate the aggregation tendency of our ADC under stress conditions of high ionic strength (150 mM NaCl) and temperature (37 °C).[27] The stability of anti-Her2-$(AF)_2$ formulated in PBS at pH 7.4 and 37 °C, was monitored during 3 weeks by size-exclusion chromatography (SEC) (Figure S10A) and SDS-PAGE (Figure S10B). Samples showed minimal to no aggregate formation (Figures 3A and S10) although we observed some smaller molecular weight peaks, which we assigned to partial proteolytical degradation of the mAb. Our results were comparable to those previously reported for other site-specific conjugates, demonstrating reduced aggregation, relative to nonspecifically conjugated ADCs.[28]

Another key parameter to determine the safety and efficacy of ADCs is their stability in serum, and particularly the premature release of the cytotoxic cargo, which can result in undesired toxicities. As a first approximation to in serum stability, we studied it *in vitro* by incubating the unconjugated (anti-Her2) and conjugated antibody (anti-Her2-$(AF)_2$) in mouse serum for 5 days at 37 °C. The amounts of total anti-Her2 antibody and ADC were determined by ELISA and LC-MS. ELISA measurements were carried out using an anti-AF mouse monoclonal antibody from Epitope Diagnostics to specifically monitor degradation of the antibody-conjugate. For LC-MS stability measurements, the amounts of anti-Her2, Her2-$(AF)_2$ conjugates, and their degradation fragments were determined, at each time point, relative to the amount of internal control antibodies, spiked in the samples prior to analysis (Figure S11A). Both ELISA and LC-MS provided comparable results indicating that more than 60% of the ADC remained intact after 5 days incubation in serum (78 ± 3% for Her2 and 62 ± 1% for Her2-AF). Importantly, no mass related to the premature release of Auristatin F-linker from the antibody was detected by LC-MS (Figure S11B). These results suggest that the observed degradation of the SEPL conjugate is not governed by premature cleavage of the payload-linker, as observed for some maleimide conjugates,[8,29] but rather due to nonspecific degradation of the IgG, common to the one suffered by the naked antibody.

***In Vitro* and *in Vivo* Evaluation.** After the physicochemical characterization of the ADCs, we focused on the study of their biological properties. First of all, we determined the effect of SEPL on the ability of the antibodies to bind their target antigen. ELISA assays demonstrated anti-Her2 conjugate obtained via SEPL, with several different cargoes, fully retained their binding affinity to human Her2 (Figure 3B).

Next, we tested the in vitro cytotoxicity of the ADC against Her2-overexpressing cell lines SKBR-3 (Figure 3C), BT474, and CALU-3 (Figure S12), and compared it with that of the free drug, the unconjugated anti-Her2 antibody, and an unrelated benchmark antibody (anti-Her1). To determine the specificity of the responses we used a control cell line devoid of Her2 expression, but with high levels of Her1 (A-431, Figure 3D). The anti-Her2 ADCs showed potent *in vitro* cytotoxicity against SKBR-3, BT474, and CALU-3 cell lines and no cytoxicity to the control A-431 cell line. Concentrations around 1 nM of the ADC were sufficient to cause an 80% loss in cell viability. On the other hand, and as previously reported, unconjugated anti-Her2 showed a small effect on cell viability (20% viability loss), and had an almost 10-fold higher EC50, illustrating the potential for improved efficacy of ADCs. These results confirmed the proposed mechanism of action, based on receptor mediated internalization of the ADC, and drug release, presumably through lysosomal degradation of the ADC. Finally, we also compared the cytotoxicity in vitro of Her2-$(AF)_2$ (DAR = 2) and Her2-$(AF)_4$ (DAR = 4) against SKBR-3 cell line (Figure S12). Both ADCs showed potent *in vitro* cytotoxicity with EC50s within the same order of magnitude.

In vivo evaluation was performed in an orthotopic model of Her2 positive breast cancer. BT474 cells were grown and implanted in the mammary fat pad of athymic mice, and allowed to grow until tumor reached sizes around 150−200 mm^3. Once tumors reached the required size, animals were

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grouped and treated with anti-Her2, anti-Her2-$(AF)_2$, the benchmark ADC T-DM1 at prescribed doses, or vehicle (PBS), and tumor volume monitored over time. The samples used in the *in vivo* experiments were characterized by HIC, SEC, and SDS-PAGE (Figures S7 and S13). Initial experiments consisted in administration of a single dose of 15 mg/kg for all compounds. PBS treated mice displayed a 5-fold increase in tumor volume over 2 weeks, while Herceptin (anti-Her2) simply caused a slight decrease in the rate of tumor growth, as previously described.[5,30] T-DM1, on the other hand, caused initial tumor stasis, but rebounded after 10 days. Only the site-specifically modified ADC (anti-Her2-$(AF)_2$) prepared by SEPL was able to cause tumor shrinkage (Figure 4A). The high efficacy of the SEPL's ADC translated into a survival advantage for animals treated with it (Figure 4B). Additionally, a dose–response study was carried out to confirm previous results and explore a range of concentrations from 1 to 15 mg/kg in a two-dose setting (Figure S14). Importantly, all treated animals remained in good health throughout the study and no weight loss, or other overt toxicity issues were detected in any of the treatment groups (Figure S14).

Finally, in vivo stability of the ADC was studied by monitoring total amount of antibody in plasma of treated animals (single 10 mg/kg intravenous dose). Total amount of antibody was monitored by sandwich ELISA for animals treated with anti-Her2, anti-Her2-$(AF)_2$, and T-DM1, and all compounds showed similar stability profiles (Figure 4C) in agreement with results observed in the in vitro experiments.

Anti-Her2-$(AF)_2$ ADC obtained via SEPL was shown to be more efficacious than the benchmark, nonspecifically conjugated T-DM1. This result is particularly interesting considering the higher drug loading of T-DM1 (average DAR 3.5)[3] versus SEPL's ADC (DAR 2), and the fact that no significant differences in stability between the different compounds were detected *in vivo*.

To conclude, we have demonstrated that SEPL successfully generates highly homogeneous site-specifically conjugated ADCs, with controllable DAR, in good yields and without collateral modifications. Importantly, we showed that our conjugation strategy ensures antigen binding is fully retained, does not negatively affect aggregation and stability profiles and that ADCs thus obtained are fully active both *in vitro* and *in vivo*.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.bioconjchem.8b00630.

Experimental details are described for the synthesis of the drug-linker compound, the expression of the antibody, and the preparation of the ADC (PDF)

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Notes

The authors declare the following competing financial interest(s): SF, AO and MVP are employees of ProteoDesign, and JLH, CC, JA and FM are employees of LEITAT.

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■ REFERENCES

(1) Polakis, P. (2016) Antibody Drug Conjugates for Cancer Therapy. *Pharmacol. Rev. 68*, 3–19.

(2) Lash, A. (2010) Making the Case for Antibody-Drug Conjugates. *In Vivo*, 32–38.

(3) Chari, R. V. J., Miller, M. L., and Widdison, W. C. (2014) Antibody–Drug Conjugates: An Emerging Concept in Cancer Therapy. *Angew. Chem., Int. Ed. 53*, 3796–3827.

(4) Junutula, J. R., Raab, H., Clark, S., Bhakta, S., Leipold, D. D., Weir, S., Chen, Y., Simpson, M., Tsai, S. P., and Dennis, M. (2008) Site-Specific Conjugation of a Cytotoxic Drug to an Antibody Improves the Therapeutic Index. *Nat. Biotechnol. 26*, 925–932.

(5) Panowski, S., Bhakta, S., Raab, H., Polakis, P., and Junutula, J. R. (2014) Site-Specific Antibody Drug Conjugates for Cancer Therapy. *mAbs 6*, 34–45.

(6) Shen, B.-Q., Xu, K., Liu, L., Raab, H., Bhakta, S., Kenrick, M., Parsons-Reponte, K. L., Tien, J., Yu, S.-F., Mai, E., et al. (2012) Conjugation Site Modulates the In Vivo Stability and Therapeutic Activity of Antibody-Drug Conjugates. *Nat. Biotechnol. 30*, 184–189.

(7) Axup, J. Y., Bajjuri, K. M., Ritland, M., Hutchins, B. M., Kim, C. H., Kazane, S. A., Halder, R., Forsyth, J. S., Santidrian, A. F., Stafin, K., et al. (2012) Synthesis of Site-Specific Antibody-Drug Conjugates Using Unnatural Amino Acids. *Proc. Natl. Acad. Sci. U. S. A. 109*, 16101–16106.

(8) Jackson, D., Atkinson, J., Guevara, C. I., Zhang, C., Kery, V., Moon, S. J., Virata, C., Yang, P., Lowe, C., Pinkstaff, J., et al. (2014) In Vitro and In Vivo Evaluation of Cysteine and Site-Specific Conjugated Herceptin Antibody-Drug Conjugates. *PLoS One 9*, 1–14.

(9) Zimmerman, E. S., Heibeck, T. H., Gill, A., Li, X., Murray, C. J., Madlansacay, M. R., Tran, C., Uter, N. T., Yin, G., Rivers, P. J., et al. (2014) Production of Site-Specific Antibody–Drug Conjugates Using Optimized Non-Natural Amino Acids in a Cell-Free Expression System. *Bioconjugate Chem. 25*, 351–361.

(10) Lhospice, F., Delphine, B., Belmant, C., Patrick, D., Aristeidis, C., Fischer, E., Laurent, G., Bonnafous, C., Viaud, N., Represa, A., et al. (2015) Site-Specific Conjugation of Monomethyl Auristatin E to Anti-CD30 Antibodies Improves Their Pharmacokinetics and Therapeutic Index in Rodent Models. *Mol. Pharmaceutics 12*, 1863–1871.

(11) Strop, P., Liu, S.-H., Dorywalska, M., Delaria, K., Dushin, R. G., Tran, T.-T., Ho, W.-H., Farias, S., Casas, M. G., Abdiche, Y., Zhou, D., et al. (2013) Location Matters: Site of Conjugation Modulates Stability and Pharmacokinetics of Antibody Drug Conjugates. *Chem. Biol. 20*, 161–167.

(12) Agarwal, P., van der Weijden, J., Sletten, E. M., Rabuka, D., and Bertozzi, C. R. (2013) A Pictet-Spengler Ligation for Protein Chemical Modification. *Proc. Natl. Acad. Sci. U. S. A. 110*, 46–51.

(13) Van Geel, R., Wijdeven, M. A., Heesbeen, R., Verkade, J. M. M., Wasiel, A. A., van Berkel, S. S., and van Delft, F. L. (2015) Chemoenzymatic Conjugation of Toxic Payloads to the Globally Conserved N-Glycan of Native mAbs Provides Homogeneous and Highly Efficacious Antibody-Drug Conjugates. *Bioconjugate Chem. 26*, 2233–2242.

(14) Swee, L. K., Guimaraes, C. P., Sehrawat, S., Spooner, E., Barrasa, M. I., and Ploegh, H. L. (2013) Sortase-Mediated Modification of αDEC205 Affords Optimization of Antigen Presentation and Immunization Against a Set of Viral Epitopes. *Proc. Natl. Acad. Sci. U. S. A. 110*, 1428–1433.

DOI: 10.1021/acs.bioconjchem.8b00630
Bioconjugate Chem. 2018, 29, 3503–3508

(15) Hackenberger, C. P., and Schwarzer, D. (2008) Chemoselective Ligation and Modification Strategies for Peptides and Proteins. *Angew. Chem., Int. Ed. 47*, 10030−10074.
(16) Dawson, P. E., Muir, T. W., Clark-Lewis, I., and Kent, S. B. (1994) Synthesis of Proteins by Native Chemical Ligation. *Science 266*, 776−779.
(17) Vila-Perelló, M., and Muir, T. W. (2010) Biological Applications of Protein Splicing. *Cell 143*, 191−200.
(18) Vila-Perelló, M., Liu, Z., Shah, N., Willis, J. A., Idoyaga, J., and Muir, T. W. (2013) Streamlined Expressed Protein Ligation Using Split Inteins. *J. Am. Chem. Soc. 135*, 286−292.
(19) Frutos, S., Jordan, J. B., Bio, M. M., Muir, T. W., Thiel, O. R., and Vila-Perelló, M. (2016) Access to Site-Specific Fc-cRGD Peptide Conjugates Through Streamlined Expressed Protein Ligation. *Org. Biomol. Chem. 14*, 9549−9553.
(20) Shah, N. H., Dann, G. P., Vila-Perelló, M., Liu, Z., and Muir, T. W. (2012) Ultrafast Protein Splicing is Common Among Cyanobacterial Split Inteins: Implications for Protein Engineering. *J. Am. Chem. Soc. 134*, 11338−11341.
(21) Doronina, S. O., Mendelsohn, B. A., Bovee, T. D., Cerveny, C. G., Alley, S. C., Meyer, D. L., Oflazoglu, E., Toki, B. E., Sanderson, R. J., Zabinski, R. F., et al. (2006) Enhanced Activity of Monomethyl Auristatin F Through Monoclonal Antibody Delivery: Effects of Linker Technology on Efficacy and Toxicity. *Bioconjugate Chem. 17*, 114−124.
(22) Meden, H., and Kuhn, W. (1997) Overexpression of the Oncogene c-erbB-2 (HER2/ neu) in Ovarian Cancer: A New Prognostic Factor. *Eur. J. Obstet. Gynecol. Reprod. Biol. 71*, 173−179.
(23) Tian, F., Lu, Y., Manibusan, A., Sellers, A., Tran, H., Sun, Y., Phuong, T., Barnett, R., Hehli, B., Song, F., DeGuzman, M. J., et al. (2014) A General Approach to Site-Specific Antibody Drug Conjugates. *Proc. Natl. Acad. Sci. U. S. A. 111*, 1766−1771.
(24) Doronina, S. O., Bovee, T. D., Meyer, D. W., Miyamoto, J. B., Anderson, M. E., Morris-Tilden, C. A., and Senter, P. D. (2008) Novel Peptide Linkers for Highly Potent Antibody-Auristatin Conjugate. *Bioconjugate Chem. 19*, 1960−1963.
(25) Wakankar, A., Chen, Y., Gokarn, Y., and Jacobson, F. S. (2011) Analytical Methods for Physicochemical Characterization of Antibody Drug Conjugates. *MAbs 3*, 161−172.
(26) Manning, M. C., Chou, D. K., Murphy, B. M., Payne, R. W., and Katayama, D. S. (2010) Stability of Protein pharmaceuticals: An Update. *Pharm. Res. 27*, 544−575.
(27) Hollander, I., Kunz, A., and Hamann, P. R. (2008) Selection of Reaction Additives Used in the Preparation of Monomeric Antibody−Calicheamicin Conjugates. *Bioconjugate Chem. 19*, 358−361.
(28) Adem, Y. T., Schwarz, K. A., Duenas, E., Patapoff, T. W., Galush, W. J., and Esue, O. (2014) Auristatin Antibody Drug Conjugate Physical Instability and the Role of Drug Payload. *Bioconjugate Chem. 25*, 656−664.
(29) Alley, S. C., Benjamin, D. R., Jeffrey, S. C., Okeley, N. M., Meyer, D. L., Sanderson, R. J., and Senter, P. D. (2008) Contribution of Linker Stability to the Activities of Anticancer Immunoconjugates. *Bioconjugate Chem. 19*, 759−765.
(30) Baselga, J., Norton, L., Albanell, J., Kim, Y.-M., and Mendelsohn, J. (1998) Recombinant Humanized Anti-HER2 Antibody (Herceptin) Enhances the Antitumor Activity of Paclitaxel and Doxorubicin against HER2/neu Overexpressing Human Breast Cancer Xenografts. *Cancer Res. 58*, 2825−2831.

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