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Biography

Barbara Bernardim was born in Guarapuava, Brazil where she received her bachelor's degree in Chemistry (2011). In the same year, she moved to Sao Paulo where she began her master's and later (2013) her doctoral studies in chemistry at the University of Sao Paulo – IQSC/USP. She performed the total synthesis of glycosidase inhibitors, as well as developing new synthetic methodologies involving diazoketones. In 2015, she joined Gonçalo Bernardes' lab at the University of Cambridge as a visiting PhD student. During her visit, Barbara discovered a new "click" reaction that allows the site-selective modification of proteins and antibodies at cysteine under mild and stable conditions. After completing her PhD (2017), Barbara was awarded a prestigious postdoctoral fellowship from FAPESP. She is currently a Royal Society Newton International Fellow at the Department of Chemistry at the University of Cambridge and also a St. John's College Research Associate.

Abstract title:

Abstract

Antibody–drug conjugates (ADCs) are emerging as promising cancer therapies.[1] While considerable improvements have been made, there is still a significant need for innovation in the design of methodologies for the installation of probes.[2] Cys-specific maleimide reagents are currently the method of choice for the construction of ADCs despite the known instability of the resulting products *in vivo*.[3-4] Herein, we describe a strategy for the irreversible and site-specific modification of proteins and antibodies using stoichiometric amounts of carbonylacrylic reagents.[5,6] This work is based on a thio-Michael type addition of native and engineered Cys to a carbonylacrylic reagent equipped with fluorophores, PEG and cytotoxic drugs moieties (Fig. 1). We started our work by modifying native full-length IgG antibodies (modification at the native cysteines present as disulfides), engineered IgGs with encoded additional cysteine residues, and smaller recombinant antibody fragments such as nanobodies. The desired Cys-adducts were obtained in >95% yield after 2 h of incubation at 37 ^{o}C using 1 equiv. of **1** per Cys. The incorporation of cytotoxic drugs was achieved in >95% yield under the same conditions. Importantly, the conjugates retained their binding specificity as confirmed by fluorescence-activated cell sorting (FACS) of live cells. We report carbonylacrylic derivatives as alternative, high-yielding, stable and attractive substrates for the creation of stable ADCs. Compared with the classic maleimide technology, the current approach is robust and stable, with improved quality and homogeneity of the conjugate product.

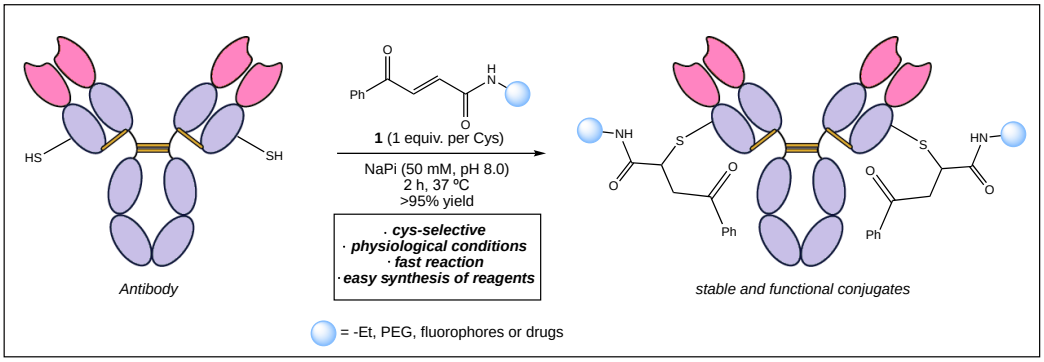

Figure 1 Synthesis of stable and functional antibody conjugates using carbonylacrylic reagents.

[1] *Br. J. Cancer,* **2016,** *114*, 362
[2] *Nat. Chem.* **2016**, *8*, 1088
[3] *Nat. Biotech.* **2012**, *30*, 631
[4] *Nat. Biotech*. **2014**, *32*, 1059
[5] *Nat. Commun.* **2016**, *7*, 13128
[6] *Nat. Protocol.* ***2019***, 14, 86