ChemPubSoc Europe

DOI: 10.1002/cmdc.201600372

CHEMMEDCHEM Communications

Introducing Glycolinkers for the Functionalization of Cytotoxic Drugs and Applications in Antibody–Drug Conjugation Chemistry

Filip S. Ekholm,[a, b] Henna Pynnönen,[a] Anja Vilkman,[a] Virve Pitkänen,[a] Jari Helin,[a] Juhani Saarinen,[a] and Tero Satomaa*[a]

Antibody–drug conjugates (ADCs) are promising alternatives to naked antibodies for selective drug-delivery applications and treatment of diseases such as cancer. Construction of ADCs relies upon site-selective, efficient and mild conjugation technologies. The choice of a chemical linker is especially important, as it affects the overall properties of the ADC. We envisioned that hydrophilic bifunctional chemical linkers based on carbohydrates would be a useful class of derivatization agents for the construction of linker–drug conjugates and ADCs. Herein we describe the synthesis of carbohydrate-based derivatization agents, glycolinker–drug conjugates featuring the tubulin inhibitor monomethyl auristatin E and an ADC based on an anti-EGFR antibody. In addition, an initial in vitro cytotoxicity evaluation of the individual components and the ADC is provided against EGFR-positive cancer cells.

Antibody–drug conjugates (ADCs) are an emerging class of cytotoxic agents for selective drug delivery applications and treatment of diseases such as cancer.[1] The large potential shown by ADCs has not gone unnoticed by pharmaceutical companies, and there are currently two ADCs on the market and a growing number in clinical trials.[2] The construction of ADCs relies on regioselective organic transformations and efficient bioconjugation technologies.[3] The challenges associated with the chemical aspects of their construction are numerous, for example: site-selective modification of multifunctional drugs, site-selective modification of antibodies, development of robust, benign and highly selective bioconjugation reactions, and optimization of the linker structure in order to achieve optimal pharmacokinetic and pharmacodynamic properties in the end-products. While much progress has been achieved in the past[4] and more recently,[5] the field as whole is still under constant development.

One of the most important aspects of ADC design is the chemical linker, which has a considerable effect on the overall properties of the ADCs. In modern ADCs, hydrophobic cytotoxic agents are almost exclusively used. The combination of hydrophobic cytotoxic agents and the commonly used relatively hydrophobic chemical linkers is suboptimal, and the addition of multiple drug–linker moieties of this kind to an antibody may be devastating with regard to the biocompatibility and pharmaceutical efficacy of the ADC. These problems are reflected in the design of ADCs, which tend to focus on the incorporation of a limited amount of drugs per antibody (typically 2–4 drugs per antibody). Furthermore, problems associated with the overexpression of efflux pump proteins which are capable of removing hydrophobic cytotoxic molecules from the intracellular environment has been encountered with multidrug-resistant cancer cells.[6]

To overcome these challenges, an increasing amount of effort has been invested in the design of hydrophilic chemical linkers that may counteract these issues.[7] We envisioned that carbohydrate-based linkers would be a viable option for ADCs, as carbohydrates are well known for their hydrophilic nature and biocompatibility. In contrast to the previously reported polyethylene glycol (PEG)[7a] and sulfonate-linker-based strategies,[7b] the carbohydrate backbone offers further structural variation possibilities through modification of the hydroxy groups which might be beneficial for fine-tuning of linker properties and construction of multifunctional linker species. To set up a suitable synthetic strategy, we decided to investigate which modification protocols would be applicable for the functionalization of simple sugars and provide cross-linking tools applicable to the construction of ADCs.

In the past, reductive amination reactions of carbohydrates have been developed as a tool for the selective modification of the reducing end of unprotected mono-, oligo-, and polysaccharides.[8] Still today, these robust reactions play a crucial role in chemical sciences, for example, in the immobilization of carbohydrates or biomolecules onto surfaces, which has been a significant contributor (e.g., through microarray experiments) to the continued advancement of our understanding of biological processes.[9] Our initial plan was to exploit this reaction protocol and turn modified reducing monosaccharides into hydrophilic bifunctional derivatization agents (glycolinkers) for the functionalization of cytotoxic agents. The aldehyde form of reducing sugars, which is in equilibrium with the hemiacetal through mutarotation, was viewed as a potential conjugation site for amine-containing cytotoxic agents. A thorough survey of bioconjugation protocols was conducted in order to select an appropriate second functional group for the glycolinkers.

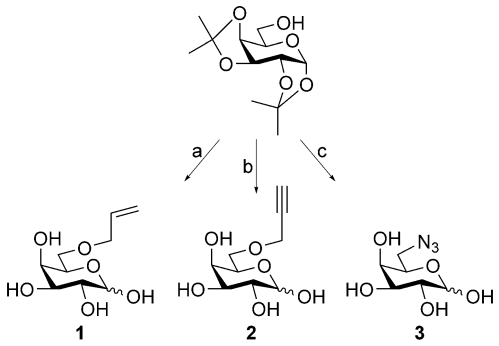
[a] Dr. F. S. Ekholm, H. Pynnönen, A. Vilkman, V. Pitkänen, Dr. J. Helin, J. Saarinen, T. Satomaa
Glykos Finland Ltd., Viikinkaari 6, 00790 Helsinki (Finland)
E-mail: tero.satomaa@glykos.fi

[b] Dr. F. S. Ekholm
Department of Chemistry, University of Helsinki, PO Box 55, A. I. Virtasen aukio 1, 00014 Helsinki (Finland)

Supporting information and the ORCID identification number(s) for the author(s) of this article can be found under http://dx.doi.org/10.1002/cmdc.201600372.

Successful bioconjugation strategies based on maleimide/thiol chemistry,[10] Staudinger ligation techniques,[11] azide–alkyne cycloaddition reactions,[12] and ketone/aldehyde-amine (oxime)[13] chemistry have been reported. Because our plans involved the functionalization of amine-containing cytotoxic agents, the methods involving oxime chemistry were neglected, and emphasis was placed on the design of glycolinkers applicable to other conjugation strategies.

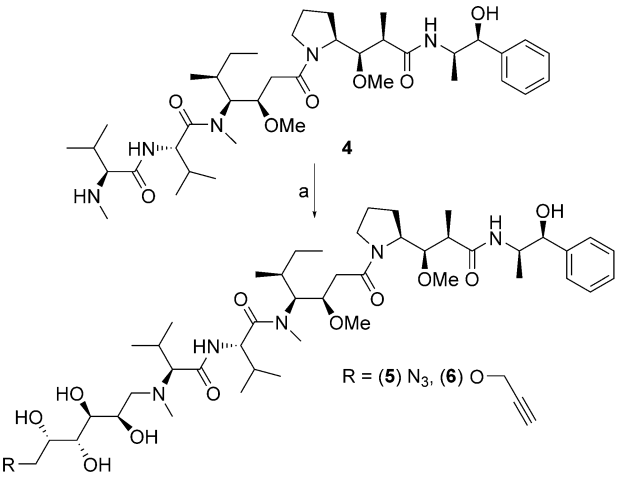
1,2:3,4-Di-*O*-isopropylidene-D-galactose was chosen as a suitable starting material because it is commercially available and the free primary hydroxy group can be modified rather easily by the use of standard synthetic protocols, thereby providing access to a vast amount of versatile conjugation species. The chemical structures of, and the short synthetic routes to three alternatives are provided in Scheme 1. In these model sub-



Scheme 1. Synthesis of bifunctional monosaccharides **1**–**3**. *Reagents and conditions*: a) 1. allyl bromide, NaH, DMF, 90%, 2. TFA, H_2O, quant.; b) 1. propargyl bromide, NaH, DMF, 91%, 2. TFA, H_2O, quant.; c) 1. TsCl, pyridine, DMF, 81%, 2. NaN_3, toluene, 68%, 3. TFA, H_2O, quant.

strates, an amine-containing drug molecule can be conjugated to the reducing end, thereby leaving the second functional group free to react with site-specifically modified antibodies, for example. Briefly, the terminal alkene in **1** can be used in thiol-ene reactions and cross-coupling metathesis reactions,[14] the terminal alkyne in **2** and can be used in azide–alkyne cycloaddition reactions,[12] and the terminal azide in **3** can be used in both azide–alkyne cycloaddition reactions and Staudinger ligation protocols.[11,12] Azide–alkyne cycloaddition chemistry is currently viewed as one of the most promising techniques for bioconjugation reactions because these functionalities are rare in naturally occurring biomolecules, in contrast to amines and thiols, for example.[15] Therefore, we decided to concentrate on the use of monosaccharides **2** and **3** in this study.

In order to compare the features of the glycolinkers with other linker alternatives, it was important to use a thoroughly tested cytotoxic agent. Monomethyl auristatin E (MMAE) was found to be a suitable choice, especially for ADC purposes. MMAE is an antineoplastic agent and an antimitotic drug (tubulin inhibitor) composed of five amino acid residues (compound **4**, Scheme 2). It is the cytotoxic agent in the approved ADC brentuximab vedotin (Adcetris), which is used in the treatment of Hodgkin's lymphoma and systemic anaplastic large-



Scheme 2. Synthesis of MMAE–glycolinker conjugates. *Reagents and conditions*: a) **2** or **3**, $NaCNBH_3$, DIPEA, DMSO, 34% (**5**), 24% (**6**).

cell lymphoma.[5a] In addition, 20 ADCs which have proceeded to clinical trials have relied on auristatin derivatives as their cytotoxic agents.[16] The secondary amine in the N-terminal residue in MMAE was modified via a reductive amination reaction with glycolinkers **2** and **3**,[17] thereby providing amphiphilic MMAE–glycolinker conjugates **5** and **6** in acceptable isolated yields. Notably, the yields of the presented protocol are similar to the overall yields obtained in the multistep synthetic sequences required for several other linker alternatives.[7,18] Further optimization of the reaction conditions was therefore not attempted at this stage. Instead, focus was placed on the NMR spectroscopic characterization of MMAE–glycolinker conjugates **5** and **6** (which proved to be challenging).

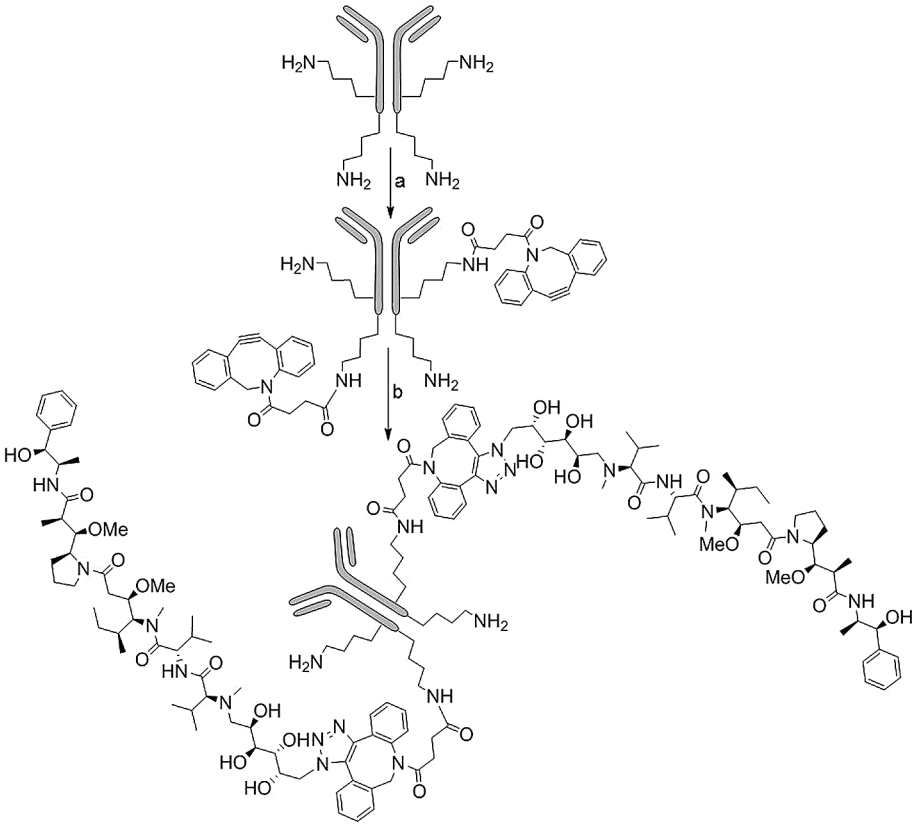
It is well known that proline and other similar amino acids can populate both the *cis* and *trans* isomeric states, in contrast to other amino acids which are present mostly as the *trans* isomer.[19] In the MMAE–glycolinker conjugates, a diastereomeric mixture containing the two forms in roughly equal proportions was observed in the NMR spectra. The signal splitting was not limited to the proline analogue [residue (2)] alone, but was seen in all of the residues, further complicating assignment. A further indication of the difficulty involved in solving the complex NMR spectra of MMAE conjugates is the fact that a full assignment has not been previously reported. While a complete assignment with accurate coupling constants is difficult to obtain due to severely overlapping signals, we were able to locate the chemical shifts for most of the signals of both diastereomers using a number of one-dimensional (^1H, ^{13}C) and two-dimensional (COSY, HSQC, TOCSY, ROESY, and HMBC) NMR spectroscopic techniques. Even with the two-dimensional spectra available, the assignment was not trivial, as a number of key signals from both diastereomers overlapped in crowded areas of the spectrum. To the best of our knowledge, the NMR spectroscopic data reported in the Supporting Information are among the most detailed to date on this important and widely used class of cytotoxic agents.

With the characterization of the MMAE–glycolinker conjugates completed, our attention was turned toward the con-

struction of a model ADC. We chose to use an in-house produced anti-EGFR antibody (cetuximab) as a model substrate, because the epidermal growth factor receptor (EGFR) is a clinically validated cancer treatment target in non-small-cell lung cancer, squamous-cell carcinoma of the head and neck, colorectal cancer, and pancreatic cancer. In fact, activation of growth factors and receptors of the EGFR family is observed in most human carcinomas.[20] In addition, EGFR is an internalizing receptor that can effectively transport the drug inside the tumor cell upon binding of a drug-loaded antibody, and is therefore a good target for ADC-based therapy.

While a number of tools for the site-selective modification of antibodies have emerged in recent years,[21] we decided that modification of lysine side chain amino groups would be sufficient for evaluation of the glycolinkers in this study. It is well known that the modification of lysine side chain amino groups produces a heterogeneous mixture of products, as similar modification protocols have been used in the construction of approved ADCs in the past, for example, in the construction of trastuzumab emtansine (Kadcyla), which is used in the treatment of metastatic breast cancer and has a drug-to-antibody ratio (DAR) of 0–8.[22] In the current study, lysine side chain amino groups were functionalized using a dibenzocyclooctyne (DBCO)-NHS-ester reagent, which resulted in the successful incorporation of DBCO units to the antibody (Scheme 3). The heterogeneous modified antibody mixture was analyzed by MALDI-TOF mass spectrometry (light chain analysis) and by labeling with Alexa Fluor 488 azide followed by spectrophotometric analysis. The results indicated a DBCO-to-antibody ratio of ~10. The DBCO-modified antibody was reacted with MMAE–glycolinker **5** by a copper-free strain-promoted alkyne–azide cycloaddition reaction (SPAAC) to yield the final ADC. The reaction between the DBCO-modified antibody and MMAE–glycolinker **5** was found to be complete as indicated by MALDI-TOF MS analysis of the antibody light chain as well as the Fc domain (Figure 1). The DAR of the product was found to be ~10 (see Supporting Information for more details).

With access to all of the individual components and the model ADC, we decided to conduct a preliminary cytotoxic evaluation of these molecules. MMAE (**4**) and MMAE–N_3–glycolinker conjugate **5** were studied in cytotoxicity assays using the human head-and-neck cancer cell line HSC-2. The MMAE–glycolinker–ADC was studied in cytotoxicity assays using the human ovarian cancer cell line SKOV-3 (Figure 2, both cell lines are EGFR-positive). MMAE was found to be highly cytotoxic to the cells with a calculated 50% growth-inhibitory concentration (IC_{50}) of 4 nM.[23] As expected, **5** was less cytotoxic, with an IC_{50} value of 12 nM, presumably reflecting decreased cell membrane permeability due to increased hydrophilic character of the glycolinker-modified drug. This might decrease non-target-related toxicities caused by drug liberated from the ADC in the body, which is one of the dose-limiting factors in clinically applied ADCs.[24] The cytotoxicity of the ADC was in the middle, with an IC_{50} value of 9 nM. The successful construction of the



Scheme 3. Synthesis of Cetuximab–glycolinker–MMAE conjugates. *Reagents and conditions*: a) NHS-DBCO, PBS, DMSO, ~10 residues; b) **5**, PBS, DMSO, 0 unreacted DBCO residues.

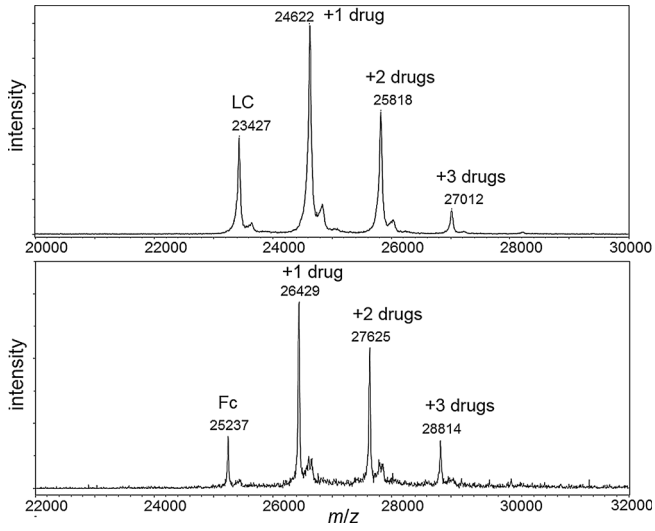


Figure 1. MALDI-TOF MS analysis of the MMAE–(DBCO-N_3-glycolinker)–ADC light chain (above) and Fc domain (below).

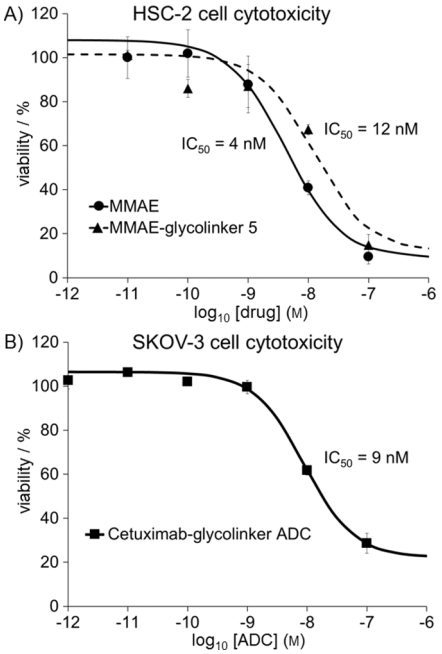


Figure 2. Cytotoxicity assays: A) Cytotoxicity of MMAE (original drug) and MMAE-glycolinker **5** against cancer cell line HSC-2. B) Cytotoxicity of anti-EGFR cetuximab–glycolinker ADC against cancer cell line SKOV-3. Error bars show standard deviation of three parallel experiments.

ADC together with its modest cytotoxicity indicates that hydrophilic bifunctional glycolinkers are applicable to the construction of ADCs.

Based on our observations, carbohydrates can be applied as chemical derivatization agents in the construction of ADCs. The potential benefits of glycolinkers are their hydrophilic character, ease of modification coupled with multiple structural variation possibilities (in comparison with the previously reported PEG, sulfonate, and glucuronide-linker strategies),[7] biocompatibility, and their natural occurrence (renewable resources). In this study, short synthetic routes leading to modified galactose substrates were used to create bifunctional glycolinkers for the derivatization of amine-containing drugs and subsequent bioconjugation reactions. The applicability of the hydrophilic glycolinkers was verified by the construction of MMAE–glycolinker conjugates and a cetuximab–glycolinker–MMAE ADC with the considerable drug-to-antibody ratio 10. The initial cytotoxic evaluation revealed a modest cytotoxicity for the model ADC and a decreased cytotoxicity for the glycolinker–MMAE–conjugate relative to free MMAE. The results of our studies suggest that carbohydrates may hold a place within future linker technologies, but more work in this area is required to determine whether they can surpass the currently applied linker protocols. The applicability of the presented glycolinkers, where the drug is permanently modified by the carbohydrate, is likely restricted to compounds that can tolerate a covalent modification with a stable linker (e.g., auristatins, including MMAE, and maytansinoids, used in the construction of Kadcyla[22]). Inspired by these findings, we have continued with the development of other types of glycolinkers for other types of cytotoxic drugs, and further optimization of the associated reaction conditions and structural properties. The results of these studies will be reported in due course.

Acknowledgements

*The authors thank Ms. Anu Karinen for the larger-scale preparation of conjugate **5**. F.S.E. thanks the Walter and Lisi Wahl Foundation for financial support.*

Keywords: antibody–drug conjugates · bioconjugation · carbohydrates · cytotoxic activity · structural characterization

[1] a) C. Peters, S. Brown, *Biosci. Rep.* **2015**, *35*, e00225; b) P. J. Carter, P. D. Senter, *Cancer J.* **2008**, *14*, 154–169; c) E. L. Sievers, P. D. Senter, *Annu. Rev. Med.* **2013**, *64*, 15–29.

[2] R. S. Zolot, S. Basu, R. P. Million, *Nat. Rev. Drug Discovery* **2013**, *12*, 259–260.

[3] a) M. R. Gordon, M. Canakci, L. Li, J. Zhuang, B. Osborne, S. Thayumanavan, *Bioconjugate Chem.* **2015**, *26*, 2198–2215; b) H. L. Perez, P. M. Cardarelli, S. Deshpande, S. Gangwar, G. M. Schroeder, G. D. Vite, R. M. Borzilleri, *Drug Discovery Today* **2014**, *19*, 869–881.

[4] a) T. Ghose, M. R. C. Path, S. P. Nigam, *Cancer* **1972**, *29*, 1398–1400; b) G. F. Rowland, G. J. O'Neill, D. A. L. Davies, *Nature* **1975**, *255*, 487–488; c) G. A. Pietersz, K. Krauer, *J. Drug Targeting* **1994**, *2*, 183–215; d) P. A. Trail, D. Willner, S. J. Lasch, A. J. Henderson, S. Hofstead, A. M. Casazza, R. A. Firestone, I. Hellström, K. E. Hellström, *Science* **1993**, *261*, 212–215.

[5] a) A. Younes, N. L. Bartlett, J. P. Leonard, D. A. Kennedy, C. M. Lynch, E. L. Sievers, A. Forero-Torres, *N. Engl. J. Med.* **2010**, *363*, 1812–1821; b) P. D. Senter, E. L. Sievers, *Nat. Biotechnol.* **2012**, *30*, 631–637; c) P. M. LoRusso, D. Weiss, E. Guardino, S. Girish, M. X. Sliwkowski, *Clin. Cancer Res.* **2011**, *17*, 6437–6447; d) A. Mullard, *Nat. Rev. Drug Discovery* **2013**, *12*, 329–332.

[6] M. Barok, H. Joensuu, J. Isola, *Breast Cancer Res.* **2014**, *16*, 209.

[7] a) R. P. Lyon, T. D. Bovee, S. O. Doronina, P. J. Burke, J. H. Hunter, H. D. Neff-LaFord, M. Jonas, M. E. Anderson, J. R. Setter, P. D. Senter, *Nat. Biotechnol.* **2015**, *33*, 733–735; b) R. Y. Zhao, S. D. Wilhelm, C. Audette, G. Jones, B. A. Leece, A. C. Lazar, V. S. Goldmacher, R. Singh, Y. Kovtun, W. C. Widdison, J. M. Lambert, R. V. J. Chari, *J. Med. Chem.* **2011**, *54*, 3606–3623.

[8] a) D. S. Dalpathado, H. Jiang, M. A. Kater, H. Desaire, *Anal. Bioanal. Chem.* **2005**, *381*, 1130 – 1137; b) G. R. Gray, *Arch. Biochem. Biophys.* **1974**, *163*, 426 – 428; c) M. G. O'Shea, M. S. Samuel, C. M. Konik, M. K. Morell, *Carbohydr. Res.* **1998**, *307*, 1 – 12.

[9] a) S. Park, J. C. Gildersleeve, O. Blixt, I. Shin, *Chem. Soc. Rev.* **2013**, *42*, 4310 – 4326; b) T. Horlacher, P. H. Seeberger, *Chem. Soc. Rev.* **2008**, *37*, 1414 – 1422; c) S. Park, M.-R. Lee, I. Shin, *Chem. Commun.* **2008**, 4389 – 4399.

[10] a) M. Arslan, T. N. Gevrek, A. Sanyal, R. Sanyal, *RSC Adv.* **2014**, *4*, 57834 – 57841; b) P. Diagaradjane, J. M. Orenstein-Cardona, N. E. Colón-Casasnovas, A. Deorukhkar, S. Shentu, N. Kuno, D. K. Schwartz, J. G. Gelovani, S. Krishnan, *Clin. Cancer Res.* **2008**, *14*, 731 – 741; c) M. E. Gindy, S. Ji, T. R. Hoye, A. Z. Panagiotopoulos, R. K. Prudhomme, *Biomacromolecules* **2008**, *9*, 2705 – 2711.

[11] a) C. Grandjean, A. Boutonnier, C. Guerreiro, J.-M. Fournier, L. A. Mulard, *J. Org. Chem.* **2005**, *70*, 7123 – 7132; b) C. I. Schilling, N. Jung, M. Miskup, U. Schepers, S. Bräse, *Chem. Soc. Rev.* **2011**, *40*, 4840 – 4871; c) H. Zhang, Y. Ma, X.-L. Sun, *Chem. Commun.* **2009**, 3032 – 3034.

[12] a) Q. Wang, T. R. Chan, R. Hilgraf, V. V. Fokin, K. B. Sharpless, M. G. Finn, *J. Am. Chem. Soc.* **2003**, *125*, 3192 – 3193; b) X. L. Sun, C. L. Stabler, C. S. Cazalis, E. L. Chaikof, *Bioconjugate Chem.* **2006**, *17*, 52 – 57; c) I. Singh, C. Freeman, F. Heaney, *Eur. J. Org. Chem.* **2011**, 6739 – 6746; d) E. M. Sletten, C. R. Bertozzi, *Acc. Chem. Res.* **2011**, *44*, 666 – 676.

[13] a) G. Thumshirn, U. Hersel, S. L. Goodman, H. Kessler, *Chem. Eur. J.* **2003**, *9*, 2717 – 2725; b) A. Dirksen, P. E. Dawson, *Bioconjugate Chem.* **2008**, *19*, 2543 – 2548; c) Y. Zeng, T. N. C. Ramya, A. Dirksen, P. E. Dawson, J. C. Paulson, *Nat. Methods* **2009**, *6*, 207 – 209.

[14] a) C. E. Hoyle, C. N. Bowman, *Angew. Chem. Int. Ed.* **2010**, *49*, 1540 – 1573; *Angew. Chem.* **2010**, *122*, 1584 – 1617; b) C. P. Ramil, Q. Lin, *Chem. Commun.* **2013**, *49*, 11007 – 11022.

[15] M. F. Debets, C. W. J. van der Doelen, F. P. J. T. Rutjes, F. L. van Delft, *ChemBioChem* **2010**, *11*, 1168 – 1184.

[16] S. Rostami, I. Qazi, R. Sikorski, *ADC Rev.* **2014**, DOI: adcreview.com/articles/doi-10-14229jadc-2014-8-1-001/.

[17] The numbering of peptide residues and positions can be found in the Supporting Information.

[18] a) P. Sarkar, C. Reichman, T. Saleh, R. B. Birge, C. G. Kalodimos, *Mol. Cell* **2007**, *25*, 413 – 426; b) M. Keller, C. Sager, P. Dumy, M. Schutkowski, G. S. Fischer, M. Mutter, *J. Am. Chem. Soc.* **1998**, *120*, 2714 – 2720; c) Q. Sui, D. Borchardt, D. K. Rabenstein, *J. Am. Chem. Soc.* **2007**, *129*, 12042 – 12048.

[19] S. Verma, D. Miles, L. Gianni, I. E. Krop, M. Welslau, J. Baselga, M. Pegram, D.-Y. Oh, V. Diéras, E. Guardino, L. Fang, M. W. Lu, S. Olsen, K. Blackwell, *N. Engl. J. Med.* **2012**, *367*, 1783 – 1791.

[20] F. Ciardiello, G. Tortora, *N. Engl. J. Med.* **2008**, *358*, 1160 – 1174.

[21] a) X. Li, T. Fang, G.-J. Boons, *Angew. Chem. Int. Ed.* **2014**, *53*, 7179 – 7182; *Angew. Chem.* **2014**, *126*, 7307 – 7310; b) J. R. Junutula, H. Raab, S. Clark, S. Bhakta, D. D. Leipold, S. Weir, Y. Chen, M. Simpson, S. P. Tsai, M. S. Dennis, Y. Lu, Y. G. Meng, C. Ng, J. Yang, C. C. Lee, E. Duenas, J. Gorrell, V. Katta, A. Kim, K. McDorman, K. Flagella, R. Venook, S. Ross, S. D. Spencer, W. L. Wong, H. B. Lowman, R. Vandlen, M. X. Sliwkowski, R. H. Scheller, P. Polakis, W. Mallet, *Nat. Biotechnol.* **2008**, *26*, 925 – 932; c) J. Y. Axup, K. M. Bajjuri, M. Ritland, B. M. Hutchins, C. H. Kim, S. A. Kazane, R. Halder, J. S. Forsyth, A. F. Santidrian, K. Stafin, Y. Lu, H. Tran, A. J. Seller, S. L. Biroc, A. Szydlik, J. K. Pinkstaff, F. Tian, S. C. Sinha, B. Felding-Habermann, V. V. Smider, P. G. Schultz, *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 16101 – 16106; d) T. Satomaa, J. Helin, F. S. Ekholm (Glykos Finland Oy), WO2014177771 A1, **2014**.

[22] M. T. Kim, Y. Chen, J. Marhoul, F. Jacobson, *Bioconjugate Chem.* **2014**, *25*, 1223 – 1232.

[23] S. O. Doronina, B. E. Toki, M. Y. Torgov, B. A. Mendelsohn, C. G. Cerveny, D. F. Chace, R. L. DeBlanc, R. P. Gearing, T. D. Bovee, C. B. Siegall, J. A. Francisco, A. F. Wahl, D. L. Meyer, P. D. Senter, *Nat. Biotechnol.* **2003**, *21*, 778 – 784.

[24] B. E. C. G. de Goeij, J. M. Lambert, *Curr. Opin. Immunol.* **2016**, *40*, 14 – 23.

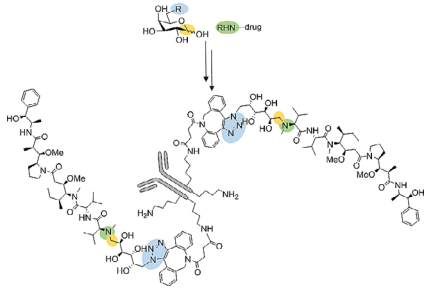
Received: July 21, 2016
Revised: September 29, 2016
Published online on ■■ ■■, 0000

COMMUNICATIONS

*F. S. Ekholm, H. Pynnönen, A. Vilkman, V. Pitkänen, J. Helin, J. Saarinen, T. Satomaa**

■■ – ■■

Introducing Glycolinkers for the Functionalization of Cytotoxic Drugs and Applications in Antibody–Drug Conjugation Chemistry



A better tether: Carbohydrates can be used as chemical derivatization agents in generating antibody–drug conjugates. The potential benefits of such glycolinkers are their hydrophilicity, relative ease of structural modification, biocompatibility, and their natural occurrence. We report new carbohydrate-based derivatization agents for the modification of amine-containing drugs and their applications in antibody–drug conjugation chemistry.