Bioconjugate Chemistry

Subscriber access provided by University of East Anglia Library

Communication

A peptide-duocarmycin conjugate targeting the Thomsen-Friedenreich antigen has potent and selective antitumour activity

Oliver Charles Cartwright, Andrew Michael Beekman,
Marco M. D. Cominetti, David A Russell, and Mark Searcey

Bioconjugate Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.bioconjchem.0c00282 • Publication Date (Web): 09 Jun 2020

Downloaded from pubs.acs.org on June 10, 2020

Just Accepted

"Just Accepted" manuscripts have been peer-reviewed and accepted for publication. They are posted online prior to technical editing, formatting for publication and author proofing. The American Chemical Society provides "Just Accepted" as a service to the research community to expedite the dissemination of scientific material as soon as possible after acceptance. "Just Accepted" manuscripts appear in full in PDF format accompanied by an HTML abstract. "Just Accepted" manuscripts have been fully peer reviewed, but should not be considered the official version of record. They are citable by the Digital Object Identifier (DOI®). "Just Accepted" is an optional service offered to authors. Therefore, the "Just Accepted" Web site may not include all articles that will be published in the journal. After a manuscript is technically edited and formatted, it will be removed from the "Just Accepted" Web site and published as an ASAP article. Note that technical editing may introduce minor changes to the manuscript text and/or graphics which could affect content, and all legal disclaimers and ethical guidelines that apply to the journal pertain. ACS cannot be held responsible for errors or consequences arising from the use of information contained in these "Just Accepted" manuscripts.

is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036
Published by American Chemical Society. Copyright © American Chemical Society. However, no copyright claim is made to original U.S. Government works, or works produced by employees of any Commonwealth realm Crown government in the course of their duties.

A peptide-duocarmycin conjugate targeting the Thomsen-Friedenreich antigen has potent and selective antitumour activity

Oliver Charles Cartwright,[†] Andrew Michael Beekman,[†] Marco M. D. Cominetti,[†] David A. Russell[‡] and Mark Searcey*[†,‡]

[†]School of Pharmacy, University of East Anglia, Norwich Research Park, Norwich, Norfolk, NR47TJ, United Kingdom
[‡]School of Chemistry, University of East Anglia, Norwich Research Park, Norwich, Norfolk, NR47TJ, United Kingdom.
*E-mail: M.Searcey@uea.ac.uk, +44 (0)1603 59 7544

ABSTRACT: Solid phase synthesis allowed the rapid generation of a peptide-drug conjugate. A peptide targeting the Thomsen-Friedenreich antigen (TFα) was conjugated to the alkylating subunit of the potent cytotoxin duocarmycin SA. The compound, containing a cathepsin B cleavable linker, was shown to be active and selective against TFα expressing tumour cell lines.

INTRODUCTION: Antibody drug conjugates (ADCs) currently dominate the targeted therapeutic landscape with seven approved therapies and over sixty others in clinical trials.[1] This approach does have some significant drawbacks, including , possibility of immunogenicity, and poor tumour penetration.[2] Peptide drug conjugates (PDCs) offer solutions to some of these drawbacks. PDCs combine the cytotoxic activity of a drug payload with the targeting ability of a specific peptide sequence. Due to the smaller size of peptides, penetration into solid tumours is improved.[3] The design, synthesis and characterisation of peptides is relatively straightforward. Methodologies to generate peptides are well established and automated systems have been developed. The coupling of drug molecules to peptides allows for complete control over the drug ratio and full structural characterisation. Additionally, peptides can be readily modified to provide the desired characteristics, such as added functionality, or imparting improved physiochemical properties.[2] As in ADC systems, linker technologies between the cytotoxic drug and the peptide can be employed to allow for efficient release of the payload at the desired site of action whilst ensuring stability during circulation. PDCs have shown success when deploying doxorubicin,[4] daunomycin[5] and kinase inhibitors,[6] utilising CD13 and HER2 targeting peptides, improving specificity and solubility issues previously seen with these payloads.

The cancer phenotype is commonly associated with aberrant glycosylation patterns. One glycan that is directly linked to cancer is the Thomsen-Friedenreich antigen (TFα or CD176).[7,8] It is a disaccharide composed of a galactose β1-3 N-acetylgalactosamine, O-linked to a glycoprotein through serine or threonine residues and commonly written as Galβ1-3GalNAc-α-O-Ser/Thr. The TFα is therapeutically attractive due to its cryptic nature in normal cells and exposure in embryonic and cancer cells.[9] The expression of the TFα has been demonstrated in 90% of primary human carcinomas, including in the lung, the breast and the pancreas.[10,11] Additionally, cancer initiating cells or cancer stem cells in the lung, liver and colon express the TFα.[12] The peptide sequence HGRFILPWWYAFSPS (TFα -peptide) is known to bind tightly to the TFα (K_d = 1.2 μM) and has been demonstrated to inhibit processes directly involved in TFα accessibility.[13]

As a payload for PDCs (and ADCs), analogues of the duocarmycins are attractive. Duocarmycin SA and yatakemycin (Figure 1) rank amongst the most potent natural cytotoxins discovered.[14,15] The cyclopropyl and prodrug *seco* forms are both naturally occurring and equipotent in most circumstances (Figure 1).[16] Studies of the binding-driven bonding model of their interaction with DNA suggest that their utility will be enhanced when targeted to tumour cells.[17,18] In fact, SYD985, an ADC that utilises a peptide linker for a duocarmycin analogue to trastuzumab and has recently been progressed to phase III clinical trial.[19]

We previously reported the preparation of a molecule that enables the use of the alkylating unit of duocarmycin SA (termed DSA) in solid phase peptide synthesis (SPPS), referred to as Fmoc-DSA(OBn)-OH.[20,21] We demonstrated the use of this uncyclised *seco*-DSA unit in SPPS, allowing incorporation into the backbone of a peptide and, by extension, this approach allows the incorporation of the amino acid structure of the DSA alkylation subunit into any defined PDC structure. We report here a PDC targeting DSA to the TFα.

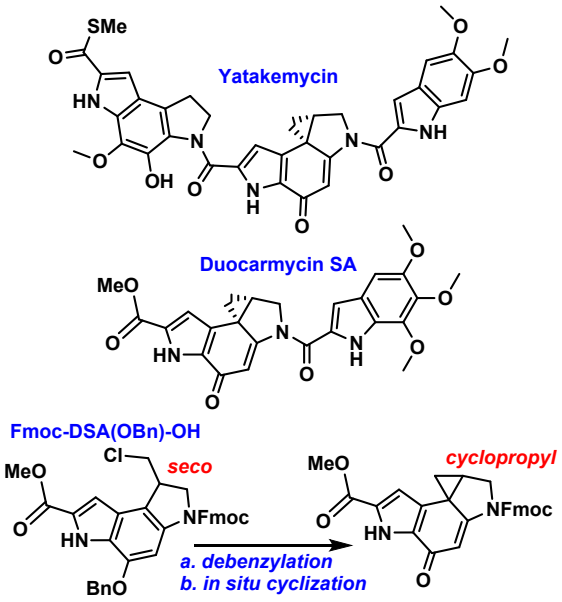
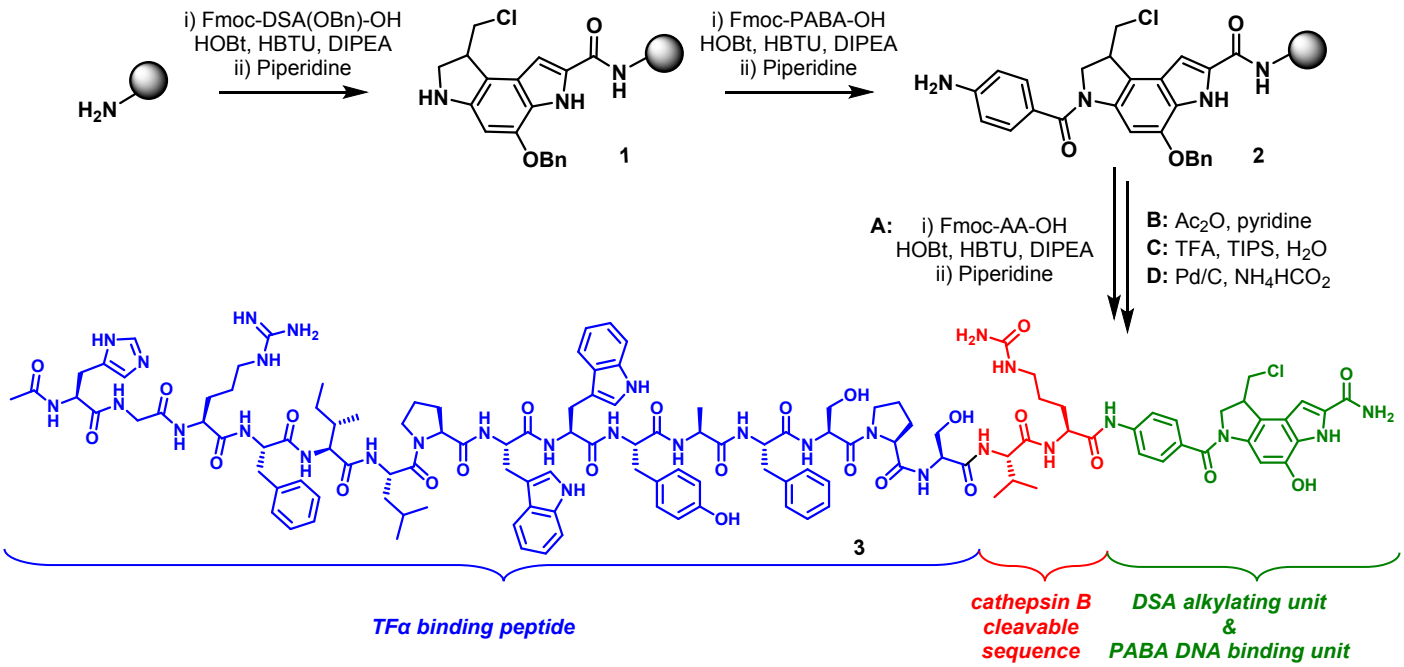


Figure 1. Structures of the natural duocarmycin SA and yatakemycin, and the solid phase equipped alkylating unit Fmoc-DSA(OBn)-OH.



Scheme 1. Solid phase peptide synthesis of peptide-drug conjugate 3. Schematic of PDC design. Cathepsin B cleavage occurs at PABA and Cit amide to release green payload.

RESULTS AND DISCUSSION: The design of our peptide-drug conjugate aimed to fully exploit the ability to be prepared on the solid phase. Previous work has shown that substitution on the C-terminus of DSA results in a loss of activity, and that a neutral C terminus is required.[20] Therefore, rink amide was chosen for the resin and Fmoc-DSA(OBn)-OH was loaded directly onto the resin, allowing for the production of a terminal amide. Studies of drug conjugate generation often debate "non-cleavable" versus "cleavable" linkers. The former case relies on the breakdown of the peptide structure by non-specific proteases whereas, in the latter scenario, linkers are designed to be cleaved by selective enzymes. A review of the literature highlights that in the case of duocarmycin payloads the cleavable sequences are superior.[22] As such, we employed the well-established[21,23] cathepsin B cleavable sequence Val-Cit,[24] possibly adding secondary targeting, with cathepsin B overexpressed in cancer cell lines.[25] This cleavable sequence is often associated with a spacing unit. We selected the *para*-aminobenzoic acid (PABA) unit for its simple incorporation in Fmoc solid phase chemistry and its ability to improve the DNA binding, and therefore efficacy, of both enantiomers of DSA.[26] Accordingly, we employed the racemic Fmoc-DSA(OBn)-OH for ease of preparation with minimal reduction in potency. Work by us[27,28] and others[29,30] suggests that lectins and conjugated lectins undergo receptor mediated endocytosis when bound to the TFα. We hoped to exploit this mechanism in the delivery of the PDC.

The payload of the peptide drug conjugate (Fmoc-DSA(OBn)-OH) was prepared as reported previously,[20] and utilised with SPPS to generate duocarmycin- TFα -peptide PDC **3** (Scheme 1). Fmoc protected NovaPEG rink amide resin was first deprotected with 20% piperidine in DMF for 10 mins twice, followed by coupling with 1.5 eq. Fmoc-DSA(OBn)-OH using HOBt, HBTU and DIPEA overnight. Subsequently, Fmoc-PABA-OH was added, moving through the sequence to Fmoc-His(Trt)-OH. The final amino acid was Fmoc deprotected and acetylated. The peptide was removed from resin with trifluoroacetic acid mediated cleavage and purification was achieved by RP-HPLC. Deprotection of the phenol to release the active duocarmycin warhead was achieved by transfer hydrogenation, utilising palladium on carbon and ammonium formate. The cleavage of the benzyl group in the presence of the extended peptide proved troublesome, requiring fresh palladium and closely monitored reaction temperatures and time, often without reproducible results. Work on new methodology to release the active payload efficiently and reliably is underway and will be disclosed in due course.

PDC **3** was evaluated against a panel of cancer cell lines commonly used in our lab to evaluate the selectivity of the designed construct. Breast cancer cell lines MCF-7 and SKBR3, lung cancer cell lines A549 and H292, colorectal carcinoma cells HCT116, fibrosarcoma cells HT1080 and acute promyelocytic leukemia cells HL-60 were utilised. The TFα is expressed in 90% of primary human carcinomas, therefore the non-cancerous human bronchial epithelial cell line 16HBE14o was also employed as negative control. All cell lines were analysed by flow cytometry to evaluate the expression of the TFα. Three cells lines, A549, H292 and 16HBE14o, showed no detectable expression of the TFα (expressed as a value of 1.0 when comparing fluorescence of the TFα antibody in the presence of the cells versus the negative control, Table 1). All other cell lines demonstrated at least a 1.7-fold fluorescence intensity increase (Table 1). All cell lines were also evaluated for their cathepsin B activity, using the cathepsin B specific Z-Arg-Arg-7-amido-4-methylcoumarin (Z-RR-AMC) assay. The cleavage of the Arg-Arg sequence was compared to the non-cancerous cell line

16HBE14o. The cancerous cell lines demonstrated increased cathepsin B activity, while cathepsin B activity was not detected in 16HBE14o cell lysates. An MTS ([3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) cell proliferation assay was used to determine the efficacy of PDC **3** against cancer cell lines. PDC **3**, benzyl protected control **4**, the resulting payload after enzyme cleavage **5**, the resulting peptide after enzyme cleavage **6**, and the analogous PDC without the cleavable sequence **7** (in which the Val-Cit-PABA sequence is removed between the TFα -peptide and DSA, Figure 2), were incubated with cell cultures for 72 hours, followed by

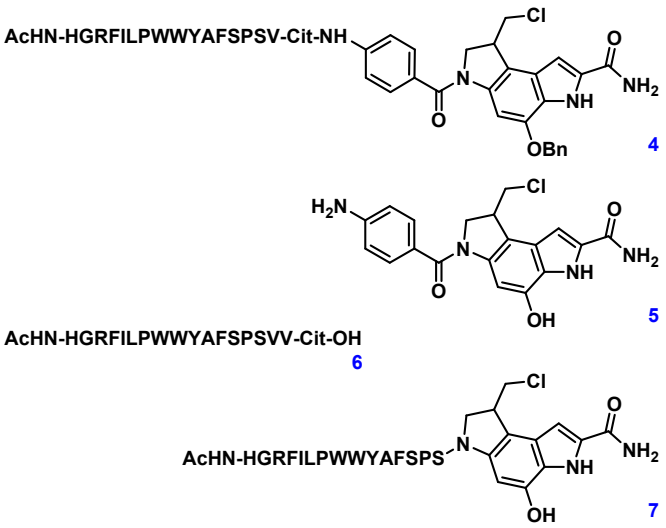


Figure 2. Control compounds; benzyl protected control **4**, enzyme cleaved payload **5**, enzyme cleaved peptide **6**, and cleavable sequence control **7**.

treatment with MTS. The results are summarized in table 1. All cell lines demonstrated sensitivity toward the DSA-PABA payload **5** in the nanomolar range. The cleaved peptide **6**, and the benzyl protected control peptide **4**, had no appreciable effect on cell proliferation (>100 μM), suggesting any observed cytotoxic activity is due to the DSA warhead. Interestingly, PDC **7**, without the cathepsin B cleavable sequence, also had no appreciable effect on cell proliferation (>100 μM), despite possessing the active DSA DNA alkylating moiety. Perhaps this PDC is either not being taken up into cells or not being broken down by proteases and peptidases in the cell. In both cases, the warhead may not be reaching the nucleus, the site of action of the duocarmycins. Cell lines that demonstrated appreciable levels of TFα were sensitive to PDC **3**. Interestingly, HCT116, which had relatively high TFα expression and cathepsin B activity, demonstrated the most sensitivity to PDC **3**, approaching similar potency to the payload **5**. Excitingly, A549, H292 and 16HBE14o, which showed no detectable TFα expression, appeared unaffected by PDC **3** up to 100 μM, suggesting TFα expression is required for the efficacy of PDC **3**. To provide further evidence that the PDC was targeting the TFα we employed a competitive binding assay. HCT116 cells (high TFα expression), MCF-7 cells (moderate TFα expression) and A549 cells (undetectable TFα expression) were incubated with and without jacalin, a protein known to tightly bind to the TFα,[31] and then exposed to cytotoxic compounds **3** and **5** (Table 2, Figure 3). Excitingly, it was observed that in the presence of jacalin **3** had a reduced efficacy on HCT116 and MCF-7 cells compared to its activity in the absence of jacalin. MCF-7 cells showed a 10-fold reduction in potency and HCT116 showed a 19-fold reduction in IC_{50}. In A549 cells **3** still showed no effect on proliferation. Additionally, jacalin had no effect on the activity of payload **5** in any of the cell lines. As a control bovine serum albumin (BSA) was used to confirm the change in efficacy of **3** was not a crowding effect. Pleasingly, the potency of PDC **3** was restored towards the HCT116 and MCF-7 cell lines, and again, unchanged in the A549 cells. These data are indicative that the TFα is playing a role in the delivery of the peptide drug conjugate.

Table 1: IC_{50} results obtained for compounds **3** and **5** to selected cancerous and non-cancerous cell lines. Errors are 95 % confidence intervals.

Cell Line	**3** (nM)	**5** (nM)	*TFα* expression[a]	Cat B activity[b]
MCF-7	864 [738; 987]	28 [24; 33]	3.4	62
SKBR3	526 [474; 617]	4 [3; 5]	2.2	77
A549	>100 μM	39 [28; 62]	1.0	96
H292	>100 μM	9 [7; 13]	1.0	59
HCT116	402 [328; 502]	57 [39; 78]	10.7	95
HT1080	8266 [6796; 10198]	154 [139; 170]	3.4	47
HL-60	1831 [927; 2976]	33 [23; 53]	1.7	64
16HBE14o	>100 μM	89 [71; 102]	1.0	1

[a]Fold increase in mean fluorescence intensity of Anti- TFα antibody treated to negative control (treated/negative). [b]Fold increase in observed fluorescence of cell line compared to 16HBE14o (cell line/16HBE14o).

Table 2: IC_{50} results obtained for compounds **3** and **5** to selected cancerous and non-cancerous cell lines in the presence of jacalin or bovine serum albumin (BSA) at 10 μM. Errors are 95 % confidence intervals

Cell Line	**3** + jacalin (nM)	IC_{50} ratio[a]	**5** + jacalin (nM)	**3** + BSA (nM)	**5** + BSA (nM)
MCF-7	9183 [7652; 11020]	10.6	21 [7; 67]	984 [707; 1370]	21 [3; 137]
A549	>100 μM	1	57 [40; 53]	>100 μM	45 [39; 52]
HCT116	7769 [6156; 9804]	19.3	58 [34; 100]	376 [191; 741]	60 [32; 113]

[a]Fold increase in IC_{50} of **3** + jacalin compared to **3** ((**3** + jacalin)/**3**)

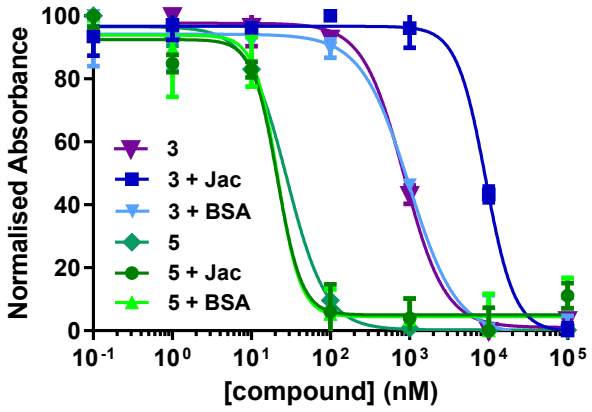


Figure 3. Competitive binding antiproliferation of MCF-7 cancer cells assay of PDC **3** and payload **5**, without added protein, or in the presence of jacalin or bovine serum albumin (BSA) at 1 µM. Y-axis is the normalized absorbance at 490 nm after treatment with MTS for 3 hr. Results are a representative experiment

The targeted delivery of ultra-potent cytotoxins has become a key element of cancer drug discovery. Incorporating payloads into peptide chains has proven an excellent way to direct cytotoxic agents. The duocarmycins, which are ultra-potent antitumour antibiotics, are yet to be realized as a suitable drug, despite their unique mode of action. The development of units suitable for solid phase peptide synthesis, both the alkylating and DNA binding units, creates a paradigm for the rapid and economical preparation of conjugates containing duocarmycins. The Fmoc-DSA(OBn)-OH unit has demonstrated great promise when deployed with a Thomsen-Friedenreich antigen binding peptide, displaying duocarmycin-dependent cytotoxicity in cancer cell lines. The TFα directing peptide drug conjugate demonstrated efficacy in cell lines which express the TFα at nanomolar concentrations and no effect on proliferation on cells that do not show TFα expression. Importantly, the PDC demonstrated reduced potency when the TFα was shielded with the known TFα binding lectin jacalin. The PDC may possess two-fold targeting, requiring both a cathepsin B cleavable linker and demonstrating a reliance on the TFα.

CONCLUSION: This work has highlighted the potential for the use of ultra-potent cytotoxins in solid phase peptide synthesis. Duocarmycin analogues can be prepared on resin, without the need for the unmasking of the active compound, allowing the preparation of increased quantities safely. Late stage liberation of the active duocarmycin has been demonstrated, just prior to cellular examination, reducing the handling of ultra-potent cytotoxins. This payload masking strategy also circumvents the known potency reduction observed when self immolative linkers are used to mask the *seco*-warhead.[23] This demonstration of the Fmoc-DSA(OBn)-OH unit forms an archetype for the production of antibody-drug, nanoparticle-drug and peptide-drug conjugates of the duocarmycins.

Conflicts of interest

There are no conflicts to declare

Acknowledgements

This work was funded in part through a BBSRC CASE award BB/L017261/1 with Novartis Pharma (OCC).

SUPPORTING INFORMATION. Full experimental descriptions of synthesis and biological experiments, and supporting figures.

Notes and references

(1) Lambert, J. M.; Berkenblit, A. Antibody–Drug Conjugates for Cancer Treatment. *Ann. Rev. Med.* **2018**, *69*, 191–207. https://doi.org/10.1146/annurev-med-061516-121357.

(2) Ma, L.; Wang, C.; He, Z.; Cheng, B.; Zheng, L.; Huang, K. Peptide-Drug Conjugate: A Novel Drug Design Approach. *Curr. Med. Chem.* **2017**, *24*, 3373–3396.

(3) Fosgerau, K.; Hoffmann, T. Peptide Therapeutics: Current Status and Future Directions. *Drug Discov. Today* **2015**, *20*, 122–128.

(4) Ai, S.; Jia, T.; Ai, W.; Duan, J.; Liu, Y.; Chen, J.; Liu, X.; Yang, F.; Tian, Y.; Huang, Z. Targeted Delivery of Doxorubicin through Conjugation with EGF Receptor–Binding Peptide Overcomes Drug Resistance in Human Colon Cancer Cells. *Br. J. Pharmacol.* **2013**, *168*, 1719–1735. https://doi.org/doi:10.1111/bph.12055.

(5) Enyedi, K. N.; Tóth, S.; Szakács, G.; Mező, G. NGR-Peptide−drug Conjugates with Dual Targeting Properties. *PLoS One* **2017**, *12*, e0178632. https://doi.org/10.1371/journal.pone.0178632.

(6) Argyros, O.; Karampelas, T.; Asvos, X.; Varela, A.; Sayyad, N.; Papakyriakou, A.; Davos, C. H.; Tzakos, A. G.; Fokas, D.; Tamvakopoulos, C. Peptide–Drug Conjugate GnRH–Sunitinib Targets Angiogenesis Selectively at the Site of Action to Inhibit Tumor Growth. *Cancer Res.* **2016**, *76*, 1181–1192. https://doi.org/10.1158/0008-5472.can-15-2138.

(7) Cao, Y.; Merling, A.; Karsten, U.; Goletz, S.; Punzel, M.; Kraft, R.; Butschak, G.; Schwartz-Albiez, R. Expression of CD175 (Tn), CD175s (Sialosyl-Tn) and CD176 (Thomsen-Friedenreich Antigen) on Malignant Human Hematopoietic Cells. *Int. J. Cancer* **2008**, *123*, 89–99.

(8) Samuel, J.; Noujaim, A. A.; MacLean, G. D.; Suresh, M. R.; Longenecker, B. M. Analysis of Human Tumor Associated Thomsen-Friedenreich Antigen. *Cancer Res.* **1990**, *50*, 4801–4808.

(9) Yu, L.-G. The Oncofetal Thomsen–Friedenreich Carbohydrate Antigen in Cancer Progression. *Glycoconjugate J.* **2007**, *24*, 411–420.

(10) Springer, G. F. T and Tn, General Carcinoma Autoantigens. *Science* **1984**, *224*, 1198–1206.

(11) Dube, D. H.; Bertozzi, C. R. Glycans in Cancer and Inflammation—Potential for Therapeutics and Diagnostics. *Nat. Rev. Drug Discov.* **2005**, *4*, 477.

(12) Lin, W.; Karsten, U.; Goletz, S.; Cheng, R.; Cao, Y. Expression of CD176 (Thomsen-Friedenreich Antigen) on Lung, Breast and Liver Cancer-initiating Cells. *Int. J. Exp. Pathol.* **2011**, *92*, 97–105.

(13) Peletskaya, E. N.; Glinsky, V. V; Glinsky, G. V; Deutscher, S. L.; Quinn, T. P. Characterization of Peptides That Bind the Tumor-Associated Thomsen-Friedenreich Antigen Selected from Bacteriophage Display Libraries. *J. Mol. Biol.* **1997**, *270*, 374–384.

(14) Yasuzawa, T.; Iida, T.; Muroi, K.; Ichimura, M.; Takahashi, K.; Sano, H. Structures of Duocarmycins, Novel Antitumor Antibiotics Produced by Streptomyces Sp. *Chem. Pharm. Bull.* **1988**, *36*, 3728–3731. https://doi.org/10.1248/cpb.36.3728.

(15) Igarashi, Y.; Futamata, K.; Fujita, T.; Sekine, A.; Senda, H.; Naoki, H.; Furumai, T. Yatakemycin, a Novel Antifungal Antibiotic Produced by Streptomyces Sp. TP-A0356. *J. Antibiot.* **2003**, *56*, 107–113.

(16) Boger, D. L.; Johnson, D. S. CC-1065 and the Duocarmycins: Understanding Their Biological Function through Mechanistic Studies. *Angew. Chemie Int. Ed.* **1996**, *35*, 1438–1474. https://doi.org/10.1002/anie.199614381.

(17) Boger, D. L.; Garbaccio, R. M. Shape-Dependent Catalysis: Insights into the Source of Catalysis for the CC-1065 and Duocarmycin DNA Alkylation Reaction. *Acc. Chem. Res.* **1999**, *32*, 1043–1052. https://doi.org/10.1021/ar9800946.

(18) Wolfe, A. L.; Duncan, K. K.; Lajiness, J. P.; Zhu, K.; Duerfeldt, A. S.; Boger, D. L. A Fundamental Relationship between Hydrophobic Properties and Biological Activity for the Duocarmycin Class of DNA-Alkylating Antitumor Drugs: Hydrophobic-Binding-Driven Bonding. *J. Med. Chem.* **2013**, *56*, 6845–6857. https://doi.org/10.1021/jm400665c.

(19) Menderes, G.; Bonazzoli, E.; Bellone, S.; Black, J.; Altwerger, G.; Masserdotti, A.; Pettinella, F.; Zammataro, L.; Buza, N.; Hui, P.; et al. SYD985, a Novel Duocarmycin-Based HER2-Targeting Antibody-Drug Conjugate, Shows Promising Antitumor Activity in Epithelial Ovarian Carcinoma with HER2/Neu Expression. *Gynecol. Oncol.* **2017**, *146*, 179–186. https://doi.org/10.1016/j.ygyno.2017.04.023.

(20) Stephenson, M. J.; Howell, L. A.; O'Connell, M. A.; Fox, K. R.; Adcock, C.; Kingston, J.; Sheldrake, H.; Pors, K.; Collingwood, S. P.; Searcey, M. Solid-Phase Synthesis of Duocarmycin Analogues and the Effect of C-Terminal Substitution on Biological Activity. *J. Org. Chem.* **2015**, *80*, 9454–9467. https://doi.org/10.1021/acs.joc.5b01373.

(21) Beekman, A. M.; Cominetti, M. M. D.; Cartwright, O. C.; Boger, D. L.; Searcey, M. A Small Molecule Drug Conjugate (SMDC) of DUPA and a Duocarmycin Built on the Solid Phase. *MedChemComm* **2019**, *10*, 2170–2174. https://doi.org/10.1039/C9MD00279K.

(22) Beekman, A. M.; Cominetti, M. M. D.; Searcey, M. Duocarmycins as Antibody–Drug Conjugate (ADC) Payloads. In *Cytotoxic Payloads for Antibody–Drug Conjugates*; The Royal Society of Chemistry, 2019; pp 187–208. https://doi.org/10.1039/9781788012898-00187.

(23) Zhang, D.; Le, H.; Cruz-Chuh, J. dela; Bobba, S.; Guo, J.; Staben, L.; Zhang, C.; Ma, Y.; Kozak, K. R.; Lewis Phillips, G. D.; et al. Immolation of P-Aminobenzyl Ether Linker and Payload Potency and Stability Determine the Cell-Killing Activity of Antibody–Drug Conjugates with Phenol-Containing Payloads. *Bioconjug. Chem.* **2018**, *29*, 267–274. https://doi.org/10.1021/acs.bioconjchem.7b00576.

(24) Dorywalska, M.; Dushin, R.; Moine, L.; Farias, S. E.; Zhou, D.; Navaratnam, T.; Lui, V.; Hasa-Moreno, A.; Casas, M. G.; Tran, T.-T. Molecular Basis of Valine-Citrulline-PABC Linker Instability in Site-Specific ADCs and Its Mitigation by Linker Design. *Mol. Cancer Ther.* **2016**, *15*, 958–970.

(25) Podgorski, I.; Sloane, B. F. Cathepsin B and Its Role (s) in Cancer Progression. *Biochem. Soc. Symp.* **2003**, *70*, 263–276.

(26) Warpehoski, M. A.; Gebhard, I.; Kelly, R. C.; Krueger, W. C.; Li, L. H.; McGovren, J. P.; Prairie, M. D.; Wicnienski, N.; Wierenga, W. Stereoelectronic Factors Influencing the Biological Activity and DNA Interaction of Synthetic Antitumor Agents Modeled on CC-1065. *J. Med. Chem.* **1988**, *31*, 590–603. https://doi.org/10.1021/jm00398a017.

(27) Obaid, G.; Chambrier, I.; Cook, M. J.; Russell, D. A. Targeting the Oncofetal Thomsen–Friedenreich Disaccharide Using Jacalin-PEG Phthalocyanine Gold Nanoparticles for Photodynamic Cancer Therapy. *Angew. Chemie Int. Ed.* **2012**, *51*, 6158–6162. https://doi.org/10.1002/anie.201201468.

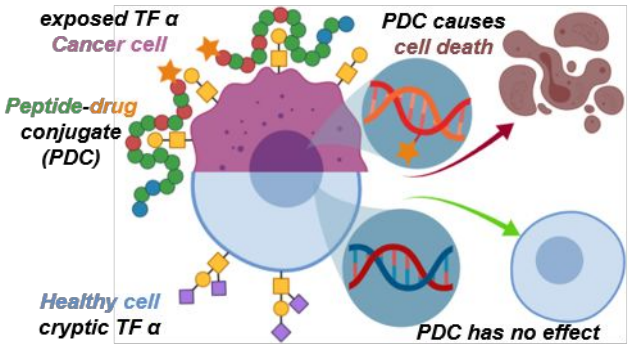
(28) Obaid, G.; Chambrier, I.; Cook, M. J.; Russell, D. A. Cancer Targeting with Biomolecules: A Comparative Study of Photodynamic Therapy Efficacy Using Antibody or Lectin Conjugated Phthalocyanine-PEG Gold Nanoparticles. *Photochem. Photobiol. Sci.* **2015**, *14*, 737–747. https://doi.org/10.1039/C4PP00312H.

(29) Yu, L.-G.; Fernig, D. G.; White, M. R. H.; Spiller, D. G.; Appleton, P.; Evans, R. C.; Grierson, I.; Smith, J. A.; Davies, H.; Gerasimenko, O. V; et al. Edible Mushroom (Agaricus Bisporus) Lectin, Which Reversibly Inhibits Epithelial Cell Proliferation, Blocks Nuclear Localization Sequence-Dependent Nuclear Protein Import . *J. Biol. Chem.* **1999**, *274*, 4890–4899. https://doi.org/10.1074/jbc.274.8.4890 .

(30) Yu, L.-G.; Fernig, D. G.; Rhodes, J. M. Intracellular Trafficking and Release of Intact Edible Mushroom Lectin from HT29 Human Colon Cancer Cells. *Eur. J. Biochem.* **2000**, *267*, 2122–2126. https://doi.org/10.1046/j.1432-1327.2000.01230.x.

(31) Jeyaprakash, A. A.; Rani, P. G.; Reddy, G. B.; Banumathi, S.; Betzel, C.; Sekar, K.; Surolia, A.; Vijayan, M. Crystal Structure of the Jacalin–T-Antigen Complex and a Comparative Study of Lectin–T-Antigen Complexes. *J. Mol. Biol.* **2002**, *321*, 637–645.

TOC Graphic


exposed TF α
Cancer cell
Peptide-drug
conjugate
(PDC)
PDC causes
cell death
Healthy cell
cryptic TF α
PDC has no effect