Drug Delivery

Linker Hydrophilicity Modulates the Anticancer Activity of RGD–Cryptophycin Conjugates

Michele Anselmi,[a, b] Adina Borbély,[a] Eduard Figueras,[a] Carmela Michalek,[a] Isabell Kemker,[a] Luca Gentilucci,[b] and Norbert Sewald*[a]

Abstract: Most anticancer agents are hydrophobic and can easily penetrate the tumor cell membrane by passive diffusion. This may impede the development of highly effective and tumor-selective treatment options. A hydrophilic β-glucuronidase-cleavable linker was used to connect the highly potent antimitotic agent cryptophycin-55 glycinate with the αβ3 integrin ligand (RGDfK). Incorporation of the self-immolative linker containing glucuronic acid results in lower cytotoxicity than that of the free payload, suggesting that hydrophilic sugar linkers can preclude passive cellular uptake. In vitro drug-release studies and cytotoxicity assays demonstrated the potential of this small molecule–drug conjugate, providing guidance for the development of therapeutics containing hydrophobic anticancer drugs.

Introduction

Many commonly used anticancer drugs do not preferentially accumulate at the tumor site, which leads to systemic side effects and to suboptimal therapeutic efficacy.[1] This limitation can be overcome by a ligand-targeted drug delivery approach.[2] Covalent conjugation of cytotoxic agents to antibodies, peptides, or small molecule ligands, capable of selective binding to receptors abundant on the tumor cell surface, enables drug accumulation at the tumor site while decreasing off-target toxicity.[3,4] In this context, nine antibody-drug conjugates (ADCs) have received marketing approval in cancer therapy so far, while many more are currently under clinical investigation.[5,6] Although ADCs have shown therapeutic benefits in clinical trials, they also display significant drawbacks, such as limited intratumor penetration, high manufacturing costs and potential immunogenicity.[7] On the other hand, small molecule–drug conjugates (SMDCs) have attracted considerable interest as a valid alternative to ADCs due to their advantageous pharmacokinetic profile, simpler and more affordable synthetic routes and lack of immunogenicity.[8] Their smaller size enables rapid and homogeneous diffusion into tissue, potentially resulting in high tumor/organ ratio.[9] Like ADCs, SMDCs are composed of a cytotoxic agent and a targeting ligand (homing device) covalently assembled across a linker, which provides sufficient stability during circulation and allows efficient drug release at the site of the disease.[10,11] Such constructs must be designed to safeguard cellular uptake of the payload for example, by receptor-mediated endocytosis[5] or to liberate the cytotoxic drug extracellularly in the tumor microenvironment.[12,13]

Among the tumor-associated receptors, the heterodimeric transmembrane glycoprotein integrin αβ3 is considered a potential tumor target due to its overexpression on cancer cell surfaces and blood vessels of several solid tumors (e.g., breast cancer, glioblastoma, pancreatic tumor, prostate carcinoma).[14,15] The αβ3 integrin subtype plays a central role in many stages of cancer progression such as angiogenesis, tumor growth, apoptosis resistance, and metastasis. Integrin αβ3 recognizes and binds the extracellular matrix (ECM) proteins through the minimal tripeptide sequence Arg-Gly-Asp (RGD).[17,18] Consequently, several cyclic RGD-bearing peptides and peptidomimetics have been prepared and conjugated to different cytotoxic agents.[19] Specifically, the cyclopentapeptide c(RGDfK) has been widely exploited as targeting ligand for imaging,[20] diagnostic[21] and drug delivery applications,[22] due to its nanomolar binding affinity and the Lys conjugation handle.[23,24]

Besides to cytotoxic agents commonly used in chemothera-
ype regimens,[25] the cryptophycins have also been recently considered as drug candidates for targeted tumor therapy.[26] Cryptophycins are natural occurring 16-membered macrocyclic depsipeptides produced by cyanobacteria.[27] This class of compounds exhibits potent cytotoxicity toward several cancer cells.
including multidrug-resistant (MDR) cells. Their strong anti-proliferative activity is based on the irreversible inhibition of the β-tubulin polymerization during mitosis, leading to cell-cycle arrest in G2/M phase and activation of apoptosis pathways. Although the expected clinical success could not be achieved using cryptophycin-52 (4) as stand-alone agent, these compounds have emerged as potent payloads in the targeted therapy approach. For example, different cryptophycin-based ADCs developed by Sanofi and Genentech have shown promising preclinical results.

Moreover, we have contributed to this field with the development of tumor targeting ADCs (31–33) and SMDCs (35–39) bearing the potent cryptophycin-55 glycinate (3) as anticancer payload. This cryptophycin derivative possesses distinct features, such as high in vitro potency, remarkable in vivo activity against MDR xenografts, excellent stability in mouse and human plasma, making it suitable for active tumor targeting.

We recently reported the development of first-generation RGD-cryptophycin conjugates (34) containing the enzymatically cleavable Val-Cit linker (X1 and X2, see Supporting Information Figure S4) and found that conjugates display high in vitro potency but poor selectivity toward M21 and M21-L human melanoma cell lines with different αvβ3 integrin expression levels. We proposed that the nonspecific passive cellular uptake of the conjugates could be associated with the high payload hydrophobicity. Nevertheless, drug-linkers with improved hydrophilicity provide optimal pharmacokinetic properties to the overall construct that may prevent aggregation and/or passive permeation across the cell membrane. To this end, the protease-sensitive β-glucuronide can be incorporated into the linker system as a hydrophilic alternative to Val-Cit linkers, to minimize the hydrophobicity and permit an efficient drug release. The β-glucuronidase is responsible for the hydrolysis of glucuronyl–O bonds and it can selectively activate glucuronide prodrugs accumulated at antigen-positive cancer cells.  

Results and Discussion

Design

Herein we report the first β-glucuronidase-cleavable conjugate equipped with the potent antimitotic agent cryptophycin-55 glycinate (Cry-55gly) and the αvβ3 integrin ligand (RGDFK), suitable for the targeted therapy of solid tumors. This drug delivery system was designed to be selectively activated by the tumor-associated enzyme β-glucuronidase present at high concentrations intracellularly in lysosomes and in necrotic tumor environment of many malignancies including lung, breast, ovarian, gastrointestinal tract carcinomas, and melanomas.

Upon enrichment and binding to integrin αvβ3, the active cytotoxic drug can be liberated from the RGD-cryptophycin conjugate both inside the target cells and in the extracellular tumor environment, from where it can diffuse into surrounding cancer cells.

The central self-immolative linker covalently connects the main components of the system, ensuring an efficient drug release after the enzymatic cleavage of the β-glucuronide trigger located at the para position on the aromatic core. The synthesis of a negative control to monitor the drug-release efficiency was envisaged by positioning the β-glucuronide (β-GlcA) in meta position on the aromatic ring of the linker. In the latter case the enzymatic cleavage is not followed by self-immolation step, thus a conjugate-intermediate with decreased activity is expected.

Synthesis

Jeffrey et al., reported the synthesis of conjugates between β-glucuronide linkers and monomethyl auristatin E (MMAE), by reacting an acetyl and methyl ester protected GlcA derivative with the cytotoxic agent. The protecting groups of the sugar moiety were removed by treatment with LiOH to provide the
drug-linker intermediate ready to be conjugated to the respective tumor targeting ligand. However, this synthetic strategy was expected unfeasible with Cry-55gly due to its instability under alkaline conditions. In a pilot experiment, treatment of Cry-55gly with LiOH/H₂O at 0 °C for 30 minutes resulted in 70% conversion into the epoxide containing cryptophycin derivative (cryptophycin-52, 4) as determined by HPLC–MS (Scheme 1A, see Supporting Information). This observation is consistent with the conversion of other cryptophycin analogues into the corresponding epoxides under various conditions (i.e., chlorohydrins are transformed into epoxides at physiological pH or in aqueous solution).

An alternative synthetic route was developed to avoid payload decomposition during synthesis, involving the exchange of the protecting groups on the carbohydrate moiety (Scheme 1B). Conjugate 1 was prepared from commercially available 4-hydroxy-3-nitrobenzaldehyde (5). Stereoselective glycosylation of 5 to acetobromo-α-D-glucuronic acid methyl ester (6) was performed under Koenigs-Knorr conditions in the presence of silver oxide as catalyst affording 7 in 93% yield. This was followed by aldehyde reduction with sodium borohydride providing 8 in 95% yield without the need of purification. The corresponding benzylic alcohol was treated with tert-butylmethylsilyle chloride and imidazole to produce the silyl ether protected derivative 9 (93%). At this stage, as reported by Grinda et al., the protecting groups of the β-glucuronide were modified via a three-step strategy to yield the fully allyl-protected carbohydrate (12). This methodology offers a stable and compatible glucuronide protection in the course of the synthesis, while the deprotection can be performed in a one-step procedure under mild conditions at the end of the synthesis. The acetyl groups were removed from 9 using sodium methoxide to afford the hydroxy-free derivative 10 (84%). Transsterification of the methyl ester with sodium allylolate gave the allyl ester 11 in 83% yield. The three allyl carbonates were introduced in the presence of a large excess of allyl chloroformate using pyridine as solvent. After three days, the fully allyl protected glucuronide 12 was obtained in 82% yield. Subsequently, nitro reduction with zinc powder under acidic conditions gave the free aniline (13, 88%) which was subsequently coupled with 5-hexynoic acid in the presence of EEDQ providing 14 (92%) with a suitable alkyne-functionalized spacer. Removal of tert-butyldimethylsilyl group was carried out with HF/pyridine to yield the free benzyl alcohol 15 (93%) which was subsequently treated with 4-nitrophenyl chloroformate and pyridine to give the activated carbonate 16 in quantitative yield. Cry-55gly αβ(3) was introduced via nucleophilic substitution in the presence of DPEA to afford the carbamate 17 in 77% yield after RP-HPLC purification. Full allyl deprotection of the glucuronide moiety was carried out using catalytic amount of tetrakis(triphenylphosphine)palladium(0) affording the Cry-55gly linker intermediate 18 in 66% yield after RP-HPLC purification. Finally, the conjugation to the targeting ligand c(RGDFK) 33, properly modified with 3-azidopropionic acid on the Lys side chain (32, see Supporting Information), was achieved by triazole formation. The copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC) was carried out at 35 °C for 24 h in the presence of alkyne 18 and the azido-cyclopeptide derivative 33, using CuSO₄ and sodium ascorbate. After purification by preparative RP-HPLC, the final conjugate 1 was obtained in 84% yield.

Following a similar synthetic approach, the conjugate 2 equipped with the β-glucuronide moiety in meta position on the linker was also prepared (negative control). Firstly, the precursor 3-(hydroxymethyl)-5-nitrophenol (20) was synthesized by reduction of the commercially available 3-hydroxy-5-nitrobenzoic acid 19 using a solution of 1.0 M BH₃·THF in THF at 0 °C (94%). The resulting benzylic alcohol of 20 was selectively protected with tert-butylmethylsilyle chloride in the presence of imidazole at 0 °C producing the protected silyl ether derivative 21 (61%). Then, the free hydroxy group in meta-position was coupled with acetyl-bromo-α-D-glucuronic acid methyl ester 6 under the same Koenigs-Knorr conditions that yielded 7, to give 22 with 80% yield. By following the same synthetic Scheme described above, the RGD–cryptophycin conjugate 2 was obtained in 43% yield. The final conjugates 1 and 2 were characterized by analytical HPLC and HRMS (see Supporting Information).

Integrin binding affinity

Conjugates 1 and 2 were evaluated for their ability to compete with vitronectin binding to the isolated αvβ₃ receptor. The binding affinity was assessed using a competitive ELISA-based assay and it was compared with the affinity of the free peptide 33 (Figure 2). Integrin binding assays were carried out by incubation of αvβ₃ integrin with increasing concentrations of the conjugates (10⁻⁵–10⁻¹⁵ M) in presence of the ECM immobilized protein vitronectin. Peptide 33 showed an IC₅₀ value of 0.81 nm, similar to that of the reference cilengitide (IC₅₀ = 0.54 nm), confirming that functionalization with 3-azidopropionic acid did not affect the integrin binding. Conjugates 1 and 2 retained good binding affinity to the receptor with IC₅₀ values in the nm range (21.9 nm and 11.7 nm, respectively) indicating that the increased size and the steric bulk of these conjugates cause only a modest decrease in affinity (Figure 2).

β-Glucuronidase-catalyzed release of cryptophycin-55 glycinate

The drug-release mechanism involves the enzymatic hydrolysis of the glycosidic bond from the linker followed by the spontaneous self-immolative process with concomitant loss of carbon dioxide and release of the active drug (Scheme 2).

The drug-release efficiency was tested by treating the conjugates 1 and 2 with E. coli β-glucuronidase (200 U mL⁻¹) at 37 °C. The release of Cry-55gly (3) was monitored over a period of 60 min by analytical HPLC, followed by analyte identification using UPLC–MS. As expected, the conjugates 1 and 2 were rapidly cleaved upon incubation with the enzyme (Figure 3). In detail, the enzymatic cleavage of 1, with para-substituted β-glucuronide moiety, generated the metabolite M1 that rapidly underwent 1,6-elimination releasing the active Cry-55gly payload (Figure 3A, full characterization and MS spectra are in
Supporting Information, Figure S3). In contrast, the β-glucuronidase mediated linker cleavage of the meta-substituted conjugate 2 led to the rapid formation of metabolite M2, but this was not followed by 1,6-elimination (self-immolative step) and the Cry-55gly was not released over time (Figure 3B). Control experiments indicated that both conjugates were stable in the

Scheme 1. A) Epoxide formation side reaction: a) LiOH, H₂O, 30 min, 0°C, 70% conversion. B) Synthesis of conjugates 1 and 2: a) Ag₂O, CH₃CN, darkness, RT, 4 h; b) NaBH₄, CH₂Cl₂/PrOH (5:1), silica gel, 0°C, 45 min; c) imidazole, TEBMSCl, CH₂Cl₂, RT, 12 h; d) MeONa 30% w/v, MeOH, 0°C, 1.5 h; e) sodium allylate 0.126M, allylic alcohol, RT, 40 min; f) allyl chloroformate, pyridine, RT, 72 h; g) zinc, MeOH/AcOH (10:1), RT, 30 min; h) 5-hexynoic acid, EEDQ, CH₂Cl₂, RT, 24 h; i) HF/pyridine 70%, THF, RT, 1 h; j) 4-nitrophenyl chloroformate, pyridine, CH₂Cl₂, 0°C—RT, 2 h; k) DIPEA, DMF, RT, 4 h; l) Pd(PPh₃)₄, morpholine, CH₂Cl₂, RT, 1 h; m) CuSO₄·5H₂O, sodium ascorbate, DMF/H₂O (1:1), 35°C, 3 h; n) BH₃·THF, THF, 0°C—RT, overnight; o) imidazole, TDBMSCl, THF, 0°C, 2 h.

Chem. Eur. J. 2021, 27, 1015–1022 www.chemeurj.org 1018 © 2020 The Authors. Published by Wiley-VCH GmbH
absence of enzyme confirming an efficient linker self-immolative process only in the presence of \(\beta\)-glucuronidase.

These results show a rapid \(t_{1/2} = 15\) min) and virtually complete enzymatic degradation of the conjugate 1 despite the relatively short linkage connecting the \(\beta\)-glucuronidase responsive linker to the cyclic integrin ligand. This is in contrast to recently published results of López Rivas et al., in which inefficient enzymatic cleavage and the lack of regained in vitro activity in the presence of \(\beta\)-glucuronidase were attributed to a suboptimal distance between the enzymatic cleavage site and the ligand in a cyclo[DKP-RGD]-GlcA-MMAE conjugate.\(^{49}\) Albeit the close similarity in terms of distance, our system contains a 1,4-triazole unit that can induce a conformational turn and provide better accessibility to \(\beta\)-glucuronidase.\(^{52}\) In a close comparison between our conjugate and the efficient system reported by López Rivas et al. (conjugate bearing a PEG4 spacer), both contain a 1,4-disubstituted triazole adjacent to the \(\beta\)-glucuronidase-cleavable moiety which points out that a conformational turn may be the key element rather than the distance.

**In vitro cytotoxicity assay**

The in vitro cytotoxic activity of the RGD-cryptophycin conjugates was tested against the \(\alpha_\text{v}\beta_3\) integrin expressing M21 human melanoma cells.\(^{53, 54}\) Cell viability was measured by resazurin assay after 2 h treatment with increasing concentrations of the free drug and conjugates 1 and 2 in the absence or presence of \(\beta\)-glucuronidase \((2\ \text{U}\ \text{well}^{-1})\) for 2 h and additional 70 h incubation (Figure 4, Table 1). As the exact \(\beta\)-glucuronidase expression level is unknown in these cancer cells, this model aimed to more closely resemble the tumor microenvir-

---

**Figure 2.** Affinities of conjugates 1, 2 and reference compound 33 to human integrin \(\alpha_\text{v}\beta_3\).

**Scheme 2.** \(\beta\)-Glucuronidase-mediated cleavage, self-immolative mechanism and Cry-55gly release from conjugates 1 and 2.
Degradation of conjugates 1 (A) and 2 (B) in the presence of β-glucuronidase. HPLC chromatograms show degradation of 1 upon incubation with E. coli β-glucuronidase (200 U mL\(^{-1}\)) in PBS at 37 °C within 60 min, as well as the formation of metabolites M1 and Cry-55gly (3), while the degradation of 2 leads to the formation of metabolite M2.

Cytotoxic effect of Cry-55gly (3), conjugates 1 and 2 against M21 human melanoma cells in the absence (A) or presence (B) of β-glucuronidase after 2 h treatment and additional 70 h incubation. Curves were obtained by nonlinear regression (four-parameter dose–response); each point represents the mean ± standard deviation of quadruplicates, and the measurements were repeated twice.

Table 1. Cytotoxicity of Cry-55gly (3) and conjugates 1 and 2 against M21 human melanoma cells in the absence or presence of β-glucuronidase (βGlu).

| Structure | Compd | Compd + βGlu |
|-----------|-------|-------------|
| Cry-55gly (3) | 4.25 ± 0.43 | 4.22 ± 0.44 |
| c(RGDK)-(p)-GlcA-Cry-55gly (1) | 309.6 ± 19.2 | 351 ± 0.55 |
| c(RGDK)-(m)-GlcA-Cry-55gly (2) | 303.0 ± 26.9 | 308.4 ± 22.0 |

[a] Data are the mean ± SD of quadruplicates, and measurements were repeated twice.

The development of new cryptophycin-based conjugates bearing spacers with improved hydrophilicity shows a great promise and potential for the targeted therapy of solid tumors.\(^{[35]}\) In this work, a β-glucuronidase-responsive linker, a hydrophilic alternative of the widely used Val-Cit linker, has been used to connect the potent antimiotic agent cryptophycin-55 glucinate with the c(RGDK) integrin ligand. A multistep synthetic route was developed to optimally tailor the central sugar-linker moiety, thus, affording a synthetic methodology compatible with the functional groups of cryptophycin-55 glucinate, susceptible to hydrolysis in alkaline reaction conditions. The β-glucuronidase-induced cleavage enabled fast and efficient release of the active payload from conjugate 1 containing a self-immo- lative linker. The conjugates showed a 70-fold decreased activity relative to the free drug in α\(_5\)β\(_3\) integrin expressing M21 human melanoma cells, suggesting that hydrophilic sugar link-
ers can preclude passive cellular uptake. Furthermore, conjugate 1 produced similar cytotoxicity as the payload when β-glucuronidase was added to the cell culture medium, underlining that extracellular linker activation liberates the active drug.

These results indicate that RGD–cryptophycin conjugates bearing β-glucuronide linker have the potential to be therapeutically effective in vivo against integrin αvβ3 overexpressing tumors with high β-glucuronidase activity. In this approach, after binding and accumulation of the conjugate at the tumor site, the free drug, that is released by a β-glucuronidase-mediated activation, can penetrate neighboring cancer cells by passive diffusion causing bystander killing. In line with recent advances, this methodology could be further applied for the development of therapeutics containing hydrophobic anticancer drugs (e.g., MMAE, maytansinoids) to prevent their passive uptake by healthy cells.

**Experimental Section**

Procedures for biological assays, supplementary figures, synthetic procedures and characterization details, along with 1H NMR, 13C NMR, HPLC, MS, and HRMS data can be found in the Supporting Information.

**Acknowledgements**

This project received funding from the European Union’s Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No. 642004 (ETN MAGI-CBULLET). We gratefully acknowledge MIUR (Project PRIN20157WW5EH) and the University of Bologna for financial support. The authors acknowledge Marco Wißbrock and Anke Nieß for technical support, Dr. Georg Falck for flow cytometry analysis, and Dr. Jens Sproß (Department of Chemistry, Bielefeld University) for mass spectrometry measurements. The M21 human melanoma cells were kindly provided by David Cheresh and The Scripps Research Institute (La Jolla, CA, USA). Open access funding enabled and organized by Projekt DEAL.

**Conflict of interest**

The authors declare no conflict of interest.

**Keywords:** antitumor agents · beta-glucuronidase · drug delivery · integrin · small molecule drug conjugates

---

[1] R. V. J. Chari, M. L. Miller, W. C. Widdison, *Angew. Chem. Int. Ed.* 2014, 53, 3796–3827; *Angew. Chem.* 2014, 126, 3872–3904.
[2] M. Srinivasarao, P. S. Low, *Chem. Rev.* 2017, 117, 12133–12164.
[3] M. Srinivasarao, C. V. Galliford, P. S. Low, *Nat. Rev. Drug Discovery* 2015, 14, 203–219.
[4] D. Böhme, A. G. Beck-Sickinger, *J. Pept. Sci.* 2015, 21, 186–200.
[5] [https://www.fda.gov/drugs/new-drugs-fda-nders-new-molecular-entities-new-therapeutic-biological-products/new-drug-therapy-approvers-2019](https://www.fda.gov/drugs/new-drugs-fda-nders-new-molecular-entities-new-therapeutic-biological-products/new-drug-therapy-approvers-2019) (Accessed: 15 April, 2020); [https://www.fda.gov/drugs/drug-approvals-and-databases/fda-granted-accelerated-approval-belantamab-mafodotin-blm-multiple-myeloma](https://www.fda.gov/drugs/drug-approvals-and-databases/fda-granted-accelerated-approval-belantamab-mafodotin-blm-multiple-myeloma) (Accessed: 28 August, 2020).
[39] A. Borbely, F. Thoreau, E. Figueras, M. Kadi, J. L. Coll, D. Boturyn, N. Sewald, Chem. Eur. J. 2020, 26, 2602 – 2605.

[40] J. Liang, R. E. Moore, E. D. Moher, J. E. Munroe, R. S. Al-awar, D. A. Hay, D. L. Varie, T. Y. Zhang, J. A. Aikins, M. J. Martinelli, C. Shih, J. E. Ray, L. L. Gibson, V. Vasudevan, L. Polin, K. White, J. Kushner, C. Simpson, S. Pugh, T. H. Corbett, Invest. New Drugs 2005, 23, 213 – 224.

[41] 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.

[42] G. E. Mudd, A. Brown, L. Chen, K. Van Rietschoten, S. Watcham, D. P. Teufel, S. Pavan, R. Lani, P. Huxley, G. S. Bennett, J. Med. Chem. 2020, 63, 4107 – 4116.

[43] S. C. Jeffrey, J. B. Andreyka, S. X. Bernhardt, K. M. Kissler, T. Kline, J. S. Lenox, R. F. Moser, M. T. Nguyen, N. M. Okeley, I. J. Stone, X. Zhang, P. D. Senter, Bioconjugate Chem. 2006, 17, 831 – 840.

[44] P. J. Burke, J. Z. Hamilton, S. C. Jeffrey, J. H. Hunter, S. O. Doronina, N. M. Okeley, J. B. Miyamoto, M. E. Anderson, I. J. Stone, M. L. Ulrich, J. K. Simmons, E. E. McKinney, P. D. Senter, R. P. Lyon, Mol. Cancer Ther. 2017, 16, 116 – 123.

[45] X. Chen, B. Wu, P. G. Wang, Curr. Med. Chem. Anticancer Agents 2003, 3, 139 – 150.

[46] M. de Graaf, E. Boven, H. W. Scheeren, H. J. Haisma, H. M. Pinedo, Curr. Pharm. Des. 2002, 8, 1391 – 1403.

[47] I. Tranoy-Opalinski, T. Legigan, R. Barat, J. Clarhaut, M. Thomas, B. Renoux, S. Papot, Eur. J. Med. Chem. 2014, 74, 302 – 313.

[48] M. Nahrwold, C. Weiß, T. Bogner, F. Mertink, J. Conradi, B. Sammet, R. Palmsino, S. Royo Gracia, T. Preuße, N. Sewald, J. Med. Chem. 2013, 56, 1853 – 1864.

[49] P. López Rivas, C. Müller, C. Breunig, T. Hechler, A. Pahl, D. Arosio, L. Belvisi, L. Pignataro, A. Dal Corso, C. Gennari, Org. Biomol. Chem. 2019, 17, 4705 – 4710.

[50] M. Grinda, J. Clarhaut, I. Tranoy-Opalinski, B. Renoux, A. Monvoisin, L. Cronier, S. Papot, ChemMedChem 2011, 6, 2137 – 2141.

[51] S. Papot, I. Tranoy, T. Tillequin, J. C. Florent, J. P. Gesson, Curr. Med. Chem. Anticancer Agents 2002, 2, 155 – 185.

[52] D. C. Schröder, O. Kracker, T. Frohů, J. Gora, M. Jewginski, A. Nieß, I. Antes, R. Latalka, A. Marion, N. Sewald, Front. Chem. 2019, 7, 155.

[53] D. A. Cheresh, R. C. Spiro, J. Biol. Chem. 1987, 262, 17703 – 17711.

[54] B. Felding-Habermann, B. M. Mueller, C. A. Romerdahl, D. A. Cheresh, J. Clin. Invest. 1992, 89, 2018 – 2022.

[55] H. Bouchard, M.-P. Brun, P. Hubert, Novel Peptidic Linkers and Cryptophycin Conjugates, Their Preparation and Their Therapeutic Use (Sanofi SA), US Pat. No. US20180369401A1, 2018.

[56] A. H. Staudacher, M. P. Brown, Br. J. Cancer 2017, 117, 1736 – 1742.

[57] A. Raposo Moreira Dias, A. Pina, A. Dean, H.-G. Lerchen, M. Caruso, F. Gasparri, I. Fraietta, S. Troiani, D. Arosio, L. Belvisi, L. Pignataro, A. Dal Corso, C. Gennari, Chem. Eur. J. 2019, 25, 1696 – 1700.

Manuscript received: July 24, 2020
Revised manuscript received: September 2, 2020
Accepted manuscript online: September 21, 2020
Version of record online: December 8, 2020