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Structural perspectives on linkers enabling cell-specific antibody-mediated targeted protein degradation

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This work is dedicated to the *Arkivoc* Commemorative Issue honoring the retirement of Prof. Hans-Gunther (Hagga) Schmalz

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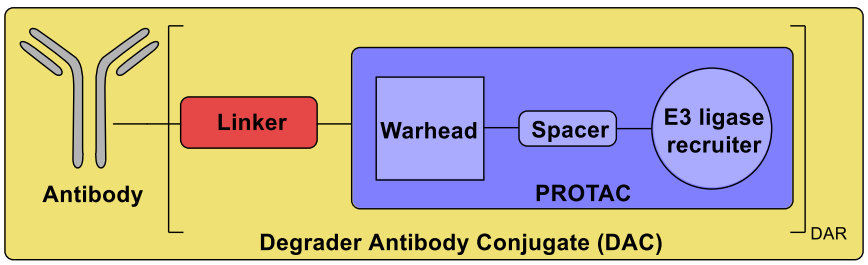
Abstract

Proteolysis-targeting chimeras (PROTACs) are considered promising alternatives to conventional small molecules. PROTACs are bifunctional molecules that are separated by a spacer that brings a target protein into the proximity of E3 ubiquitin ligases, thereby mediating target protein ubiquitination and degradation through the proteasome. PROTACs are powerful due to their catalytic function, which has a strong biological effect, and they work using sub-stoichiometric concentrations, even when the binding affinity to the target protein is low. However, non-specific delivery poses substantial risks for side effects. Therefore, degrader-antibody-conjugates (DACs) have been developed as a new class of antibody-drug-conjugates (ADCs) to specifically deliver the PROTAC to the cell type of interest. In the rational design of DAC conjugates, the linker moiety has the potential to regulate many pharmacological aspects, including the mode of release, the attachment of the functional payload, the drug-antibody ratio (DAR), and the chemical properties of hydrophilicity and stability. In this mini-review, we have explored which chemical linkers have been used in the DACs published to date. Finally, we provide an outlook on promising DAC linker designs aimed at further refining DAC versatility.



Keywords: PROTACs, degrader-antibody conjugates, ADCs, linker structures

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1. Introduction

Proteolysis-targeting chimeras (PROTACs) are hetero-bifunctional small molecules that mediate targeted protein degradation via the ubiquitin–proteasome system. PROTACs have shown promising results, offering significant advantages over conventional small-molecule therapies.[1] These degraders can target non-enzymatic proteins, that were previously considered undruggable.[1] Other advantages involve their catalytic function, enabling sub-stoichiometric concentrations by degrading target proteins iteratively, reducing side effects[2] and drug resistance caused by high-dose treatments[3,4]. Further, PROTACs exhibit sustained target engagement and prolonged efficacy following drug washout, outperforming small-molecule inhibitors in temporal activity.[5] Importantly, numerous PROTACs have entered clinical testing.[6] As a result, PROTACs are receiving growing interest due to their therapeutic potential and mechanism of action.

However, because these catalytic protein-degrading compounds have strong biological effects and there is a risk of off-target delivery, this could lead to severe adverse events.[6] Furthermore, PROTACs often exhibit low cellular uptake despite high lipophilicity because their relatively large size (typically 800-1000 Da) reduces aqueous solubility. The hydrophobic bifunctional components further drive excessive lipophilicity, causing membrane retention and poor partitioning into cells.[7] While some PROTACs exhibit good oral bioavailability and cellular uptake[8], these properties are not guaranteed for all PROTAC designs and targets, limiting their applicability. Therefore, advanced cell-specific delivery methods are necessary for desired and efficient cell-specific uptake to increase efficiency and limit side effects. Antibody-drug conjugates (ADCs) can increase solubility and stability, reduce metabolic clearance, and simultaneously deliver the drug to target cells.[9] Recently, a new class of ADCs has been developed: degrader-antibody-conjugates (DACs).[10–12] Such DACs comprise a therapeutic PROTAC conjugated to a transmembrane receptor-binding antibody (Figure 1), facilitating cell-type-specific uptake by lysosomal internalization.[13] The linker connecting the PROTAC to the antibody is a critical determinant of dosage, stability, and controlled release[14], making it a central design element in optimizing antibody-mediated PROTAC delivery.

In this mini-review, we introduce the structural properties of PROTACs and DACs, highlight recent strategies in DAC linker design, and discuss future perspectives for improving their efficacy through linker enhancements. As

both the PROTACs and DACs are bifunctional molecules, we distinguish the connecting moieties by nomenclature using a spacer for PROTACs and a linker for DACs. While we briefly discuss the importance of the spacer, this review focuses on the linker structures currently being utilized in DACs. Therefore, the following inclusion criteria were applied to focus the research scope and enable a detailed understanding of the structural linker properties of recent DAC modalities. Only PROTAC-based DACs with disclosed structures of chemically conjugated linkers were considered, and the included studies were published in peer-reviewed journals between the first reported DAC in 2019 and the time of writing in early 2026, as found on PubMed. Of note, while many pharmaceutical companies are developing DACs, we included only those that met our inclusion criteria.

1.1. Structural properties of PROTACs and DACs

PROTACs consist of a ubiquitin E3 ligase recruiter, covalently linked to a protein targeting warhead (Figure 1). The warhead can be derived from a well-studied high-affinity inhibitor, such as small molecule ligand JQ1 for the Bromodomain-containing protein (BRD) transcription/chromatin remodeling factors, which has led to the development of multiple PROTAC derivatives.[15,16] Noteworthy, the warhead can be a small-molecule binder that binds only its target protein and, by itself, does not inhibit enzymatic function.[17] Furthermore, even low-affinity small-molecule inhibitors can be potently used in PROTACs.[18] Most existing PROTACs recruit the E3 ubiquitin ligases Von Hippel-Linda (VHL) or Cereblon (CRBN) via the VHL032 or thalidomide ligands, respectively. These ligases mediate polyubiquitination of available lysines, which flag the protein for proteasomal degradation by recognition of its ubiquitin receptors. The warhead and E3 ligase recruiting ligand are conjugated through a spacer of which the length and composition can greatly influence PROTAC efficiency.[19] The spacer is the main adjustable component for PROTAC optimization.[14] Spacer rigidity is critical in PROTAC design, ranging from flexible polyethylene glycol (PEG) or alkyl chains to more rigid structures such as aromatic rings or piperazine-based spacers.[20,21] The structure choice can also be based on adjusting solubility or lipophilicity. PEG and piperazine rings can enhance solubility, while adding alkyl groups can increase lipophilicity.[21] The latter is important as large PROTAC molecules are notoriously cell-impermeable[22], however, excessive lipophilicity also hampers membrane permeability, for instance by membrane retention.[22] Antibody-mediated PROTAC delivery may overcome this limitation, making PROTAC design focus on degradation efficiency and selectivity rather than on improving solubility and lipophilicity.

Noteworthy, the DACs published to date have all been designed to target cancer cells. While this mini review therefore focuses on these anti-cancer DACs, the strategy is not limited to oncology. Similar to the broad applicability observed with emerging PROTACs, DACs may also be employed to target other cell types. The scope of this mini-review is to discuss the structural and biochemical properties of DAC linker molecules.

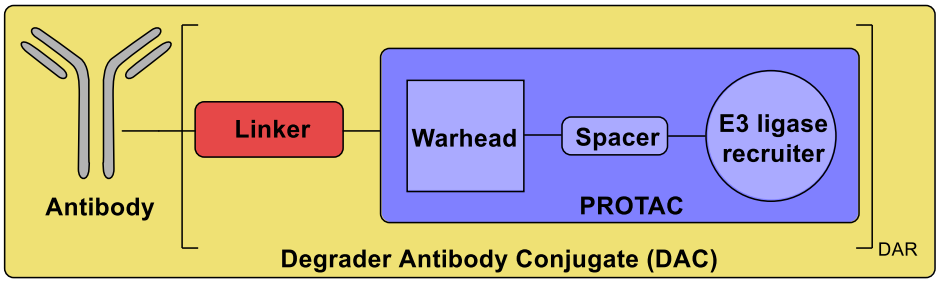


Figure 1. A bispecific PROTAC contains a warhead that binds the protein of interest, a ligand that recruits an E3 ubiquitin ligase, and a spacer (in blue) connecting these components. Degrader antibody conjugates (DACs) combine an antibody that recognizes a receptor on the target cell type with a PROTAC payload, joined together by a linker (in red). An important aspect of DAC linker structure involves balancing stability with intracellular drug release and influencing the payload measured by the drug-antibody-ratio (DAR).

DAC linkers, therefore, define the mode and timing of payload release, influencing both the stability in circulation and the sub-cellular site of release. Unstable linkers can cause premature drug release, and therefore non-specific delivery, while the lack of drug release may compromise efficiency due to the antibody's dependence on full lysosomal degradation. Previously designed ADCs utilized non-cleavable or cleavable linkers, which are protease-, pH-, or redox-sensitive.[14] In the following sections, we discuss the structural properties of recently used DAC linkers and propose future linker optimizations.

2. Recent Advances in DAC Linkers

The first DACs were published by Pillow[12] and Maneiro[11] in 2019 and 2020, building upon the highly potent BRD4-degrading PROTACs. Not long after, the groups of Dragovich[10,23,24], Wang[25], Machinaga[26], Tai[27], and Chan[28] also presented newly designed DACs, predominantly targeting BRD4 or the estrogen receptor (ER) (table 1). We will discuss the published works ordered by the release triggers of the linkers. As such, the majority of the reported DACs employ either redox-sensitive glutathione (GSH)-cleavable or protease-sensitive cathepsin B-cleavable peptide linkers. In addition, a few ester- or diphosphate-based, cleavable linker systems, and some covalent, non-cleavable linkers have been described (Table 1). We annotated the DACs by assigning numbers to the PROTACs and letters to the linkers. For instance, DAC **4b** and **4k** harbor the same PROTAC, however, they utilize a GSH-sensitive or a phosphatase-sensitive linker, respectively. As this review focuses on DAC linkers and for reduced complexity, we did not take the antibody target into account. Importantly, one DAC number is often conjugated to multiple antibodies, actually resulting in more DACs. Of note, all DAC linkers have been coupled to free or reduced cysteine residues on the antibody for a drug-antibody ratio (DAR) of two, four or, in most cases, six PROTAC equivalents per antibody.

Table 1. DACs described in this review. The number refers to the PROTAC, and the letter indicates the linker. The release type, release trigger, linker motif, PROTAC target, E3 ligase recruiting ligand, antibody targets, drug-antibody-ratio (DAR), and references are depicted per DAC. The DACs and linker motifs are linked with the corresponding figures by the DAC number and linker letters. Three different Cathepsin B linker structures (Figure 3A) are annotated by (i), (ii), or (iii).

DAC	Release Type	Release Trigger	Linker motif	Target	E3 Ligase	Antibody target	DAR	Ref.
1a	Redox	GSH	2-mercaptopropan-1-ol	BRD4	VHL	STEAP1, CLL1	6	[23]
2a	Redox	GSH	2-mercaptopropan-1-ol	BRD4	VHL	STEAP1, CLL1	6	[23]

3a	Redox	GSH	2-mercaptopropan-1-ol	BRD4	VHL	STEAP1, CLL1	2 or 6	[12,24]
1b	Redox	GSH	2-mercapto-2-methyl-propan-1-ol	BRD4	VHL	STEAP1, CLL1	6	[23]
2b	Redox	GSH	2-mercapto-2-methyl-propan-1-ol	BRD4	VHL	STEAP1, CLL1	6	[23]
3b	Redox	GSH	2-mercapto-2-methyl-propan-1-ol	BRD4	VHL	STEAP1, CLL1	2 or 6	[24]
4b	Redox	GSH	2-mercapto-2-methyl-propan-1-ol	BRD4	VHL	STEAP1, CLL1	6	[24]
1c	Redox	GSH	3-mercaptobutan-2-ol	BRD4	VHL	STEAP1, CLL1	6	[23]
2c	Redox	GSH	3-mercaptobutan-2-ol	BRD4	VHL	STEAP1, CLL1	6	[23]
3c	Redox	GSH	3-mercaptobutan-2-ol	BRD4	VHL	STEAP1, CLL1	2	[24]
4c	Redox	GSH	3-mercaptobutan-2-ol	BRD4	VHL	STEAP1, CLL1	6	[24]
5c	Redox	GSH	3-mercaptobutan-2-ol	ER	VHL	HER2, CD22	6	[10]
1d	Protease	Cathepsin B	(i)cBu Cit PAB(CO)	BRD4	VHL	STEAP1, CLL1	2 or 6	[23]
6d	Protease	Cathepsin B	(i)cBu Cit PAB(CO)	BRD4	VHL	STEAP1, CLL1, HER2	6	[23]
7d	Protease	Cathepsin B	(i)cBu Cit PAB(CO)	BRD4	VHL	STEAP1, CLL1, HER2	6	[23]
2d	Protease	Cathepsin B	(i)cBu Cit PAB(CO)	BRD4	VHL	STEAP1, CLL1	6	[23]
8d	Protease	Cathepsin B	(i)cBu Cit PAB(CO)	BRD4	VHL	STEAP1, HER2	6	[23]
9d	Protease	Cathepsin B	(i)cBu Cit PAB(CO)	BRD4	VHL	STEAP1, CLL1, HER2	2 or 6	[23]
3d	Protease	Cathepsin B	(i)cBu Cit PAB(CO)	BRD4	VHL	STEAP1, CLL1	6	[24]
4d	Protease	Cathepsin B	(i)cBu Cit PAB(CO)	BRD4	VHL	STEAP1, CLL1	6	[24]
10d	Protease	Cathepsin B	(i)cBu Cit PAB(CO)	BRD4	VHL	STEAP1, CLL1	2	[24]
11d	Protease	Cathepsin B	(i)cBu Cit PAB(CO)	BRD4	VHL	STEAP1, CLL1	6	[24]
12d	Protease	Cathepsin B	(i)cBu Cit PAB(CO)	BRD4	VHL	STEAP1, CLL1	6	[24]
13e	Protease	Cathepsin B	(i)cBu Cit PAB	BRD4	VHL	STEAP1, CLL1	6	[24]
14f	Protease	Cathepsin B	(i)Val Cit PAB(CO)	RIPK2	VHL	HER2, IL4	4	[28]
15g	Protease	Cathepsin B	(iii)Val Cit PAB	ER	XIAP	HER2, B7H4	6	[10]
15g’	Protease	Cathepsin B	(iii)Val Cit PAB	ER	XIAP	HER2	6	[10]
16h	Protease	Cathepsin B	(i)cBu Cit	BRD4	VHL	STEAP1, CLL1, HER2	6	[23]
8h	Protease	Cathepsin B	(i)cBu Cit	BRD4	VHL	STEAP1, HER2	6	[23]
17i	Protease	Cathepsin B	(ii)Phe Gly	BRD4	VHL	PSMA	2	[27]
18i	Protease	Cathepsin B	(ii)Phe Gly	BRD2-4	CRBN	CEACAM6	4	[26]
19i	Protease	Cathepsin B	(ii)Phe Gly	BRD2-4	CRBN	CEACAM6	4	[26]
20j	Hydrolase	Esterase	Ester	BRD4	VHL	HER2	4	[11]

20k	Hydrolase	Esterase	Ester	BRD4	VHL	ROR1	6	[25]
4l	Protease	Phosphatase	Diphosphate	BRD4	VHL	STEAP1, CLL1	6	[24]
5l	Protease	Phosphatase	Diphosphate	BRD4	VHL	HER2, CD22	6	[10]
16m	Non-cleavable	None	Covalent	BRD4	VHL	STEAP1, CLL1, HER2	6	[23]
6m	Non-cleavable	None	Covalent	BRD4	VHL	STEAP1, CLL1, HER2	6	[23]
7m	Non-cleavable	None	Covalent	BRD4	VHL	STEAP1, CLL1, HER2	6	[23]

2.1. GSH cleavable linkers

Four independent studies reported DACs comprising GSH-cleavable redox-sensitive linkers, exploiting the significantly higher GSH concentration in the cytosol relative to plasma. This cytosolic GSH enables rapid intracellular release of the payload while maintaining an adequate plasma half-life. To fine-tune the balance between stability and release efficiency, methylation of the ethane linkage can modulate the steric environment of the disulfide bond, as demonstrated by DAC linkers **b** and **c** (Figure 2A).[12,23,24] Notably, this linker system can be attached to the VHL ligand of the final PROTAC under mild conditions. The PROTAC linker conjugate can then be linked to the antibody's cysteine side chain via a disulfide bond. After internalization, the disulfide can be reduced by GSH, releasing the labile PROTAC-linker conjugate. The residual PROTAC is ultimately cleaved by intramolecular cyclization (Figure 2A). Five different PROTACs have been coupled to DACs using GSH-sensitive linkers (Figure 2B). Interestingly, multiple studies have shown that tumor cells and the tumor microenvironment produce more GSH, thereby increasing the efficiency of GSH-dependent release.[29,30]

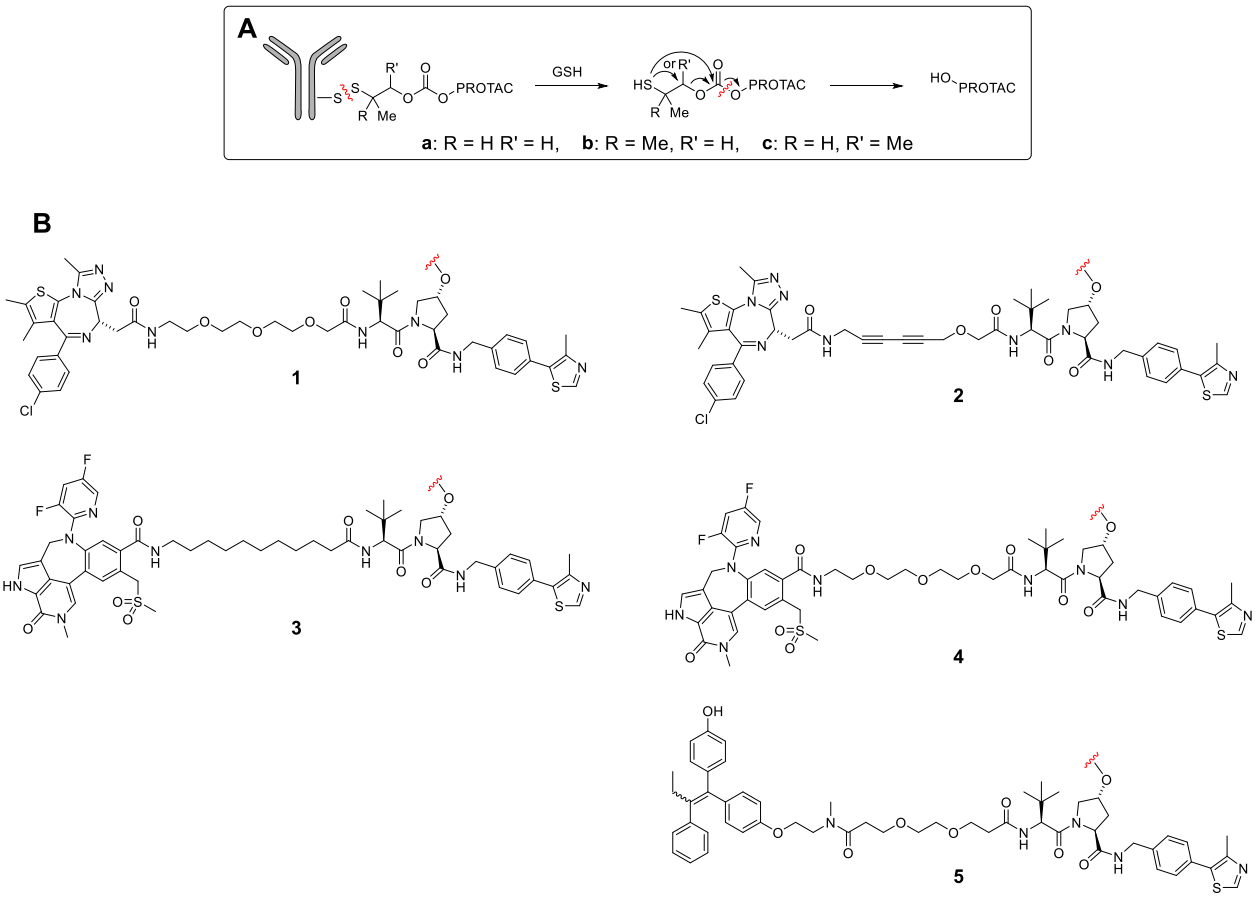
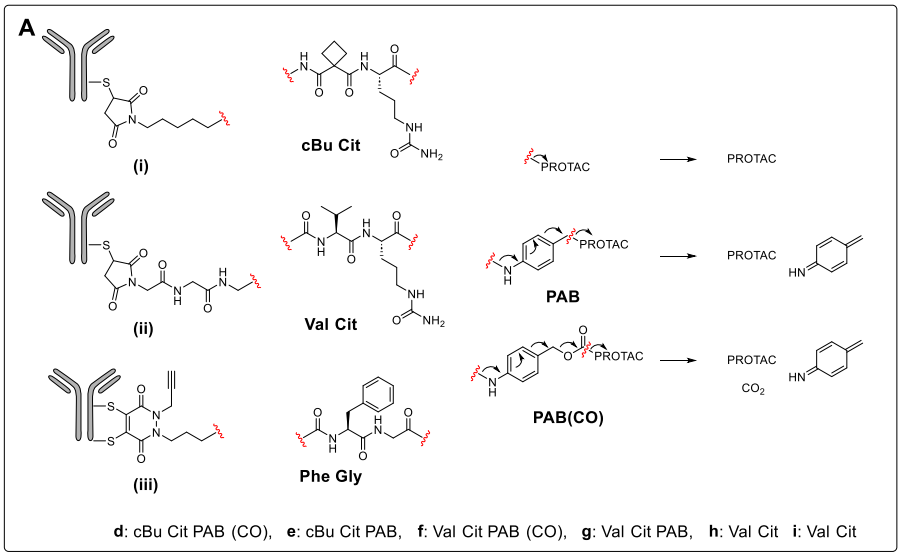


Figure 2. The glutathione (GSH)-cleavable linkers and two possible release mechanisms (A) connecting an antibody with PROTACs exemplified in (B). Cytosolic abundant GSH cleaves the N-terminal disulfide bond first, whereafter the C-terminal ether linkage is cleaved through hydrolysis, releasing the PROTAC (A). Various PROTACs (B) have been connected to antibodies using GSH-linkers, all of which were coupled to the hydroxyl site of the hydroxyproline on the VHL E3 ligase ligand, shown in (B) in red.

2.2. Cathepsin B cleavable linkers

Cathepsin B is a lysosomal cysteine protease that recognizes specific dipeptide linker motifs and cleaves the C-terminal amide bond. When incorporated into a DAC, this either directly releases the PROTAC or, through a secondary reaction, utilizes a labile para-aminobenzyl (PAB)-PROTAC conjugate. Frequently, a carbonate or carbamate group is incorporated between the benzylic position and the PROTAC, enabling the concomitant release of carbon dioxide, which further drives the release. The degrader moiety is commonly attached through an alcohol or amine functionality (Figure 3A). There are currently 20 published DAC variants (excluding variants with different antibody targets) that harbor Cathepsin-sensitive motifs. Like in classical ADCs[31], the widely used protease-cleavable motifs in DACs include the Val-Cit and cBu-Cit dipeptides (Figure 3). Notably, DAC **15g** and **15g'** are the only DACs harboring a PROTAC that recruits the E3 ubiquitin ligase XIAP, while all other PROTACs bind VHL or CRBN. These Cathepsin B-linkers were conjugated to the PROTAC on the E3 ligase ligand, the target protein ligand, or, for compounds **9**, **12**, and **13**, on the spacer moiety (Figure 3B).


A
(i)
cBu Cit
(ii)
Val Cit
(iii)
Phe Gly
PROTAC
PROTAC
PAB
PAB(CO)
CO2
d: cBu Cit PAB (CO), e: cBu Cit PAB, f: Val Cit PAB (CO), g: Val Cit PAB, h: Val Cit i: Val Cit

B

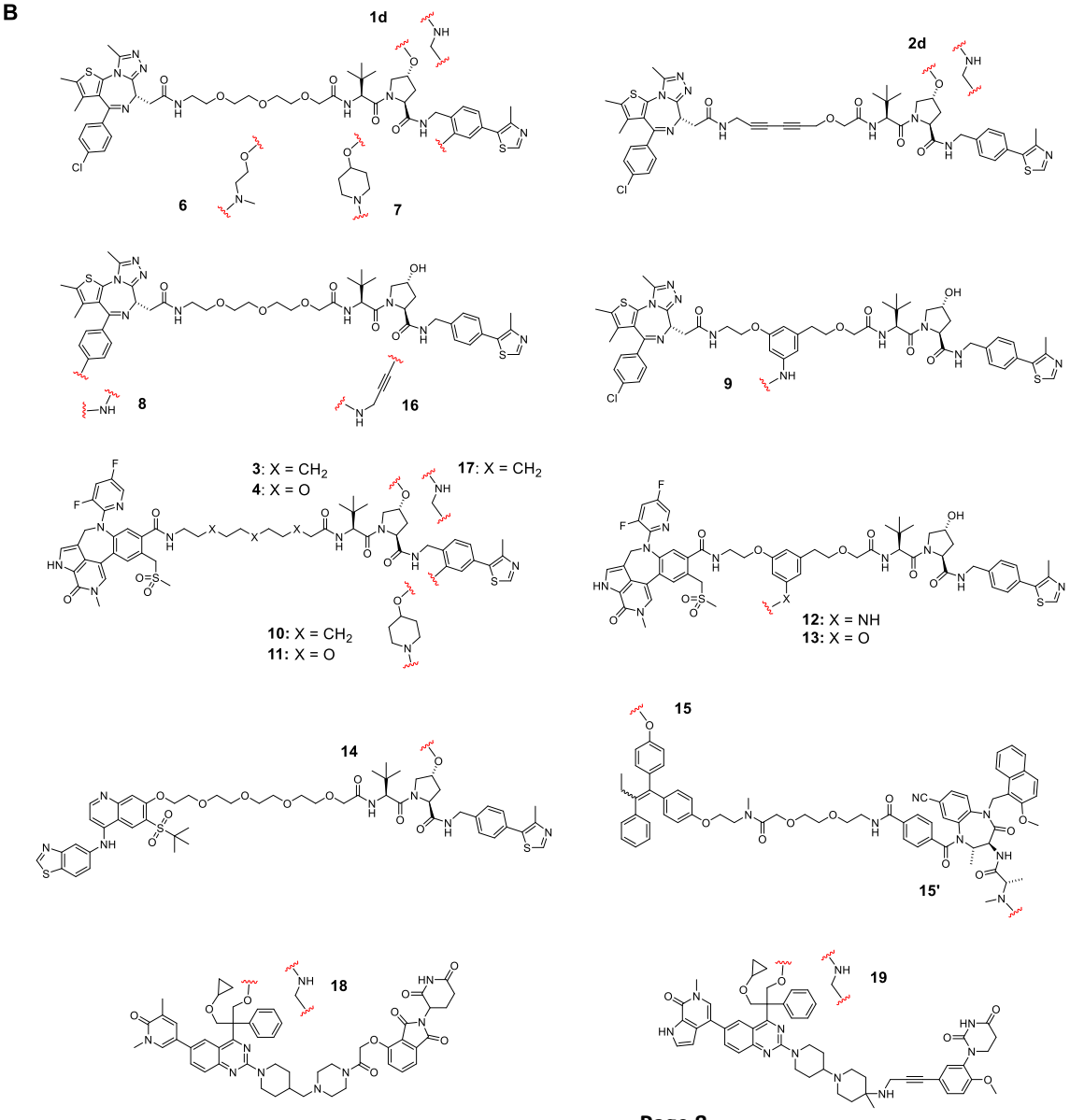

1d
6
7
2d
8
16
9
3: X = CH2
4: X = O
17: X = CH2
10: X = CH2
11: X = O
12: X = NH
13: X = O
14
15
15'
18
19

Figure 3. Cathepsin B cleavable DAC-linker moieties (A) and examples of published PROTACs that utilized these linkers (B). The release mechanism (A) is based on the cysteine protease Cathepsin B, which cleaves the C-terminal amide bond of the Val-Cit, cBu-Cit, or Phe-Gly dipeptides. Additionally, the linker can involve the para-aminobenzyl (PAB) self-immolative spacer with or without a carbonate or carbamate group at the benzylic position. All the published PROTACs connected to Cathepsin B-linkers are exemplified in B, with the conjugation site indicated in red.

2.3. Esterase cleavable linkers

In certain cases, degrader antibody conjugates utilize a labile ester linkage, which is connected to the hydroxyproline moiety of the degrader (Figure 4B). The esterases cleave after the C-terminal carbonyl group of the linker, releasing the PROTAC (Figure 4A). While this strategy can facilitate controlled release and PROTAC activation, the inherent susceptibility of ester bonds to hydrolysis poses a challenge, often exhibiting limited stability in plasma.[32] This instability can potentially reduce the *in vivo* half-life of the conjugate and affects its overall therapeutic efficacy. Furthermore, its release mechanism depends on proteolytic activity via non-specific esterases in the cytosol and lysosomes.[32] The actual lysosomal cleavage is relatively low, which may hamper the applicability of ester-based linkers in DAC development.[32] Consequently, there are only two published DAC variants[11,25] that uses ester linkers (Figure 4B).

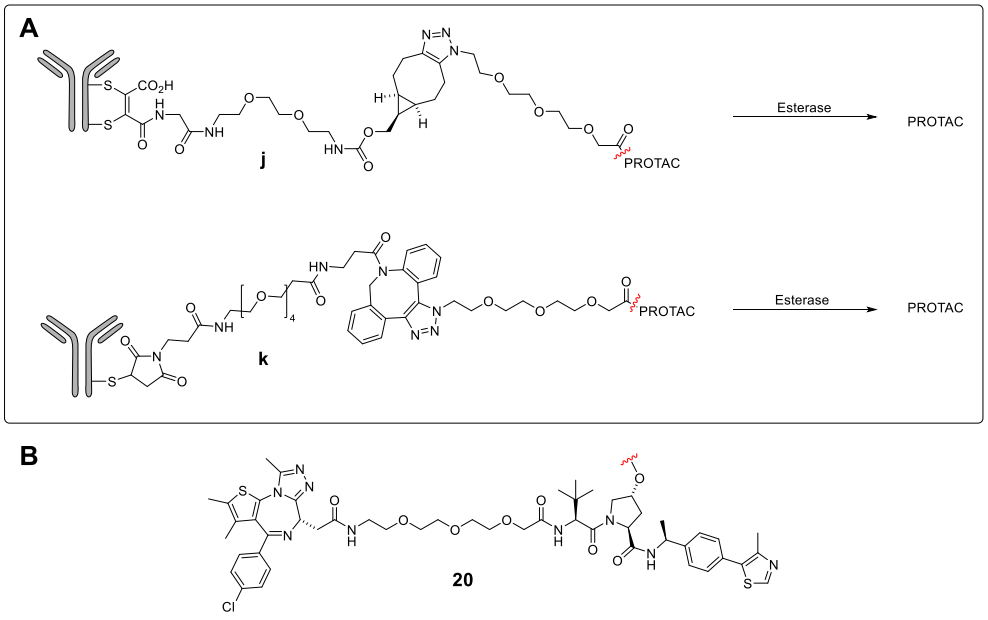


Figure 4. Ester linkers can be used for esterase-mediated release of the degrader (A). Two DACs, **20j** and **20k**, which both use the same PROTAC (B), have been described with ester-based linkers (A). The degraders are conjugated to the linkers on their hydroxyproline moiety.

2.4. Diphosphate linkers

Phosphatases recognize diphosphate linker motifs. In a sequential reaction, diphosphatases or pyrophosphatases recognize the diphosphate group to cleave the labile unit and release the monophosphate-

PROTAC conjugate. Subsequently, the monophosphate recognizing phosphatases release the active PROTAC (Figure 5A). In the case of DAC **4l** and **5l**, the phosphate linker moiety is connected to the hydroxyproline unit of the VHL binder. Importantly, diphosphate linkers are stable in the circulation and predominantly released within the target cell. The two diphosphate linker DAC variants are coupled to two different antibodies each and show a DAR of six (table 1).[10,24] Interestingly, as specific phosphatases are overexpressed within the tumor microenvironment,[33] the use of diphosphatase linkers could improve the selective release of the anti-cancer degrader payload.

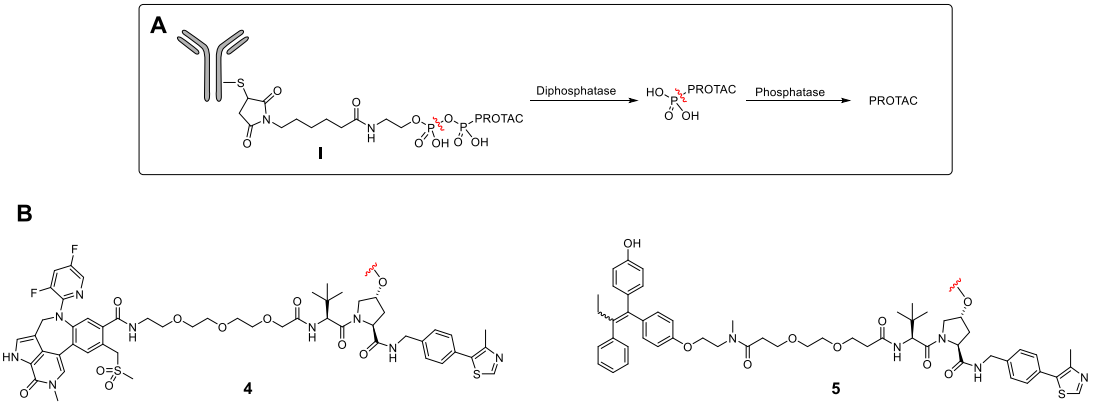


Figure 5. Diphosphate linkers are cleaved by diphosphatases/pyrophosphatases and phosphatases (A). Two DAC variants made use of such linkers, both coupled to the hydroxyproline of the VHL ligand on the PROTAC (B).

2.5. Covalent linkers

Finally, some strategies employ non-cleavable linkers, in which the degrader is covalently attached to an antibody. In these designs, the cysteine-linker-degrader construct is released upon lysosomal degradation of the antibody (Figure 6). Utilizing non-cleavable linkers results in highly stable conjugates in circulation, reducing side-effects caused by non-specific cell targeting. However, the release efficacy of the degrader is lower compared to ADCs using cleavable linkers,[34] due to lysosomal dependency and therapeutic loss through early extracellular trafficking. Notably, linker and PROTAC are not cleaved, therefore limiting the possible attachment site to the non-binding periphery of the PROTAC.

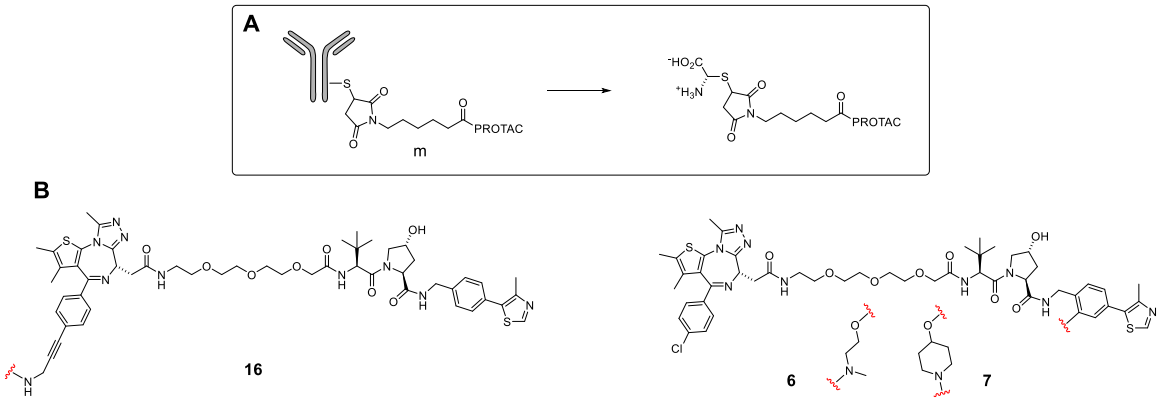


Figure 6. Non-cleavable linkers can be used to develop stable DACs. Upon lysosomal degradation of the antibody the PROTAC is released (A). Three DACs have been published using non-cleavable amino-acid-based linker moieties (A and B).

Considering the structural properties of the DAC linkers, we now briefly compare some functional characteristics of the different linker types. First, the acquired stability of protease-, phosphatase-, and esterase-sensitive linkers is moderate, whereas covalent linkers exhibit very high plasma stability. In contrast, GSH-redox–sensitive linkers are prone to premature release in plasma without additional modifications[35], and even with chemical adjustments these issues persist.[36] Second, the intracellular release mechanisms vary substantially among linker types: covalent linkers represent the most challenging release modality because they depend entirely on complete degradation, whereas redox-sensitive linkers may facilitate efficient cytosolic release owing to high intracellular GSH concentrations. The protease-, esterase-, and phosphatase-sensitive linkers instead provide more controlled lysosomal release. Finally, regarding hydrophilicity, phosphatase linkers stand out due to their pronounced polarity, resulting in high solubility and improved antibody conjugation and manufacturing efficiency, ultimately leading to higher DAR values.[37] Noteworthy, it is difficult to generalize across these different linker types, as their behavior strongly depends on the conjugated molecule. In addition, emerging linker modalities are being developed that further enhance these functional properties, as discussed in the following chapter.

3. A Future Outlook on DAC Linkers

As the field of DACs is expanding, we have explored which DAC linker motifs are currently in use. Given that the linker is a critical determinant of the release profile and conjugate stability, and is readily tunable, we propose using alternative DAC linker architectures to enable more versatile applications. This section exemplifies some future approaches for DAC development.

To start with, using additional Cathepsin B-recruiting, dipeptidic motifs such as Val-Ala may allow fine tuning of the release rate and selectivity driven by Cathepsin B-mediated cleavage, offering a means to optimize intracellular activation profiles for specific degrader-antibody conjugates.[38] Dependent on the amino acids used, Cathepsin B-mediated cleavage rate is highly adjustable. As such, Dubowchik and colleagues measured the in vitro Cathepsin B-mediated release rate for different dipeptide substrates, ranging from 8 minutes using Phe-Lys to 2040 minutes with Trp-Cit.[38] While the conventional approach has used dipeptide motifs for peptide-based linkers, a recent study found that next-generation peptide linkers may consist of multitiple amino acids.[31]

As an alternative to peptide-based Cathepsin B-cleavable linkers, future DACs may harbor β-glucuronide linkers[39], which are currently not utilized as DAC linkers (Table 1). The β-glucuronide linker is cleaved by β-glucuronidase in endosomes and lysosomes. These linkers are considered more hydrophilic than the amino-acid-based Cathepsin B-linkers, thereby allowing a greater hydrophobic payload as conjugates.[39] To increase payload attachment, one might also use β-glucuronide to achieve a higher DAR while maintaining solubility. However, to achieve the highest DAR, we envision using multifunctional branched linkers to increase payload attachment.[40]

In terms of antibody functionalization, all DACs make use of cysteines to attach the linker. As this conjugation approach is limited by cysteine availability, one might consider targeting lysines to achieve higher

DARs. However, the disadvantage is non-specific antibody binding, causing higher functional variance, hampering pharmacokinetic responses, and further drug development. Another approach is the use of engineered site-specific antibodies harboring non-natural amino acids with functional groups for linker coupling.[41] For example, one could incorporate a keto-amino acid such as p-acetyl-l-phenylalanine.[42] This would result in homogeneous DACs with possibly increased payload attachment.

Interestingly, pH-sensitive linkers have not been utilized in published DACs, despite their advantageous properties for tumor targeting. Their release mechanism is independent of a single protein, reducing the likelihood of mutation-driven drug resistance. As such, hydrazone linkers may be useful as they are stable in neutral pH but are cleaved in acidic environments (pH=4.5-5.5).[9] Hence, the payload release can be controlled in the lysosomes. Noteworthy, there are multiple pharmaceutical companies which have developed DACs incorporating hydrazone linkers, however, the exact linker structures and pharmacokinetic outcomes are non-disclosed. Therefore, we did not include any non-peer reviewed modalities in this mini-review.

While current efforts in the DAC field primarily focus on PROTACs, these degraders mainly act on cytosolic and nuclear proteins. An emerging alternative capable of targeting membrane and extracellular proteins are lysosome-targeting chimeras (LYTACs).[43] LYTACs are bifunctional molecules that bind a target protein in the extracellular compartment and a membrane receptor, mediating lysosomal trafficking. Although LYTACs can be designed for cell specificity by exploiting cell-type–specific receptors, more generalizable versions may be developed through antibody conjugation strategies.[44] Consequently, while outside the scope of our investigation, the structural principles discussed in this mini-review could also be applicable to other targeting modalities.

In summary, for additional fine-tuning of the payload release, we propose using alternative dipeptidic linker moieties for Cathepsin B-mediated release, β-glucuronide linkers for increased hydrophilicity, branched linker moieties for increased DARs, engineering specific antibodies for enhanced linker coupling, and pH-sensitive linkers. Overall, this mini-review examined the linker architectures and characteristics of current DACs and outlined potential future linker designs that could enhance their versatility.

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Felix Blüm received his B.Sc. from the University of Cologne in 2019, working on carbon-monoxide-releasing molecules. He continued his M.Sc. studies in Cologne, including a research internship at the Leibniz University Hannover, and completed his Master's degree with a focus on asymmetric catalysis. Following his studies, he worked at the life-science start-up Prosion. In 2023, he joined the group of Prof. H.-G. Schmalz for his doctoral studies in the field of targeted protein degradation.

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