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Research paper

Design and optimization of oestrogen receptor PROTACs based on 4-hydroxytamoxifen

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A B S T R A C T

In the last four decades, treatment of oestrogen receptor positive (ER+) breast cancer (BCa), has focused on targeting the estrogenic receptor signaling pathway. This signaling function is pivotal to sustain cell proliferation. Tamoxifen, a competitive inhibitor of oestrogen, has played a major role in therapeutics. However, primary and acquired resistance to hormone blockade occurs in a large subset of these cancers, and new approaches are urgently needed. Aromatase inhibitors and receptor degraders were approved and alternatively used. Yet, resistance appears in the metastatic setting. Here we report the design and synthesis of a series of proteolysis targeting chimeras (PROTACs) that induce the degradation of estrogen receptor alpha in breast cancer MCF-7 (ER+) cells at nanomolar concentration. Using a warhead based on 4-hydroxytamoxifen, bifunctional degraders recruiting either cereblon or the Von Hippel Lindau E3 ligases were synthesized. Our efforts resulted in the discovery of **TVHL-1**, a potent ERα degrader (DC_{50}: 4.5 nM) that we envisage as a useful tool for biological study and a platform for potential therapeutics.

1. Introduction

The oestrogen receptor (ER) is a transcription factor that plays a crucial role in many fundamental biological processes as well as in several diseases such as osteoporosis, neurodegeneration and cancer [1].

ER is an important target in the treatment and prevention of breast cancer since approximately 70% of human breast cancers are hormone-dependent and ER-positive (ER+) [2]. Hence, ER alpha (ERα) has proven to be the main target for endocrine therapy in breast cancer treatment, with such hormone therapies effective and reducing the progression of the disease.

Selective estrogen receptor modulators (SERMs) mainly encompass drugs that act as estrogen antagonists, binding to ER and modulating or inhibiting its activity. Tamoxifen is a SERM developed in 1966 that was approved for metastatic breast cancer treatment in 1973 [3]. Since then, Tamoxifen has remained the primary chemotherapeutic strategy for treating ER + breast cancer. Other relevant SERMs include Toremifene, with a structure related to Tamoxifen, and Raloxifene, a nonsteroidal drug used to reduce the risk of breast cancer with lower toxicity than Tamoxifen (Fig. 1) [4]. The most active metabolite of Tamoxifen is 4-Hydroxytamoxifen (**4OHT**) [5], with a similar affinity for ERs as the natural substrate but a prolonged half-life and persistence in tissues [6]. **4OHT** is metabolized in the liver by enzymes including CYP2D6 and CYP3A4, along with other active metabolites such as Endoxifen (Fig. 1) [7]. Although Tamoxifen is the most widely used treatment for ER + breast cancer, there are many side effects associated with this chemotherapy, as well as resistance mechanisms that lead to the treatment becoming ineffective. In these cases, ERα activates transcription even with oestrogen deprivation and/or in the presence of SERMs. Moreover, it increases the risk of developing endometrial cancer and it is classified as a carcinogen by the FDA [8].

Selective estrogen receptor degraders (SERDs) were developed as an alternative to address these issues by improving the efficacy of ER downregulation. The proposed mechanism of action for SERDs is the

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induction of ER protein misfolding, leading to a proteasome-dependent ER degradation [9]. Fulvestrant (Fig. 1) is, to date, the most important drug with this mechanism of action and remains the only FDA approved SERD that is currently administered to breast cancer patients, though it has to be administered weekly *via* intramuscular injections due to its poor bioavailability [10]. The clinical success of Fulvestrant suggests that degradation of ER is beneficial to patients with ER + breast cancer and has motivated intensive research towards SERDs with improved pharmacokinetic properties. The development of orally bioavailable SERDs was reported in clinical development [11] and novel potent SERDs have been recently described [12].

Proteolysis targeting chimeras (PROTACs) are heterobifunctional compounds that induce the degradation of a protein by promoting a protein-protein interaction (PPI) between the target and a native E3 ligase. This interaction induces the ubiquitination and subsequent degradation of the protein by the proteasome (Fig. 2). The potential of this methodology, first described in 2001 [13], was rapidly valued by the scientific community, evolving into the development of many bifunctional compounds based on this pharmacological approach [14–16].

The first ERα PROTAC, developed by Crews, Deshaies and co-workers [17] was based on the natural hormone, namely 17β-estradiol (E2) and a phosphopeptide to recruit the E3 ligase. This proof of concept had many technical issues that were overcome over the years. The discovery of the Von Hippel–Lindau tumour suppressor (pVHL) and its activity as a degrader of hypoxia-inducible factors (HIFs) allowed improvement of the E3 ligase binders [18]. Crews' group designed a pentapeptide derived from HIF-1α protein (PROTAC B, Fig. 3), that could be used to recruit the E3 ligase complex [19]. Independently, Kim's group also designed ERα PROTACs based on E2 and short peptides (8aa and 5aa) derived from HIF-1α protein [20]. These peptides, linked to E2, afforded PROTACs that were used as probes for angiogenesis [21]. Later on, the linker and the attachment point were optimized [22,23], but the relatively poor ERα degradation efficiency led them to construct a "two headed-PROTAC" that was more efficient (IC_{50} = 0.12 μM) [24]. Other types of protein degraders were developed, as in 2011, hybrid molecules called Specific and Nongenetic IAPs-dependent Protein Erasers (SNIPERS) were synthesized [25].

The next step was the development of bifunctional compounds using warheads different to the natural hormone. A SNIPER linking **4OHT** and methylbestatin (MeBS) -which binds the ubiquitin ligase cIAP1 (cellular inhibitor of apoptosis proteins 1)- was able to degrade ERα and promoted necrotic cell death in breast cancer cells [26]. The efficiency of the degrader was largely improved using other IAP antagonists. Naito's groups developed SNIPER(ER)-87 (Fig. 3) and SNIPER(ER)-110 [27] linking **4OHT** with derivatives of the IAP inhibitor LCL161 developed by Novartis [28].

The biological relevance of ER degradation fostered the interest of many pharmaceutical companies that patented several families of ER PROTACs. GlaxoSmithKline (GSK) reported compounds based on a Raloxifene derivative linked to a IAP ligand (Fig. 3) that degraded DC_{50} at concentrations lower than 1 μM [29]. AstraZeneca patented a family of PROTACs based on their SERD for ER+/HER2– breast cancer (AZD9496) [30]. The most advanced clinical candidates were developed by Arvinas [31]. They developed compounds based on indole, tetrahydronaphthalene or tetrahydroisoquinoline derivatives as estrogen warheads linked to thalidomide or Von-Hippel-Lindau (VHL) as E3 ligase binders [32], and one of these compounds (ARV-471, Fig. 3) is currently in a Phase 2 clinical study. More recently, Accutar Biotech patented ER-Protacs based of **4OHT** [33]. Nevertheless, these results did not stop the improvement of new ERα degraders. Recent advances include PROTACs using Raloxifene as ERα binder and VHL as E3 recruiter [34], as well as the conjugation of ERα PROTACs to antibodies to provide alternative delivery methods [35]. A detailed review on ER degraders has been published recently [36].

The importance of this strategy within oncology is indisputable; thus, further exploration of PROTAC-induced degradation of ERα, as well as the understanding of its mechanism of action in BCa treatment, will be essential for transferring this technology to the clinical setting. There is a need for potent, small molecule-type ERα PROTACs that are easily accessible and available to the scientific community, so by investigating the linking of E3 ligase binders to best-in-class small molecule inhibitors such as **4OHT**, we contemplated the preparation of powerful compounds to be used for research in BCa and may act as a platform for clinical advance.

In this work, we describe the efficient synthesis of a series of novel ERα PROTACs based on **4OHT** as warhead. We have studied different linker compositions, as well as distinct pomalidomide derivatives and VHL ligands as E3 ligase recruiters. Our efforts resulted in the discovery of **TVHL-1**, a potent ERα degrader (DC_{50}: 4.5 nM) that we foresee as a useful tool for biology research.

2. Results and discussion

Our retrosynthetic analysis for the synthesis of the PROTACs envisaged a convergent synthesis based on a copper catalyzed azide-alkyne

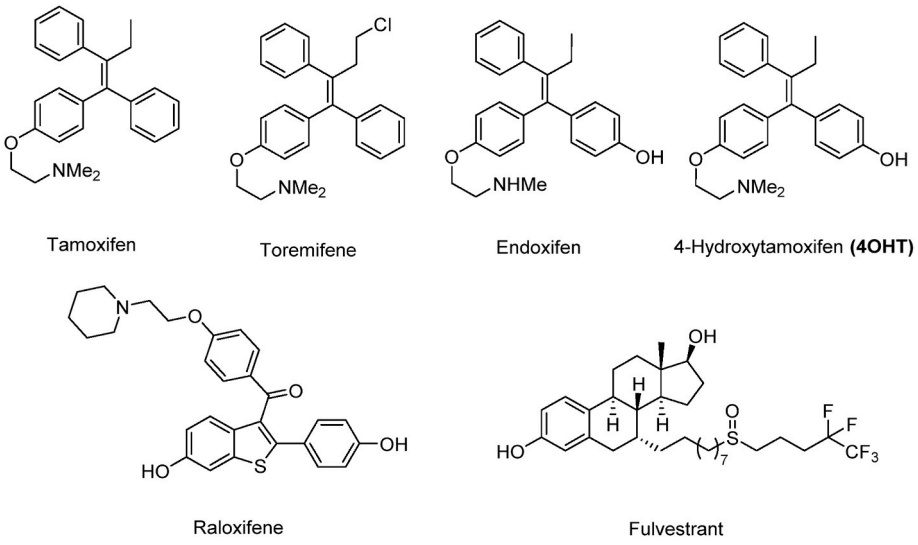


Fig. 1. Structures of representative selective oestrogen receptor modulators (SERMs) and degraders (SERDs) and active metabolites.

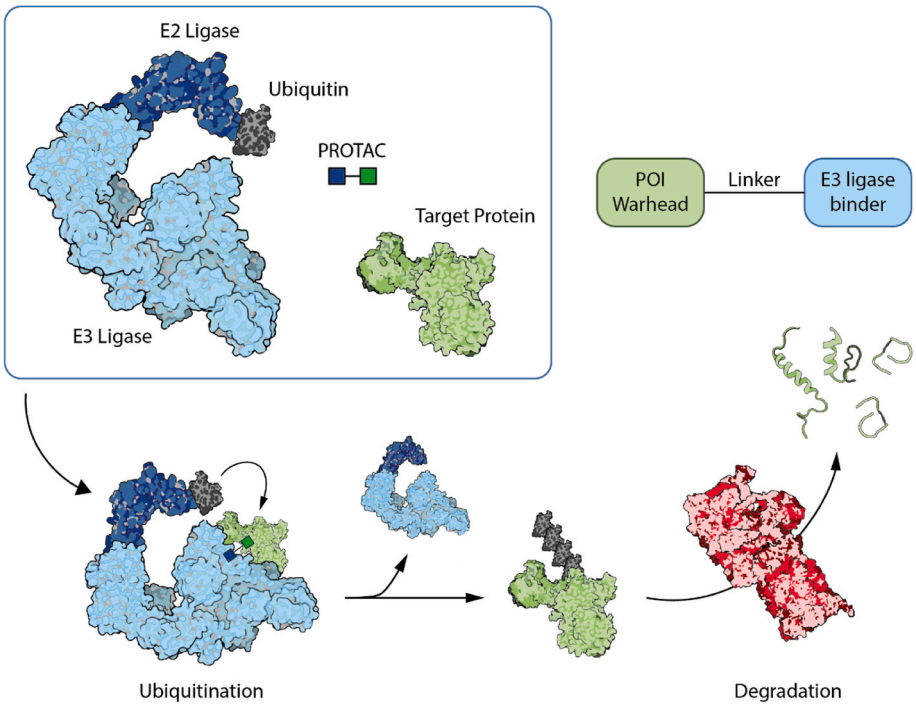


Fig. 2. Schematic representation of the PROTAC mechanism of action and general structure of a PROTAC.

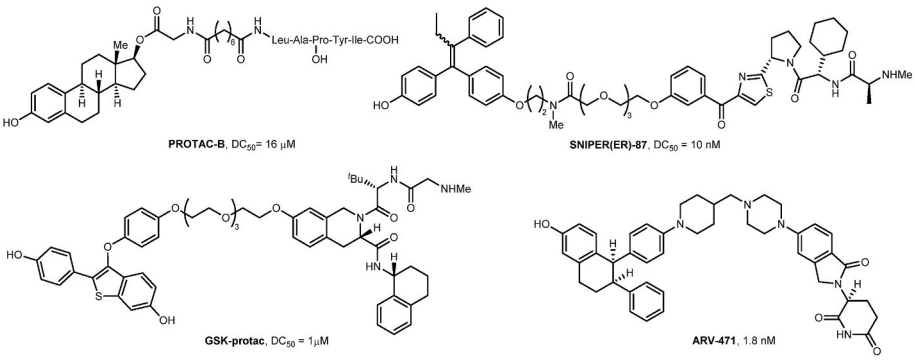


Fig. 3. Structures of representative previously described ER protacs.

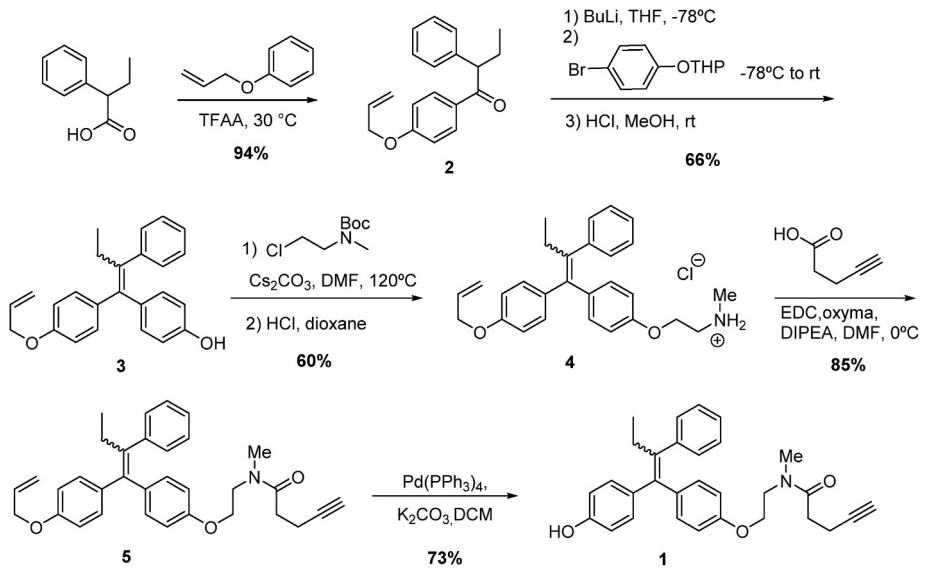
cycloaddition (CuAAC) to couple two advanced intermediates. Inspired on our previous work on p38 degraders [37] we planned to couple *via* click reaction the E3 ligase ligand bearing a terminal azide with an appropriate **4OHT** derivative bearing a terminal alkyne group [38] (Fig. 5).

The interaction of **4OHT** with the active site of ERα has been investigated in-depth over the past 50 years and there are exhaustive SAR studies carried out on each part of the compound structure [39]. Based on these studies, we identified the dimethylamino side chain as the best attachment site for a linker. We designed **4OHT** derivative **1** as the key acetylenic partner and a family of thalidomide analogs derivatized with a terminal azide to recruit the cereblon (CRBN) E3 ligase. Later on, the introduction of the VHL ligand into our design followed a similar approach; VHL-ligand bearing fragments were prepared with a terminal azide that could be used in a CuAAC cycloaddition.

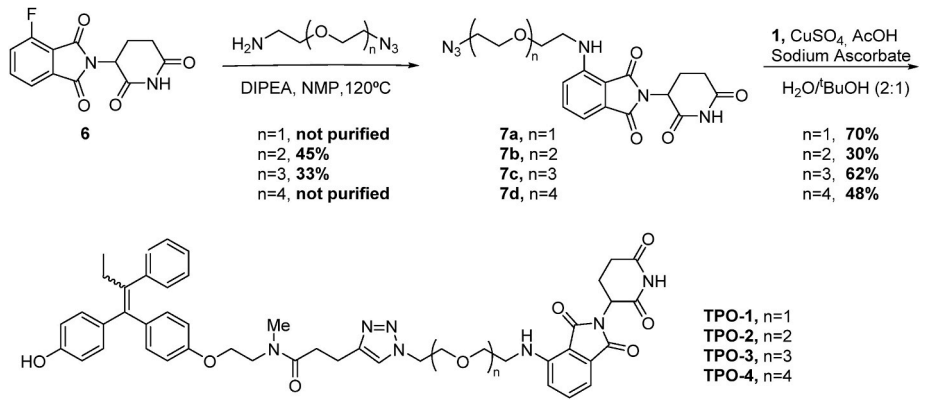
There are several routes in the literature to synthesize **4OHT**. We selected an allyl group as a convenient phenol protecting group and designed a route starting with a Friedel-Crafts acylation [40] between 2-phenylbutanoic acid and allyloxy benzene to afford ketone **2** (Scheme 1). Addition of 4-tetrahydropyranyloxy phenyl lithium to this ketone, followed by deprotection of the THP group gave the mono-protected intermediate **3** in good yield. The Williamson ether formation with N-protected *N*-methyl-2-chloroethanamine allowed the introduction of the tamoxifen side-chain. After removal of the Boc group, the amide formation with 4-pentynoic acid gave **4OHT** fragment **5**. Palladium catalyzed deprotection of the allyl ether in basic conditions afforded the acetylenic **4OHT** fragment **1** as a mixture of isomers.

To perform the CuAAC, it was necessary to synthesize the corresponding azido partners. Condensation of 4-fluorophthalic anhydride with 3-aminopiperidine-2,6-dione afforded the fluorothalidomide **6** in excellent yield. The SNAr reaction of **6** with four commercially available amino-azides with different lengths of PEG linkers afforded the azido intermediates **7a-d**. Finally, the click reaction of these azides with the acetylenic **4OHT** derivative **1** afforded the potential PROTACs **TPO-1-4** in moderate to high yields (Scheme 2).

This facile diversification allowed the examination of a range of linker lengths for our starting candidate design. Western blotting data (Fig. 6A) showed that **TPO-1** and **TPO-3** compounds were effective inducing ERα degradation at a range between 1 and 5 μM in MCF-7 ER + BCa cells. **TPO-3** compound was selected because it showed substantial degradation at 5 μM, and a consistent concentration/activity relation confirmed by triplicates. We studied the kinetics of the degradation



Scheme 1. Synthesis of the **4-OHT** derivative with a terminal alkyne (**1**).



Scheme 2. Synthesis of PROTACs **TPO-1-4** derived from 4-hydroxytamoxifen and pomalidomide.

process using **TPO-3** and no substantial degradation was observed during the first 12 h (Fig. 6B), but the maximum degradation was achieved after 24 h of treatment.

In order to determine the DC_{50} values (concentration to achieve 50% of protein degradation), MCF-7 cells were treated with **TPO-3** at different concentrations during 24 h. Quantification of ERα levels normalized to GAPDH levels showed a DC_{50} value of 11.94 nM (Fig. 6C).

To demonstrate that the observed degradation effect is CRBN-dependent, a non-effective **TPO-3-Me** compound was synthesized, (see Supplementary material) possessing a modified version of the pomalidomide fragment methylated at the glutarimide nitrogen, which blocks the binding activity towards CRBN [41]. **TPO-3-Me** did not degrade ERα at a concentration of 1 μM (Fig. 4D), but some degradation was observed at higher concentrations.

To demonstrate that ERα degradation is through hijacking the ubiquitin-proteasome system, a series of rescue experiments were performed (Fig. 6E) using the proteasome inhibitor Bortezomib (BTZ) [42]. Pre-treatment of MCF-7 cells with this proteasome inhibitor for 1 h,

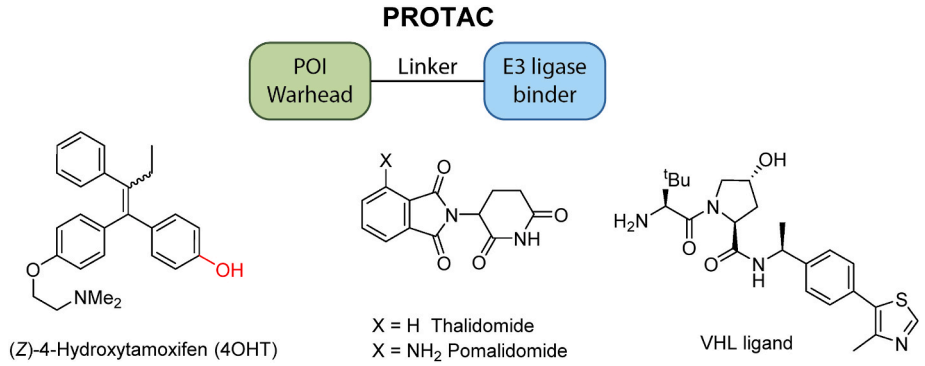


Fig. 4. General approach of ERα PROTACs. **4OHT** was selected as warhead, Pomalidomide and VHL ligand were selected as E3 ligase binders.

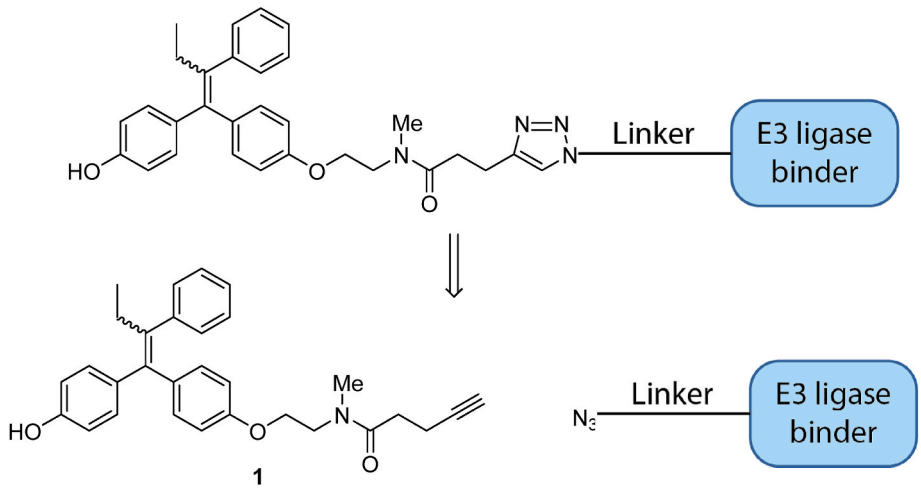


Fig. 5. Retrosynthetic analysis of the ER-PROTACs.

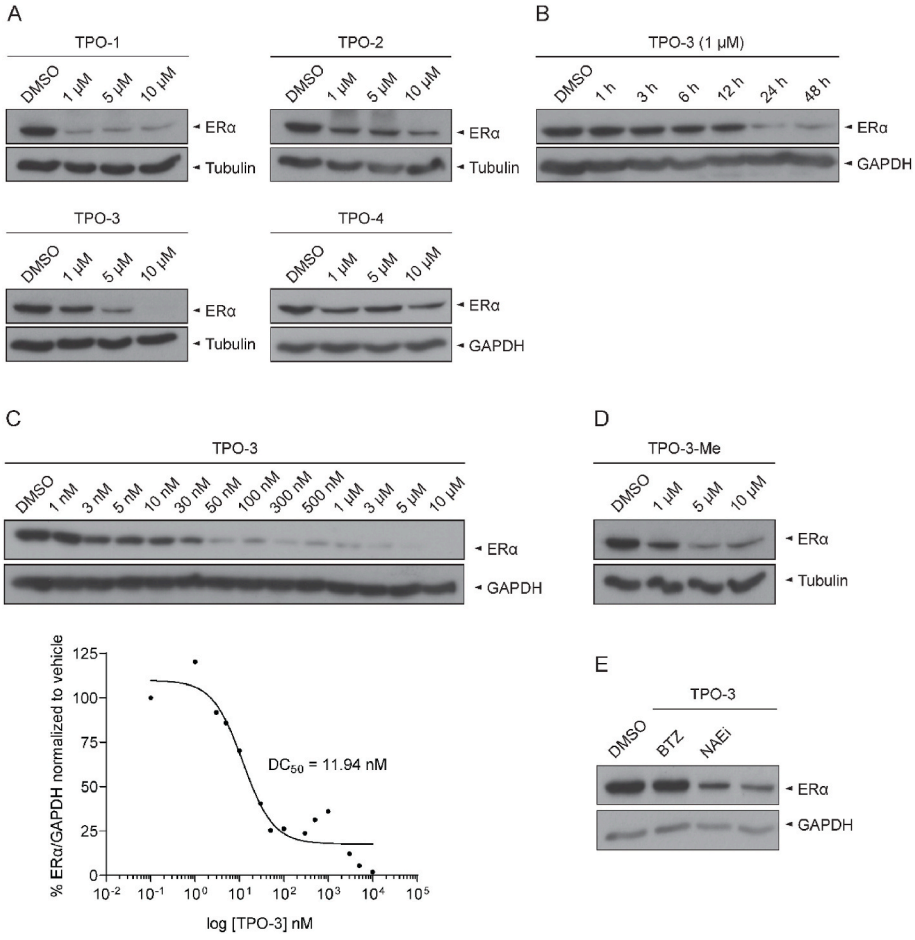


Fig. 6. Degradation of ERα induced by compounds **TPO-1-4. A)** MCF-7 cells were treated with **TPO-1-4** at different concentrations during 24 h, and cell lysates were analyzed by immunoblotting**. B)** MCF-7 cells were treated with 1 μM **TPO-3** during different time periods (1–48 h). Control cells were treated with DMSO for 48 h. **C)** MCF-7 cells were treated with **TPO-3** at a wide range of concentrations during 24 h (representative results from 2 biological replicates). DC_{50} value for **TPO-3** compound, based on quantification of ERα levels normalized to GAPDH levels in MCF-7 cells. **D)** MCF-7 cells were treated with the inactive compound **TPO-3-Me** at different concentrations for 24 h. **E)** MCF-7 cells were pre-treated for 1 h with Bortezomib 1 μM or MLN4924 1 μM, and then treated with **TPO-3** 1 μM during 12 h.

prevented ERα degradation by **TPO-3**, confirming that the mechanism of action is dependent on proteasome activity. Moreover, pre-treatment with MLN4924, a NEED8-activating enzyme inhibitor (NAEi) [43], also reduced the extent of degradation caused by **TPO-3**, suggesting that ERα degradation is mostly mediated by E3 ligase activity. However, the persistence of some small degree of degradation suggests that alternative mechanisms other than the Ubiquitin-Proteasome pathway may also participate. This observation is consistent with the degradation observed using **TPO-3-Me** at higher concentrations.

Unfortunately, although the degradation of ERα by treatment with **TPO-3** was good, reproducibility in subsequent experiments became an issue, and we could not identify the origin of this low reliability. This lack of reproducibility led us to explore other analogs by replacing the CRBN E3 ligase ligand for the VHL ligand.

Both Cereblon and VHL E3 ligase ligands have been successfully employed in the design of protein degraders [15,16]. To explore if recruitment of the VHL E3 ligase could afford more efficient ERα degradation, we extended our initial scope to cover compounds that

bear the VHL ligand instead of thalidomide derivatives. A different set of molecular linkers attached to the VHL ligand were synthesized, all bearing a terminal azide group (Scheme 3). Fragments **9a-b** were prepared by direct amidation of the VHL ligand using 4-azidobutanoic acid and 6-azidohexanoic acid. Fragments **11a-c** were prepared by first attaching a terminal Boc-protected amino acid and performing a second amidation with the corresponding terminal azido acid. Analogous to thalidomide derivatives **7a-d**, VHL-based degraders were assembled using a CuAAC, yielding compounds **TVHL-1-5** in moderate to good yields (Scheme 3).

The efficacy of compounds **TVHL-1-5** degrading ERα was tested by Western blotting (Fig. 7A). Compounds **TVHL-1** and **TVHL-2** both show substantial degradation of ERα at a concentration of 10 nM.

The DC_{50} values for **TVHL-1** and **TVHL-2**, the two most potent compounds of the series, were determined by quantification of Western blotting data. MCF-7 cells were treated with **TVHL-1** and **TVHL-2** at different concentrations during 24 h, and ERα protein levels normalized to GAPDH protein levels were plotted against PROTAC concentration to obtain DC_{50} values of 4.5 nM and 5.3 nM respectively (Fig. 7B and C). Remarkably, **TVHL-1** and **-2** effect was consistently observed in other ER + BCa cell populations such as T47D (Fig. S1; Supplementary material).

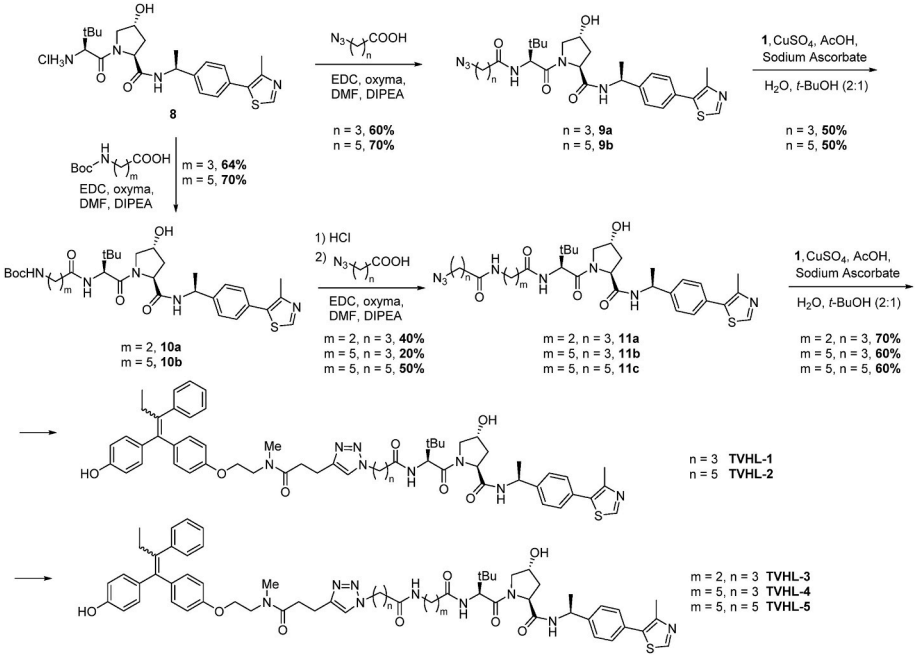
PROTACs, as bifunctional molecules, often show a decrease in activity at high concentrations. The so-called "hook effect" appears when binary interactions compete with the tertiary complex formation [43]. In our case, a small hook effect was observed at 10 μM (Fig. 7B, C and Fig. S1) so we did not test higher concentrations.

A series of rescue experiments were performed (Fig. 7D) to explore the mechanism of action of the new set of compounds. Pre-treatment with the proteasome inhibitor Carfilzomib for 30 min before PROTAC treatment for 6 h rescued ERα from being degraded, proving that degradation was proteasome dependent. Moreover, pre-treatment with MLN4924 (NAEi) [44], also prevented degradation of ERα, confirming that binding to the VHL ligand is essential for the mechanism of action.

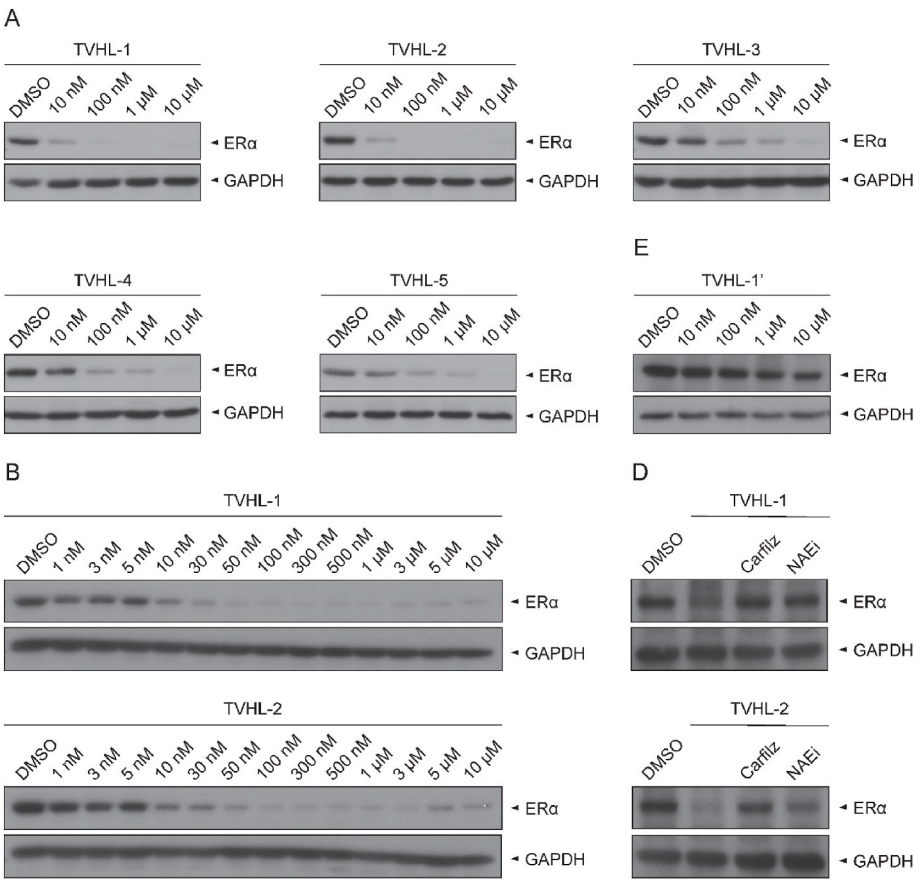
In parallel, compound **TVHL-1'** (Scheme 4) was synthesized using the diastereoisomer of the VHL ligand with the inverted hydroxyl stereocenter [45]. The compound was not able to degrade ERα (Fig. 7E), showing that disruption of the binding to VHL protein prevents degradation. These experiments show that recruitment of the VHL E3 ligase is essential for **TVHL-1** and **TVHL-2** induced degradation of ERα. These results are consistent with the well-known mechanism of action of bifunctional degraders, proving **TVHL-1** and **TVHL-2** act as *bona fide* PROTACs.

3. Conclusions

We designed and synthesized bifunctional degraders that target ERα, using a derivative of 4-hydroxytamoxifen (**4OHT**) for recruitment of the target protein and cereblon (CRBN) or the Von Hippel Lindau (VHL) ligands for the recruitment of the E3 ligase. Initial designs encompassed the use of pomalidomide, a thalidomide derivative that binds to CRBN as the E3 ligase recruiter, identifying **TPO-3** as the most active of the series. However, the synthesis of a series of compounds using the VHL ligand as E3 ligase recruiter afforded more potent and reliable PROTACs. **TVHL-1** and **TVHL-2** were selected as the best ERα protein degraders, having DC_{50} values of 4.5 nM and 5.3 nM respectively. Thus, the use of the ER-PROTAC induces ER-depletion comparable to a genetic ER-knockout and to a higher magnitude than short hairpin RNAs. Importantly, the magnitude and the timeline for this effect can be controlled in time both in cultured cells as well as, potentially, in vivo. This is in sharp contrast to current genetic tricks such as homologous recombination modifications in the *ESR1* gene loci or by means of CRISPR/Cas9. In these latter cases, time control requires inducible systems (TET-ON/OFF or CRE-ERT2 recombinase) that are known for leakiness or potential incompatibilities experimental side effects (tamoxifen, and to a lesser degree E2, are the activators of CRE-ERT2). In summary, these reagents have the potential to provide a pivotal tool to test the role and to functionally validate ER and its interactions with both molecular mechanisms as well as drugs currently being tested or in discovery phase for ER + BCa. This study builds on the importance of the oestrogen receptor in the context of ER + BCa, and describes an efficient synthesis of highly potent ERα degraders, which have applicability in biological research and may become a platform for new drug development given their effect and solubility.



Scheme 3. Synthesis of ERα PROTACs **TVHL-1-5** derived from tamoxifen and VHL ligand.



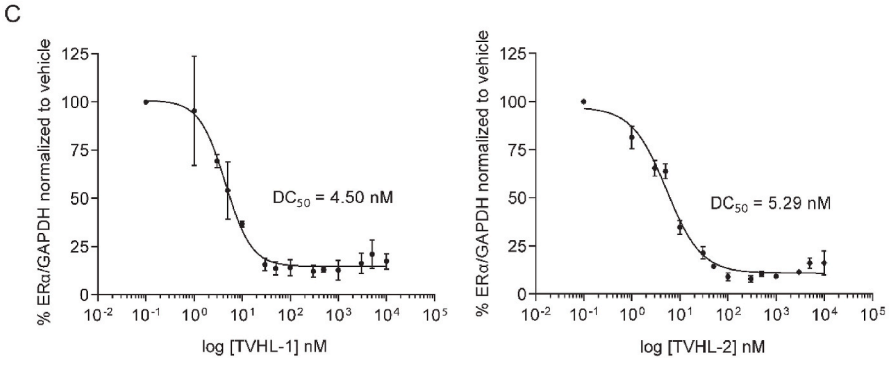
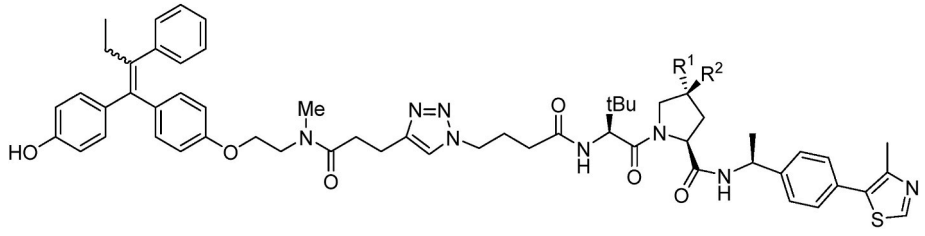


Fig. 7. Degradation of ERα induced by compounds **TVHL-1-5**. **A)** MCF-7 cells were treated with **TVHL-1-5** at different concentrations for 24 h, and cell lysates were analyzed by immunoblotting. **B)** MCF-7 cells were treated with **TVHL-1** or **TVHL-2** at a wide range of concentrations during 24 h (representative results from triplicates). **C)** DC_{50} values for **TVHL-1** and **TVHL-2** compounds, based on quantification of ERα levels normalized to GAPDH levels in MCF-7 cells, by triplicates. **D)** MCF-7 cells were pre-treated for 30 min with Carfilzomib 1 μM or MLN4924 1 μM, and then treated with **TVHL-1** or **TVHL-2**500 nM during 6 h. **E)** MCF-7 cells were treated with the inactive diastereoisomer of **TVHL-1** (**TVHL-1′**) at different concentrations for 24 h.



R^1 = OH, R^2 = H, **TVHL-1**
R^1 = H, R^2 = OH, **TVHL-1'**

Scheme 4. Structure of **TVHL-1** and **TVHL-1′**.

4. Experimental section

4.1. Chemistry. General

All compounds were chemically synthesized, purified by chromatography and characterized by ^{1}H NMR, ^{13}C NMR, IR and HRMS. Compounds were of a purity ≥95% as determined by HRMS and ^{13}C NMR spectroscopy, or by HPLC/Ms. NMR spectra were recorded at 23 °C on a Varian Mercury 400 or Varian 500 apparatus. ^{1}H NMR and ^{13}C NMR spectra were referenced either to relative internal TMS or to residual solvent peaks. Signal multiplicities in the ^{13}C NMR spectra were assigned by HSQC or DEPT experiments. IR spectra were recorded in a Thermo Nicolet Nexus FT-IR apparatus. Melting points were determined using a Büchi M − 540 apparatus. HRMS were recorded in a LTQ-FT Ultra (Thermo Scientific) using nanoelectrospray technique. HPLC chromatography was performed on Hewlett-Packard 1050 equipment with UV detection using a Kinetix EVO C18 50 × 4.6 mm, 2.6 μm column (Standard gradient: 10 mM NH_4CO_3/MeCN (95:5) – (0:100)).

The following compounds were prepared following standard or reported procedures: 2-(4-bromophenoxy)tetrahydro-2H-pyran [46], *tert*-butyl(2-chloroethyl)(methyl)carbamate, 2-(2,6-dioxopiperidin-3-yl)-4-fluoroisoindoline-1,3-dione (**6**) [47], 4-fluoro-2-(1-methyl-2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (**6-Me**) [23], (2R,4R)-1-((S)-2-amino-3,3-dimethylbutanoyl)-4-hydroxy-*N*-((S)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide hydrochloride (**8**) and (2S,4S)-1-((S)-2-amino-3,3-dimethylbutanoyl)-4-hydroxy-*N*-((S)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide hydrochloride [34].

4.2. Acronyms

DCM, Dichloromethane; TFAA, trifluoroacetic anhydride; THF, tetrahydrofurane; EtOAc, ethyl acetate; EDC, 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide; oxyma, ethyl cyano(hydroxyimino)acetate; DMF, *N*,*N*-dimethylformamide; NMP, *N*-methyl-2-pyrrolidone; DIPEA, *N*,*N*-Diisopropylethylamine; HATU, (1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxide hexafluorophosphate.

4.3. Experimental procedures

1-(4-(Allyloxy)phenyl)-2-phenylbutan-1-one (2). To a flask charged with 2-phenylbutanoic acid (10 g, 60.9 mmol) was added (allyloxy)benzene (8.3 mL, 60.9 mmol) and then TFAA (9.5 mL, 67.0 mmol). The mixture was stirred for 2.5 h at 30 °C, then added dropwise to a saturated solution of $KHCO_3$ and washed with DCM. The combined organic layers were then washed with brine, dried over $MgSO_4$, then concentrated under reduced pressure to afford **2** as a colorless oil, which solidified upon standing (16 g, 94% yield). Mp: 43 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.99–7.89 (m, 2H), 7.33–7.26 (m, 4H), 7.23–7.15 (m, 1H), 6.91–6.84 (m, 2H), 6.01 (ddt, J = 17, 11, 5 Hz, 1H), 5.39 (dq, J = 17, 2 Hz, 1H), 5.29 (dq, J = 11, 1 Hz, 1H), 4.55 (dt, J = 5, 2 Hz, 2H), 4.39 (t, J = 7 Hz, 1H), 2.19 (dt, J = 14, 7 Hz, 1H), 1.84 (dt, J = 14, 7 Hz, 1H), 0.89 (t, J = 7 Hz, 3H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 198.6, 162.2, 140.0, 132.5, 130.9, 130.1, 128.8, 128.2, 126.8, 118.1, 114.3, 68.8, 55.1, 27.1, 12.3 ppm. IR (film): 3026, 1666, 1597, 1258, 1020, 744 cm^{-1}. HRMS (ESI): calc. for $[C_{19}H_{21}O_2]^+$: 281.15361; found: 281.15354.

(*E*/*Z*)-4-(1-(4-(Allyloxy)phenyl)-2-phenylbut-1-en-1-yl)phenol (3). To a flask charged with a solution of 2-(4-bromophenoxy)tetrahydro-2H-pyran (6.9 g, 26.8 mmol) in THF (15 mL) at −78 °C was added dropwise *n*-BuLi (2.5 M, 10.7 mL, 26.8 mmol). The mixture was maintained between −72 and −68 °C for 45 min. Then, a solution of 1-(4-(allyloxy)phenyl)-2-phenylbutan-1-one (**2**, 5.0 g, 18.0 mmol) in THF (15 mL) was added dropwise. The mixture was allowed to reach rt, stirred overnight and worked up with water and ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure to afford 11.7 g of the tertiary alcohol intermediate as a brown oil. This crude intermediate was dissolved in methanol (40 mL), and conc. HCl (2.0 mL, 26 mmol) was added dropwise at rt. After 1 h, NaOH (1 M, 20 mL) was added until pH 9, diluted with H_2O and extracted with ethyl acetate. The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure to afford a brown oil that was purified by flash column chromatography. The desired product **3** eluted at 7% EtOAc/hexanes as an off-white solid, as 1:1 mixture of E/Z stereoisomers (6.12 g, 66% yield). Mp: 120 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.12–7.00 (m, 7H), 6.84–6.80 (m, 2H), 6.67–6.63 (m, 2H), 6.42–6.37 (m, 2H), 6.01 (ddt, J = 17, 11, 5 Hz, 1H), 5.36 (dq, J = 17, 2 Hz, 1H), 5.23 (dq, J = 11, 1 Hz, 1H), 4.48 (dt, J = 5, 2 Hz, 2H), 2.41 (q, J = 7 Hz, 2H), 0.85 (t, J = 7 Hz, 3H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.3*, 156.5, 154.2*, 153.3, 142.6*, 142.6, 141.1*, 141.1, 137.7*, 137.7, 136.4*, 136.4, 136.0*, 135.9, 133.4*, 133.3, 132.1*, 131.9, 130.8*, 130.5, 129.7, 127.8, 125.9, 117.7*, 117.5, 114.9*, 114.2, 114.2*, 113.5, 68.8*, 68.6, 29.0*, 29.0, 13.6 ppm. (* denote extra signals belonging to the other isomer). IR (film): 3395, 2964, 1606, 1506, 1231, 844 cm^{-1}. HRMS (ESI): calc. for $[C_{25}H_{25}O_2]^+$: 357.18491; found: 357.18526.

(*E*/*Z*)-2-(4-(1-(4-(allyloxy)phenyl)-2-phenylbut-1-en-1-yl)phenoxy)-*N*-methylethan-1-amine hydrochloride (4). 4-(1-(4-(Allyloxy)phenyl)-2-phenylbut-1-en-1-yl)phenol (**3**, 166 mg, 0.47 mmol), Cs_2CO_3 (445 mg, 1.40 mmol) and DMF (1 mL) were stirred for 20 min at 120 °C. *tert*-Butyl (2-chloroethyl)(methyl)carbamate (108 mg, 0.56 mmol) was added dropwise and the reaction mixture was stirred overnight at 120 °C. Work-up with ethyl acetate and water gave an organic phase that was washed with brine and copper sulfate, dried over $MgSO_4$, and concentrated under reduced pressure. The crude, composed mainly by (*E*/*Z*)-2-(4-(1-(4-(allyloxy)phenyl)-2-phenylbut-1-en-1-yl)phenoxy)-*N*-Boc-*N*-methylethan-1-amine (150 mg, 0.29 mmol) was dissolved in 4 N HCl/dioxane (2 mL) and stirred at rt for 1 h. The reaction crude was concentrated under reduced pressure and purified by flash column chromatography, the product **4** eluted at 3% MeOH/DCM as a white thick oil (72 mg, 0.17 mmol, 60% yield over 2 steps). ^{1}H NMR (400 MHz, Methanol-d4) δ 7.11–6.97 (m, 7H), 6.95–6.90 (m, 1H), 6.85–6.81 (m, 1H), 6.76–6.69 (m, 1H), 6.67–6.63 (m, 1H), 6.59–6.53 (m, 1H), 6.50–6.42 (m, 1H), 5.94 (dddt, J = 42.4, 17.3, 10.5, 5.1 Hz, 1H), 5.37–5.06 (m, 2H), 4.50–4.44 (m, 2H), 4.33–4.27* (m, 2H), 4.21 (dd, J = 5.5, 4.4 Hz, 2H), 4.07–4.02* (m, 2H), 3.41–3.35 (m, 2H), 3.27* (dd, J = 7.1, 3.0 Hz, 2H), 2.72 (s, 3H), 2.65* (s, 3H), 2.38 (p, J = 7.5 Hz, 2H), 0.82 (td, J = 7.4, 1.3 Hz, 3H). ^{13}C NMR (101 MHz, cd3od) δ 187.1, 186.3*, 186.3, 185.4*, 172.1, 171.9*, 171.0, 170.8*, 167.4, 167.4*, 166.9, 165.6, 165.2*, 163.2, 163.1*, 161.3, 161.1, 159.9, 159.7*, 159.0, 159.0, 157.1, 157.0, 155.3, 155.2*, 145.6, 145.5, 143.6, 143.5, 142.8, 142.7, 98.0, 97.8, 92.5, 92.3, 77.7, 77.7, 62.0, 61.9, 58.1, 58.0, 42.0. (* denote extra signals belonging to the other isomer) IR (ATR): 1692, 1390, 1356, 1160, 1118 cm^{-1}. HRMS (ESI): calc. $[C_{28}H_{32}NO_2]^+$: 414.24276; found: 414.24250.

(*E*/*Z*)-*N*-(2-(4-(1-(4-(Allyloxy)phenyl)-2-phenylbut-1-en-1-yl)phenoxy)ethyl)-*N*-methylpent-4-ynamide (7). A solution of pent-4-ynoic acid (32 mg, 0.33 mmol), ethyl cyano(hydroxyimino)acetate (oxyma®) (70 mg, 0.50 mmol), EDC·HCl (95 mg, 0.50 mmol) in DMF (2 mL) was stirred at 0 °C under nitrogen. DIPEA (115 μL, 0.66 mmol) was added dropwise. After 15 min, a solution of 2-(4-(1-(4-(allyloxy)phenyl)-2-phenylbut-1-en-1-yl)phenoxy)-*N*-methylethan-1-amine (**4**, 150 mg, 0.36 mmol) was added to the reaction mixture and stirred overnight. Ethyl acetate and water were added, the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine and copper sulfate, dried over $MgSO_4$, and then concentrated under reduced pressure. The crude was purified by flash column chromatography. The desired product **5** eluted at 25% ethyl acetate/hexanes as a yellowish solid (136 mg, 0.28 mmol, 85% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.20–7.07 (m, 7H), 6.96–6.83 (m, 2H), 6.80–6.72 (m, 2H), 6.59–6.49 (m, 2H), 6.03 (dddt, J = 40.1, 17.2, 10.6, 5.3 Hz, 1H), 5.49–5.17 (m, 2H), 4.59–4.33 (m, 2H), 4.19–3.94 (m, 2H), 3.71 (ddt, J

= 41.8, 13.6, 5.3 Hz, 2H), 3.22–2.95 (m, 3H), 2.75–2.39 (m, 6H), 2.02–1.91 (m, 1H), 0.96–0.89 (m, 3H). ^{13}C NMR (101 MHz, CDCl3) δ 171.7, 171.5*, 157.5, 157.4*, 156.7, 156.5*, 156.1*, 142.7, 141.3, 137.8, 136.7, 136.5*, 136.3*, 136.0*, 133.5, 132.2, 132.1*, 132.0, 130.9, 130.8, 130.7, 129.8, 128.0, 126.1, 126.1, 117.8, 117.6*, 114.4, 114.1, 113.6, 113.3, 83.8, 83.7*, 83.6*, 83.5*, 69.0, 68.8*, 68.7*, 66.9, 66.6*, 65.3*, 65.0*, 63.2, 49.3, 49.2*, 48.3*, 37.6, 37.6*, 34.2*, 34.1*, 32.7, 32.6*, 32.3*, 32.2*, 29.2, 14.5, 13.7. (* denote extra signals belonging to the other isomer). IR (ATR): 1649, 1503, 1231 cm^{-1}. HRMS (ESI): calc. $[C_{33}H_{36}NO_3]^+$: 494.26898; found: 494.26897.

(*E/Z*)-*N*-(2-(4-(1-(4-hydroxyphenyl)-2-phenylbut-1-en-1-yl)phenoxy)ethyl)-*N*-methylpent-4-ynamide (1). A suspension of *N*-(2-(4-(1-(4-(allyloxy)phenyl)-2-phenylbut-1-en-1-yl)phenoxy)ethyl)-*N*-methylpent-4-ynamide (**5**, 75 mg, 0.15 mmol), $Pd(PPh_3)_4$ (9 mg, 0.01 mmol), K_2CO_3 (63 mg, 0.46 mmol) and methanol (1 mL) was stirred under nitrogen at room temperature for 4 h. Ethyl acetate and NH_4Cl sat were added, the aqueous layer was re-extracted using ethyl acetate (x2) and the combined organic layers washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The crude was purified by flash column chromatography, compound **1** was eluted at 50% ethyl acetate/hexanes as an colorless thick oil (48 mg, 0.11 mmol, 73% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.19–7.05 (m, 7H), 6.89–6.67 (m, 4H), 6.54–6.44 (m, 2H), 4.16–3.92 (m, 2H), 3.81–3.58 (m, 2H), 3.21–2.94 (m, 3H), 2.76–2.43 (m, 6H), 1.98–1.89 (m, 1H), 0.92 (td, J = 7.4, 1.8 Hz, 3H). ^{13}C NMR (101 MHz, CDCl3) δ 171.8, 171.6*, 157.3, 157.0*, 156.5*, 156.1*, 155.0, 154.0*, 142.8, 142.7*, 141.4, 141.2*, 141.1*, 137.9, 136.8, 136.3*, 136.0, 135.7*, 132.2, 132.1, 130.9, 130.8, 130.8, 129.8, 128.0, 126.1, 126.0, 115.2, 114.5, 114.0, 113.3, 83.7, 83.7*, 83.5*, 83.5*, 68.9, 66.9, 66.6*, 65.3*, 65.0*, 49.3, 49.3*, 48.4*, 37.7, 37.7*, 34.2*, 32.7, 32.6*, 32.3*, 32.2*, 29.2, 14.5, 13.8. (* denote extra signals belonging to the other isomer). IR (ATR): 1778, 1603, 1500 cm^{-1}. HRMS (ESI): calc. $[C_{30}H_{32}NO_3]^+$: 454.23767; found: 454.23743.

4-((2-(2-azidoethoxy)ethyl)amino)-2-(2,6-dioxopiperidin-3-yl) isoindoline-1,3-dione (7a). To a solution of 2-(2,6-dioxopiperidin-3-yl)-4-fluoroisoindoline-1,3-dione (**4**, 96 mg, 0.35 mmol) in NMP (4 mL) in a boiling tube was added 2-(2-azidoethoxy)ethan-1-amine (50 mg, 0.38 mmol) and DIPEA (121 μL, 0.69 mmol), sealed, then heated to 120 °C for 3 h. Ethyl acetate and water were added to the reaction mixture, then the aqueous layer was re-extracted using ethyl acetate (x2), the combined organic layers washed with brine (x3), $CuSO_4$ (x2) and brine (x1), dried over $MgSO_4$, and then concentrated under reduced pressure. The crude was filtered through silica (1% MeOH/DCM) and used without further purification.

4-((2-(2-(2-azidoethoxy)ethoxy)ethyl)amino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (7b). The product was prepared following the procedure described for **7a**, starting from **4** (300 mg, 1.09 mmol) and 2-(2-(2-azidoethoxy)ethoxy)ethan-1-amine (209 mg, 1.2 mmol). The crude was purified by flash column chromatography, compound **7b** was eluted at 2% MeOH/DCM as a yellow solid (210 mg, 0.49 mmol, 45% yield). Mp: 103 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.68 (s, 1H), 7.45 (ddd, J = 8.6, 7.1, 0.6 Hz, 1H), 7.06 (dd, J = 7.1, 0.6 Hz, 1H), 6.89 (d, J = 8.5 Hz, 1H), 6.47 (t, J = 5.7 Hz, 1H), 4.98–4.87 (m, 1H), 3.74–3.68 (m, 2H), 3.67–3.61 (m, 6H), 3.45 (q, J = 5.5 Hz, 2H), 3.35 (dd, J = 5.6, 4.5 Hz, 2H), 2.89–2.65 (m, 3H), 2.11–2.04 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.6, 169.4, 168.7, 167.7, 146.9, 136.1, 132.5, 116.9, 111.7, 110.3, 70.7, 70.7, 70.1, 69.6, 50.7, 48.9, 42.4, 31.4, 22.8. IR (ATR): 3092, 2857, 2092, 1692 cm^{-1}. HRMS (ESI): calc. $[C_{19}H_{23}N_6O_6]^+$: 431.16736; found: 431.16719.

4-((2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethyl)amino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (7c). The product was prepared following the procedure described for **7a**, starting from **4** (75 mg, 0.27 mmol) and 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethan-1-amine (65 mg, 0.30 mmol). The crude was purified by flash column chromatography, compound **7c** was eluted at 1% MeOH/DCM as a yellow solid (43 mg, 0.09 mmol, 33% yield). Mp: 85 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.46 (NH, bs, 1H), 7.47 (dd, J = 9, 7 Hz, 1H), 7.08 (d, J = 7 Hz, 1H), 6.91 (d, J = 9 Hz, 1H), 6.47 (NH, t, J = 6 Hz, 1H), 4.91 (dd, J = 12, 5 Hz, 1H), 3.71 (t, J = 5 Hz, 2H), 3.68–3.62 (m, 10H), 3.46 (q, J = 5 Hz, 2H), 3.36 (t, J = 5 Hz, 2H), 2.90–2.66 (m, 3H), 2.13–2.06 (m, 1H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.4, 169.4, 168.6, 167.7, 146.9, 136.1, 132.6, 116.9, 111.7, 110.3, 70.8, 70.8, 70.1, 69.6, 50.8, 49.0, 42.5, 31.5, 22.9 ppm. IR (ATR): 3390, 2107, 1698, 1624, 1324, 1115 cm^{-1}. HRMS (ESI): calc. for $[C_{21}H_{27}O_7N_6]^+$: 475.1936; found: 475.1937.

4-((14-azido-3,6,9,12-tetraoxatetradecyl)amino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (7d). The product was prepared following the procedure described for **7a**, starting from **4** (100 mg, 0.36 mmol) and 14-azido-3,6,9,12-tetraoxatetradecan-1-amine (106 mg, 0.40 mmol). The crude was filtered through silica (1% MeOH/DCM) and used without further purification.

4-((2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethyl)amino)-2-(1-methyl-2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (7c-Me). The product was prepared following the procedure described for **7a**, starting from 4-fluoro-2-(1-methyl-2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (**4-Me**, 100 mg, 0.34 mmol) and 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethan-1-amine (84 mg, 0.37 mmol). The crude was purified by flash column chromatography, the product was eluted at 1% MeOH/DCM as a yellow thick oil (40 mg, 0.08 mmol, 24% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (ddd, J = 8.6, 7.1, 0.6 Hz, 1H), 7.08 (dd, J = 7.1, 0.6 Hz, 1H), 6.91 (d, J = 8.4 Hz, 1H), 6.46 (t, J = 5.9 Hz, 1H), 4.95–4.84 (m, 1H), 3.77–3.57 (m, 14H), 3.46 (q, J = 5.6 Hz, 2H), 3.39–3.32 (m, 2H), 3.19 (s, 0H), 2.99–2.90 (m, 1H), 2.79–2.70 (m, 2H), 2.11–2.04 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.4, 169.5, 169.1, 167.9, 146.9, 136.1, 132.6, 116.8, 111.7, 110.5, 70.8, 70.1, 69.6, 50.8, 49.7, 42.5, 32.0, 27.3, 22.2. IR (ATR): 2869, 2096, 1701 cm^{-1}. HRMS (ESI): calc. $[C_{22}H_{29}N_6O_7]^+$: 489.20922; found: 489.20883.

4.4. General procedure of the copper-catalyzed azide-alkyne cycloaddition (CuAAC) reaction

A flask was charged with *N*-(2-(4-(1-(4-(allyloxy)phenyl)-2-phenylbut-1-en-1-yl)phenoxy)ethyl)-*N*-methylpent-4-ynamide (**1**, 1 eq.), the corresponding azido partner (1 eq.), $CuSO_4$ (0.1 eq.), sodium ascorbate (0.2 eq.), H_2O/*tert*-butanol 2:1 (0.026 M), and acetic acid (2 eq.) was sonicated until a homogeneous suspension was observed and stirred overnight at 30 °C. Brine and ethyl acetate were added to the reaction mixture, the aqueous layer extracted once with ethyl acetate, dried over $MgSO_4$ and concentrated under reduced pressure. The corresponding crude was purified by flash column chromatography.

3-(1-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)-*N*-(2-(4-(1-(4-hydroxyphenyl)-2-phenylbut-1-en-1-yl)phenoxy)ethyl)-*N*-methylpropanamide (TPO-1). The product was prepared following the general procedure from alkyne **1** and 4-((2-(2-azidoethoxy)ethyl)amino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (**7a**, 50 mg, 0.07 mmol). The crude was purified by flash column chromatography; the product eluted at 3% MeOH/DCM yielding **TPO-1** (40 mg, 0.05 mmol, 70% yield) as a yellow solid. Mp: 99–108 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73–7.62 (m, 1H), 7.50–7.37 (m, 1H), 7.17–7.00 (m, 7H), 6.87–6.64 (m, 5H), 6.51–6.39 (m, 3H), 5.04–4.92 (m, 1H), 4.53–4.41 (m, 2H), 4.11–3.90 (m, 2H), 3.85–3.74 (m, 2H), 3.69–3.56 (m, 4H), 3.44–3.35 (m, 2H), 3.12–2.90 (m, 5H), 2.85–2.68 (m, 6H), 2.50–2.40 (m, 2H), 2.14–2.06 (m, 1H), 0.90 (t, J = 7.4 Hz, 3H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.0*, 172.9, 172.0*, 172.0, 169.6, 169.1*, 169.1, 167.8, 157.3*, 157.0*, 156.5*, 156.1, 155.4*, 155.3*, 154.4*, 154.4, 147.2, 147.1, 146.7, 142.8*, 142.7, 141.0*, 140.9, 138.0, 136.8*, 136.7*, 136.3, 136.2, 135.8*, 135.6*, 135.4*, 135.2, 132.7, 132.1, 132.1, 130.8, 130.7, 129.8, 127.9, 126.0, 123.2, 116.8, 115.2, 114.5, 114.1, 113.3, 113.3, 112.0, 110.7, 70.6*, 69.9, 69.4, 66.7*, 66.4, 65.7*, 65.4, 50.4, 49.6, 49.1, 48.2*, 48.1, 42.3, 37.6*, 37.5*, 34.6*, 34.5, 32.9*, 32.9*, 32.5*, 32.5, 31.5, 29.1, 23.1, 21.2*, 21.2*, 20.9*, 20.9, 13.8 ppm. (* denote extra signals belonging to the other isomer). IR (ATR): 1686, 1625,

1496, 723 cm^{-1}. HRMS (ESI): calc. $[C_{47}H_{50}N_7O_8]^+$: 840.37154; found: 840.36991.

3-(1-(2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)-*N*-(2-(4-(1-(4-hydroxyphenyl)-2-phenylbut-1-en-1-yl)phenoxy)ethyl)-*N*-methylpropanamide (TPO-2). The product was prepared following the general procedure starting from alkyne **1** and 4-((2-(2-(2-azidoethoxy)ethoxy)ethyl)amino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (**7b**, 29 mg, 0.07 mmol). The crude was purified by flash column chromatography; the product eluted at 3% MeOH/DCM yielding **TPO-2** (18 mg, 0.02 mmol, 30% yield) as a yellow solid. Mp: 105–113 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50–7.35 (m, 2H), 7.09–6.95 (m, 7H), 6.85–6.57 (m, 5H), 6.45–6.32 (m, 3H), 4.83 (ddt, J = 11.4, 3.2, 2.0 Hz, 1H), 4.40–4.28 (m, 2H), 4.04–3.83 (m, 2H), 3.79–3.71 (m, 2H), 3.66–3.46 (m, 8H), 3.35 (dq, J = 5.3, 3.0 Hz, 2H), 3.04–2.83 (m, 5H), 2.79–2.59 (m, 5H), 2.47–2.32 (m, 2H), 2.03–1.98 (m, 1H), 0.86–0.80 (m, 3H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.7*, 172.6*, 172.6*, 172.5, 171.6, 169.5, 168.9, 167.8, 157.3*, 157.0*, 156.5*, 156.1, 155.4*, 155.4*, 154.5*, 154.5, 146.9, 146.7, 142.8*, 142.7, 141.1*, 140.9, 138.0, 136.8*, 136.7*, 136.4, 136.2, 135.7*, 135.6*, 135.3*, 135.2, 132.6, 132.1, 132.1, 130.8, 130.7, 129.8, 127.9, 126.0, 123.1, 116.9, 115.2, 114.5, 114.0, 113.3, 113.3, 111.8, 110.4, 70.7, 70.6, 69.7, 69.4, 66.6*, 66.3*, 65.7*, 65.4, 50.2, 49.3*, 49.2, 49.1*, 48.2*, 48.2, 42.4, 37.6*, 37.5*, 34.5*, 34.4, 32.9*, 32.8*, 32.5*, 32.4, 31.5, 29.1, 22.9, 21.2*, 21.2*, 20.9*, 20.9, 13.8 ppm. (* denote extra signals belonging to the other isomer). IR (ATR): 1698, 1503, 738 cm^{-1}. HRMS (ESI): calc. $[C_{49}H_{54}N_7O_9]^+$: 884.39775; found: 884.39630.

3-(1-(2-(2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethoxy)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)-*N*-(2-(4-(1-(4-hydroxyphenyl)-2-phenylbut-1-en-1-yl)phenoxy)ethyl)-*N*-methylpropanamide (TPO-3). The product was prepared following the general procedure starting from alkyne **1** and 4-((2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethyl)amino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (**7c**, 23 mg, 0.05 mmol). The crude was purified by flash column chromatography; the product eluted at 4% MeOH/DCM yielding **TPO-3** (23 mg, 0.03 mmol, 62% yield) as a yellow solid. Mp: 84–89 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53–7.40 (m, 2H), 7.17–7.00 (m, 7H), 6.91–6.63 (m, 5H), 6.47 (ddd, J = 6.7, 5.6, 2.9 Hz, 3H), 4.90 (dd, J = 11.8, 5.4 Hz, 1H), 4.40–4.24 (m, 2H), 4.10–3.81 (m, 2H), 3.81–3.50 (m, 14H), 3.43 (q, J = 5.4 Hz, 2H), 3.14–2.91 (m, 5H), 2.91–2.66 (m, 5H), 2.51–2.40 (m, 2H), 2.12–2.06 (m, 1H), 0.90 (td, J = 7.4, 1.7 Hz, 3H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.6*, 172.5, 171.5, 169.4, 168.7, 167.8, 157.3*, 156.5, 155.3*, 154.4, 146.9, 146.8, 142.7, 141.1, 138.0, 136.2, 132.6, 132.1, 132.1, 130.8, 130.7, 129.8, 127.9, 126.0, 123.1*, 123.0, 117.0, 115.2, 114.5, 114.1, 113.3, 111.8, 110.4, 93.6, 70.8, 70.7, 70.7, 70.6, 69.6, 66.7*, 66.4, 50.1, 49.2*, 49.0, 48.3*, 48.2, 42.5, 37.6*, 37.6*, 34.5*, 34.4, 32.9*, 32.5, 31.6, 29.1, 22.9, 21.0, 13.8. (* denote extra signals belonging to the other isomer) ppm. IR (ATR): 1701, 1622, 1114, 729 cm^{-1}. HRMS (ESI): calc. $[C_{51}H_{58}N_7O_{10}]^+$: 928.42397; found: 928.42301.

3-(1-(14-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-3,6,9,12-tetraoxatetradecyl)-1H-1,2,3-triazol-4-yl)-*N*-(2-(4-(1-(4-hydroxyphenyl)-2-phenylbut-1-en-1-yl)phenoxy)ethyl)-*N*-methylpropanamide (TPO-4). The product was prepared following the general procedure from alkyne **1** and 4-((14-azido-3,6,9,12-tetraoxatetradecyl)amino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (**7d**, 34 mg, 0.07 mmol). The crude was purified by flash column chromatography; the product eluted at 4% MeOH/DCM yielding **TPO-4** (31 mg, 0.03 mmol, 48% yield) as a yellow solid. Mp: 112–120 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56–7.43 (m, 2H), 7.20–7.02 (m, 7H), 6.92–6.78 (m, 2H), 6.77–6.64 (m, 2H), 6.53–6.43 (m, 2H), 4.97–4.82 (m, 1H), 4.40–4.25 (m, 2H), 4.09–3.88 (m, 2H), 3.79–3.48 (m, 18H), 3.43 (dt, J = 5.6, 2.8 Hz, 2H), 3.17–2.63 (m, 10H), 2.50–2.41 (m, 2H), 2.11–2.04 (m, 1H), 0.91–0.86 (m, 3H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.5, 171.5, 169.4, 168.7, 167.8, 157.4*, 157.0*, 156.5*, 156.1, 155.3*, 154.4, 147.0, 146.8, 142.7, 141.1*, 141.0, 138.0, 136.4, 136.2, 135.8*, 135.4, 132.6, 132.1, 132.1, 131.0, 130.7, 129.8, 129.0, 127.9, 126.0, 122.9, 117.0, 115.2, 114.5, 114.1, 113.3, 113.2, 111.8, 110.4, 70.8, 70.8, 70.7, 70.6, 70.5, 69.6, 66.7*, 66.4*, 66.0*, 65.7, 50.1, 49.3*, 49.2*, 49.0, 48.3*, 48.3, 42.5, 37.7*, 37.6*, 34.5*, 34.4, 32.9*, 32.0*, 31.5*, 31.1, 29.5*, 29.1, 22.9*, 22.8, 21.1, 13.7 ppm. (* denote extra signals belonging to the other isomer). IR (ATR): 1701, 1619, 1108, 729 cm^{-1}. HRMS (ESI): calc. $[C_{53}H_{62}N_7O_{11}]^+$: 972.45018; found: 972.44833.

(2*S*,4*R*)-1-((*S*)-2-(4-azidobutanamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (9a). A flask charged with 4-azidobutanoic acid (63 mg, 0.49 mmol), ethyl cyano(hydroxyimino)acetate (oxyma®) (111 mg, 0.74 mmol), EDC·HCl (149 mg, 0.74 mmol) and DMF (3.5 mL) was purged under nitrogen and stirred at 0 °C and DIPEA (126 μL, 0.12 mmol) was added dropwise. After 15 min (2*R*,4*R*)-1-((*S*)-2-amino-3,3-dimethylbutanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl) phenyl)ethyl)pyrrolidine-2-carboxamide hydrochloride (**8**, 250 mg, 0.49 mmol) was added to the reaction mixture and stirred overnight. Ethyl acetate and water were added, then the aqueous layer was re-extracted using ethyl acetate, the combined organic layers washed with brine and copper sulfate, dried over $MgSO_4$, and then concentrated under reduced pressure. The crude was purified by flash column chromatography; the product eluted at 10% methanol/DCM yielding **9a** (50 mg, 60% yield) as a white solid. Mp: 133 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.67 (s, 1H), 7.44–7.32 (m, 5H), 6.34 (dd, J = 9.0, 3.1 Hz, 1H), 5.07 (p, J = 7.0 Hz, 1H), 4.69 (t, J = 7.8 Hz, 1H), 4.56 (d, J = 8.7 Hz, 1H), 4.50 (s, 1H), 4.03 (dt, J = 11.5, 1.9 Hz, 1H), 3.61 (dd, J = 11.2, 3.9 Hz, 1H), 3.31 (td, J = 6.5, 1.9 Hz, 2H), 2.51 (s, 3H), 2.46 (s, 1H), 2.36–2.19 (m, 2H), 2.10–1.99 (m, 1H), 1.87 (pd, J = 6.9, 3.7 Hz, 2H), 1.47 (d, J = 7.1 Hz, 3H), 1.03 (s, 9H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.6, 172.0, 169.8, 150.5, 148.6, 143.2, 131.1, 129.7, 126.6, 70.1, 58.7, 57.8, 56.8, 50.8, 49.0, 35.7, 35.3, 33.1, 26.6, 24.8, 22.3, 16.2 ppm. IR(ATR): 3272, 2971, 2098, 1614, 1066 cm^{-1}. HRMS (ESI): calculated for $[C_{25}H_{34}N_7O_4S]^+$: 528.2387, found: 528.2378.

(2*S*,4*S*)-1-((*S*)-2-(4-azidobutanamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (9a′). The product was prepared following the procedure described for 9a, starting from (2S,4S)-1-((*S*)-2-amino-3,3-dimethylbutanoyl)-4-hydroxy-*N*-((S)-1-(4-(4-methylthiazol-5-yl) phenyl)ethyl)pyrrolidine-2-carboxamide hydrochloride (20 mg, 0.04 mmol), 4-azidobutanoic acid (5.3 mg, 0.04 mmol). The crude was purified by flash column chromatography; the product eluted at 10% methanol/DCM yielding **9a'** as a white solid (16 mg, 70% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.68 (d, J = 1.2 Hz, 1H), 7.57–7.44 (m, 1H), 7.38 (dd, J = 8.8, 6.1 Hz, 3H), 6.09 (s, 1H), 5.07 (p, J = 7.1 Hz, 1H), 4.58 (d, J = 8.9 Hz, 1H), 4.46 (s, 1H), 3.94 (d, J = 11.2 Hz, 1H), 3.79 (dd, J = 11.2, 4.7 Hz, 1H), 3.24 (td, J = 6.5, 3.1 Hz, 2H), 2.52 (d, J = 11.1 Hz, 4H), 2.46–2.11 (m, 4H), 1.99–1.72 (m, 2H), 1.50 (d, J = 7.0 Hz, 3H), 1.01 (s, 9H). HRMS (ESI): calculated for $[C_{27}H_{38}O_4N_7S]^+$: 556.27005, found: 556.26957.

(2*S*,4*R*)-1-((*S*)-2-(6-azidohexanamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (9b). The product was prepared following the procedure described for 9a, starting from amine **8** (100 mg, 0.21 mmol) and 6-azidohexanoic acid (27 mg, 0.21 mmol). The crude was purified by flash column chromatography; the product eluted at 10% methanol/DCM yielding **9b** (85 mg, 70% yield) as a white solid. Mp: 71–80 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.66 (s, 1H), 7.43 (d, J = 7.9 Hz, 1H), 7.41–7.32 (m, 5H), 6.27 (d, J = 8.8 Hz, 1H), 5.07 (p, J = 7.0 Hz, 1H), 4.57 (d, J = 8.9 Hz, 1H), 4.49 (s, 1H), 4.01 (dt, J = 11.4, 1.9 Hz, 1H), 3.90 (s, 1H), 3.61 (dd, J = 11.2, 3.9 Hz, 1H), 3.23 (t, J = 6.8 Hz, 2H), 2.50 (s, 3H), 2.44 (ddd, J = 12.7, 7.6, 4.8 Hz, 1H), 2.16 (t, J = 7.4 Hz, 2H), 2.02 (ddd, J = 12.7, 8.1, 2.4 Hz, 1H), 1.68–1.50 (m, 4H), 1.46 (d, J = 6.9 Hz, 3H), 1.42–1.29 (m, 2H), 1.02 (s, 9H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.3, 172.0, 169.9, 150.5, 148.5, 143.2, 131.0, 129.6, 126.6, 70.0, 58.7, 57.6, 56.8, 51.3, 48.9, 36.2, 35.7, 35.3, 28.6, 26.5, 26.4,

25.1, 22.3, 16.2 ppm. IR(ATR): 3265, 2971. 2095, 1687, 1614, 1066 cm^{-1}. HRMS (ESI): calculated for $[C_{29}H_{42}N_7O_4S]^+$: 584.3014, found: 584.3009.

***tert*-Butyl (3-(((*S*)-1-((2*S*,4R)-4-hydroxy-2-(((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-3-oxopropyl)carbamate (10a).** A flame-dried flask was loaded with hydrochloride **8** (250 mg, 0.52 mmol), 3-((*tert*-butoxycarbonyl)amino)propanoic acid (92 mg, 0.52 mmol), DIPEA (0.4 mL, 2.08 mmol) and DMF (1 mL) and stirred 15 min at rt. HATU (235 mg, 0.62 mmol) was added and reaction mixture was stirred overnight. Ethyl acetate and water were added to the reaction mixture, then the aqueous layer was re-extracted using ethyl acetate, the combined organic layers washed with brine, $CuSO_4$ and dried over $MgSO_4$, and then concentrated under reduced pressure. The product was purified by silica flash column chromatography, eluted at 5% MeOH/DCM yielding **10a** as a white solid (204 mg, 64% yield). Mp: 107 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.67 (s, 1H), 7.49 (d, *J* = 7.8 Hz, 1H), 7.43–7.32 (m, 4H), 6.61 (d, *J* = 8.7 Hz, 1H), 5.08 (p, *J* = 7.1 Hz, 1H), 4.75 (t, *J* = 8.0 Hz, 1H), 4.56 (d, *J* = 8.5 Hz, 1H), 4.50 (s, 1H), 4.09 (d, *J* = 11.4 Hz, 1H), 3.60 (dd, *J* = 11.4, 3.6 Hz, 1H), 3.45–3.26 (m, 2H), 2.52 (s, 5H), 2.40–2.29 (m, 1H), 2.15–2.04 (m, 1H), 1.47 (d, *J* = 6.9 Hz, 3H), 1.42 (s, 9H), 1.04 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.2, 169.8, 156.3, 150.5, 148.6, 143.3, 131.7, 131.0, 129.7, 126.6, 79.6, 70.3, 58.6, 57.9, 57.0, 49.0, 37.0, 36.4, 35.7, 35.2, 28.6, 26.6, 22.3, 16.2 ppm. IR (ATR): 3305, 2967, 1619, 1523, 1163 cm^{-1}. HRMS (ESI): calculated for $[C_{31}H_{46}N_5O_6S]^+$: 616.31633, found: 616.31548.

***tert*-Butyl (6-(((*S*)-1-((2*S*,4R)-4-hydroxy-2-(((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-6-oxohexyl)carbamate (10b).** A flask charged with 6-((*tert*-butoxycarbonyl)amino)hexanoic acid (153 mg, 0.66 mmol), ethyl cyano(hydroxyimino)acetate (oxyma) (141 mg, 0.99 mmol), EDC·HCl (189 mg, 0.99 mmol) and DMF (4.4 mL) was purged under nitrogen and stirred at 0 °C and DIPEA (288 μL, 1.65 mmol) was added dropwise. After 15 min, hydrochloride **8** (318 mg, 0.66 mmol) was added to the reaction mixture and stirred overnight. Ethyl acetate and water were added, then the aqueous layer was re-extracted using ethyl acetate, the combined organic layers washed with brine and copper sulfate, dried over $MgSO_4$, and then concentrated under reduced pressure. The crude was purified by flash column chromatography, the product eluted at 10% methanol/DCM yielding **10b** (300 mg, 70% yield) as a white solid. Mp: 74 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.66 (s, 1H), 7.47 (d, *J* = 7.8 Hz, 1H), 7.38 (t, *J* = 6.2 Hz, 4H), 6.26 (d, *J* = 8.7 Hz, 1H), 5.08 (p, *J* = 7.1 Hz, 1H), 4.72 (t, *J* = 7.9 Hz, 1H), 4.56 (d, *J* = 8.7 Hz, 1H), 4.52–4.47 (m, 1H), 4.13–4.01 (m, 1H), 3.70–3.54 (m, 1H), 3.07 (d, *J* = 7.3 Hz, 2H), 2.51 (s, 4H), 2.27–2.04 (m, 3H), 1.61 (s, 2H), 1.51–1.37 (m, 14H), 1.34–1.23 (m, 2H), 1.04 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.8, 172.3, 169.8, 156.2, 150.4, 148.6, 143.3, 131.0, 129.7, 126.6, 79.3, 70.1, 58.6, 57.7, 56.8, 48.9, 40.5, 36.4, 35.6, 35.1, 29.8, 28.6, 26.7, 26.3, 25.3, 22.4, 16.2. IR(ATR): 3304, 2927, 1622, 1530, 1163 cm^{-1}. HRMS (ESI): calculated for $[C_{34}H_{52}N_5O_6S]^+$: 658.36328, found: 658.36249.

(2*S*,4*R*)-1-((*S*)-2-(3-(4-azidobutanamido)propanamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (11a). Compound 10a (268 mg, 0.44 mmol) was dissolved in 4 N HCl/dioxane (5 mL) and stirred at rt for 3 h. The reaction crude was concentrated under reduced to afford the corresponding hydrochloride as an off white solid. The product was used without further purification (241 mg, quant.).

A flame-dried flask was loaded with previous hydrochloride (232 mg, 0.42 mmol), 4-azidobutanoic acid (55 mg, 0.42 mmol), DIPEA (0.3 mL, 1.68 mmol) and DMF (1.7 mL), then HATU (176 mg, 0.46 mmol) was added and reaction mixture was stirred overnight. Ethyl acetate and water were added to the reaction mixture, then the aqueous layer was re-extracted using ethyl acetate, the combined organic layers washed with brine, $CuSO_4$ and dried over $MgSO_4$, and then concentrated under reduced pressure. The crude was purified by column chromatography; the product eluted at 5% MeOH/DCM yielding **11a** (104 mg, 40% yield) as a white solid. Mp: 79 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.67 (s, 1H), 7.43–7.33 (m, 5H), 6.70 (d, *J* = 8.7 Hz, 1H), 6.46 (t, *J* = 6.1 Hz, 1H), 5.09 (p, *J* = 7.0 Hz, 1H), 4.72 (t, *J* = 8.1 Hz, 1H), 4.56 (d, *J* = 8.7 Hz, 1H), 4.51 (dt, *J* = 4.2, 2.1 Hz, 1H), 4.06 (dt, *J* = 11.5, 1.8 Hz, 1H), 3.62 (dd, *J* = 11.4, 3.6 Hz, 1H), 3.59–3.36 (m, 2H), 3.32 (td, *J* = 6.6, 1.7 Hz, 2H), 2.55–2.41 (m, 5H), 2.35 (ddd, *J* = 15.0, 7.5, 4.9 Hz, 1H), 2.29–2.19 (m, 2H), 2.11 (ddt, *J* = 13.6, 8.0, 1.9 Hz, 1H), 1.88 (qd, *J* = 8.0, 7.6, 6.3 Hz, 2H), 1.47 (d, *J* = 6.9 Hz, 3H), 1.04 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.5, 172.4, 172.2, 169.7, 150.5, 148.7, 143.2, 131.7, 131.1, 129.7, 126.6, 70.3, 58.6, 58.2, 57.0, 51.0, 49.0, 36.0, 36.0, 35.9, 35.2, 33.3, 26.7, 24.9, 22.3, 16.2. IR(ATR): 3295, 2998, 2090, 1623, 1012 cm^{-1}. HRMS (ESI): calculated for $[C_{30}H_{43}N_8O_5S]^+$: 627.30716, found: 627.30480.

(2*S*,4*R*)-1-((*S*)-2-(6-(4-azidobutanamido)hexanamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (11b). Compound 10b (166 mg, 0.12 mmol) was dissolved in 4 N HCl/dioxane (5 mL) and stirred at rt for 3 h. The reaction crude was concentrated under reduced to afford the corresponding hydrochloride as an off white solid. The product was used without further purification (150 mg, quant.).

A flame-dried flask was loaded with the previous hydrochloride (150 mg, 0.25 mmol), 4-azidobutanoic acid (33 mg, 0.25 mmol), DIPEA (0.25 mL, 1.00 mmol) and DMF (1 mL), then HATU (104 mg, 0.28 mmol) was added and reaction mixture was stirred overnight. Ethyl acetate and water were added to the reaction mixture, then the aqueous layer was re-extracted using ethyl acetate, the combined organic layers washed with brine, $CuSO_4$ and dried over $MgSO_4$, and then concentrated under reduced pressure. The product was purified by silica flash column chromatography; eluted at 5% MeOH/DCM, yielding **11b** as a white solid (33 mg, 20% yield). Mp: 71–80 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.67 (s, 1H), 7.48–7.33 (m, 5H), 6.33 (d, *J* = 8.8 Hz, 1H), 5.95 (t, *J* = 5.8 Hz, 1H), 5.06 (dt, *J* = 13.7, 7.1 Hz, 1H), 4.70 (t, *J* = 8.0 Hz, 1H), 4.57 (d, *J* = 8.7 Hz, 1H), 4.49 (s, 1H), 4.14–4.01 (m, 1H), 3.60 (dd, *J* = 11.3, 3.7 Hz, 1H), 3.32 (t, *J* = 6.5 Hz, 2H), 3.20 (q, *J* = 6.9 Hz, 2H), 2.51 (s, 3H), 2.49–2.41 (m, 1H), 2.23 (q, *J* = 7.5 Hz, 2H), 2.08 (dtd, *J* = 13.9, 8.1, 7.1, 2.9 Hz, 1H), 1.89 (p, *J* = 6.9 Hz, 2H), 1.59 (dt, *J* = 10.5, 7.5 Hz, 2H), 1.46 (dd, *J* = 6.9, 2.7 Hz, 5H), 1.37–1.19 (m, 2H), 1.03 (s, 9H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.7, 172.1, 172.0, 169.9, 150.5, 148.6, 143.3, 131.0, 129.7, 126.6, 70.1, 58.7, 57.7, 56.9, 48.9, 39.4, 36.2, 35.9, 35.3, 33.3, 29.2, 26.6, 26.3, 25.1, 25.0, 22.3, 16.2 ppm. IR(ATR): 3275, 2918, 2094, 1622, 1531, 834 cm^{-1}. HRMS (ESI): calculated for $[C_{33}H_{49}N_8O_5S]^+$: 669.35411, found: 669.35288.

(2*S*,4*R*)-1-((*S*)-2-(6-(6-azidohexanamido)hexanamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (11c). Compound **10b** (166 mg, 0.12 mmol) was dissolved in 4 N HCl/dioxane (5 mL) and stirred at rt for 3 h. The reaction crude was concentrated under reduced to afford the corresponding hydrochloride as an off white solid. The product was used without further purification (150 mg, quant.).

A flame-dried flask was loaded with previous hydrochloride (150 mg, 0.25 mmol), 6-azidohexanoic acid (40 mg, 0.25 mmol), DIPEA (0.25 mL, 1.00 mmol) and DMF (1 mL), then HATU (104 mg, 0.28 mmol) was added and reaction mixture was stirred overnight. Ethyl acetate and water were added to the reaction mixture, then the aqueous layer was re-extracted using ethyl acetate, the combined organic layers washed with brine, $CuSO_4$ and dried over $MgSO_4$, and then concentrated under reduced pressure. The product was purified by silica flash column chromatography; eluted at 5% MeOH/DCM yielding **11c** as a white solid (88 mg, 50% yield). Mp: 81–75 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.65 (d, *J* = 1.3 Hz, 1H), 7.51 (d, *J* = 7.9 Hz, 1H), 7.39–7.31 (m, 4H), 6.47 (d, *J* = 8.6 Hz, 1H), 6.04 (t, *J* = 5.8 Hz, 1H), 5.11–4.99 (m, 1H), 4.65 (t, *J* = 8.0 Hz, 1H), 4.52 (d, *J* = 8.8 Hz, 1H), 4.48 (d, *J* = 5.8 Hz, 1H), 3.99 (d, *J* = 11.3 Hz, 1H), 3.61 (dd, *J* = 11.2, 3.7 Hz, 1H), 3.29–3.19 (m, 2H), 3.15 (q, *J* = 6.7 Hz, 2H), 2.48 (d, *J* = 1.7 Hz, 4H), 2.38–2.29 (m, 1H), 2.21–2.03 (m, 5H), 1.65–1.51 (m, 6H), 1.49–1.17 (m, 9H), 1.01 (s, 9H)

ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.8, 173.1, 171.9, 170.1, 150.5, 148.5, 143.4, 131.6, 130.9, 129.6, 126.5, 70.0, 58.9, 57.8, 56.9, 51.3, 48.8, 39.2, 36.4, 36.2, 36.0, 35.2, 29.2, 28.6, 26.6, 26.4, 26.2, 25.3, 25.1, 22.2, 16.1 ppm. IR(ATR): 3306, 2921, 2094, 1622, 1531, 1081 cm^{-1}. HRMS (ESI): calculated for $[C_{35}H_{53}N_8O_5S]^+$: 697.38541, found: 697.38439.

(2*S*,4*R*)-4-hydroxy-1-((*S*)-2-(4-(4-(3-((2-(4-((*E*)-1-(4-hydroxyphenyl)-2-phenylbut-1-en-1-yl)phenoxy)ethyl)(methyl)amino)-3-oxopropyl)-1H-1,2,3-triazol-1-yl)butanamido)-3,3-dimethylbutanoyl)-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (TVHL-1). The product was prepared following the general procedure from alkyne 1 and (2*S*,4*R*)-1-((*S*)-2-(4-azidobutanamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-((S)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (**9a**, 25 mg, 0.04 mmol). The crude was purified by flash column chromatography; the product eluted at 10% MeOH/DCM yielding **TVHL-1** as a white solid (25 mg, 50% yield). Mp: 96–103 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.67 (s, 1H), 7.42–7.30 (m, 4H), 7.19–6.95 (m, 8H), 6.80 (dd, *J* = 8.6, 3.1 Hz, 2H), 6.73 (dd, *J* = 8.2, 5.5 Hz, 1H), 6.66 (dd, *J* = 8.3, 5.7 Hz, 1H), 6.42 (dd, *J* = 38.3, 8.3 Hz, 2H), 5.15–5.03 (m, 1H), 4.72 (dq, *J* = 8.6, 4.0 Hz, 1H), 4.49 (t, *J* = 6.2 Hz, 2H), 4.20–3.97 (m, 4H), 3.97–3.83 (m, 1H), 3.76–3.65 (m, 1H), 3.66–3.54 (m, 2H), 3.12–2.89 (m, 5H), 2.75 (ddt, *J* = 54.6, 23.9, 6.7 Hz, 2H), 2.51 (s, 3H), 2.52–2.37 (m, 3H), 2.14 (s, 1H), 2.04–1.86 (m, 3H), 1.47 (dd, *J* = 6.9, 4.5 Hz, 3H), 1.05 (d, *J* = 2.8 Hz, 9H), 0.94–0.85 (m, 3H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 207.0*, 172.4*, 172.4*, 172.3, 172.2, 172.1, 172.0*, 170.0, 169.9, 157.2*, 156.7*, 156.3*, 155.9*, 155.4, 154.5, 150.4, 148.4, 147.2*, 146.9, 143.3, 143.3*, 142.6*, 142.5, 141.1*, 140.9, 137.8*, 137.7*, 137.7*, 137.7, 137.3*, 136.9*, 136.6, 135.5*, 135.5*, 135.1*, 135.0, 132.0, 132.0*, 131.9, 131.7*, 130.8, 130.6, 130.6, 129.7, 129.5, 127.8, 126.5, 125.9, 122.4*, 122.4*, 122.3, 115.2, 114.5, 114.0*, 113.9, 113.2*, 113.1, 70.1, 66.4*, 66.1*, 65.5*, 65.2, 58.6*, 58.5, 58.4*, 58.4*, 58.3, 56.8, 49.2*, 49.1*, 48.9, 48.6, 48.5*, 48.3, 37.7*, 37.7, 36.1*, 36.0*, 35.1, 35.0*, 35.0*, 34.3*, 34.2, 32.7, 32.7*, 32.2, 32.2, 32.0, 32.0*, 31.9, 29.0, 26.6, 25.9, 25.8*, 22.2, 21.1, 16.1, 13.6 ppm. IR(ATR): 2955.1, 1621.1, 1505.6, 1234.7, 727.4 cm^{-1}. HRMS (ESI): calculated for $[C_{57}H_{69}O_7N_8S]^+$: 1009.50044, found: 1009.50085.

(2*S*,4*S*)-4-hydroxy-1-((*S*)-2-(4-(4-(3-((2-(4-((E)-1-(4-hydroxyphenyl)-2-phenylbut-1-en-1-yl)phenoxy)ethyl)(methyl)amino)-3-oxopropyl)-1H-1,2,3-triazol-1-yl)butanamido)-3,3-dimethylbutanoyl)-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (TVHL-1′). The product was prepared following the general procedure from alkyne **1** and (2S,4S)-1-((*S*)-2-(4-azidobutanamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-((S)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (**9a′**, 8.5 mg, 0.015 mmol). The crude was purified by flash column chromatography; the product was eluted at 15% MeOH/DCM yielding **TVHL-1'** as a white wax (9 mg, 60%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.79–8.67 (m, 1H), 7.42–7.25 (m, 4H), 7.20–7.02 (m, 8H), 6.84–6.64 (m, 4H), 6.51–6.36 (m, 2H), 5.04 (dt, *J* = 14.3, 7.2 Hz, 1H), 4.61 (t, *J* = 8.4 Hz, 1H), 4.50 (dd, *J* = 11.4, 7.1 Hz, 2H), 4.21–3.90 (m, 3H), 3.83–3.60 (m, 5H), 3.08–2.94 (m, 5H), 2.87–2.60 (m, 2H), 2.52–2.41 (m, 4H), 2.39–1.96 (m, 6H), 1.26 (d, *J* = 2.6 Hz, 3H), 1.05–0.99 (m, 9H), 0.91 (t, *J* = 7.3 Hz, 3H). HRMS (ESI): calculated for $[C_{57}H_{69}O_7N_8S]^+$: 1009.50044; found: 1009.49884.

(2*S*,4*R*)-4-hydroxy-1-((*S*)-2-(6-(4-(3-((2-(4-((E)-1-(4-hydroxyphenyl)-2-phenylbut-1-en-1-yl)phenoxy)ethyl)(methyl)amino)-3-oxopropyl)-1H-1,2,3-triazol-1-yl)hexanamido)-3,3-dimethylbutanoyl)-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (TVHL-2). The product was prepared following the general procedure from alkyne **1** and (2*S*,4*R*)-1-((*S*)-2-(6-azidohexanamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-((S)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (**9b**, 26 mg, 0.04 mmol). The crude was purified by flash column chromatography; the product eluted at 10% MeOH/DCM yielding **TVHL-2** as a white solid (22 mg, 50% yield). Mp: 82–90 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.68 (s, 1H), 7.44–7.33 (m, 5H), 7.20–7.00 (m, 8H), 6.81 (t, *J* = 8.6 Hz, 1H), 6.70 (dt, *J* = 21.9, 8.0 Hz, 2H), 6.48 (dd, *J* = 11.4, 8.4 Hz, 1H), 6.31 (t, *J* = 8.5 Hz, 1H), 5.16–5.01 (m, 1H), 4.71 (q, *J* = 9.1, 8.6 Hz, 1H), 4.64–4.54 (m, 1H), 4.49 (s, 1H), 4.11–3.53 (m, 8H), 3.13–2.90 (m, 5H), 2.87–2.59 (m, 2H), 2.52 (s, 3H), 2.50–2.37 (m, 3H), 2.27–2.01 (m, 3H), 1.75–1.52 (m, 4H), 1.47 (t, *J* = 6.8 Hz, 3H), 1.18 (dd, *J* = 14.6, 7.5 Hz, 2H), 1.07–0.99 (m, 9H), 0.91 (td, *J* = 7.4, 1.8 Hz, 3H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.5*, 173.4, 172.4*, 172.4*, 172.3, 172.0*, 171.9, 169.9, 157.2*, 156.8*, 156.4*, 155.9, 155.3*, 154.4, 150.4, 148.5, 146.8, 143.2, 142.6, 141.1*, 141.0*, 140.9, 137.8*, 137.8, 136.9*, 136.7*, 136.5, 135.6*, 135.5*, 135.2*, 135.2, 131.9, 131., 130.8, 130.6, 129.7, 129.6, 127.8, 126.5, 125.9, 121.8, 115.2, 114.5, 113.9*, 113.9, 113.2*, 113.1, 70.0, 66.5*, 66.2*, 65.5*, 65.1, 58.7, 57.7, 56.9, 49.7, 49.2*, 49.1*, 48.9, 48.3*, 48.3, 37.7*, 37.6*, 35.8, 35.3, 34.3*, 34.2, 32.8*, 32.7, 32.4*, 32.4, 29.8, 29.0, 26.5, 25.8*, 25.8, 24.6, 22.2, 21.1, 16.1, 13.6 ppm. IR(ATR): 2958, 1622, 1506, 1237, 727 cm^{-1}. HRMS (ESI): calculated for $[C_{59}H_{73}O_7N_8S]^+$: 1037.53174, found: 1037.53229.

(2*S*,4*R*)-4-hydroxy-1-((*S*)-2-(3-(4-(4-(3-((2-(4-((*E*)-1-(4-hydroxyphenyl)-2-phenylbut-1-en-1-yl)phenoxy)ethyl)(methyl)amino)-3-oxopropyl)-1H-1,2,3-triazol-1-yl)butanamido)propanamido)-3,3-dimethylbutanoyl)-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (TVHL-3). The product was prepared following the general procedure from alkyne 1 and (2*S*,4*R*)-1-((*S*)-2-(3-(4-azidobutanamido)propanamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (11a) (25 mg, 0.04 mmol). The crude was purified by flash column chromatography; the product was eluted at 10% MeOH/DCM yielding TVHL-3 as a white solid (30 mg, 70% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.70 (s, 1H), 7.66 (s, 1H), 7.38 (s, 5H), 7.19–6.99 (m, 8H), 6.89–6.62 (m, 4H), 6.49 (d, *J* = 9.0 Hz, 2H), 5.10 (s, 1H), 4.62 (s, 2H), 4.43 (s, 1H), 4.20–3.37 (m, 8H), 3.36–2.65 (m, 9H), 2.59–2.11 (m, 9H), 2.01 (d, *J* = 31.9 Hz, 5H), 1.49–1.33 (m, 3H), 1.03 (d, *J* = 3.8 Hz, 9H), 0.91 (td, *J* = 7.6, 2.2 Hz, 3H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.2, 171.8, 170.3, 156.3, 155.8, 155.4, 154.5, 143.6, 142.5, 141.1, 140.9, 137.7, 137.0, 136.6, 135.6, 135.2, 131.9, 130.6, 129.7, 127.8, 126.6, 125.9, 115.3, 114.6, 114.0, 113.2, 70.2, 67.1, 58.8, 58.0, 57.2, 48.8, 48.4, 46.0, 36.8, 36.2, 36.0, 35.4, 32.7, 29.0, 26.6, 22.4, 13.6 ppm. IR (ATR): 2963, 1620, 1506, 1170, 727 cm^{-1}. Mp: 98 °C. HRMS (ESI): calculated for $[C_{60}H_{74}O_8N_9S]^+$: 1080.53756, found: 1080.53782.

(2*S*,4*R*)-4-hydroxy-1-((*S*)-2-(6-(4-(4-(3-((2-(4-((*E*)-1-(4-hydroxyphenyl)-2-phenylbut-1-en-1-yl)phenoxy)ethyl)(methyl)amino)-3-oxopropyl)-1H-1,2,3-triazol-1-yl)butanamido)hexanamido)-3,3-dimethylbutanoyl)-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (TVHL-4). The product was prepared following the general procedure from alkyne 1 and (2S,4R)-1-((*S*)-2-(6-(4-azidobutanamido)hexanamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-((S)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (**11b**, 25 mg, 0.04 mmol). The crude was purified by flash column chromatography, the product was eluted at 10% MeOH/DCM yielding **TVHL-4** as a white solid (28 mg, 60% yield). Mp:70–75 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.68 (s, 1H), 7.51–7.29 (m, 5H), 7.20–7.00 (m, 7H), 6.86–6.79 (m, 1H), 6.75–6.65 (m, 2H), 6.48 (ddd, *J* = 14.9, 8.4, 4.2 Hz, 2H), 6.39–6.33 (m, 1H), 5.09 (td, *J* = 7.2, 4.1 Hz, 1H), 4.71 (q, *J* = 8.0 Hz, 1H), 4.62 (dd, *J* = 9.3, 7.5 Hz, 1H), 4.49 (s, 1H), 4.22–3.81 (m, 6H), 3.62 (dd, *J* = 9.7, 6.1 Hz, 2H), 3.15 (d, *J* = 6.7 Hz, 2H), 3.12–2.90 (m, 5H), 2.90–2.59 (m, 2H), 2.52 (s, 3H), 2.50–2.39 (m, 3H), 2.17 (tt, *J* = 19.2, 6.6 Hz, 3H), 2.10–1.96 (m, 4H), 1.58 (dq, *J* = 20.8, 6.9 Hz, 2H), 1.48–1.44 (m, 3H), 1.44–1.38 (m, 2H), 1.32–1.26 (m, 2H), 1.03 (d, *J* = 5.7 Hz, 9H), 0.91 (td, *J* = 7.4, 1.4 Hz, 3H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.8, 171.9, 171.7, 169.9, 157.2*, 156.3, 155.3*, 154.4, 150.4, 148.5, 143.3, 142.5, 141.1*, 140.9, 137.8*, 137.7, 136.9*, 136.5, 135.6*, 135.2, 132.0, 131.9, 130.8, 130.6, 129.7, 129.6, 127.8, 126.5, 125.9, 122.3, 115.3, 115.2, 114.5, 113.9, 113.2, 69.9, 66.5*, 66.1*, 65.3*, 65.0, 58.7, 57.5, 57.0, 49.0, 48.9, 48.3, 39.2, 37.6*, 37.5*, 36.0, 35.3, 34.2*, 34.1, 32.7, 32.6, 29.0, 28.8, 26.5, 26.1, 26.0, 24.8, 22.2,

21.0, 16.1, 13.6 ppm. IR(ATR): 2925, 1624, 1237, 830 cm^{-1}. HRMS (ESI): calculated for $[C_{63}H_{80}O_8N_9S]^+$: 1122.58451, found: 1122.58502.

(2*S*,4*R*)-4-hydroxy-1-((*S*)-2-(6-(6-(4-(3-((2-(4-((*E*)-1-(4-hydroxyphenyl)-2-phenylbut-1-en-1-yl)phenoxy)ethyl)(methyl)amino)-3-oxopropyl)-1H-1,2,3-triazol-1-yl)hexanamido)hexanamido)-3,3-dimethylbutanoyl)-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (TVHL-5). The product was prepared following the general procedure from alkyne **1** and (2*S*,4*R*)-1-((*S*)-2-(6-(6-azidohexanamido)hexanamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-((S)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (**11c**, 25 mg, 0.04 mmol). The crude was purified by flash column chromatography, the product was eluted at 10% MeOH/DCM yielding **TVHL-5** as a white solid (25 mg, 60% yield). Mp: 80 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.68 (s, 1H), 7.46–7.29 (m, 6H), 7.19–6.99 (m, 7H), 6.87–6.77 (m, 1H), 6.76–6.60 (m, 2H), 6.54–6.38 (m, 2H), 5.99 (dd, J = 13.9, 6.4 Hz, 1H), 5.09 (td, J = 7.2, 3.7 Hz, 1H), 4.72 (q, J = 8.2 Hz, 1H), 4.65–4.56 (m, 1H), 4.49 (d, J = 10.9 Hz, 1H), 4.12–3.57 (m, 8H), 3.17 (p, J = 6.9 Hz, 2H), 3.12–2.89 (m, 5H), 2.75 (ddt, J = 58.3, 26.4, 6.6 Hz, 2H), 2.52 (d, J = 2.1 Hz, 3H), 2.50–2.40 (m, 3H), 2.28–2.03 (m, 5H), 1.73–1.52 (m, 6H), 1.50–1.36 (m, 5H), 1.32–1.11 (m, 4H), 1.07–1.00 (m, 9H), 0.91 (td, J = 7.4, 1.8 Hz, 3H) ppm.^{13}C NMR (101 MHz, $CDCl_3$) δ 173.9, 173.1, 172.2*, 172.0, 169.8, 156.3, 155.4*, 154.5, 150.4, 148.5, 146.9, 143.2, 142.5, 141.2, 137.8, 136.9*, 136.5, 135.4, 131.9, 130.9, 130.6, 129.7, 129.6, 127.8, 126.5, 125.9, 121.8, 115.2, 114.5, 113.9, 113.1, 69.9, 66.5*, 66.2, 58.6, 57.6, 56.9, 49.7, 49.1*, 48.9, 48.3, 39.2, 37.7*, 37.6, 36.2, 36.0, 35.8, 35.2, 32.8, 29.8, 29.0, 28.9, 26.5, 26.0, 25.9, 24.9, 24.8, 22.2, 21.1, 16.1, 13.6 ppm. IR(ATR): 2931, 1626, 1051 cm^{-1}. HRMS (ESI): calculated for $[C_{65}H_{84}O_8N_9S]^+$: 1150.61581, found: 1150.61685.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2022.114770.

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