From Inhibition to Degradation: Targeting the Anti-apoptotic Protein Myeloid Cell Leukemia 1(MCL1)

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ABSTRACT

Protein-protein interactions (PPIs) have emerged as significant targets for therapeutic development, owing to their critical nature in diverse biological processes. An ideal PPI-based target is the protein myeloid cell leukemia 1 (MCL1), a critical pro-survival factor in cancers such as multiple myeloma where MCL1 levels directly correlate to disease progression. Current strategies for halting the anti-apoptotic properties of MCL1 revolve around inhibiting its

sequestration of pro-apoptotic factors. Existing inhibitors disrupt endogenous regulatory proteins, however this strategy actually leads to an increase of MCL1 protein levels. Here we show the development of heterobifunctional small molecules capable of selectively targeting MCL1 using a Proteolysis Targeting Chimera (PROTAC) methodology leading to successful degradation. We have confirmed the involvement of the E3 ligase CUL4A-DDB1 cereblon (CRBN) ubiquitination pathway, making these PROTACs a first step toward a new class of anti-apoptotic BCL-2 family protein degraders.

INTRODUCTION

Myeloid cell leukemia 1 (MCL1) is a pro-survival protein overexpressed in a variety of different cancers and is of tremendous therapeutic interest.[1,2] MCL1 is involved in complex protein-protein interactions (PPIs) involving pro-apoptotic factors Bim, Bak, and Bax.[3] These anti-apoptotic interactions prevent the activation of caspase cascades, promoting cell survival. Due to this anti-apoptotic nature, MCL1 has been recognized as a vital survival factor in human cancers such as lymphoma, leukemia, breast cancer, and multiple myeloma (MM) – where levels of MCL1 directly correlate to disease progression.[4] The ability of MCL1 to silence apoptotic pathways allows it to circumvent the typical clearance mechanisms of cells, and is therefore often overexpressed by tumor cells in order to gain a survival advantage.[3]

MCL1 is a challenging drug target as it is amongst the 85% of proteins in the human genome which have been deemed 'undruggable' – a term used to describe protein targets which are currently chemically intractable, making them onerous to drug.[5,6] Despite this label, there have been some successes, and MCL1 inhibitors have been developed with a variety of structural

geometries, including peptides, macrocycles, and boronic acids, although the majority of progress has been experienced with small molecule leads.[7-10] These compounds modulate MCL1 through competitive inhibition of interactions with its pro-apoptotic targets, by disrupting "hot spots" of the PPI interfaces.[11]

PPIs are difficult to target with small molecule therapeutics due to their shallow and relatively featureless binding regions.[12] The majority of compounds that target MCL1 either occupy the flat binding site of the BH3 groove,[7,8,12,13] an allosteric binding site,[14] or indirectly influence MCL1 via downstream effects through tyrosine kinase and HDAC inhibition[15-16] or CDK9 degradation.[17] Nonetheless, MCL1 has gained much attention as a target for anti-cancer therapeutics,[1,2,18] and several organizations have acute programs targeting MCL1 with compounds AMG 176, AMG 397 (Amgen), AZD 5991 (Astra Zeneca), and S 64315 (Servier) currently undergoing clinical trials.[7,8,12,13,19-23]

One inherent pitfall of inhibition techniques is the dependence on the on/off rates of these compounds within binding pockets. Inhibitors require very small dissociation constants and slow metabolic clearance to ensure maximum efficacy.[11] These challenges make discovering effectively potent inhibitors increasingly difficult for new, unexplored drug targets. A new paradigm in the field of drug discovery aims to perturb PPIs via proximity mediated manipulation.[24] The literature has seen a recent surge in applications towards selective protein degradation, especially since seminal reports utilizing Proteolysis Targeting Chimera (PROTAC) technology.[25-29] Numerous pharmaceutical companies now have protein degrader programs, with some compounds beginning to enter clinical trials. [30-31]

PROTACs are small molecule conjugates which tether target proteins and E3 ligases through heterobifunctional poles, inducing ubiquitination and labeling proteins for proteasomal degradation (Figure 1).[24,26,32] The intermolecular recruitment of E3 ligases facilitates a gain-of-function response in the local environment by covalently linking ubiquitin subunits to lysine residues of the target protein. Examples of suitable E3 ligases are the von Hippel-Lindau (VHL) protein[33] with ligands developed by the Crews lab,[34] as well as cereblon (CRBN) which is targeted by thalidomide and related analogues.[35]

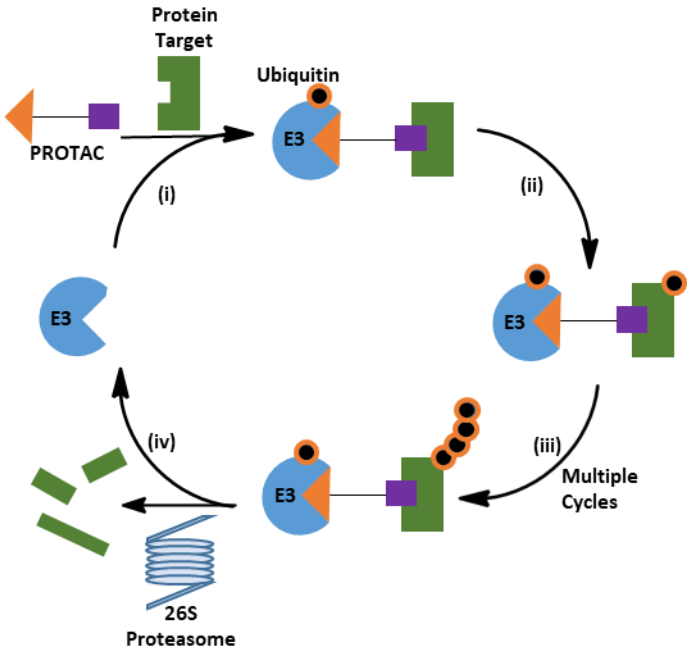


Figure 1. (a) Ubiquitin-Proteasome Pathway (UPP) schematic. (i) PROTAC poles coordinate the protein target and an E3 ubiquitin ligase to form a ternary complex. (ii) E3 ligase transfers ubiquitin onto the protein target. (iii) Ubiquitination occurs several times, polyubiquitinating the target protein. (iv) Ternary complex dissociates and PROTAC is recycled. Polyubiquitinated protein undergoes degradation via 26S proteasomes.

PROTAC-mediated degradation of proteins is beneficial over direct inhibition as it only requires the ternary complex to exist long enough to ubiquitinate the target protein.[36] Upon ubiquitination of its target, the PROTAC and E3 ligase can then dissociate from the ternary complex and be recycled to take part in future ubiquitination events, enabling catalytic degradation cycles.[37] Herein, we demonstrate the development of novel PROTACs capable of inducing degradation of the anti-apoptotic protein MCL1, through a proteasome-mediated pathway.

RESULTS AND DISCUSSION

The MCL1-binding pole of the PROTACs was motivated by the MCL1 inhibitor A-1210477 (Figure 2), which has an inhibition constant (K_i) of 0.45 nM.[7] The CRBN-binding portion was inspired by thalidomide analogs which bind to CRBN via the thalidomide binding domain (TBD).[35,38] CRBN is capable of recruiting DDB1, forming the requisite complex for ubiquitination to occur. Notably, thalidomide, lenalidomide, and pomalidomide all recruit CRBN, and are existing clinical therapies for MM. These immunomodulatory imide drugs (IMiDs) have similar affinities for CRBN, with K_d values of 250 nM, 178 nM, and 157 nM respectively.[39]

The linker length and pole composition of PROTACs affect characteristics such as cell permeability,[40] solubility,[41] and physical distance.[42] The distance between targeting molecules is vital for effective recruitment and positioning of target proteins and E3 ligases. We chose 4-hydroxythalidomide as the CRBN pole and synthesized biotinylated affinity probes in order to confirm whether this would still be an appropriate CRBN-targeting ligand once conjugated to various linkers. In order to facilitate rapid syntheses of numerous biotin probes we employed

pentafluorophenyl (Pfp) esters of biotin. The 4-hydroxythalidomide motif was conjugated to biotin through various terminal diamine linkers, and these probes were utilized in immunoprecipitation (IP) experiments and visualized for CRBN binding. These affinity experiments allow for the qualitative visualization of binary target engagement and are a powerful diagnostic tool for linker length validation.[43] Binding to endogenous CRBN was accommodated when the hydroxythalidomide pole was conjugated to biotin through the polyethylene glycol (PEG) linker 4,7,10-trioxa-1,13-tridecanediamine (Supplementary Figure 1).

Recent reports showed that the glutarimide portion of thalidomide family members is sufficient for CRBN binding to occur.[35] We applied this biotinylated affinity probe strategy to glutarimide to determine whether this would be a suitable, minimalized CRBN pole. Two glutarimide-biotin conjugates were synthesized to study which would afford better binding (**2, 3**). In our IP experiments, neither glutarimide probe expressed effective binding, suggesting that the entire thalidomide ligand is required to accommodate potential linkers required for PROTAC synthesis.

We investigated MCL1 ligand binding using the same biotinylation methodology. Previously reported SAR studies suggested that certain substitutions at the aryl piperazine of A-1210477 could still afford binding to the BH3 groove of MCL1.[7] Our IP experiments revealed that conjugation of biotin through the aryl piperazine (**8**) yielded modest MCL1 binding (Figure 2b). Substitution of the morpholine moiety to create an isosteric, piperazine-linked biotin probe (**17**) significantly improved MCL1 binding (Figure 2c). This interaction provided evidence that this newly implemented motif occupied an appropriate exit vector for conjugating linkers. It is noteworthy that N-alkylation of the indole twists the carboxylate in the 2-position out of the plane of the indole ring, improving the hydrogen bond interaction of the MCL1 ligand with Arg 263, affording more efficient binding within the BH3 groove.[44]

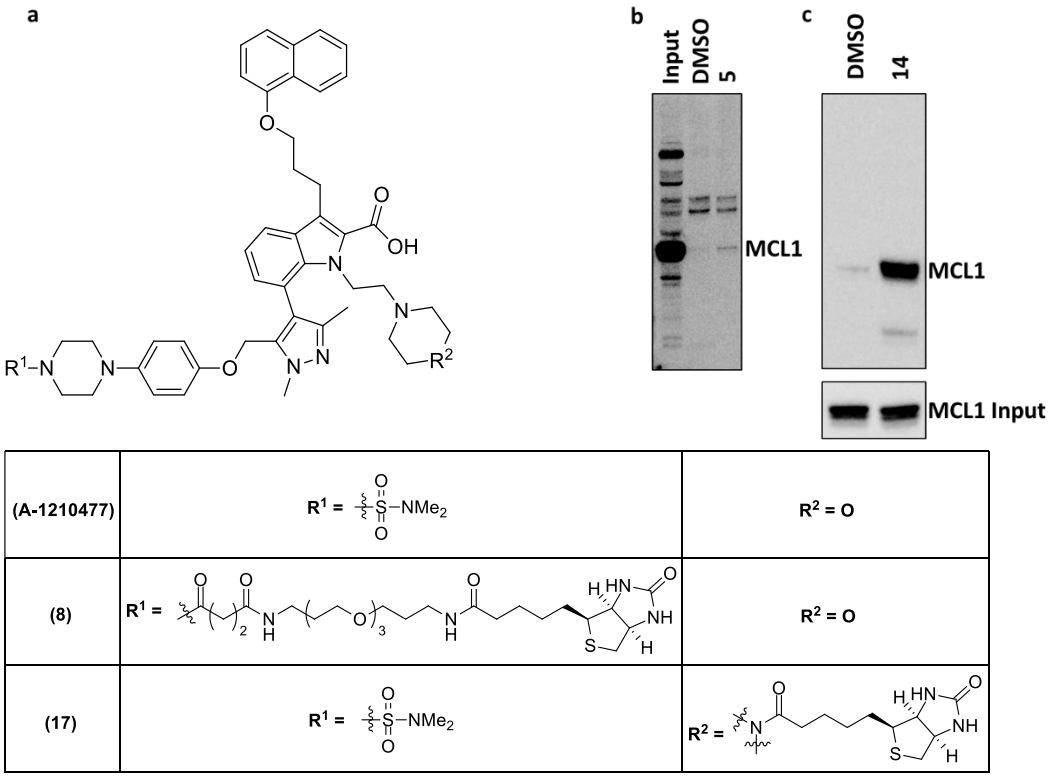


Figure 2. Biotinylation affinity studies for MCL1 ligands. (a) Chemical structures of MCL1 inhibitor A-1210477 (AbbVie) and biotinylated affinity probes **8** and **17**. (b-c) Streptavidin immunoprecipitation with **8** and **17** followed by immunoblot for MCL1 to visualize the formation of the streptavidin-MCL1 complex. The input shows endogenous MCL1 expression in OPM2 cell lysate.

Published docking studies demonstrated that the free carboxylic acid at the 2-position of the indole of A-1210477 was responsible for anchoring the molecule into the BH3 groove.[7] To confirm this claim, and due to synthetic accessibility, our initial PROTAC had the CRBN pole coupled to A-1210477 through a diaminobutane linker at the carboxylic acid (**dMCL1-3**). Our experimental findings coroborated the literature results as no MCL1 degradation was observed.

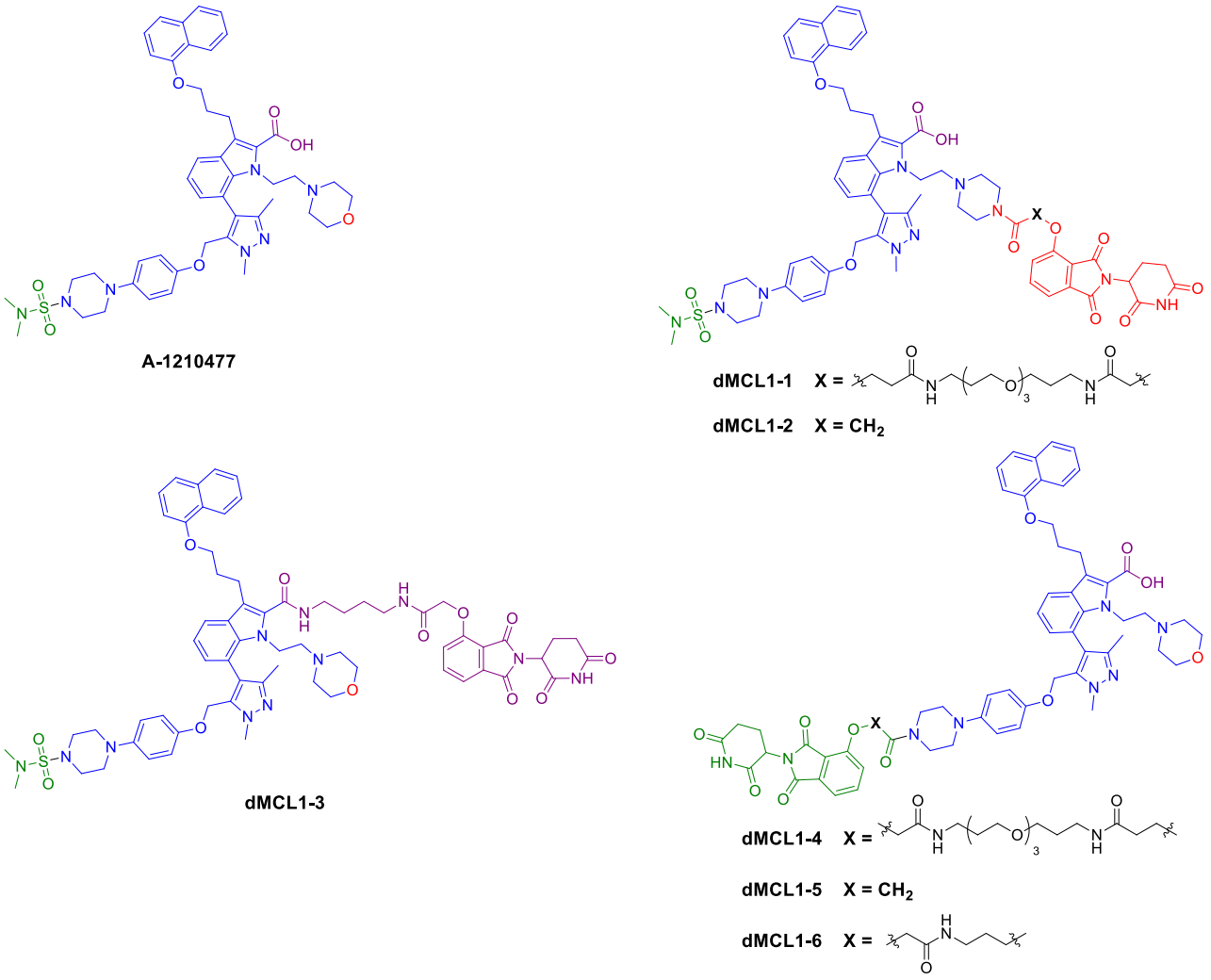


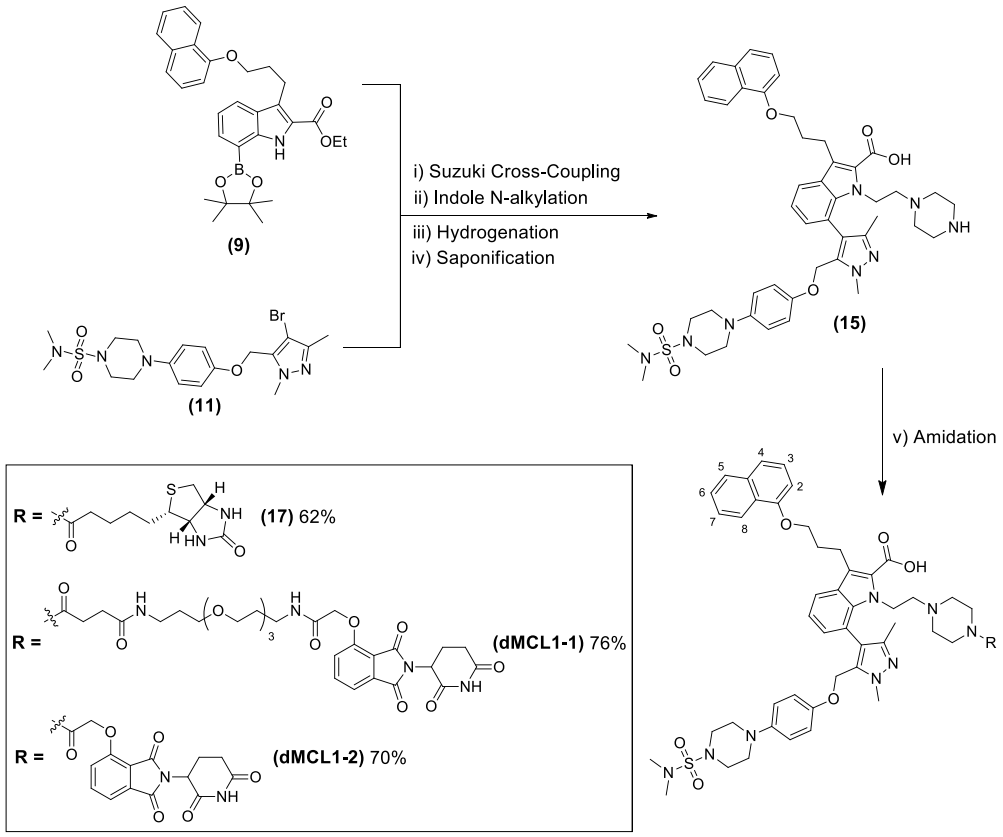
Figure 3. Chemical structures of MCL1 degraders synthesized in this work and MCL1 inhibitor A-1210477 (AbbVie).

Our PROTAC iterations which were linked directly through the aryl piperazine (**dMCL1-4/5/6**, Figure 3) provided underwhelming degradation. *In vitro* degradation studies in the MM cell line OPM2 revealed that conjugates linked through the aryl piperazine ring accommodated MCL1 degradation, but with modest to poor efficacy. Our results indicated that coupling the CRBN pole through this aryl piperazine made the ligand sterically inaccessible for effective binding within the BH3 groove, consistent with our biotin-probe results (Supplementary Figure

1). Due to the aryl piperazine ring's contribution to the subnanomolar MCL1 affinity,[7] this motif was maintained for the multi-gram scale intermediate synthesis required for extending our own SAR study (Scheme 1).

Influenced by our previous results, we next investigated substitution of the morpholine ring of A-1210477 as a point of conjugation. Crystallographic data shows that the morpholine ring extends out of the BH3 groove into the solvent space, providing a greater effect on solubility than MCL1 binding affinity.[7] Coupling this information with the knowledge of the other suboptimal conjugation sites, we redesigned our MCL1 ligand synthesis. The terminal sulfamide moiety was maintained, and installed prior to a convergent Suzuki cross coupling, establishing the main skeleton of the ligand (Scheme 1). Indole N-alkylation was performed with a benzyl protected piperazine derivative, affording an appropriate linker to occupy an effective exit vector from the BH3 groove. This installed a protected secondary amine protruding from the core of the BH3 groove, available for subsequent couplings upon deprotection. The benzyl group was liberated through hydrogenation, followed by saponification of the ethyl ester with lithium hydroxide to provide **15**. While Wurz and coworkers use click-chemistry to rapidly form bispecific conjugates,[45] we utilized a pentafluorophenyl activated ester of 4-hydroxthalidomide, to form the requisite amide bonds. Notably, this advanced coupling intermediate was simply purified via trituration with diethyl ether and was rapidly prepared for similar final coupling steps. This Pfp coupling approach was used to conjugate the MCL1 ligand to the CRBN pole through a known PEG extension[46] (**dMCL1-1**), as well as directly to the newly installed piperazine linker (**dMCL1-2**) (Figure 3). The distance between the targeting poles was varied to examine the role of linker length on ternary complex formation and ubiquitination efficacy.

Scheme 1. Synthetic routes for MCL1 biotinylated affinity probes and PROTACs.[a]



[a]Reagents and conditions: (i) $Pd(dppf)Cl_2$, Cs_2CO_3, DMF, 82%; (ii) 1-benzyl-4-(2-chloroethyl)piperazine dichloride, Cs_2CO_3, DMF, 42%; (iii) H_2, Pd/C, DMF, quantitative; (iv) LiOH, H_2O, MeOH, 99%; (v) pentafluorophenyl ester of **R**, DIPEA, DMF, 62-76%.

To elucidate the effect physical distance and linker conformation have on ternary complex formation, **dMCL1-2** was employed in IP experiments using purified His-tagged MCL1. Formation of the ternary complex [CRBN-**dMCL1-2**-MCL1] was confirmed by IP of His-tagged MCL1, followed by an immunoblot for CRBN (Figure 4a). Upon immunoprecipitation of MCL1-His, CRBN was only successfully recruited when cells were treated with **dMCL1-2**, implying that the conjugate is capable of bringing MCL1 and CRBN within close proximity.

In order to gain an understanding of possible structures of this key ternary complex, we employed atomistic simulations using the Modeling Employing Limited Data (MELD) approach.[47] We simulated the formation of the ternary complex of CRBN and MCL1 with either **dMCL1-1** or **dMCL1-2**. We added restraints to the simulations in order to: (1) keep the structures of CRBN and MCL1 close to their respective X-ray crystallographic structures; and (2) guide the ligand poles to their respective binding pockets on CRBN or MCL1, ensuring that the bound conformations are identical to previous crystallographic studies,[7] so that the major factors being compared are the conformation of the linker and the relative orientation of CRBN and MCL1. MELD replica exchange simulations were carried out in triplicate for 200 ns (Supplementary Information). We regard these simulations as preliminary, but several trends are evident and consistent across replicates. Both **dMCL1-1** and **dMCL1-2** are found to bridge between CRBN and MCL1, bringing them into close proximity (Figure 4b). For both ligands, there is substantial flexibility in the relative orientation between CRBN and MCL1 (Figure 4c). Additionally, the ligand poles are deeply bound within their respective binding sites and there is extensive contact between CRBN and MCL1 (Figure 4d). Although these trends are similar for both ligands, we observed a number of differences between the simulations with **dMCL1-1** and **dMCL1-2**.

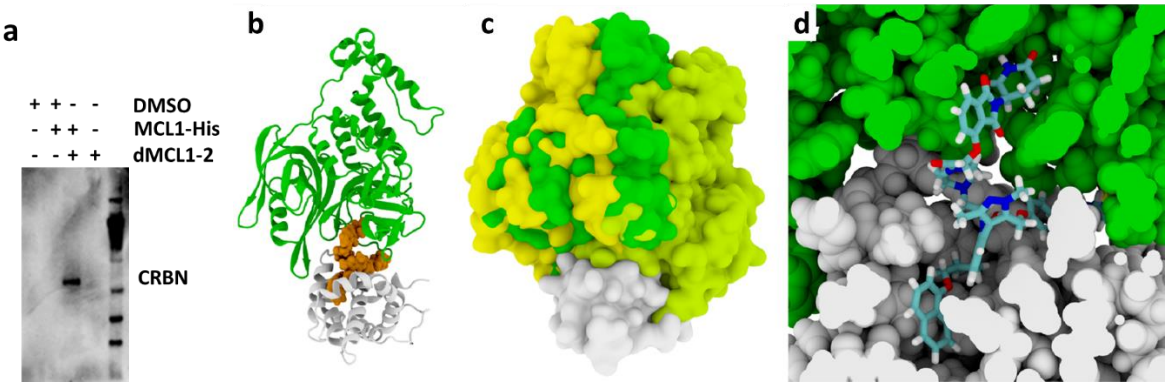


Figure 4. Ternary complex formation and overview of MELD simulation results. (a) Immunoblot for CRBN after a 4 hour pre-treatment of MCL1-His with DMSO or **dMCL1-2**, subsequent exposure to OPM2 cell lysate, followed by IP of MCL1-His and bound ternary complexes. (b) PROTAC **dMCL1-2** (orange) binds to MCL1 (white) and CRBN (green), bridging the two proteins in close proximity. (c) Sample of spatial orientations of CRBN (yellow, light green, dark green) relative to MCL1 (white). (d) PROTAC **dMCL1-2** is deeply bound to both the BH3 groove of MCL1 (white) and the thalidomide binding domain of CRBN (green). PDB ID entries 4tzc was used for CRBN, and 5vkc was used for MCL1.

We investigated the degree of ligand extension in our simulations by measuring the distance between selected atoms on each of the PROTAC poles (CRBN pole: glutarimide nitrogen atom, MCL1 pole: C5 on the naphthalene ring). In the **dMCL1-1** ternary complex, the ligand extension varies from 24–33 Å with a mean distance of 28 Å, whereas the **dMCL1-2** complex varies between from 19–26 Å with a mean distance of 23 Å. Although the larger extension of **dMCL1-1** is consistent with the longer linker, the difference of 5 Å is considerably less than expected if the linker structures were fully extended (Figure 3) due to the tendency of **dMCL1-1** to coil between the two proteins. These results are consistent with previous findings that linker length plays a significant role in degradation efficiency.[48]

There is substantial flexibility in the relative orientation between CRBN and MCL1 (Figure 5). We observe large variations in the twist and tilt angles for both linkers, with somewhat more variability for **dMCL1-1** compared to **dMCL1-2** (particularly for the tilt angle), consistent with the longer linker. Despite some overlap, the orientational distributions for **dMCL1-1** and **dMCL1-2** are different, which would potentially present different faces of MCL1 towards the ubiquitin ligase, in turn affecting ubiquitylation kinetics that may explain the difference in

efficacy between the two ligands. A recent study by Nowak and co-workers successfully used orientational preferences to design a PROTAC specific for the Bromodomain and Extra Terminal (BET) domain of BRD4 over the homologous domains from BRD2 and BRD3.[49] In that same work, they also report several crystal structures of unusually low resolution (ca. 4–6 Å) for some linker-target combinations, which might be consistent with substantial conformational heterogeneity.

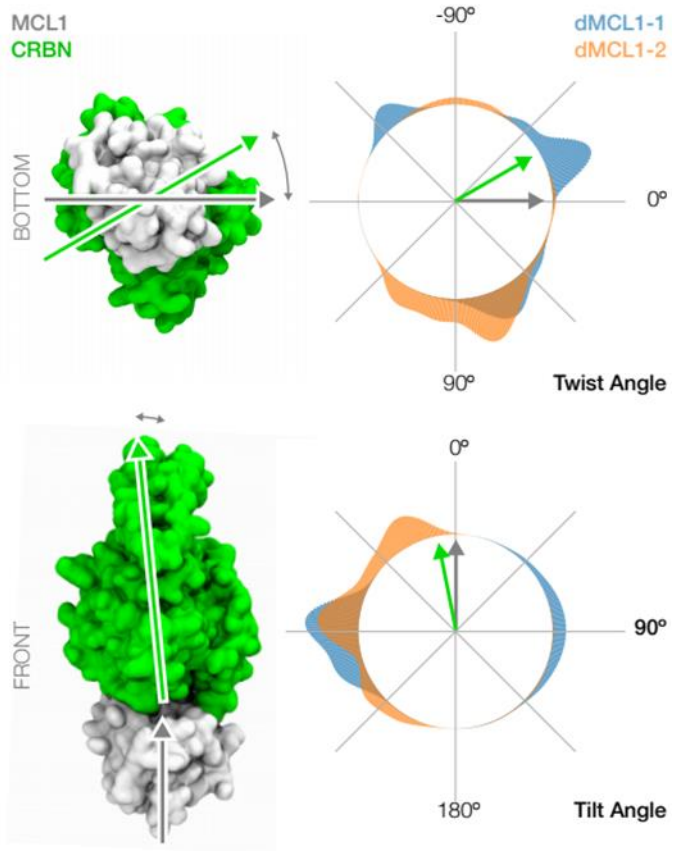


Figure 5. Graphical representation of relative orientations of CRBN with respect to MCL1 observed for either **dMCL1-1** (blue) or **dMCL1-2** (orange) in MELD binding simulations. Vectors were defined between the center of mass of each protein and a selected amino acid on the surface for CRBN (green arrows) and MCL1 (grey arrows). The distribution of twist (top)

and tilt (bottom) angles were visualized using kernel density estimation using a von Mises circular kernel. PDB ID entries 4tzc was used for CRBN, and 5vkc was used for MCL1.

Taken together, these observations indicate that structural heterogeneity and geometry may affect the ubiquitination and degradation of MCL1, although detailed mechanistic understanding must be improved before general predictive models suitable for rational design can be made. Consistent with previous work, these results underscore the sensitivity of binding and ubiquitination to the nature of the linker and its site of attachment.

To observe the degree of MCL1 polyubiquitination upon formation of the ternary complex, we performed *in vitro* ubiquitination assays of OPM2 cell lysate incubated with **dMCL1-1** or **dMCL1-2** (Figure 6a). MCL1 contains 5 lysine residues which participate in ubiquitination.[50] E3 ligases transfer ubiquitin subunits to residues 5/40/136/194/197K, forming various polyubiquitin chains which allow MCL1 to be recognized by the 26S proteasome for degradation. Ubiquitinylation assays were performed using a commercial kit (Abcam) to confirm ubiquitination via CRBN recruitment by visualizing MCL1 ubiquitin units in the immunoblots. These assays suggest that both linker lengths accommodate MCL1 ubiquitination, but to differing degrees. The shorter distance between poles of **dMCL1-2** yielded a greater extent of MCL1 ubiquitination versus the extended spacer of **dMCL1-1** (Figure 6a).

These finding support that modifications in linker length and composition affect ubiquitination efficacy. These results also provided evidence that our optimized conjugation site was suitable for positioning MCL1 within an appropriate proximity to CRBN for ubiquitination to occur, as predicted by our biotinylation experiments. We employed the established PROTAC **dBET1**, developed by Bradner for the degradation of BRD4,[28] as a positive control for ubiquitination.

The level of MCL1 ubiquitination observed with **dMCL1-1** and **dMCL1-2** were comparable to the ubiquitination of BRD4 by **dBET1** (Figure 6b).

Upon confirming ternary complex formation and MCL1 ubiquitination, degradation capabilities of **dMCL1-2** were assessed using a commercial degradation kit (Enzo). The 26S proteasome, the degradation machinery of the UPP, is susceptible to degeneration after only a few hours in these *in vitro* conditions, therefore only a 3 hour incubation period could be employed. Marked decreases in MCL1 levels were observed at 500 nM in HeLa cells compared to DMSO controls, providing evidence of successful UPP mediated degradation *in vitro* (Figure 6c). Endogenous MCL1 degradation was reduced at treatments of 1 μM, compared to 500 nM. This observation may be a characteristic pharmacological property of PROTACs known as the 'hook effect' where higher concentrations of bifunctional degraders prevent degradation as binary complex formation outcompetes against ternary complex formation due to saturation of protein binding sites by the large number of available ligands.[24]

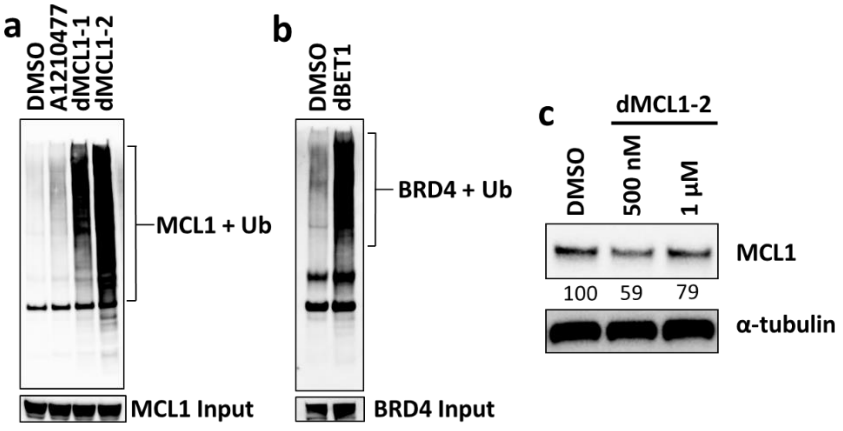


Figure 6. Ubiquitination and *in vitro* degradation studies of MCL1 degraders. (a) Immunoprecipitation (IP) of MCL1 from *in vitro* ubiquitinylation assays with immunoblotting for ubiquitin. (b) IP of BRD4 from *in vitro* ubiquitinylation assays and immunoblotting for ubiquitin.

(c) Immunoblot for MCL1 after 3 hours of *in vitro* degradation conditions with DMSO or **dMCL1-2** in HeLa cell lysate. Degradation is reported as a percentage of protein abundance relative to the DMSO control.

We next investigated the mechanistic dependency on proteasome function and CRBN using biochemical and gene editing techniques respectively (Figure 7). First, we rescued **dMCL1-2** induced MCL1 degradation using the FDA approved proteasome inhibitor bortezomib. The increased MCL1 levels in the combination treatment of cells with **dMCL1-2** and Bortezomib confirm the necessity for proteasome activity in this mode of action (Figure 7a). It is noteworthy that the loss of proteasome activity prevents natural MCL1 ubiquitination events from occurring via the endogenous MCL1 ubiquitin ligase E3 (Mule), thereby baseline MCL1 levels increase.[50] To definitively establish the CRBN dependence of this degradation mechanism, a CRBN knockout cell line of OPM2$^{CRBN-/-}$ was generated using CRISPR Cas9 gene editing and this CRBN deficient MM cell line was treated with A-1210477 or **dMCL1-2**. No degradation of MCL1 was observed with A-1210477 independent of cell line (Figure 7c), consistent with its known inhibitory mode of action. Instead, A-1210477 binds tightly in the BH3 pocket, competitively inhibiting Mule and resulting in increased MCL1 levels *in vitro* (lanes 2-5 and 11-14), a prevalent pitfall of A-1210477.[51] Treatment of OPM2^{WT} cells with **dMCL1-2** afforded MCL1 degradation at nanomolar concentrations (lanes 6-9). Considering **dMCL1-2** also occupies the BH3 groove, this degrader will displace Mule from the BH3 groove as well, preventing some level of natural ubiquitination from occurring. Therefore, there is marked degradation of both isoforms of MCL1 with **dMCL1-2** relative to the stabilization of MCL1 that occurs from occupation of the BH3 groove, as is seen in lanes 2-5. Moreover, **dMCL1-2** induces

apoptosis at 250 and 500 nM after a 24 hours treatment in 1% FBS as revealed by cleavage of Caspase-3 (Figure 8).

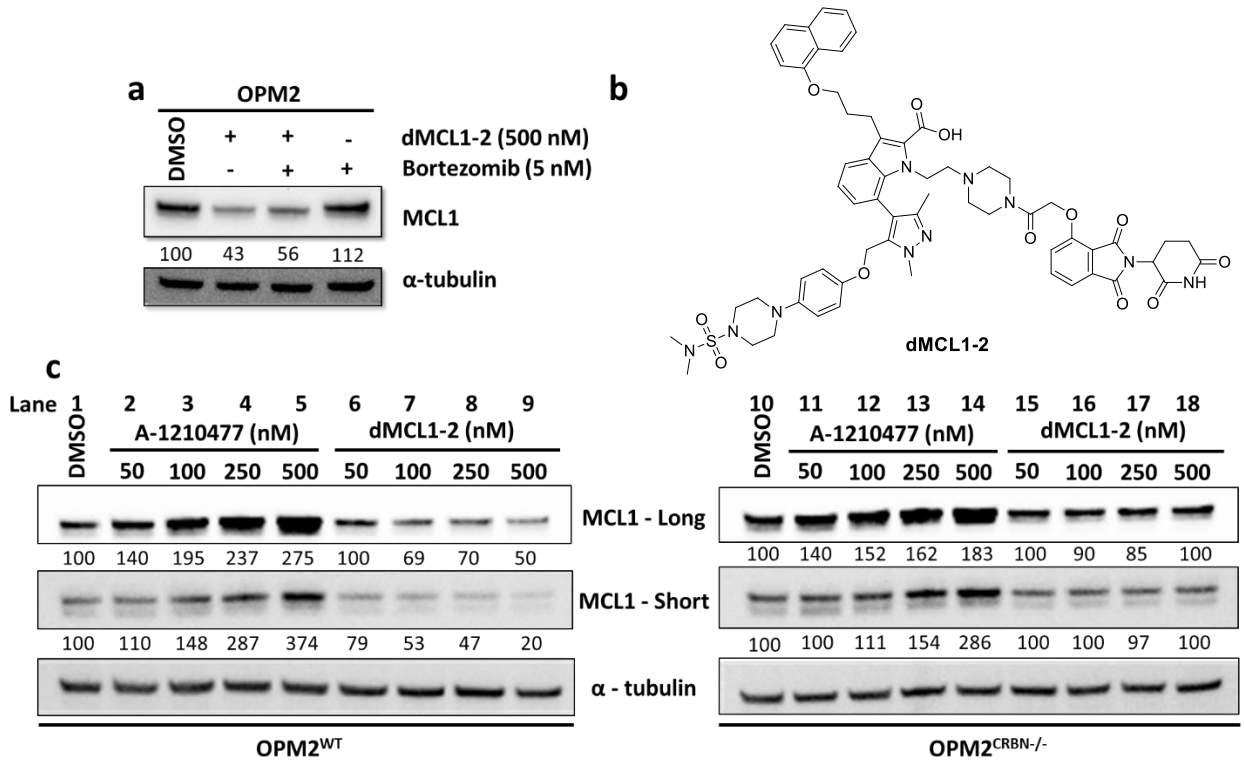


Figure 7. Cellular degradation assays of **dMCL1-2**. (a) Immunoblot for MCL1 after 3 hours of treatment with DMSO, **dMCL1-2**, and/or bortezomib in OPM2 cells. (b) Chemical structure of **dMCL1-2**. (c) Biochemical analysis of **dMCL1-2** mediated MCL1 degradation. Immunoblot for MCL1 long and short isoforms after 48 hours of treatment with DMSO, **dMCL1-2**, or A-1210477 in $OPM2^{WT}$ and $OPM2^{CRBN-/-}$ cells.

In contrast, the same treatment of $OPM2^{CRBN-/-}$ cells with **dMCL1-2** was ineffective (Figure 7c). MCL1 levels experienced insignificant changes in abundance when CRBN is absent (lanes 15-18). Consistent with our proposed mode of action, $OPM2^{CRBN-/-}$ cells no longer possess the capability to form the CUL4A-DDB1 complex and ubiquitinate MCL1, preventing any degradation from occurring, and supporting the UPP degradation mechanism of **dMCL1-2**. Even

whilst MCL1 was not degraded in OPM2$^{CRBN-/-}$ cells, some cell death still occurred (Supplementary Figure 2), consistent with the activity of A-1210477 alone. Notably, in preliminary metabolic stability studies using human liver microsomes, both A-1210477 and **dMCL1-2** have similar $T_{1/2}$ values of 21.7 and 20.6 minutes respectively (Supplementary Table 1).

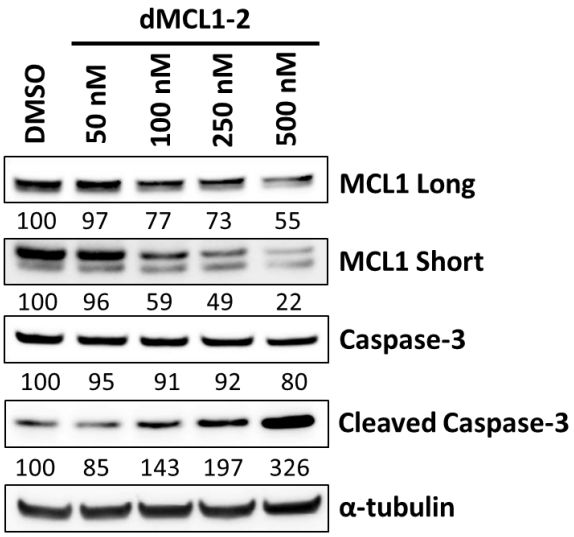


Figure 8. Apoptosis induction factor assay. Cleaved Caspase-3 was probed by immunoblot of OPM2 cells treated with **dMCL1-2** for 24 hours with 1% FBS.

MCL1 has been recognized as chemically intractable due to its flat and featureless BH3 groove. MCL1 is also considered a very difficult protein to target due to its rapid turnover rate, under two hours in most cell lines and conditions.[52] To further comprehend the degradation effect of this class of compounds against natural MCL1 degradation, we performed control treatments of OPM2 and MM1.S cells with **dMCL1-2** and cycloheximide (Figure 9a). Cycloheximide (CHX) inhibits protein translation, providing insight into the natural turnover of MCL1. This also clarifies the extent of chemically induced degradation occurring by following

MCL1 abundance in the presence and absence of **dMCL1-2**. Upon treatment of OPM2 and MM1.S whole cells with CHX, MCL1 levels were depleted much more rapidly in the presence of **dMCL1-2** than in its absence in both cell lines (Figure 9a). Decreased protein expression was observed at timepoints as early as 30 minutes after treatment with CHX. This finding does not coincide with the normal depletion of MCL1 expression of cells treated with CHX alone, indicating that **dMCL1-2** is indeed influencing chemically induced degradation beyond that of the natural MCL1 turnover. The same experiment was performed on the genetically engineered OPM2$^{CRBN-/-}$ cell line (Figure 9b). Consistent with the former experiment, there were marked differences in MCL1 levels in OPM2^{WT} cells treated with CHX in the presence versus absence of **dMCL1-2**. Levels of MCL1 in OPM2$^{CRBN-/-}$ cells degraded consistently over all timepoints of CHX treatment regardless of the presence of **dMCL1-2**, again confirming the necessity for CRBN in this proteasome-mediated degradation mode of action.

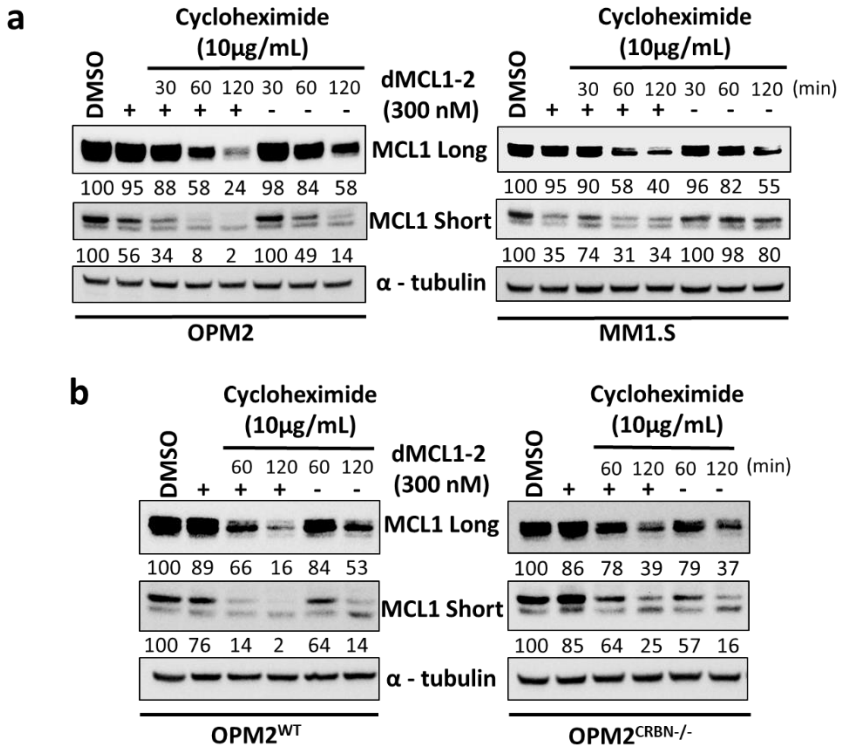


Figure 9. Cyclohexamide (CHX) chase assays to compare MCL1 degradation to normal protein turnover. (a) Immunoblot for MCL1 after 72 hour treatment with DMSO or **dMCL1-2** and then treated for the last 30, 60, or 120 minutes with CHX in OPM2 or MM1.S cells. (b) Immunoblot for MCL1 after 72 hour treatment with DMSO or **dMCL1-2** and then treated for the last 60 or 120 minutes with CHX in $OPM2^{WT}$ or $OPM2^{CRBN-/-}$ cells.

CONCLUSION

Most proximity mediated protein degradation efforts in the literature to date have focused on targeting tumour driving proteins such as CDK9,[17] kinases,[53] and bromodomain and extra terminal (BET) proteins.[27-30a] Our SAR studies have yielded **dMCL1-2**, the first demonstration of a PROTAC which effectively enhances proximity between MCL1 and the E3 ligase CRBN, inducing direct ubiquitination of MCL1 and labeling it for proteasomal degradation at nanomolar concentrations (Figure 7c).Through biotinylated affinity studies we qualitatively assessed binding capabilities in a rapid manner, guiding the synthetic design. Our investigations have shown that these compounds can form ternary complexes between CRBN and MCL1 (Figures 4 & 5), necessary for PROTAC mediated degradation. The CRBN and proteasome dependency of this mechanism have also been confirmed through a CRISPR knockout of CRBN as well as biochemical studies with bortezomib. Preliminary computational studies provide a dynamic prediction of the intimate interactions between MCL1 and CRBN when tethered through **dMCL1-2**. These simulations are consistent with experimental observations of MCL1 ubiquitination and degradation, revealing extensive contacts between MCL1 and CRBN, which corroborates the sensitivity of the linker and its attachment location.

Through our systematic design strategy, computational modelling, and molecular biology guided SAR studies, we have developed novel PROTACs which target the anti-apoptotic MCL1. These compounds effectively recruit the CUL4A-DDB1 CRBN E3 ligase pathway to degrade MCL1 at nanomolar concentrations, activating the cellular apoptosis machinery. As new MCL1 ligands continue to be developed and optimized, this chemical probe technology will provide a firm foundation for future MCL1 PROTAC iterations to be discovered. This report is a fundamental first step toward the development of a new class of anti-apoptotic BCL-2 family protein degraders which will be powerful tools for studying this family of anti-apoptotic proteins and provide a more in-depth understanding into their mechanisms of action.

EXPERIMENTAL SECTION

All chemical reactions were carried out under a nitrogen atmosphere with dry solvents under anhydrous conditions, unless otherwise noted. Reagents were purchased at the highest commercial quality and used without further purification, unless otherwise stated. Tetrahydrofuran (THF) and toluene were distilled immediately before use from sodium-benzophenone. “Dri-Solv” EMD Millipore grade DMF was used. A kdScientific KDS-210 syringe pump was used for dropwise additions of reagents. Triturations were performed using a VWR Model 75T Ultrasonic Cleaner. Solvents were removed in vacuo using either a Buchi R-300 rotavapor (equiped with an I-300 Pro Interface, B-300 Base Heating Bath, Welch 2037B-01 DryFast pump and VWR AD15R-40-V11B Circulating Bath), or Biotage V-10 evaporator, or Kugelrohr short path distillation apparatus. Reactions were monitored by thin layer chromatography (TLC) carried out on Merck glass silica gel plates ($60F_{254}$) using UV light as a visualizing agent and iodine and/or phosphomolybdic acid stain as developing agents. Manual flash chromatography was performed using Silicycle SiliaFlash F60 silica gel (particle size

0.040–0.063 mm, 230-400 mesh) as well as for automated flash chromatography. Solvents for silica gel chromatography were used as supplied by Sigma-Aldrich. Automated flash chromatography was performed on a Biotage Isolera instrument, equipped with a UV detector and Biotage Dalton mass detector. Chromatograms were recorded at 254 and 280 nm. High-resolution mass spectra (HRMS) and low-resolution mass spectra (LRMS) were obtained using Agilent 6520 Accurate-Mass QTOF LC/MS and GenTech 5890 Series II SSQ 7000 instruments respectively. Final compounds tested in experiments were evaluated after analytical high performance liquid chromatography (HPLC) performed on an Agilent 1260 Infinity LC equipped with an Agilent 1260 autosampler, an Agilent 1260 multi-wavelength UV detector, and an Agilent 1260 automated fraction collector with a Poroshell 120 EC-C18 4.6 x 50 mm 2.7 µm column coupled with a Poroshell 120 EC-C18 4.6x5 mm 2.7 µm UHPLC guard column. Purification was run with a flow rate of 1.5 mL/min. Solvents (H2O, acetonitrile, isopropanol) containing 0.1% trifluoroacetic acid (TFA) were used. The following gradient was used at 60ºC: Method:, 5-95% MeCN in water, 0-20 min. The purity of final compounds was evaluated using the analytical LCMS system described above, and compound purity was >95%. ^{1}H and ^{13}C Nuclear Magnetic Resonance (NMR) spectra were recorded on Bruker RDQ 400 (BBFO probe), Bruker DRX 400 (BBO probe), Bruker DRY 400 (BBO probe), or Bruker CFI 600 (BBO probe) spectrometers. The following abbreviations are used to designate multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad.

Materials. Compounds **9**, tert-butyl 4-(4-((4-bromo-1,3-dimethyl-1H-pyrazol-5-yl)methoxy)phenyl)piperazine-1-carboxylate, and ethyl 7-(1,3-dimethyl-5-((4-(piperazin-1-yl)phenoxy)methyl)-1H-pyrazol-4-yl)-1-(2-morpholinoethyl)-3-(3-(naphthalen-1-yloxy)propyl)-1H-indole-2-carboxylate were prepared according to the procedure of Souers.[7] Perfluorophenyl

1-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)-2,17-dioxo-7,10,13-trioxa-3,16-diazaicosan-20-oate was prepared by a known procedure.[46] A-1210477 was purchased from Selleckchem and used without further purification.

N-(1-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)-2-oxo-7,10,13-trioxa-3-azahexadecan-16-yl)-5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamide (**1**). To a mixture of 4,7,10-trioxa-1,13-tridecanediamine (0.81g, 3.7 mmol) and Et_3N (0.20 ml, 1.4 mmol) was added a solution of **16** (0.15 g, 0.37 mmol) in DMF (6 ml) at 0° C over 1 hour. The mixture was allowed to stir at ambient temperature for 1 hour more and was then concentrated *in vacuo*. The residue was triturated with Et_2O (2 x 7 ml x 2 hours) furnishing 0.17 g of the corresponding mono-protected amine (99%) as a white powder. ^{1}H NMR (400 MHz, CD_3OD) δ 4.53-4.48 (m, 1H), 4.34-4.30 (m, 1H), 3.70-3.58 (m, 13H), 3.54 (t, J=6.0 Hz, 2H), 3.32 (t, J=7.1 Hz, 2H), 3.30-3.20 (m, 1H), 2.98-2.83 (m, 4H), 2.71 (d, J=12.7 Hz, 1H), 2.22 (t, J=7.3 Hz, 1H), 1.88-1.57 (m, 9H), 1.50-1.42 (m, 2H). All other data was in agreement with literature.[54]

A solution of this mono-protected amine (45 mg, 0.10 mmol), DIPEA (0.035 ml, 0.20 mmol) and **21** (0.035 g, 0.089 mmol) in DMF (1.5 ml) was stirred at ambient temperature for 2 hours. The solvent was removed *in vacuo* and the residue was purified by silica gel flash chromatography (10-20% MeOH in $CHCl_3$) to afford 0.029 g (43%) of the title compound as a 1:1 mixture of diastereomers. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.32 (d, J = 24.0 Hz, 1H), 7.74 (t, J = 7.9 Hz, 1H), 7.60 (t, J = 5.8 Hz, 1H), 7.54 (d, J = 7.3 Hz, 1H), 7.22 (d, J = 8.4 Hz, 1H), 6.67 (t, J = 5.6 Hz, 1H), 6.34 (d, J = 5.7 Hz, 1H), 5.87 – 5.74 (m, 1H), 5.06 – 4.94 (m, 1H), 4.66 (s, 2H), 4.47 (dd, J = 7.8, 4.9 Hz, 1H), 4.28 (dd, J = 8.0, 4.6 Hz, 1H), 3.68 – 3.53 (m, 12H), 3.51 – 3.41 (m, 3H), 3.31 (q, J = 6.1 Hz, 2H), 3.12 (td, J = 7.3, 4.5 Hz, 1H), 2.82 (dtd, J = 18.5, 11.6, 10.8, 4.1

Hz, 4H), 2.72 (d, J = 12.9 Hz, 1H), 2.20 – 2.07 (m, 3H), 1.86 (p, J = 6.5 Hz, 2H), 1.75 (p, J = 6.0 Hz, 2H), 1.67 – 1.55 (m, 3H), 1.39 (p, J = 7.4 Hz, 2H). ^{13}C NMR (101 MHz, CDCl3) δ 173.0, 172.1, 172.0, 172.0, 169.0, 169.0, 166.9, 166.7, 166.0, 164.0, 154.6, 137.0, 133.5, 119.8, 119.8, 118.1, 117.3, 70.4, 70.1, 69.9, 69.8, 68.7, 68.2, 68.2, 61.8, 61.8, 60.2, 55.6, 50.7, 49.3, 40.5, 40.5, 37.7, 37.6, 36.5, 35.9, 35.9, 31.5, 29.3, 28.8, 28.8, 28.2, 28.2, 28.1, 28.0, 25.5, 25.5, 22.6. HRMS (ESI) m/z calc. for $[C_{35}H_{48}N_6O_{11}S + H]^+$ = 783.2994, found 738.3005.

N-(2,6-dioxopiperidin-3-yl)-5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamide (**2**). A solution of 3-amino-piperidine-2,6-dione hydrochloride (0.066 g, 0.40 mmol), DIPEA (0.21 ml, 0.155 g, 1.20 mmol) and **16** (0.165 g, 0.040 mmol) in DMF (3.0 ml) was stirred at ambient temperature overnight. The solvent was removed *in vacuo* and the residue was redissolved in DMF (3 ml) and stirred with K_2CO_3 (0.150 g, 1.1 mmol) for 2 hours. The inorganic salts were filtered off, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel flash chromatography (20% MeOH in $CHCl_3$) to afford 0.125 g (88%) of the title compound. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.77 (s, 1H), 8.14 (d, J = 8.3 Hz, 1H), 6.38 (d, J = 24.5 Hz, 2H), 4.53 (q, J = 8.4 Hz, 1H), 4.30 (dd, J = 7.8, 5.0 Hz, 1H), 4.13 (ddd, J = 7.4, 4.4, 1.8 Hz, 1H), 3.10 (ddd, J = 8.6, 6.1, 4.3 Hz, 1H), 2.82 (dd, J = 12.4, 5.0 Hz, 1H), 2.71 (ddd, J = 18.0, 10.6, 8.2 Hz, 1H), 2.57 (d, J = 12.4 Hz, 1H), 2.13 (td, J = 7.3, 1.9 Hz, 2H), 1.90 (td, J = 10.6, 9.4, 4.9 Hz, 2H), 1.67 – 1.41 (m, 4H), 1.40 – 1.26 (m, 2H). ^{13}C NMR (101 MHz, CD_3OD) δ 174.8, 173.4, 172.3, 164.8, 61.8, 60.3, 55.5, 49.6, 39.6, 35.1, 30.7, 28.0, 28.0, 25.2, 24.2. HRMS (ESI) m/z calc. for $[C_{15}H_{22}N_4O_4S + H]^+$ = 355.1435, found 355.1437.

N1-(2,6-dioxopiperidin-3-yl)-N4-(15-oxo-19-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)-4,7,10-trioxa-14-azanonadecyl)succinamide (**3**). To a mixture of 4,7,10-trioxa-1,13-tridecanediamine (0.81g, 3.7 mmol) and Et_3N (0.20 ml, 1.4 mmol) was added

a solution of **16** (0.15 g, 0.37 mmol) in DMF (6 ml) at 0° C over 1 hour. The mixture was allowed to stir at ambient temperature for 1 hour more and was then concentrated *in vacuo*. The residue was triturated with Et_2O (2 x 7 ml x 2 hours) furnishing 0.17 g of the corresponding mono-protected amine (99%) as a white powder. ^{1}H NMR (400 MHz, CD_3OD) δ 4.53-4.48 (m, 1H), 4.34-4.30 (m, 1H), 3.70-3.58 (m, 13H), 3.54 (t, J=6.0 Hz, 2H), 3.32 (t, J=7.1 Hz, 2H), 3.30-3.20 (m, 1H), 2.98-2.83 (m, 4H), 2.71 (d, J=12.7 Hz, 1H), 2.22 (t, J=7.3 Hz, 1H), 1.88-1.57 (m, 9H), 1.50-1.42 (m, 2H). All other data was in agreement with literature.[54]

A solution of this mono-protected amine (0.040 g, 0.09 mmol), DIPEA (0.23 ml, 0.168 g, 1.30 mmol) and succinic anhydride (0.036 g, 0.089 mmol) in DMF (3.0 ml) was stirred at ambient temperature overnight. The solvent was removed *in vacuo* and the residue was triturated with a 1:1 $CHCl_3/Et_2O$ mixture (2 x 10ml) for 15 minutes, the product was then pelleted through centrifugation of the resultant gel. The wet solid was dried *in vacuo* to furnish 0.15 g (76%) of the corresponding acid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.00 (br s, 1H), 7.79 (t, J=5.9 Hz, 1H), 7.73 (t, J=5.8 Hz, 1H), 6.40 (s, 1H), 6.34 (s, 1H), 4.35-4.28 (m, 1H), 4.16-4.10 (m, 1H), 3.58-3.48 (m, 8H), 3.46 (t, J=6.3 Hz, 4H), 3.15-3.03 (m, 5H), 2.84 (dd, J=12.5, 5.1 Hz, 1H), 2.31 (t, J=6.7 Hz, 2H), 2.05 (t, J=6.9 Hz, 2H), 1.65-1.58 (m, 5H), 1.55-1.25 (m, 6H).

To a solution of the free acid (0.15 g, 0.27 mmol) and DIPEA (0.17 ml, 0.129 g, 1.0 mmol) in DMF (1.5 ml) cooled to 0° C was added pentafluorophenyl trifluoroacetate (0.11 g, 0.070 ml, 0.41 mmol) over 1 minute and the mixture was stirred at 0° C for an additional 30 minutes. The reaction mixture was allowed to warm to ambient temperature and after 2 hours the solvent was removed *in vacuo* and the residue was triturated with Et_2O (2 x 7 ml) for 30 minutes, the product was then pelleted through centrifugation of the resultant gel. The wet solid was dried *in vacuo* to afford 0.12 g (54%) of the corresponding pentfluorophenyl-ester. This intermediate was used

without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.71 (t, J=5.9 Hz, 1H), 6.44 (t, J=5.8 Hz, 1H), 5.63 (s, 1H), 4.83 (s, 1H), 4.55-4.48 (m, 1H), 4.36-4.30 (m, 1H), 3.70-3.55 (m, 12H), 3.43-3.35 (m, 4H), 3.20-3.15 (m, 1H), 3.05 (t, J=6.4 Hz, 2H), 2.93 (dd, J=12.7, 4.8 Hz, 1H), 2.72 (d, J=12.7 Hz, 1H), 2.63 (t, J=6.8 Hz, 2H), 2.22-2.17 (m, 2H), 1.95-1.65 (m, 10H).

A solution of this activated ester (0.12 g, 0.17 mmol), DIPEA (0.10 ml, 0.748 g, 0.58 mmol) and 3-amino-piperidine-2,6-dione hydrochloride (0.030 g, 0.18 mmol) in DMF (2.0 ml) was stirred at ambient temperature for 2 hours. The solvent was removed *in vacuo* and the residue was triturated with Et_2O (2 x 5 ml) for 30 minutes. The white powder (0.13 g) was redissolved in DMF (1.5 ml) and stirred with K_2CO_3 (0.050 g, 0.36 mmol) for 1 hour. The inorganic salts were filtered off, the filtrate was concentrated *in vacuo* and the residue was triturated with a 1:1 $CHCl_3/Et_2O$ mixture (2 x 5ml) for 30 minutes, the product was then pelleted through centrifugation of the resultant gel. The wet solid was dried *in vacuo* to afford 0.098 g (83%) of the title compound. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.77 (s, 1H), 8.20 (d, J = 8.2 Hz, 1H), 7.77 (dt, J = 23.8, 5.5 Hz, 2H), 6.49 – 6.28 (m, 2H), 4.53 (q, J = 8.4 Hz, 1H), 4.31 (dd, J = 7.7, 5.0 Hz, 1H), 4.14 (ddd, J = 7.7, 4.4, 1.9 Hz, 1H), 3.57 – 3.45 (m, 8H), 3.39 (t, J = 6.4 Hz, 4H), 3.32 (s, 2H), 3.08 (p, J = 6.7 Hz, 5H), 2.83 (dd, J = 12.4, 5.1 Hz, 1H), 2.72 (ddd, J = 18.0, 9.5, 7.6 Hz, 1H), 2.62 – 2.56 (m, 1H), 2.42 – 2.27 (m, 4H), 2.05 (t, J = 7.4 Hz, 2H), 1.90 (tt, J = 7.3, 4.1 Hz, 2H), 1.62 (q, J = 6.7 Hz, 4H), 1.55 – 1.43 (m, 3H), 1.37 – 1.24 (m, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 173.4, 172.7, 172.3, 171.9, 171.5, 163.1, 70.2, 70.0, 68.5, 68.5, 61.5, 59.6, 55.9, 49.4, 36.2, 36.1, 35.6, 31.3, 31.2, 31.2, 29.8, 29.8, 28.7, 28.5, 25.7, 24.8. HRMS (ESI) m/z calc. for $[C_{29}H_{48}N_6O_9S + H]^+$ = 657.3276, found 657.3295.

N-(3-(2-(2-(3-aminopropoxy)ethoxy)ethoxy)propyl)-5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamide (**4**). To a mixture of 4,7,10-trioxa-1,13-

tridecanediamine (0.81g, 3.7 mmol) and Et_3N (0.20 ml, 1.4 mmol) was added a solution of **16** (0.15 g, 0.37 mmol) in DMF (6 ml) at 0° C over 1 hour. The mixture was allowed to stir at ambient temperature for 1 hour more and was then concentrated *in vacuo*. The residue was triturated with Et_2O (2 x 7 ml x 2 hours) furnishing 0.17 g of the corresponding mono-protected amine (99%) as a white powder. ^{1}H NMR (400 MHz, CD_3OD) δ 4.53-4.48 (m, 1H), 4.34-4.30 (m, 1H), 3.70-3.58 (m, 13H), 3.54 (t, J=6.0 Hz, 2H), 3.32 (t, J=7.1 Hz, 2H), 3.30-3.20 (m, 1H), 2.98-2.83 (m, 4H), 2.71 (d, J=12.7 Hz, 1H), 2.22 (t, J=7.3 Hz, 1H), 1.88-1.57 (m, 9H), 1.50-1.42 (m, 2H). All other data was in agreement with literature.[54]

4,20-dioxo-24-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)-9,12,15-trioxa-5,19-diazatetracosan-1-oic acid (**5**). A solution of **4** (0.16 g, 0.36 mmol), DIPEA (0.23 ml, 0.168 g, 1.30 mmol) and succinic anhydride (0.036 g, 0.089 mmol) in DMF (3.0 ml) was stirred at ambient temperature overnight. The solvent was removed *in vacuo* and the residue was triturated with a 1:1 $CHCl_3/Et_2O$ mixture (2 x 10ml) for 15 min, the product was then pelleted through centrifugation of the resultant gel. The wet solid was dried *in vacuo* to furnish 0.15 g (76%) of the corresponding acid. ^{1}H NMR (400 MHz, d-DMSO) δ 12.00 (br s, 1H), 7.79 (t, *J*=5.9 Hz, 1H), 7.73 (t, *J*=5.8 Hz, 1H), 6.40 (s, 1H), 6.34 (s, 1H), 4.35-4.28 (m, 1H), 4.16-4.10 (m, 1H), 3.58-3.48 (m, 8H), 3.46 (t, *J*=6.3 Hz, 4H), 3.15-3.03 (m, 5H), 2.84 (dd, *J*=12.5, 5.1 Hz, 1H), 2.31 (t, *J*=6.7 Hz, 2H), 2.05 (t, *J*=6.9 Hz, 2H), 1.65-1.58 (m, 5H), 1.55-1.25 (m, 6H).

Perfluorophenyl 4,20-dioxo-24-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)-9,12,15-trioxa-5,19-diazatetracosan-1-oate (**6**). A solution of the free acid **5** (0.15 g, 0.27 mmol) and DIPEA (0.17 ml, 0.129 g, 1.0 mmol) in DMF (1.5 ml) was cooled to 0° C. Pentafluorophenyl trifluoroacetate (0.11 g, 0.070 ml, 0.41 mmol) was added over 1 minute and the mixture was stirred at 0° C for an additional 30 minutes. The reaction mixture was allowed to

warm to ambient temperature and after 2 hours the solvent was removed *in vacuo* and the residue was triturated with Et_2O (2 x 7 ml) for 30 min, the product was then pelleted through centrifugation of the resultant gel. The wet solid was dried *in vacuo* to afford 0.12 g (54%) of the corresponding pentfluorophenyl-ester. This intermediate was used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.71 (t, *J*=5.9 Hz, 1H), 6.44 (t, *J*=5.8 Hz, 1H), 5.63 (s, 1H), 4.83 (s, 1H), 4.55-4.48 (m, 1H), 4.36-4.30 (m, 1H), 3.70-3.55 (m, 12H), 3.43-3.35 (m, 4H), 3.20-3.15 (m, 1H), 3.05 (t, *J*=6.4 Hz, 2H), 2.93 (dd, *J*=12.7, 4.8 Hz, 1H), 2.72 (d, J=12.7 Hz, 1H), 2.63 (t, *J*=6.8 Hz, 2H), 2.22-2.17 (m, 2H), 1.95-1.65 (m, 10H).

Benzyl 7-(5-((4-(4-(4,20-dioxo-24-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)-9,12,15-trioxa-5,19-diazatetracosan-1-oyl)piperazin-1-yl)phenoxy)methyl)-1,3-dimethyl-1H-pyrazol-4-yl)-1-(2-morpholinoethyl)-3-(3-(naphthalen-1-yloxy)propyl)-1H-indole-2-carboxylate (**7**). A solution of the activated ester **6** (0.016 g, 0.022 mmol), **28** (0.018 g, 0.022 mmol), and DIPEA (0.012 ml, 0.066 mmol) in DMF (1.5 ml) was stirred at ambient temperature overnight. The solvent was removed *in vacuo* and the residue was redissolved in DMF (1.5 ml) and stirred with K_2CO_3 (0.009 g, 0.066 mmol) for 1 hour. The inorganic salts were filtered off, the filtrate was concentrated *in vacuo* and the residue was purified by silica gel flash chromatography (10-15% MeOH in $CHCl_3$) to afford 0.020 g (66%) of the title compound. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (m, 1H), 7.82 (m, 1H), 7.71 (dd, *J* = 8.1, 1.3 Hz, 1H), 7.56 – 7.40 (m, 5H), 7.35 (dddd, *J* = 8.5, 6.8, 4.9, 3.1 Hz, 4H), 7.09 (dd, *J* = 15.1 Hz, 1H), 6.97 (dd, *J* = 7.1, 1.2 Hz, 1H), 6.91 (t, *J* = 5.6 Hz, 1H), 6.87 – 6.72 (m, 5H), 6.68 (dd, *J* = 7.6, 1.0 Hz, 1H), 6.09 (s, 1H), 5.39 – 5.29 (m, 3H), 4.81 (q, *J* = 11.6 Hz, 2H), 4.61 – 4.47 (m, 2H), 4.38 – 4.27 (m, 2H), 4.01 (t, *J* = 6.2 Hz, 2H), 3.96 (s, 3H), 3.73 (t, *J* = 5.1 Hz, 2H), 3.70 – 3.51 (m, 18H), 3.41 – 3.25 (m, 6H), 3.15 (td, *J* = 7.3, 4.5 Hz, 1H), 3.02 (dt, *J* = 15.6, 4.8 Hz, 4H), 2.91 (dd, *J* = 12.8,

5.0 Hz, 1H), 2.82 – 2.64 (m, 3H), 2.62 – 2.49 (m, 2H), 2.26 – 2.07 (m, 13H), 1.82 – 1.75 (m, 4H), 1.74 – 1.61 (m, 4H), 1.46 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.9, 172.2, 170.6, 163.5, 162.3, 154.7, 152.6, 146.6, 146.0, 137.0, 136.0, 135.6, 134.5, 130.2, 128.9, 128.7, 128.5, 127.5, 126.6, 126.3, 125.9, 125.7, 125.5, 125.1, 122.0, 120.5, 120.0, 120.0, 119.3, 118.7, 116.8, 115.7, 104.7, 70.5, 70.5, 70.1, 70.1, 70.0, 69.6, 67.6, 67.0, 66.7, 61.9, 60.1, 59.9, 58.4, 55.6, 53.2, 50.8, 50.6, 45.4, 42.3, 41.8, 40.5, 37.8, 37.5, 36.9, 35.9, 31.2, 30.7, 29.1, 28.9, 28.5, 28.2, 28.1, 25.6, 22.1, 12.4. HRMS (ESI) m/z calc. for $[C_{75}H_{96}N_{10}O_{12}S + H]^+$ = 1361.7003, found 1361.7008.

7-(5-((4-(4-(4,20-dioxo-24-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)-9,12,15-trioxa-5,19-diazatetracosan-1-oyl)piperazin-1-yl)phenoxy)methyl)-1,3-dimethyl-1H-pyrazol-4-yl)-1-(2-morpholinoethyl)-3-(3-(naphthalen-1-yloxy)propyl)-1H-indole-2-carboxylic acid (**8**). Compound **7** (0.017 g, 0.013 mmol) was hydrogenated in DMF (2.5 mL) over 10 % palladium on carbon (0.010 g) for 3 hours. After filtering through a 0.2 μm syringe filter the clear solution was concentrated *in vacuo*. The residue was purified by silica gel flash chromatography (25% MeOH in $CHCl_3$) to afford 0.011 g (70%) of the title compound. ^{1}H NMR (600 MHz, DMSO) δ 8.22 – 8.19 (m, 1H), 7.86 – 7.83 (m, 1H), 7.79 (t, *J* = 5.6 Hz, 1H), 7.74 (t, *J* = 5.6 Hz, 1H), 7.70 (d, *J* = 8.0 Hz, 1H), 7.52 – 7.47 (m, 2H), 7.43 (d, *J* = 8.3 Hz, 1H), 7.36 (t, *J* = 7.9 Hz, 1H), 7.03 (t, *J* = 7.5 Hz, 1H), 6.87 (dd, *J* = 11.3, 7.3 Hz, 2H), 6.84 – 6.76 (m, 4H), 6.41 (t, *J* = 1.9 Hz, 1H), 6.35 (s, 1H), 4.89 – 4.78 (m, 2H), 4.54 (s, 1H), 4.28 (dd, *J* = 7.8, 5.1 Hz, 1H), 4.18 (t, *J* = 6.3 Hz, 3H), 4.11 (ddd, *J* = 7.7, 4.4, 1.8 Hz, 1H), 3.84 (s, 3H), 3.53 (t, *J* = 5.0 Hz, 4H), 3.49 (dd, *J* = 5.9, 3.4 Hz, 4H), 3.45 (dd, *J* = 5.8, 3.5 Hz, 5H), 3.41 (d, *J* = 5.1 Hz, 4H), 3.37 (td, *J* = 6.4, 3.2 Hz, 6H), 3.09 – 3.02 (m, 5H), 2.95 (t, *J* = 5.0 Hz, 2H), 2.88 (t, *J* = 5.1 Hz, 2H), 2.80 (dd, *J* = 12.4, 5.1 Hz, 1H), 2.58 – 2.51 (m, 3H), 2.29 (t, *J* = 7.0 Hz, 2H), 2.20 (p, *J* = 6.4 Hz,

2H), 2.03 (ddt, *J* = 11.4, 8.4, 3.7 Hz, 8H), 1.95 (s, 3H), 1.59 (p, *J* = 6.9 Hz, 6H), 1.47 (m, 3H), 1.33 – 1.21 (m, 2H). HRMS (MALDI) m/z calc. for $[C_{68}H_{90}N_{10}O_{12}S + K]^+$ = 1309.6092, found 1309.6097.

1-(4-((4-bromo-1,3-dimethyl-1H-pyrazol-5-yl)methoxy)phenyl)piperazine (**10**). A solution of tert-butyl 4-(4-((4-bromo-1,3-dimethyl-1H-pyrazol-5-yl)methoxy)phenyl)piperazine-1-carboxylate (1.25 g, 2.70 mol) in DCM (25 mL) was cooled to 0° C and trifluoroacetic acid (5.0 mL, 7.66 g, 67 mmol) was added over 1 minute and the mixture was allowed to stir at ambient temperature for 2 hours. The reaction mixture was concentrated *in vacuo*, redissolved in DCM (40 ml), washed with a $NaHCO_3$ solution (*aq*, sat, 10 ml), brine, and dried over Na_2SO_4. The organic extract was concentrated *in vacuo* to furnish 1.01 g (99%) of the title compound and was used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.96 – 6.89 (m, 4H), 5.00 (s, 2H), 3.89 (s, 3H), 3.09 (s, 8H), 2.81 (s, 1H), 2.24 (s, 3H).

4-(4-((4-bromo-1,3-dimethyl-1H-pyrazol-5-yl)methoxy)phenyl)-N,N-dimethylpiperazine-1-sulfonamide (**11**). To a solution of amine **10** (0.900 g, 2.46 mmol) and triethylamine (3.77 mL, 2.74 g, 27 mmol) in THF (90 mL) was added *N,N,*-dimethylsulfamoyl chloride (1.32 mL, 1.77 g, 12.3 mmol) and was the mixture was stirred at ambient temperature for 3 hours. The reaction mixture was partitioned between EtOAc (700 mL) and a saturated solution of $NaHCO_3$ (350 mL) The organic layer was washed with brine, dried with Na_2SO_4, and then concentrated *in vacuo*. The residue was purified by automated silica gel flash chromatography with 5% MeOH in $CHCl_3$ to afford 0.88 g (77%) of the title compound as a yellow-white foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.98 – 6.87 (m, 4H), 5.00 (s, 2H), 3.88 (s, 3H), 3.45 – 3.36 (m, 4H), 3.17 – 3.11 (m, 4H), 2.88 (s, 6H), 2.24 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 152.4, 146.0, 145.9, 135.8, 118.8,

115.8, 95.7, 77.2, 59.8, 50.5, 46.4, 38.3, 37.6, 12.1. HRMS (EI) m/z calc. for [$C_{18}H_{26}BrN_5O_3S$] = 471.0940, found 471.0928.

Ethyl 7-(5-((4-(4-(N,N-dimethylsulfamoyl)piperazin-1-yl)phenoxy)methyl)-1,3-dimethyl-1H-pyrazol-4-yl)-3-(3-(naphthalen-1-yloxy)propyl)-1H-indole-2-carboxylate (**12**). A mixture of the boronic ester **9** (0.73 g, 1.45 mmol), bromide **11** (0.60 g, 1.26 mmol), Cs_2CO_3 (1.03 g, 3.12 mmol) and Pd(dppf)Cl_2 (0.209 g, 0.26 mmol) in DMF (12 ml) was stirred at 99° C for 4 hours. Solvent was removed *in vacuo* and the residue was diluted with $CHCl_3$ (15 ml) and stirred at ambient temperature for 15 minutes. The inorganic salts were filtered off, the filtrate was concentrated *in vacuo* and the residue was purified twice consecutively by silica gel flash chromatography (EtOAc and 2.5% MeOH in $CHCl_3$ respectively) to afford 0.75 g (52%) of the title compound. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.88 (s, 1H), 8.35 – 8.30 (m, 1H), 7.81 (dd, *J* = 6.8, 2.5 Hz, 1H), 7.72 (dt, *J* = 7.7, 3.6 Hz, 1H), 7.54 – 7.44 (m, 2H), 7.42 (d, *J* = 8.3 Hz, 1H), 7.35 (dd, *J* = 8.3, 7.5 Hz, 1H), 7.18 – 7.14 (m, 2H), 6.81 – 6.74 (m, 5H), 4.79 (s, 2H), 4.29 (s, 1H), 4.23 (t, *J* = 6.2 Hz, 3H), 3.97 (s, 3H), 3.43 (dd, *J* = 8.2, 6.7 Hz, 2H), 3.40 – 3.35 (m, 4H), 3.10 – 3.04 (m, 4H), 2.86 (s, 6H), 2.37 (p, *J* = 6.5 Hz, 2H), 2.23 (s, 3H), 1.27 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.9, 154.8, 152.5, 146.1, 146.0, 135.7, 135.2, 134.5, 128.3, 127.5, 127.1, 126.3, 125.9, 125.8, 125.1, 124.3, 123.7, 122.1, 120.4, 120.0, 120.0, 118.6, 117.1, 116.9, 116.2, 104.6, 77.3, 67.7, 60.6, 60.1, 50.4, 46.4, 38.3, 36.7, 30.5, 21.6, 14.4, 12.4. HRMS (ESI) m/s calc. for $[C_{42}H_{48}N_6O_6S + H]^+$ = 765.3429, found 765.3418.

Ethyl 1-(2-(4-benzylpiperazin-1-yl)ethyl)-7-(5-((4-(4-(N,N-dimethylsulfamoyl)piperazin-1-yl)phenoxy)methyl)-1,3-dimethyl-1H-pyrazol-4-yl)-3-(3-(naphthalen-1-yloxy)propyl)-1H-indole-2-carboxylate (**13**). A mixture of indole **12** (0.65 g, 0.85 mmol), 1-benzyl-4-(2-chloroethyl)piperazine dichloride (0.44 g, 1.41 mmol), Cs_2CO_3 (1.70 g, 5.23 mmol) and TBAI

(0.074 g, 0.20 mmol) in DMF (20 ml) was stirred at 80° C for 48 hours. Solvent was removed *in vacuo* and the residue was diluted with $CHCl_3$ (15 ml) and stirred at ambient temperature for 15 minutes. The inorganic salts were filtered off, the filtrate was concentrated *in vacuo* and the residue was purified twice consecutively by silica gel flash chromatography (5% MeOH in $CHCl_3$ and 20% THF in EtOAc respectively) to afford 0.38 g of the starting material **12** and 0.28 g (80% BRSM) of the title compound. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.41 – 8.36 (m, 1H), 7.85 – 7.80 (m, 1H), 7.73 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.51 (tt, *J* = 6.6, 3.2 Hz, 2H), 7.44 (d, *J* = 8.2 Hz, 1H), 7.36 (dd, *J* = 8.3, 7.5 Hz, 1H), 7.33 – 7.22 (m, 6H), 7.09 (dd, *J* = 8.0, 7.1 Hz, 1H), 6.96 (dd, *J* = 7.1, 1.2 Hz, 1H), 6.85 – 6.76 (m, 5H), 4.88 – 4.75 (m, 2H), 4.54 (ddd, *J* = 14.2, 8.5, 5.9 Hz, 1H), 4.40 – 4.28 (m, 3H), 4.24 (t, *J* = 6.2 Hz, 2H), 3.94 (s, 3H), 3.46 (s, 2H), 3.42 – 3.33 (m, 6H), 3.11 – 3.05 (m, 4H), 2.88 (s, 6H), 2.36 (q, *J* = 6.9 Hz, 6H), 2.21 (t, *J* = 5.7 Hz, 5H), 2.13 (s, 3H), 1.40 (t, *J* = 7.1 Hz, 3H). HRMS (ESI) m/s calc. for $[C_{55}H_{66}N_8O_6S + H]^+$ = 967.4899, found 967.4905.

Ethyl 7-(5-((4-(4-(N,N-dimethylsulfamoyl)piperazin-1-yl)phenoxy)methyl)-1,3-dimethyl-1H-pyrazol-4-yl)-3-(3-(naphthalen-1-yloxy)propyl)-1-(2-(piperazin-1-yl)ethyl)-1H-indole-2-carboxylate (**14**). A suspension of **13** (0.216 g, 0.25 mmol) and 10% palladium on carbon (10 mol%) in DMF (3.5 mL) was stirred under a hydrogen atmosphere at ambient temperature for 15 hours, after which an additional 10 mol% palladium on carbon was added and stirred for another 10 hours. The solvent was removed and the resultant black solid was redissolved in chloroform, vacuum filtered, passed through a 0.45 µm syringe filter, and concentrated *in vacuo* to afford 0.208 g (quantitative) of the dark grey solid which was used without further purification. ^{1}H NMR (400 MHz, MeOD) δ 8.26 – 8.22 (m, 1H), 7.83 – 7.78 (m, 2H), 7.50 – 7.44 (m, 2H), 7.44 – 7.39 (m, 1H), 7.35 – 7.30 (m, 1H), 7.10 (dd, *J* = 8.0, 7.1 Hz, 1H), 6.98 (dd, *J* = 7.1, 1.1 Hz, 1H),

6.83 – 6.80 (m, 2H), 6.78 (dd, *J* = 7.7, 1.0 Hz, 1H), 6.73 – 6.68 (m, 2H), 4.92 – 4.86 (m, 2H), 4.45 (d, *J* = 14.4 Hz, 1H), 4.36 – 4.28 (m, 3H), 4.20 (t, *J* = 6.0 Hz, 2H), 3.98 (s, 3H), 3.43 (td, *J* = 7.1, 2.7 Hz, 2H), 3.32 – 3.28 (m, 4H), 3.08 – 3.02 (m, 7H), 3.01 (s, 6H), 2.39 – 2.30 (m, 6H), 2.29 – 2.13 (m, 3H), 2.11 (s, 3H), 1.37 (t, *J* = 7.1 Hz, 3H). HRMS (ESI) m/s calc. for $[C_{48}H_{60}N_8O_6S + H]^+$ = 877.4429, found 877.4420.

7-(5-((4-(4-(N,N-dimethylsulfamoyl)piperazin-1-yl)phenoxy)methyl)-1,3-dimethyl-1H-pyrazol-4-yl)-3-(3-(naphthalen-1-yloxy)propyl)-1-(2-(piperazin-1-yl)ethyl)-1H-indole-2-carboxylic acid (**15**). To a solution of free amine **14** (0.208 g, 0.24 mmol) in THF (1.9 mL) and MeOH (1.9 mL) was added a solution of LiOH (0.0076 g, 0.32 mmol) in MeOH (1.0 mL). The mixture was stirred at 66° C overnight. The reaction was quenched with a solution of NH_4Cl (0.045 g) in H_2O (0.1 mL), and was stirred at ambient temperature for 1 hour. The mixture was neutralized with dropwise additions of 0.13 M HCl. Solvent was removed *in vacuo* and the residue was triturated with $CHCl_3$. The inorganic salts were filtered off, the filtrate was concentrated *in vacuo* to afford 0.199 g (99%) of the title compound which was used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (d, *J* = 7.9 Hz, 1H), 7.73 (dd, *J* = 24.0, 7.8 Hz, 2H), 7.47 – 7.31 (m, 3H), 7.28 (d, *J* = 6.9 Hz, 1H), 7.09 (t, *J* = 7.6 Hz, 1H), 6.90 (d, *J* = 7.1 Hz, 1H), 6.71 (s, 5H), 4.86 – 4.62 (m, 3H), 4.36 (s, 1H), 4.17 (s, 2H), 3.91 (s, 3H), 3.42 – 3.25 (m, 6H), 2.98 (s, 4H), 2.85 (s, 9H), 2.52 – 2.21 (m, 8H), 2.10 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 154.8, 152.4, 146.7, 145.9, 136.0, 134.4, 129.2, 128.4, 127.4, 126.3, 126.0, 125.7, 125.0, 122.1, 120.0, 119.8, 119.6, 119.4, 118.6, 115.7, 115.5, 104.7, 67.9, 59.8, 57.5, 50.3, 46.3, 43.4, 38.2, 36.9, 30.7, 21.4, 12.5. HRMS (ESI) m/z calc. for $[C_{46}H_{56}N_8O_6S + H]^+$ = 849.4116, found 849.4137.

Perfluorophenyl 5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanoate (**16**). To a suspension of biotin (0.057 g, 0.23 mmol) in DMF (2 mL), was added Et_3N (0.073 g, 0.10 mL, 0.73 mmol) under stirring at ambient temperature. Pentafluorophenyl trifluoroacetate (0.078 g, 0.48 mL, 0.28 mmol) was added to the transparent solution over 1 minute and the reaction mixture was stirred for 2 hours. The mixture was concentrated *in vacuo*, and purified by trituration with diethyl ether to afford 0.059 g (64%) of the title compound as a white powder. ^{1}H NMR (400 MHz, d-DMSO) δ ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.90 (s, 1H), 4.65 (s, 1H), 4.56 (ddt, *J* = 7.8, 5.1, 1.3 Hz, 1H), 4.39 – 4.33 (m, 1H), 3.22 (dt, *J* = 8.8, 5.6 Hz, 1H), 2.98 (dd, *J* = 12.9, 5.1 Hz, 1H), 2.77 (d, *J* = 13.0 Hz, 1H), 2.73 (t, *J* = 7.3 Hz, 2H), 1.93 – 1.68 (m, 3H), 1.72 – 1.48 (m, 1H). All other data was in agreement with literature.[55]

7-(5-((4-(4-(N,N-dimethylsulfamoyl)piperazin-1-yl)phenoxy)methyl)-1,3-dimethyl-1H-pyrazol-4-yl)-3-(3-(naphthalen-1-yloxy)propyl)-1-(2-(4-(5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanoyl)piperazin-1-yl)ethyl)-1H-indole-2-carboxylic acid (**17**). A solution of **15** (0.021 g, 0.025 mmol), DIPEA (0.01 ml, 0.007 g, 0.057 mmol) and **16** (0.099 g, 0.024 mmol) in DMF (1.0 ml) was stirred at ambient temperature overnight. The mixture was concentrated *in vacuo* and the residue was purified by silica gel flash chromatography (7.5-25% MeOH in 1:1 $CHCl_3$:DCM) to afford 0.016 g (62%) of the title compound. ^{1}H NMR (400 MHz, 15% MeOD in $CDCl_3$) δ 8.20 – 8.11 (m, 1H), 7.66 – 7.56 (m, 2H), 7.35 – 7.27 (m, 2H), 7.24 (t, *J* = 4.1 Hz, 1H), 7.17 (t, *J* = 7.9 Hz, 1H), 6.98 – 6.91 (m, 1H), 6.78 (dd, *J* = 7.2, 1.2 Hz, 1H), 6.69 – 6.54 (m, 5H), 4.72 – 4.61 (m, 2H), 4.38 (d, *J* = 12.8 Hz, 1H), 4.33 (br, s, 1H), 4.22 – 4.15 (m, 1H), 4.12 (dd, *J* = 7.9, 4.6 Hz, 1H), 4.06 (t, *J* = 6.3 Hz, 2H), 3.86 (s, 7H), 3.79 (s, 3H), 3.38 (br, s, 1H), 3.31 (br, s, 1H), 3.03 – 2.94 (m, 1H), 2.94 – 2.85 (m, 4H), 2.74 (ddd, *J* = 12.8, 5.1, 1.3 Hz, 1H), 2.69 (s, 6H), 2.56 (d, *J* = 12.8 Hz, 1H), 2.11 (m, 8H),

1.95 (s, 3H), 1.59 – 1.31 (m, 5H), 1.22 (p, J = 7.6 Hz, 2H). ^{13}C NMR (101 MHz, 15% MeOD in $CDCl_3$) δ 172.1, 155.1, 152.8, 146.3, 136.7, 136.5, 134.8, 130.0, 129.5, 127.7, 126.6, 126.2, 126.1, 125.4, 122.3, 120.9, 120.3, 119.7, 119.2, 116.4, 115.9, 105.0, 68.2, 62.2, 60.5, 60.1, 58.0, 55.8, 53.3, 52.9, 50.7, 46.6, 45.1, 42.3, 41.1, 40.6, 38.4, 37.0, 32.7, 30.9, 28.8, 28.8, 28.5, 25.2, 21.9, 12.3. HRMS (ESI) m/z calc. for $[C_{56}H_{70}N_{10}O_8S2 + H]^+$ = 1075.4892, found 1075.4873.

4-Hydroxythalidomide (**18**). A solution of 3-hydroxyphthalic anhydride (0.50 g, 3.03 mmol), 3-aminopiperidine-2,6-dione hydrochloride (0.50g, 3.05 mmol) and Et_3N (0.70 ml, 9.70 mmol) in DMF (10 ml) was stirred under reflux for 4 hours. After adding DCC (0.75 g, 3.60 mmol) the reaction mixture was refluxed for an additional 72 hours, cooled to ambient temperature, filtered, and concentrated *in vacuo*. The residue was purified by silica gel flash chromatography (10% MeOH in $CHCl_3$) to afford 1.15 g of a white powder consisting of the target product and corresponding urea. It was recrystallized from $CHCl_3$ to give 0.62 g (83% yield) of the title compound. ^{1}H NMR (400 MHz, DMSO) δ 11.16 (s, 1H), 11.08 (s, 1H), 7.66 (dd, J = 8.4, 7.2 Hz, 1H), 7.33 (d, J = 7.2 Hz, 1H), 7.26 (d, J = 8.3 Hz, 1H), 5.08 (dd, J = 12.9, 5.4 Hz, 1H), 2.89 (ddd, J = 17.4, 14.0, 5.4 Hz, 1H), 2.64 – 2.53 (m, 2H), 2.07 – 1.99 (m, 1H). All other data was in agreement with literature.[56]

Benzyl 2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetate (**19**). A suspension of phenol **18** (0.71 g, 2.86 mmol), Ph_3P (1.12 g, 4.29 mmol), and benzyl glycolate (0.50 g, 3.0 mmol) in THF (30 ml) was cooled to 0° C and DBAD (0.69 g, 3.0 mmol) was added in portions over 1 minute. After 5 minutes the mixture was allowed to warm to ambient temperature and was stirred overnight. The mixture was quenched with water (25 ml) and diluted with EtOAc (15 ml). After stirring for 15 minutes the layers were separated and the aqueous layer was extracted with EtOAc (3 x 30 ml) and $CHCl_3$ (30 ml). The combined organic extracts

were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel flash chromatography (EtOAc/Hex 1:1 - 2:1) to afford 0.96 g (79%) of the title compound. ^{1}H NMR (402 MHz, $CDCl_3$) δ 8.04 (s, 1H), 7.63 (dd, *J* = 8.4, 7.3 Hz, 1H), 7.53 (dd, *J* = 7.4, 0.7 Hz, 1H), 7.44 – 7.30 (m, 5H), 7.09 (dd, *J* = 8.4, 0.7 Hz, 1H), 5.25 (s, 2H), 5.01 – 4.92 (m, 3H), 2.96 – 2.69 (m, 3H), 2.20 – 2.09 (m, 1H). All other data was in agreement with literature.[56]

2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetic acid (**20**). Compoiund **19** (0.26 g, 0.62 mmol) was hydrogenated in DMF (12 mL) over 10 % palladium on carbon (0.020 mg) for 1.5 hours. After filtering through a 0.2 µm syringe filter the clear solution was concentrated *in vacuo* to furnish 0.24 g (99%) of the title compound (containing some amount of DMF). ^{1}H NMR (400 MHz, DMSO) δ 13.23 (s, 1H), 11.10 (s, 1H), 7.80 (dd, *J* = 8.5, 7.3 Hz, 1H), 7.48 (d, *J* = 7.2 Hz, 1H), 7.40 (d, *J* = 8.6 Hz, 1H), 5.11 (dd, *J* = 12.9, 5.4 Hz, 1H), 4.99 (s, 2H), 2.69 – 2.54 (m, 3H), 2.10 – 2.01 (m, 1H). All other data was in agreement with literature.[56]

Perfluorophenyl 2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetate (**21**). To a solution of acid **20** (0.148 g, 0.45 mmol) in DMF (6 mL), was added DIPEA (0.118 g, 0.16 mL, 0.92 mmol) with stirring and the solution was cooled to 0 °C. Pentafluorophenyl trifluoroacetate (0.187 g, 0.12 mL, 0.67 mmol) was then added. The reaction mixture was allowed to come to ambient temperature for 2 h. The mixture was concentrated *in vacuo*, and purified by trituration in diethyl ether to afford 0.175 g (79 %) of the title compound as a white powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (s, 1H), 7.76 (dd, *J* = 8.4, 7.3 Hz, 1H), 7.63 (dd, *J* = 7.4, 0.7 Hz, 1H), 7.26 (dd, *J* = 8.5, 0.8 Hz, 1H), 5.34 (d, *J* = 1.4 Hz, 2H), 5.04 – 4.97 (m, 1H), 2.98 – 2.74 (m, 3H), 2.22 – 2.14 (m, 1H). HRMS (ESI) m/z calc. for $[C_{21}H_{11}F_5N_2O_7 + Na]^+$ = 521.0379, found 521.0368. All other data was in agreement with literature.[46]

7-(5-((4-(4-(N,N-dimethylsulfamoyl)piperazin-1-yl)phenoxy)methyl)-1,3-dimethyl-1H-pyrazol-4-yl)-1-(2-(4-(1-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)-2,18-dioxo-7,10,13-trioxa-3,17-diazahenicosan-21-oyl)piperazin-1-yl)ethyl)-3-(3-(naphthalen-1-yloxy)propyl)-1H-indole-2-carboxylic acid (dMCL1-1). To a solution of the liberated acid **15** (0.034 g, 0.040 mmol) in DMF (2 mL) was added DIPEA (0.03 mL, 0.04 g, 0.313 mmol) and perfluorophenyl 1-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)-2,17-dioxo-7,10,13-trioxa-3,16-diazaicosan-20-oate (0.035 g, 0.044 mmol)[44] and was allowed to stir at ambient temperature overnight. The reaction mixture was concentrated *in vacuo* and the residue was purified by silica gel flash chromatography (5%-20% MeOH in $CHCl_3$) to afford 0.041 g (69%) of the title compound as a colorless oil. ^{1}H NMR (400 MHz, 15% CD_3OD in $CDCl_3$) δ 9.62 (s, 1H), 8.36 – 8.28 (m, 1H), 7.81 – 7.77 (m, 1H), 7.73 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.69 (dd, *J* = 8.4, 7.3 Hz, 1H), 7.59 (t, *J* = 5.8 Hz, 1H), 7.51 (d, *J* = 7.4 Hz, 1H), 7.49 – 7.44 (m, 2H), 7.40 (d, *J* = 8.3 Hz, 1H), 7.33 (t, *J* = 7.9 Hz, 1H), 7.17 (d, *J* = 8.4 Hz, 1H), 7.11 (t, *J* = 7.6 Hz, 1H), 6.94 (dd, *J* = 7.2, 1.2 Hz, 1H), 6.83 – 6.68 (m, 5H), 6.55 (t, *J* = 5.4 Hz, 1H), 5.00 (ddd, *J* = 9.9, 5.4, 2.1 Hz, 1H), 4.94 – 4.77 (m, 3H), 4.64 (s, 2H), 4.39 (m, 1H), 4.25 – 4.16 (m, 2H), 3.96 (s, 3H), 3.63 (dq, *J* = 9.1, 4.2 Hz, 7H), 3.58 (tt, *J* = 5.8, 2.3 Hz, 5H), 3.54 – 3.44 (m, 5H), 3.37 (dt, *J* = 9.5, 5.1 Hz, 8H), 3.28 (p, *J* = 5.8, 4.8 Hz, 2H), 3.06 (dd, *J* = 6.3, 3.7 Hz, 4H), 2.85 (s, 6H), 2.83 – 2.72 (m, 3H), 2.52 (s, 1H), 2.46 – 2.19 (m, 11H), 2.13 (s, 4H), 1.87 (p, *J* = 6.4 Hz, 2H), 1.72 (p, *J* = 6.1 Hz, 2H). ^{13}C NMR (101 MHz, 15% CD_3OD in $CDCl_3$) δ 172.0, 171.6, 170.3, 168.6, 166.9, 166.6, 166.0, 165.1, 154.8, 154.6, 152.3, 146.8, 146.1, 137.0, 136.0, 135.9, 134.5, 133.6, 130.2, 129.4, 129.2, 127.4, 126.3, 126.0, 125.8, 125.1, 123.6, 122.1, 120.5, 120.0, 119.8, 119.3, 118.7, 118.1, 117.3, 116.2, 115.6, 104.9, 70.4, 70.1, 70.0, 69.5, 68.8, 68.3, 67.9, 59.8, 56.4, 52.1, 51.8, 50.3, 49.3, 46.3, 43.7, 41.3, 40.2, 38.3, 37.6, 37.0, 36.6, 31.4, 31.1, 30.8, 29.3, 28.9, 28.2,

22.7, 21.7, 12.5. HRMS (ESI) m/z calc. for $[C_{75}H_{92}N_{12}O_{17}\ S+\ Na]^+$ = 1487.6316, found 1487.6327.

7-(5-((4-(4-(N,N-dimethylsulfamoyl)piperazin-1-yl)phenoxy)methyl)-1,3-dimethyl-1H-pyrazol-4-yl)-1-(2-(4-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetyl)piperazin-1-yl)ethyl)-3-(3-(naphthalen-1-yloxy)propyl)-1H-indole-2-carboxylic acid (**dMCL1-2**). To a solution of the liberated acid **15** (0.05 g, 0.059 mmol) in DMF (1 mL) was added DIPEA (0.05 mL, 0.3 mmol) and **21** (0.033 g, 0.065 mmol) and was allowed to stir at ambient temperature overnight. The reaction mixture was purified by silica gel chromatography (5% - 25% MeOH:$CHCl_3$) to afford 0.029 g (43%) of the title compound. A portion of this product (0.011 g) was subsequently purified using HPLC Method A to yield the title compound (0.003 g) as a yellow oil before biological evaluation. ^{1}H NMR (600 MHz, 15% CD_3OD in $CDCl_3$) δ 8.30 (dt, *J* = 7.0, 3.0 Hz, 1H), 7.76 – 7.71 (m, 2H), 7.53 (td, *J* = 7.8, 3.8 Hz, 1H), 7.46 – 7.40 (m, 3H), 7.36 (dd, *J* = 8.3, 2.5 Hz, 1H), 7.32 – 7.27 (m, 1H), 7.11 (t, *J* = 7.6 Hz, 1H), 7.00 (dd, *J* = 29.2, 8.4 Hz, 1H), 6.92 (dd, *J* = 7.1, 2.6 Hz, 1H), 6.73 (dd, *J* = 33.4, 8.2 Hz, 5H), 4.95 – 4.82 (m, 3H), 4.78 (dd, *J* = 11.6, 8.3 Hz, 1H), 4.63 (d, *J* = 38.6 Hz, 2H), 4.38 (s, 1H), 4.18 (t, *J* = 6.3 Hz, 2H), 3.94 (d, *J* = 9.9 Hz, 3H), 3.76 (d, *J* = 60.6 Hz, 1H), 3.52 (s, 3H), 3.41 (d, *J* = 11.1 Hz, 1H), 3.38 – 3.27 (m, 5H), 3.01 (dt, *J* = 8.0, 3.9 Hz, 4H), 2.83 (t, *J* = 1.2 Hz, 6H), 2.81 – 2.74 (m, 1H), 2.70 (d, *J* = 15.4 Hz, 2H), 2.56 (s, 2H), 2.40 (s, 2H), 2.37 – 2.28 (m, 4H), 2.12 (s, 3H), 2.02 (d, *J* = 12.1 Hz, 1H). ^{13}C NMR (151 MHz, 15% CD_3OD in $CDCl_3$) δ 171.9, 171.8, 168.8, 168.8, 166.7, 165.6, 165.6, 165.4, 165.3, 164.9, 164.8, 154.9, 154.9, 154.7, 152.3, 152.1, 146.9, 146.0, 145.9, 136.5, 136.5, 136.1, 136.0, 134.4, 133.6, 130.0, 129.5, 129.1, 129.1, 127.4, 126.4, 126.0, 126.0, 125.6, 125.1, 123.9, 122.0, 122.0, 120.5, 120.0, 119.7, 119.2, 119.2, 118.7, 118.6, 117.4, 117.3, 117.0, 116.8, 116.2, 116.1, 115.7, 115.6, 104.9, 67.9, 67.4, 60.0, 59.8,

56.4, 56.3, 52.3, 51.2, 50.3, 50.1, 49.2, 49.2, 46.3, 46.2, 43.5, 43.5, 41.1, 40.6, 38.2, 37.0, 37.0, 31.3, 30.7, 22.5, 21.7, 12.4. HRMS (ESI) m/z calc. for $[C_{61}H_{66}N_{10}O_{12}S+ H]^+$ = 1163.4655, found 1163.4665.

Dimethyl 3-hydroxyphthalate (**22**). To a solution of phthalic anhydride (0.548 g, 3.34 mmol) in methanol (15 mL) was added a solution of para-toluenesulfonic acid (0.0062g, 1.0 wt%) in methanol (0.2 mL) and refluxed at 65°C for 8 hours. The mixture was concentrated in vacuo to half its volume before being neutralized with triethylamine and cooled to 0°C. A cold ethereal solution of *N*-mitroso-*N*-methylurea (NMU) (0.503 g, 4.9 mmol) was carefully added to the phthalate solution and allowed to stir for 5 minutes at 0°C. The solvent was removed in vacuo and the residue was purified by silica gel flash chromatography (5% MeOH in $CHCl_3$) to afford 0.402 g (57%) of the title compound. ^{1}H NMR (402 MHz, $CDCl_3$) δ 10.57 (s, 1H), 7.47 (dd, J = 8.5, 7.4 Hz, 1H), 7.10 (dd, J = 8.5, 1.2 Hz, 1H), 6.97 (dd, J = 7.4, 1.2 Hz, 1H), 3.94 (s, 3H), 3.90 (s, 3H). All other data was in agreement with literature.[57]

Benzyl (4-aminobutyl)carbamate (**23**). 1,4-diamino butane (7.29 g, 83 mmol) and benzyl chloroformate (2.82 g, 2.33 ml, 16.6 mmol) in chloroform (85 ml) were reacted to afford 2.6 g (54%) of the title compound following literature procedure.[51] ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 – 7.30 (m, 5H), 5.12 (s, 2H), 4.98 (s, 1H), 3.24 (q, *J* = 6.5 Hz, 2H), 2.74 (t, *J* = 6.5 Hz, 2H), 1.58 (p, *J* = 6.7 Hz, 2H), 1.53 – 1.45 (m, 2H). All other data was in agreement with literature.[58]

Benzyl (4-(2-chloroacetamido)butyl)carbamate (**24**). To a solution of **23** (2.6 g, 11.8 mmol) and Et_3N (2.5 ml, 17.6 mmol) in DCM (100 ml) was added a solution of chloroacetyl chloride (1.6 g, 1.13 ml, 14.2 mmol) in DCM (20 ml) over 1 hour at 0°C and the mixture was stirred at 0°C for 1 hour more. The reaction mixture was then warmed to ambient temperature and stirred

overnight. The resultant inorganic salt was filtered and the filtrate was consecutively washed with a citric acid solution (aq, sat, 2x20 ml) and water (2 x 20 ml), dried over K_2CO_3 and concentrated in vacuo. The dark brown solid was purified by silica gel flash chromatography (5% MeOH in $CHCl_3$) to afford 2.70 g (77%) of the title compound. ^{1}H NMR (402 MHz, $CDCl_3$) δ 7.42 – 7.29 (m, 5H), 6.64 (s, 1H), 5.11 (s, 2H), 4.85 (s, 1H), 4.05 (s, 2H), 3.34 (q, J = 6.4 Hz, 2H), 3.24 (q, J = 6.3 Hz, 2H), 1.61 – 1.53 (m, 4H). All other data was in agreement with literature.[58]

Benzyl (4-(2-((1,3-dioxo-1,3-dihydroisobenzofuran-4-yl)oxy)acetamido)butyl)carbamate (**25**). To a solution of **24** (1.06 g, 3.54 mmol) in MeCN (25 ml) was added **22** (0.74 g, 3.54 mmol) followed by Cs_2CO_3 (2.90 g, 8.85 mmol) and the mixture was stirred at 80°C overnight. After cooling to ambient temperature, the orange solution was decanted, and the resultant white powder was consecutively washed with acetonitrile (35 ml) and $CHCl_3$ (35 ml) and the combined organic extracts was concentrated in vacuo. The residue was purified by silica gel flash chromatography (5 % MeOH in $CHCl_3$) to afford 0.93 g (58%) of the title compound. ^{1}H NMR (400 MHz, $CDCl_3$) 7.65 (d, J = 7.3 Hz, 1H), 7.47 (dd, J = 8.4, 7.3 Hz, 1H), 7.42-7.30 (m, 5H), 7.14 (d, J = 8.3 Hz, 1H), 7.07 (br s, 1H), 5.10 (s, 2H), 4.87 (br s, 1H), 4.61 (s, 2H), 3.93 (s, 3H), 3.90 (s, 3H), 3.38 – 3.30 (m, 2H), 3.25-3.15 (m, 2H), 1.65-1.45 (m, 4H).

To a solution of the product (0.93 g, 1.97 mmol) in EtOH (25 ml) was added a solution of NaOH (0.24 g, 6.0 mmol) in water (1.8 ml) and the mixture was stirred under reflux for 2 hours. After cooling to ambient temperature, the solvents were removed in vacuo, the residue was diluted with H_2O (30 ml), acidified with 1.3 N HCl (*aq*) to pH 3-4 and extracted with $CHCl_3$ (3x30ml). The combined organic extracts were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. This free diacid was heated in Ac_2O (30 ml) at 111ºC for 1 hour. The

solution was concentrated in vacuo and the residue was purified by silica gel flash chromatography (10% THF in EtOAc) to afford 0.40 g (48 %) of the corresponding anhydride. ^{1}H NMR (400 MHz, $CDCl_3$) 7.89 (t, J = 7.8 Hz, 1H), 7.66 (d, J = 7.6 Hz, 1H), 7.40-7.25 (m, 6H), 7.23 (br s, 1H), 5.10 (s, 2H), 4.89 (br s, 1H), 4.68 (s, 2H), 3.44 – 3.39 (m, 2H), 3.30-3.22 (m, 2H), 1.67-1.60 (m, 4H).

Benzyl (4-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamido)butyl)carbamate (**26**). A mixture of anhydride **25** (0.40 g, 0.94 mmol), 3-amino-piperidine-2,6-dione hydrochloride (0.18 g, 1.08 mmol), and Et_3N (0.2 ml, 1.5 mmol) in THF (15 ml) was stirred under reflux for 3 hours. After adding CDI (0.17 g, 1.04 mmol) the reaction mixture was refluxed overnight, cooled to ambient temperature and concentrated *in vacuo*. The residue was purified by silica gel flash chromatography (5% MeOH in $CHCl_3$) to afford 0.49 g (96%) of the title compound. ^{1}H NMR (400 MHz, MeOD) δ 7.91 (s, 1H), 7.82 (dd, J = 8.5, 7.4 Hz, 1H), 7.56 (dd, J = 7.4, 0.6 Hz, 1H), 7.45 (dd, J = 8.5, 0.7 Hz, 1H), 7.39 – 7.26 (m, 4H), 5.15 (dd, J = 12.5, 5.5 Hz, 1H), 5.07 (s, 2H), 4.78 (d, J = 3.7 Hz, 2H), 3.16 (t, J = 6.6 Hz, 2H), 2.87 (ddd, J = 18.8, 14.3, 5.0 Hz, 1H), 2.81 – 2.66 (m, 2H), 2.19 – 2.08 (m, 1H), 1.60 (dddd, J = 9.6, 7.1, 5.2, 2.9 Hz, 2H). ^{13}C NMR (101 MHz, MeOD) δ 173.1, 169.9, 168.5, 166.9, 166.5 – 166.3, 161.9, 154.9, 137.1, 136.8, 133.5, 128.0, 127.5, 127.3, 120.5, 118.0, 116.6, 78.0, 73.2, 68.1, 65.9, 49.2, 40.0, 38.4, 30.8, 26.8, 26.1, 22.2. HRMS (ESI) m/z calc. for $[C_{27}H_{28}N_4O_8 + H]^+$ = 537.1980, found 537.1981.

N-(4-aminobutyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide (**27**). **26** (0.15 g, 0.27 mmol) was hydrogenated over 10% $Pd(OH)_2$/C (30 mg) in DMF (6 ml) overnight. 10 mg of the catalyst was added and the hydrogenation was continued for 8 hours more. The reaction mixture was filtered and the solvent was removed *in vacuo* by short path

Kugelrohr distillation. The free amine (0.12 g, quantitative, contains some DMF) was used for next step without additional purification. ^{1}H NMR (400 MHz, MeOD) δ 7.84 (dd, *J* = 8.5, 7.4 Hz, 1H), 7.56 (dd, *J* = 7.4, 0.6 Hz, 1H), 7.47 (dd, *J* = 8.5, 0.7 Hz, 1H), 5.15 (dd, *J* = 12.5, 5.5 Hz, 1H), 4.78 (s, 2H), 3.40 (t, *J* = 6.4 Hz, 2H), 2.97 (t, *J* = 6.6 Hz, 2H), 2.92-2.70 (m, 3H), 2.22 – 2.12 (m, 1H), 1.80-1.63 (m, 4H).

7-(5-((4-(4-(N,N-dimethylsulfamoyl)piperazin-1-yl)phenoxy)methyl)-1,3-dimethyl-1H-pyrazol-4-yl)-N-(4-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamido)butyl)-1-(2-morpholinoethyl)-3-(3-(naphthalen-1-yloxy)propyl)-1H-indole-2-carboxamide (**dMCL1-3**). A solution of **A-1210477** (0.012 g, 0.0142 mmol) and N,N'-dicyclohexylcarbodiimide (DCC) (0.004 g, 0.0171 mmol) in DMF (1.5 ml) was stirred at ambient temperature for 30 minutes. A solution of pentafluorophenol (0.009 g, 0.0171 mmol) in DMF (0.5 ml) was added and the mixture was stirred overnight. The solvent and all volatile compounds were removed *in vacuo*. Half of the residue (containing 0.007 mmol of the corresponding PFP ester) was redissolved in DMF (0.5 ml) and added to a solution of free amine **27** (2.7 mg, 0.0066 mmol), DIPEA (0.0035 ml, 0.003 g, 0.020 mmol) in DMF (0.9 ml) and the mixture was stirred at ambient temperature overnight. The solvent was removed *in vacuo* and the residue was purified by silica gel flash chromatography (10% MeOH in $CHCl_3$) to afford 7.3 mg (95% yield) of the title compound. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.36 (s, 1H), 8.24 – 8.30 (m, 1H), 7.80 (dt, *J* = 7.2, 2.4 Hz, 1H), 7.73 – 7.66 (m, 2H), 7.57 – 7.51 (m, 2H), 7.50 – 7.41 (m, 3H), 7.37 – 7.31 (m, 1H), 7.18 – 7.05 (m, 3H), 6.95 (d, *J* = 7.1 Hz, 1H), 6.87 – 6.75 (m, 5H), 4.96 – 4.76 (m, 3H), 4.66 – 4.57 (m, 2H), 4.25 – 4.37 (m, 1H), 4.17 (t, *J* = 5.9 Hz, 3H), 3.97 (d, *J* = 1.6 Hz, 3H), 3.51 (d, *J* = 4.7 Hz, 4H), 3.43 – 3.36 (m, 4H), 3.33 – 3.17 (m, 6H), 3.06 – 3.14 (m, 4H), 2.87 (s, 6H), 2.83 – 2.70 (m, 2H), 2.67 – 2.54 (m, 1H), 2.42 – 2.33 (m, 2H), 2.24 – 8.30

(m, 1H), 2.23 – 2.04 (m, 9H), 1.46 (s, 4H). HRMS (MALDI) m/z calc. for $[C_{65}H_{75}N_{11}O_{12}S + Na]^+$ = 1256.5210, found 1256.5243.

Benzyl 7-(1,3-dimethyl-5-((4-(piperazin-1-yl)phenoxy)methyl)-1H-pyrazol-4-yl)-1-(2-morpholinoethyl)-3-(3-(naphthalen-1-yloxy)propyl)-1H-indole-2-carboxylate (**28**). To a solution of ethyl 7-(1,3-dimethyl-5-((4-(piperazin-1-yl)phenoxy)methyl)-1H-pyrazol-4-yl)-1-(2-morpholinoethyl)-3-(3-(naphthalen-1-yloxy)propyl)-1H-indole-2-carboxylate (0.254 g, 0.33 mmol)[7] in BnOH (25 mL) was added NaH (0.012 g, 0.5 mmol) at 0° C under stirring. The solution was allowed to warm to ambient temperature, and after 1 hour was stirred at 99° C overnight. Ethanol which was produced was removed *in vacuo*, and the reaction was heated for 6 hours more, cooled to ambient temperature and quenched by stirring with a NH_4Cl solution (*aq*, sat, 0.5 mL) for 15 minutes. The solvent was removed *in vacuo* using a Kugelrohr short path distillation apparatus. The residue was stirred with $CHCl_3$ (50 mL), filtered and concentrated *in vacuo*. The solid was purified by silica gel flash chromatography (10-15 % MeOH in $CHCl_3$) to afford 0.27 g (97%) of the title compound. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.36 – 8.30 (m, 1H), 7.83 – 7.77 (m, 1H), 7.72 (dd, *J* = 8.1, 1.3 Hz, 1H), 7.52 – 7.39 (m, 5H), 7.34 (m, 4H), 7.10 (t, *J* = 7.6 Hz, 1H), 6.97 (dd, *J* = 7.2, 1.2 Hz, 1H), 6.89 – 6.73 (m, 4H), 6.66 (d, *J* = 7.4 Hz, 1H), 5.40 – 5.27 (m, 2H), 4.81 (q, *J* = 11.6 Hz, 2H), 4.57 (ddd, *J* = 14.1, 8.2, 6.1 Hz, 1H), 4.35 (ddd, *J* = 14.0, 8.2, 5.8 Hz, 1H), 4.00 (t, *J* = 6.2 Hz, 2H), 3.96 (s, 3H), 3.55 (t, *J* = 4.7 Hz, 4H), 3.36 – 3.25 (m, 2H), 3.19 (s, 8H), 2.27 – 2.01 (m, 11H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.3, 154.7, 152.7, 146.6, 145.9, 137.0, 136.0, 135.6, 134.5, 130.2, 128.9, 128.7, 128.6, 127.5, 126.6, 126.3, 125.9, 125.7, 125.6, 125.1, 122.0, 120.5, 120.0, 120.0, 119.4, 118.8, 116.7, 115.7, 104.7, 67.6, 67.0, 66.8, 59.8, 58.4, 53.2, 49.7, 44.7, 42.2, 36.9, 30.8, 22.1, 12.4. HRMS (ESI) m/z calc. for $[C_{51}H_{56}N_6O_5 + H]^+$ = 833.4385, found 833.4398.

Tert-butyl (3-(2-(2-(3-aminopropoxy)ethoxy)ethoxy)propyl)carbamate (**29**). 4,7,10-trioxa-1,13-tridecanediamine (7.5 g, 34 mmol) and di-tert-butyl dicarbonate (1.5 g, 6.9 mmol) were reacted in 1.4-dioxane following a literature procedure to afford 2.62 g (99%) of the title compound. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.10 (br s, 1H), 3.70-3.52 (m, 14H), 3.28-3.20 (m, 2H), 2.82 (t, *J*=7.6 Hz, 2H), 1.80-1.70 (m, 4H), 1.46 (s, 9H). All other data was in agreement with literature.[59]

4-(benzyloxy)-4-oxobutanoic acid (**30**). Succinic anhydride (2.0 g, 20 mmol) and benzyl alcohol (2.38 g, 2.27 ml, 22 mmol) were reacted in DCM (20 ml) following a literature procedure to afford 3.20 g (77%) of the title compound. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 – 7.33 (m, 5H), 5.18 (s, 2H), 3.26 (t, *J* = 6.6 Hz, 2H), 2.76 (t, *J* = 6.6 Hz, 2H). All other data was in agreement with literature.[60]

Benzyl 2,2-dimethyl-4,19-dioxo-3,9,12,15-tetraoxa-5,18-diazadocosan-22-oate (**31**). A solution of **30** (0.42 g, 2.18 mmol) was dissolved in DCM (15 mL) and cooled to 0°C. Oxalyl chloride (0.26 mL, 0.381 g, 3.0 mmol) was added along with 2 drops of DMF and allowed to come to ambient temperature with stirring for 1 hour. The reaction mixture was then concentrated *in vacuo*, redissolved in benzene and filtered through a cotton plug. The filtrate was concentrated *in vacuo* to afford the corresponding acyl chloride. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45-7.30 (m, 5H), 5.18 (s, 2H), 3.26 (t, *J*=7.6 Hz, 2.76 (t, *J*=7.6 Hz 2H).

The acyl chloride was then redissolved in DCM (3 mL) and added dropwise to a 0°C solution of **29** (0.693 g, 2.2 mmol) and DIPEA (0.93 mL, 0.685 g, 5.3 mmol) in DCM (10 mL). The reaction mixture was stirred at 0°C for 30 minutes and then brought to ambient temperature overnight. The mixture was concentrated *in vacuo* and the residue was purified by silica gel flash

chromatography (5% MeOH in $CHCl_3$) to afford 0.850 g (81%) of the title compound as a faint yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 – 7.31 (m, 5H), 6.38 (s, 1H), 5.14 (s, 2H), 5.00 (s, 1H), 3.69 – 3.50 (m, 12H), 3.38 (q, *J* = 6.0 Hz, 2H), 3.23 (q, *J* = 6.4 Hz, 2H), 2.74 (t, *J* = 7.0 Hz, 2H), 2.49 (t, *J* = 7.0 Hz, 2H), 1.77 (s, *J* = 6.1 Hz, 4H), 1.45 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.7, 171.2, 156.0, 135.9, 128.5, 128.1, 128.1, 78.8, 70.5, 70.4, 70.1, 70.0, 69.9, 69.4, 66.3, 38.4, 37.9, 30.8, 29.6, 29.6, 28.9, 28.4. HRMS (ESI) m/z calc. for $[C_{26}H_{42}N_2O_8 + Na]^+$ = 533.2833, found 533.2838.

Benzyl 1-amino-14-oxo-4,7,10-trioxa-13-azaoctadecan-18-oate (**32**). To a solution of **31** (0.402 g, 0.787 mmol) in DCM (10 mL) was added TFA (1.8 mL, 2.68 g, 23.5 mmol) dropwise and was allowed to stir for 2 hours. The reaction mixture was concentrated *in vacuo*, redissolved in DCM and stirred with a saturated solution of $NaHCO_3$ (2 mL) for 30 minutes. The organic layer was separated, washed with brine, dried with Na_2SO_4, concentrated *in vacuo* to afford 0.120 g (quantitative) of the title compound as a yellow oil which was unstable under storage was used immediately without further purification. ^{1}H NMR (401 MHz, $CDCl_3$) δ 9.57 (s, 2H), 7.40 – 7.30 (m, 5H), 6.55 (s, 1H), 5.13 (s, 2H), 3.74 (t, *J* = 5.3 Hz, 2H), 3.69 – 3.65 (m, 2H), 3.62 (sept, *J* = 3.6, 2.9 Hz, 4H), 3.57 (td, *J* = 4.0, 2.4 Hz, 2H), 3.47 (t, *J* = 5.6 Hz, 2H), 3.31 (t, *J* = 6.7 Hz, 2H), 3.20 (q, *J* = 5.5 Hz, 2H), 2.71 (dd, *J* = 7.3, 5.9 Hz, 2H), 2.53 (dd, *J* = 7.2, 5.9 Hz, 2H), 1.93 (p, *J* = 5.3 Hz, 2H), 1.72 (p, *J* = 6.4 Hz, 2H).

Benzyl 1-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)-2,17-dioxo-7,10,13-trioxa-3,16-diazaicosan-20-oate (**33**). To a solution of **32** (0.120 g, 0.293 mmol) in DMF (5 mL) was added DIPEA (0.11 mL, 0.08 g, 0.586 mmol) and **21** (0.126 g, 2.4 mmol) and was stirred for 2 hours. The solvent was removed *in vacuo* and the residue was purified by silica gel flash chromatography (5% MeOH in $CHCl_3$) to afford 0.152 g (86%) of the title compound as a

yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.97 (s, 1H), 7.75 (ddd, *J* = 8.4, 7.4, 1.1 Hz, 1H), 7.56 (d, *J* = 7.3 Hz, 2H), 7.41 – 7.29 (m, 5H), 7.21 (d, *J* = 8.4 Hz, 1H), 6.43 (d, *J* = 5.8 Hz, 1H), 5.13 (s, 2H), 5.03 – 4.95 (m, 1H), 4.65 (d, *J* = 1.6 Hz, 2H), 3.67 – 3.54 (m, 12H), 3.52 – 3.44 (m, 2H), 3.35 (q, *J* = 6.2 Hz, 2H), 2.92 – 2.69 (m, 5H), 2.48 (t, *J* = 7.0 Hz, 2H), 2.16 (dq, *J* = 8.3, 3.5 Hz, 1H), 1.88 (p, *J* = 6.3 Hz, 2H), 1.80 – 1.71 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.9, 171.3, 171.1, 168.2, 166.7, 166.1, 154.6, 137.0, 135.9, 135.9, 133.6, 128.5, 128.1, 128.1, 119.7, 118.2, 117.4, 77.2, 70.4, 70.1, 70.0, 69.7, 68.8, 68.3, 66.4, 49.4, 37.8, 36.5, 31.4, 30.9, 29.7, 29.3, 28.8, 22.7. HRMS (ESI) m/z calc. for $[C_{36}H_{44}N_4O_{12} + Na]^+$ = 747.2848, found 747.2827.

Perfluorophenyl 1-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)-2,17-dioxo-7,10,13-trioxa-3,16-diazaicosan-20-oate (**34**). A suspension of **33** (0.075 g, 0.103 mmol) and palladium on carbon (0.02 g) in DMF (2 mL) was stirred under a hydrogen atmosphere for 1 hour. The suspension was filtered through a cotton plug and a 0.45 μm syringe filter. The filtrate was concentrated *in vacuo* and then redissolved in DMF (2 mL). DIPEA (0.08 mL, 0.06 g, 0.424 mmol) and pentafluorophenyl trifluoroacetate (0.03 mL, 0.04 mL, 0.158 mmol) were added and the reaction mixture was stirred for 1 hour. The solvent was removed *in vacuo* and the residue was triturated in diethyl ether (7 mL) for 30 minutes. The red residue was purified by silica gel flash chromatography (1:1 EtOAc:THF) to afford 0.035 g (41%) of the title compound. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.89 (s, 1H), 7.75 (dd, *J* = 8.4, 7.3 Hz, 1H), 7.61 – 7.54 (m, 2H), 7.20 (dd, *J* = 8.5, 0.6 Hz, 1H), 6.55 (t, *J* = 5.6 Hz, 1H), 5.01 – 4.95 (m, 1H), 4.68 – 4.61 (m, 2H), 3.68 – 3.55 (m, 12H), 3.55 – 3.41 (m, 2H), 3.38 (qd, *J* = 5.7, 2.0 Hz, 2H), 3.06 – 3.01 (m, 2H), 2.93 – 2.85 (m, 1H), 2.84 – 2.74 (m, 2H), 2.59 (t, *J* = 7.0 Hz, 2H), 2.19 – 2.14 (m, 1H), 1.88 (q, *J* = 6.5 Hz, 2H), 1.77 (p, *J* = 6.1 Hz, 2H).

Benzyl 7-(5-((4-(4-(1-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)-2,18-dioxo-7,10,13-trioxa-3,17-diazahenicosan-21-oyl)piperazin-1-yl)phenoxy)methyl)-1,3-dimethyl-1H-pyrazol-4-yl)-1-(2-morpholinoethyl)-3-(3-(naphthalen-1-yloxy)propyl)-1H-indole-2-carboxylate (**35**). A solution of **28** (0.016 g, 0.019 mmol), DIPEA (0.014 ml, 0.010 g, 0.080 mmol) and **34** (0.015 g, 0.019 mmol) in DMF (1.0 ml) was stirred at ambient temperature for 2 hours. The solvent was removed *in vacuo* and the residue was redissolved in DMF (1.0 ml) and stirred with K_2CO_3 (0.011 g, 0.080 mmol) for 1 hour. The inorganic salts were filtered off, the filtrate was concentrated *in vacuo* and the residue was purified by silica gel flash chromatography (5% MeOH in $CHCl_3$) to afford 0.019 g (69%) of the title compound. ^{1}H NMR (400 MHz, 15% CD_3OD in $CDCl_3$) δ 9.44 (s, 1H), 8.35 – 8.29 (m, 1H), 7.85 – 7.79 (m, 1H), 7.75 – 7.69 (m, 2H), 7.60 (t, *J* = 5.9 Hz, 1H), 7.55 (d, *J* = 7.3 Hz, 1H), 7.53 – 7.41 (m, 5H), 7.39 – 7.30 (m, 4H), 7.20 (d, *J* = 8.4 Hz, 1H), 7.12 – 7.07 (m, 1H), 6.97 (dd, *J* = 7.1, 1.2 Hz, 1H), 6.85 – 6.75 (m, 4H), 6.67 (dd, *J* = 7.6, 1.0 Hz, 1H), 6.62 (t, *J* = 5.7 Hz, 1H), 5.39 – 5.28 (m, 2H), 5.04 – 4.97 (m, 1H), 4.81 (q, *J* = 11.5 Hz, 2H), 4.65 (d, *J* = 1.4 Hz, 2H), 4.57 (td, *J* = 14.1, 12.7, 5.3 Hz, 1H), 4.34 (dt, *J* = 14.2, 7.1 Hz, 1H), 4.01 (t, *J* = 6.2 Hz, 2H), 3.96 (s, 3H), 3.75 (t, *J* = 5.2 Hz, 2H), 3.70 – 3.42 (m, 20H), 3.39 – 3.26 (m, 4H), 3.01 (dt, *J* = 11.0, 5.0 Hz, 4H), 2.92 – 2.76 (m, 3H), 2.62 (dt, *J* = 74.5, 6.9 Hz, 4H), 2.26 – 2.06 (m, 12H), 1.88 (p, *J* = 6.4 Hz, 2H), 1.77 (p, *J* = 6.2 Hz, 2H). ^{13}C NMR (151 MHz, 15% CD_3OD in $CDCl_3$) δ 172.3, 171.4, 170.5, 168.4, 166.7, 166.6, 166.0, 166.0, 162.3, 154.7, 154.6, 152.5, 146.6, 146.0, 137.0, 135.9, 135.6, 134.5, 133.6, 130.2, 128.9, 128.7, 128.6, 128.5, 127.4, 126.4, 126.3, 125.9, 125.7, 125.5, 125.1, 122.0, 120.5, 120.0, 119.9, 119.7, 119.3, 118.6, 118.1, 117.3, 116.7, 115.6, 104.6, 70.4, 70.4, 70.1, 70.0, 69.5, 68.7, 68.2, 67.5, 66.9, 66.7, 59.8, 58.3, 53.1, 50.8, 50.6, 49.3, 45.3, 42.1, 41.7, 37.5, 36.9, 36.5,

31.5, 31.3, 30.7, 29.2, 28.8, 28.6, 22.7, 22.1, 12.3. HRMS (ESI) m/s calc. for $[C_{80}H_{92}N_{10}O_{16} + Na]^+$ = 1471.6585, found 1471.6633.

7-(5-((4-(4-(1-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)-2,18-dioxo-7,10,13-trioxa-3,17-diazahenicosan-21-oyl)piperazin-1-yl)phenoxy)methyl)-1,3-dimethyl-1H-pyrazol-4-yl)-1-(2-morpholinoethyl)-3-(3-(naphthalen-1-yloxy)propyl)-1H-indole-2-carboxylic acid (**dMCL1-4**). Compound **35** (0.016 g, 0.011 mmol) was hydrogenated in DMF (1.0 mL) over 10 % palladium on carbon (0.006 g) for 2 hours. After filtering through a 0.2 μm syringe filter the clear solution was concentrated *in vacuo*. The residue was purified by silica gel flash chromatography (10—20% MeOH in $CHCl_3$) to afford 0.007g (47%) of the title compound. ^{1}H NMR (400 MHz, 15% CD_3OD in $CDCl_3$) δ 9.42 (s, 1H), 8.41 – 8.29 (m, 1H), 7.85 – 7.78 (m, 1H), 7.78 – 7.69 (m, 2H), 7.59 (t, *J* = 5.8 Hz, 1H), 7.54 (d, *J* = 7.3 Hz, 1H), 7.53 – 7.46 (m, 2H), 7.42 (d, *J* = 8.2 Hz, 1H), 7.35 (t, *J* = 8.0 Hz, 1H), 7.19 (d, *J* = 8.4 Hz, 1H), 7.13 (t, *J* = 7.6 Hz, 1H), 6.94 (dd, *J* = 7.2, 1.2 Hz, 1H), 6.81 (m, 4H), 6.61 (t, *J* = 5.3 Hz, 1H), 5.03 – 4.97 (m, 1H), 4.96 – 4.81 (m, 3H), 4.65 (d, *J* = 1.4 Hz, 2H), 4.35 (m, 1H), 4.29 – 4.20 (m, 2H), 3.97 (s, 3H), 3.79 – 3.42 (m, 22H), 3.43 – 3.30 (m, 4H), 3.00 (p, *J* = 4.7 Hz, 4H), 2.94 – 2.74 (m, 4H), 2.70 (t, *J* = 6.9 Hz, 3H), 2.52 (t, *J* = 6.8 Hz, 2H), 2.47 – 2.25 (m, 7H), 2.14 (s, 4H), 1.88 (p, *J* = 6.4 Hz, 2H), 1.77 (p, *J* = 6.2 Hz, 2H). HRMS (ESI) m/s calc. for $[C_{73}H_{86}N_{10}O_{16} + Na]^+$ = 1381.6115, found 1381.6077.

Benzyl 7-(5-((4-(4-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetyl)piperazin-1-yl)phenoxy)methyl)-1,3-dimethyl-1H-pyrazol-4-yl)-1-(2-morpholinoethyl)-3-(3-(naphthalen-1-yloxy)propyl)-1H-indole-2-carboxylate (**36**). A solution of **28** (0.024 g, 0.029 mmol), DIPEA (0.017 ml, 0.013 g, 0.10 mmol) and **21** (0.013 g, 0.029 mmol) in DMF (1 ml) was stirred at ambient temperature for 1 hour. The solvent was

removed *in vacuo* and the residue was redissolved in DMF (1.5 ml) and stirred with K_2CO_3 (20 mg, 0.145 mmol) for 2 hours to remove all residual pentafluorophenol. The potassium salt was filtered and DMF was removed *in vacuo*. The residue was purified by silica gel flash chromatography (5% MeOH in $CHCl_3$) to afford 0.028 g (84%) of the title compound. ^{1}H NMR (400 MHz, DMSO) δ 11.10 (s, 1H), 8.16 (s, 1H), 7.85 (dd, *J* = 7.7, 1.5 Hz, 1H), 7.80 – 7.70 (m, 2H), 7.54 – 7.42 (m, 6H), 7.41 – 7.30 (m, 5H), 7.12 – 7.05 (m, 1H), 6.95 (dd, *J* = 7.1, 1.2 Hz, 1H), 6.88 – 6.74 (m, 5H), 5.38 – 5.29 (m, 2H), 5.21 (s, 2H), 5.11 (dd, *J* = 12.9, 5.4 Hz, 1H), 4.90 – 4.79 (m, 2H), 4.48 (dt, *J* = 14.0, 7.1 Hz, 1H), 4.19 (dt, *J* = 14.0, 7.0 Hz, 1H), 4.02 (t, *J* = 6.3 Hz, 2H), 3.87 (s, 3H), 3.57 (t, *J* = 5.0 Hz, 4H), 3.37 (m, 4H), 3.23 (t, *J* = 7.6 Hz, 2H), 3.06 (s, 2H), 2.96 (m, 2H), 2.93 – 2.84 (m, 1H), 2.69 – 2.53 (m, 2H), 2.15 – 1.91 (m, 12H). ^{13}C NMR (101 MHz, DMSO) δ 173.2, 170.3, 167.2, 165.5, 162.1, 156.1, 154.5, 152.3, 145.5, 137.0, 136.8, 136.4, 136.2, 134.5, 133.6, 130.2, 129.2, 129.0, 128.8, 128.6, 127.9, 127.0, 126.8, 126.6, 125.6, 125.5, 125.1, 121.9, 120.7, 120.6, 120.4, 120.2, 118.8, 118.3, 117.5, 116.7, 116.0, 115.9, 105.5, 67.8, 66.8, 66.7, 66.6, 60.0, 58.2, 53.2, 50.3, 50.0, 49.3, 44.5, 42.2, 41.7, 40.5, 37.0, 31.4, 30.8, 22.5, 22.0, 12.5. HRMS (ESI) m/z calc. for $[C_{66}H_{66}N_8O_{11} + H]^+$ = 1147.4924, found 1147.4944.

7-(5-((4-(4-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetyl)piperazin-1-yl)phenoxy)methyl)-1,3-dimethyl-1H-pyrazol-4-yl)-1-(2-morpholinoethyl)-3-(3-(naphthalen-1-yloxy)propyl)-1H-indole-2-carboxylic acid (**dMCL1-5**). Compound **36** (0.026 g, 0.023 mmol) was hydrogenated in DMF (2 mL) over 10 % palladium on carbon (0.008 g) overnight. After filtering through a 0.2 µm syringe filter the clear solution was concentrated *in vacuo*. The residue was purified by silica gel flash chromatography (10—15% MeOH in $CHCl_3$) to afford 0.017 g (70%) of the title compound. ^{1}H NMR (400 MHz, DMSO) δ 11.10 (s, 1H), 8.25 – 8.19 (m, 1H), 7.89 – 7.83 (m, 1H), 7.80 – 7.69 (m, 2H), 7.55 – 7.41 (m, 4H), 7.37 (t, *J* = 8.2

Hz, 2H), 7.06 (t, *J* = 7.6 Hz, 1H), 6.94 – 6.77 (m, 6H), 5.21 (s, 2H), 5.11 (dd, *J* = 12.9, 5.4 Hz, 1H), 4.92 – 4.79 (m, 2H), 4.55 (dt, *J* = 14.0, 7.0 Hz, 1H), 4.20 (t, *J* = 6.4 Hz, 3H), 3.87 (s, 3H), 3.57 (t, *J* = 5.1 Hz, 4H), 3.43 (t, *J* = 4.8 Hz, 5H), 3.30 (t, *J* = 7.4 Hz, 3H), 3.07 (s, 2H), 2.96 (m, 2H), 2.94 – 2.84 (m, 1H), 2.63 – 2.54 (m, 2H), 2.22 (dt, *J* = 13.4, 6.5 Hz, 2H), 2.06 (m, 7H), 1.98 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 173.2, 170.4, 167.3, 165.7, 165.4, 164.1, 156.1, 154.6, 152.3, 146.2, 145.5, 137.0, 136.4, 136.3, 134.5, 133.6, 129.6, 128.8, 127.9, 126.9, 126.7, 125.7, 125.5, 123.7, 122.0, 120.6, 120.4, 120.2, 120.1, 119.0, 118.3, 117.2, 116.6, 116.0, 115.9, 105.4, 68.0, 66.6, 66.4, 60.0, 58.3, 53.2, 50.3, 50.0, 49.2, 44.4, 41.9, 41.7, 37.0, 31.4, 30.9, 22.5, 21.9, 12.6. HRMS (ESI) m/z calc. for $[C_{59}H_{60}N_8O_{11} + H]^+$ = 1057.4454, found 1057.4470.

4-((tert-butoxycarbonyl)amino)butanoic acid (**37**). A solution of γ-aminobutyric acid (1.0 g, 9.7 mmol) and 1M NaOH (9.7 mL, 9.7 mmol) in THF (15 mL) was cooled to 0°C. Di-tert-butyl dicarbonate (2.75 g, 12.6 mmol) was added dropwise and allowed to stir at ambient temperature overnight. The solvent was removed *in vacuo*, and the clear residue was dissolved in water (10 mL) and washed with diethyl ether (10 mL). The aqueous layer was acidified to a pH of 2 with 1 M HCl and immediately extracted with three washes of diethyl ether (10 mL). The combined organic extracts were dried with Na_2SO_4, filtered and concentrated *in vacuo*. The clear residue was purified by silica gel flash chromatography (EtOAc) to afford 1.855 g (94%) of the pure white title compound. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.23 (s, 1H), 4.72 (s, 1H), 3.21 (t, *J* = 6.7 Hz, 2H), 2.42 (t, *J* = 7.2 Hz, 2H), 1.86 (q, *J* = 7.0 Hz, 2H), 1.47 (s, 9H). All other data was in agreement with literature.[61]

Benzyl 7-(5-((4-(4-(4-((tert-butoxycarbonyl)amino)butanoyl)piperazin-1-yl)phenoxy)methyl)-1,3-dimethyl-1H-pyrazol-4-yl)-1-(2-morpholinoethyl)-3-(3-(naphthalen-1-yloxy)propyl)-1H-indole-2-carboxylate (**38**). A solution of **37** (0.0058 g, 0.029

mmol) and DIPEA (0.019 g, 0.025 mL, 0.14 mmol) in DMF (0.5 mL) was cooled in an ice bath. Oxyma-Pure (0.005 g, 0.036 mmol) and DIC (0.0045 g, 0.0056 mL, 0.036 mmol) were added and stirred at 0°C for 30 minutes. Compound **28** (0.02g, 0.024 mmol) was added and the mixture was stirred at ambient temperature overnight. The mixture was concentrated *in vavuo*, redissolved in dichloromethane to filter off diisopropylurea. The filtrate was concentrated and the residue was purified by flash chromatography (5% MeOH in $CHCl_3$) to afford 0.0155 g (65%) of the solid yellow title compound. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.34 – 8.30 (m, 1H), 7.84 – 7.80 (m, 1H), 7.72 (dd, *J* = 8.1, 1.2 Hz, 1H), 7.54 – 7.41 (m, 5H), 7.36 (dddd, *J* = 8.6, 7.0, 5.1, 3.2 Hz, 4H), 7.10 (dd, *J* = 8.0, 7.1 Hz, 1H), 6.97 (dd, *J* = 7.1, 1.2 Hz, 1H), 6.86 – 6.76 (m, 4H), 6.68 (dd, *J* = 7.6, 1.1 Hz, 1H), 5.39 – 5.30 (m, 2H), 4.82 (q, *J* = 11.6 Hz, 2H), 4.57 (dt, *J* = 14.3, 7.3 Hz, 1H), 4.37 (dt, *J* = 15.2, 7.0 Hz, 1H), 4.01 (t, *J* = 6.3 Hz, 2H), 3.97 (s, 3H), 3.76 (t, *J* = 5.1 Hz, 2H), 3.62 – 3.50 (m, 6H), 3.30 (t, *J* = 7.6 Hz, 2H), 3.21 (q, *J* = 6.5 Hz, 2H), 3.02 (q, *J* = 5.0 Hz, 4H), 2.41 (t, *J* = 7.3 Hz, 2H), 2.26 – 2.16 (m, 4H), 2.13 (d, *J* = 5.0 Hz, 7H), 1.88 (q, *J* = 7.0 Hz, 2H), 1.46 (s, 9H).HRMS (ESI) m/z calc. for $[C_{60}H_{71}N_7O_8 + H]^+$ = 1018.5437 found 1018.5408.

Benzyl 7-(5-((4-(4-(4-aminobutanoyl)piperazin-1-yl)phenoxy)methyl)-1,3-dimethyl-1H-pyrazol-4-yl)-1-(2-morpholinoethyl)-3-(3-(naphthalen-1-yloxy)propyl)-1H-indole-2-carboxylate (**39**). A solution of **38** (0.015g, 0.015 mmol) in DCM (0.2 mL) and TFA (0.1 mL, 0.46M, 0.47 mmol) was stirred for 4 hours. The mixture was brought to a pH of 10 using a saturated solution of $NaHCO_3$ with vigorous stirring. The aqueous layer was extracted with DCM, and the combined organic extracts were concentrated *in vacuo*. The residue was purified by silica gel flash chromatography (10% MeOH in DCM with 1% Et_3N) to afford 0.0098g (73%) of the solid yellow title compound. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.31 (dd, *J* = 7.8, 1.9 Hz, 1H),

7.81 (dd, *J* = 7.3, 2.0 Hz, 1H), 7.70 (dd, *J* = 8.0, 1.3 Hz, 1H), 7.56 – 7.27 (m, 9H), 7.08 (dd, *J* = 8.1, 7.1 Hz, 1H), 6.95 (dd, *J* = 7.1, 1.2 Hz, 1H), 6.86 – 6.72 (m, 4H), 6.69 – 6.62 (m, 1H), 5.39 – 5.25 (m, 2H), 4.80 (q, *J* = 11.5 Hz, 2H), 4.55 (dt, *J* = 14.3, 7.0 Hz, 1H), 4.32 (ddd, *J* = 14.0, 8.1, 5.8 Hz, 1H), 4.00 (t, *J* = 6.2 Hz, 2H), 3.95 (s, 3H), 3.75 (t, *J* = 4.7 Hz, 2H), 3.60 (s, 2H), 3.53 (t, *J* = 4.7 Hz, 4H), 3.33 – 3.22 (m, 2H), 2.99 (m, 9H), 2.84 (s, 1H), 2.49 – 2.43 (m, 1H), 2.23 – 2.07 (m, 10H), 1.85 (m, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 162.3, 154.7, 154.7, 146.5, 145.9, 137.0, 135.9, 135.6, 134.5, 130.2, 128.8, 128.7, 128.5, 127.4, 126.3, 125.8, 125.7, 125.5, 125.0, 122.0, 120.4, 120.0, 119.9, 119.3, 118.7, 116.8, 115.7, 104.7, 67.6, 66.9, 66.7, 59.9, 58.3, 53.2, 45.7, 42.2, 36.9, 31.9, 31.2, 30.7, 29.6, 29.3, 22.6, 22.0, 14.0, 12.3, 8.7. HRMS (ESI) m/z calc. for $[C_{55}H_{63}N_7O_6 + H]^+$ = 918.4913, found 918.4926.

Benzyl 7-(5-((4-(4-(4-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamido)butanoyl)piperazin-1-yl)phenoxy)methyl)-1,3-dimethyl-1H-pyrazol-4-yl)-1-(2-morpholinoethyl)-3-(3-(naphthalen-1-yloxy)propyl)-1H-indole-2-carboxylate (**40**). A solution of **39** (0.03 g, 0.031 mmol), DIPEA (0.016 mL, 0.12 g, 0.090 mmol) and **21** (15 mg, 0.031 mmol) in DMF (1.5 mL) was stirred at ambient temperature overnight. The solvent was removed *in vacuo* and the residue was purified by silica gel flash chromatography (EtOAc to 5-10% MeOH in $CHCl_3$) to afford 0.037 mg (97%) of the title compound. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.36 (s, 1H), 8.33 – 8.28 (m, 1H), 7.82 – 7.79 (m, 1H), 7.74 (dd, *J* = 8.4, 7.4 Hz, 1H), 7.70 (dd, *J* = 8.1, 1.2 Hz, 1H), 7.61 (m, 1H), 7.56 (dd, *J* = 7.3, 0.5 Hz, 1H), 7.51 – 7.40 (m, 5H), 7.38 – 7.28 (m, 4H), 7.20 (dd, *J* = 8.4, 0.7 Hz, 1H), 7.08 (dd, *J* = 8.0, 7.1 Hz, 1H), 6.95 (dd, *J* = 7.1, 1.2 Hz, 1H), 6.91 – 6.74 (m, 4H), 6.66 (dd, *J* = 7.6, 1.0 Hz, 1H), 5.38 – 5.27 (m, 2H), 4.90 (dd, *J* = 12.1, 5.5 Hz, 1H), 4.85 – 4.75 (m, 2H), 4.70 – 4.59 (m, 2H), 4.55 (dt, *J* = 14.1, 7.1 Hz, 1H), 4.31 (dt, *J* = 14.4, 7.1 Hz, 1H), 3.97 (m, 6H), 3.54 (m, 8H), 3.36 (dt, *J* = 13.2, 6.4 Hz, 1H),

3.28 (t, J = 7.7 Hz, 2H), 3.19 – 3.09 (m, 2H), 2.96 – 2.81 (m, 3H), 2.79 – 2.61 (m, 2H), 2.44 (dtd, J = 29.7, 15.5, 7.2 Hz, 2H), 2.25 – 2.03 (m, 12H), 1.96 (tt, J = 14.0, 6.9 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.2, 170.9, 168.5, 166.9, 166.5, 166.2, 162.3, 154.7, 154.6, 152.8, 146.6, 145.8, 137.0, 136.9, 135.9, 135.6, 134.5, 133.6, 130.2, 128.9, 128.7, 128.6, 128.5, 127.4, 126.5, 126.3, 125.9, 125.7, 125.5, 125.5, 125.1, 122.0, 120.5, 120.0, 119.9, 119.9, 119.3, 118.9, 118.3, 117.5, 116.7, 115.6, 104.6, 77.3, 68.4, 67.5, 67.0, 66.7, 59.8, 58.3, 53.2, 50.9, 50.8, 49.3, 45.2, 42.2, 41.4, 38.7, 36.9, 31.3, 30.7, 30.0, 24.5, 22.8, 22.1, 12.4. HRMS (ESI) m/z calc. for $[C_{70}H_{73}N_9O_{12} + H]^+$ = 1232.5451, found 1232.5417.

7-(5-((4-(4-(4-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamido)butanoyl)piperazin-1-yl)phenoxy)methyl)-1,3-dimethyl-1H-pyrazol-4-yl)-1-(2-morpholinoethyl)-3-(3-(naphthalen-1-yloxy)propyl)-1H-indole-2-carboxylic acid (**dMCL1-6**). **40** (0.037 g, 0.030 mmol) was hydrogenated in DMF (1.5 mL) over 10 % palladium on carbon (0.010 g) overnight. After filtering through a 0.2 μm syringe filter the clear solution was concentrated *in vacuo*. The residue was purified by silica gel flash chromatography (10—15% MeOH in $CHCl_3$) to afford 0.031 g (91%) of the title compound. ^{1}H NMR (400 MHz, 15% CD_3OD in $CDCl_3$) δ 8.22 – 8.17 (m, 1H), 7.74 – 7.61 (m, 4H), 7.45 – 7.42 (m, 1H), 7.38 – 7.32 (m, 2H), 7.22 (t, J = 7.9 Hz, 1H), 7.18 – 7.13 (m, 1H), 7.00 (dd, J = 8.0, 7.1 Hz, 1H), 6.82 (dd, J = 7.1, 1.2 Hz, 1H), 6.77 – 6.62 (m, 5H), 4.87 – 4.80 (m, 1H), 4.73 (d, J = 3.6 Hz, 2H), 4.60 – 4.52 (m, 3H), 4.11 (t, J = 6.3 Hz, 2H), 3.85 (d, J = 4.7 Hz, 6H), 3.57 – 3.44 (m, 7H), 3.37 – 3.21 (m, 5H), 2.92 (s, 2H), 2.89 – 2.81 (m, 2H), 2.74 – 2.59 (m, 3H), 2.34 (td, J = 7.4, 2.8 Hz, 4H), 2.26 – 2.18 (m, 4H), 1.99 (s, 4H), 1.83 (p, J = 7.2 Hz, 2H). ^{13}C NMR (151 MHz, 15% CD_3OD in $CDCl_3$) δ 171.1, 170.9, 168.4, 166.8, 166.8, 166.4, 166.2, 165.6, 154.8, 154.6, 152.5, 147.0, 145.9, 137.1, 135.8, 135.4, 134.5, 133.5, 131.5, 131.4, 129.4, 129.0, 127.4, 126.3, 126.0, 125.7,

125.0, 122.7, 122.1, 120.4, 119.9, 119.9, 119.4, 118.9, 118.9, 118.2, 117.5, 116.0, 115.5, 104.8, 68.4, 67.9, 65.0, 59.6, 56.2, 51.9, 50.8, 50.8, 50.7, 50.6, 49.2, 45.1, 41.4, 40.7, 38.7, 38.6, 37.0, 31.2, 30.8, 30.0, 29.7, 24.4, 22.8, 21.7, 12.5. HRMS (ESI) m/z calc. for $[C_{63}H_{67}N_9O_{12} + H]^+$ = 1142.4982, found 1142.4968.

Biological Evaluation. The human MM cell lines MM1.S and OPM2 were purchased from ATCC and DSMZ respectively. Cell lines were maintained in RPMI 1640 medium (Invitrogen) with *l*-glutamine and containing 10% FBS (Invitrogen), 100 U/mL of penicillin, 100 µg/mL of streptomycin (Invitrogen), and 100 µg/mL of Normocin. The $OPM2^{CRBN-/-}$ cell line was generated using CRISPR Cas9 gene editing. OPM2 cells were transduced with lentiviral vectors expressing Cas9 and delivering guide RNA (gRNA) targeting CRBN exon 2 (lentiCRISPR v2, Addgene) (DNA target sequence TTTATCCTTATGTGGGCCGA).

Functional Analysis. Cell proliferation was assessed using CellTiter-96 Cell Viability Assay (Promega) according to the manufacturer's protocol. Absorbance was detected in a multimode plate reader (Beckman Coulter). Cells were seeded in 96 well plates and treated with PROTACs or A-1210477 at different concentrations and for various times as indicated in the text.

Immunoblotting. Horseradish peroxidase (HRP) monoclonal antibody-linked bands in SDS-Gels (NuPage 4-12% Bis-Tris Gel or 3-8% Tris-Acetate Gel, Invitrogen) were imaged using a BioRad ChemiDoc imaging system and band intensities were analysed using ImageLab software and quantified using ImageJ software. Bands were reported as a relative amount as the ratio of each protein band relative to the lane's DMSO vehicle control. Both bands of the short MCL1 isoforms were used for quantification calculations.

Ternary Complex Immunoprecipitation. Tagged MCL1 was pre-incubated with **dMCL1-2**, assuring that the MCL1 pole had appropriate opportunity to bind to exogenous MCL1. The binary complex was then incubated with OPM2 cell lysate to sequester CRBN and form the necessary ternary complex implicated in UPP modulation.[62] 750 ng of purified MCL1-His (ProSpec, PRO-1202) was incubated with either **dMCL1-2** (500 nM) or DMSO at 4°C for 1 hr. OPM2 cell lysate was added and incubated overnight at 4°C. 20 µl of prewashed TALON metal affinity resin (Clontech) were added to the assay and incubated for 1 hr at 4°C. The TALON resin was washed three times with RIPA buffer, and the reactions were quenched with 4x SDS-PAGE gel loading buffer and samples were separated on SDS-PAGE followed by immunoblot with anti-CRBN mAb.

***in vitro* MCL1 and BRD4 Ubiquitinylation.** 500,000 OPM2 cells/assay were first pelleted at 2800 rpm and then lysed with radioimmunoprecipitation assay (RIPA) lysis buffer on ice for 30 minutes, followed by centrifugation at 14,000 rpm for 15 minutes. The supernatant was collected and assays were prepared with an Abcam ubiquitinylation assay kit (ab139471). According to the manufacturer's protocol, the reaction mixture was incubated at 37°C for 3 hours and then the total volume was increased to 200µl with RIPA buffer. Immunoprecipitaion experiments were performed with 2 µg of MCL1 mAb (Santa Cruz) or BRD4 mAb (Bethyl) for each condition and incubated overnight at 4°C. 20 µl of protein A/G beads (Santa Cruz Biotechnology) were added and incubated for 1 hr at 4°C followed by three washes with RIPA buffer. The reactions were quenched with 4x SDS-PAGE gel loading buffer and samples were separated on SDS-PAGE followed by immunoblot with either anti-ubiquitin mAb (Abcam) or anti-MCL1 mAb (ADI-AAP-240) and anti-BRD4 mAb (Bethyl) for inputs and controls.

***in vitro* MCL1 Degradation.** HeLa cell lysate was exposed to an ubiquitin conjugating kit (Enzo, USA) and treated with the designated drug compounds at 37°C for 3 hours and then quenched with 4x SDS-PAGE gel loading buffer. Samples were separated on SDS-PAGE, followed by immunoblot analysis with anti-MCL1 mAb (Enzo Life Sciences) and anti-α-tubulin mAb (Cell Signaling) for inputs.

***in vitro* Cellular Assays.** $OPM2^{WT}$, $OPM2^{CRBN-/-}$ and MM1.S cells were cultured in RPMI 1640 complete medium (Invitrogen) then seeded in 6-well or 96-well plates. Cells were treated with PROTACs or A-1210477 for various lengths of time, followed either by cell viability assays (Promega) or immunoblot analysis with anti-MCL1 mAb (Enzo Life Sciences) and anti-α-tubulin mAb (Cell Signaling) for inputs.

ASSOCIATED CONTENT

Supporting Information. This material is available free of charge on the ACS Publication Website at http://pubs.acs.org. Spectroscopic data for all compounds; detailed biological assay protocols; biotinylation binding studies for **1**, **2**, **3**, and **8**; *in vitro* pharmacokinetic data for **dMCL1-2**; computational modelling; molecular formula strings.

Accession Codes. PDB ID codes 4tzc and 5vkc were used for CRBN and MCL1 respectively. Authors will release the atomic coordinates and experimental data upon article publication.

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ABBREVIATIONS USED

Arg, arginine; Bak, Bcl-2 homologous antagonist/killer; Bax, Bcl-2-like protein 4; BCL-2, B-cell lymphoma 2; BET, bromodomain and extra terminal; BH3, Bcl-2 homology domain 3; Bim, Bcl-2-like protein 11; BRD4, bromodomain-containing protein 4; Cas 9, CRISPR associated protein 9; CHX, cycloheximide; CDI, carbonyldiimidazole; CDK9, cyclin-dependent kinase 9; CRBN, cereblon; CRISPR, clustered regularly interspaced short palindromic repeats; DCC, *N,N'*-dicyclohexylcarbodiimide; DIPEA, *N,N,*-diisopropylethylamine; DMSO, dimethyl sulfoxide; HDAC, Histone Deacetylase; IMiDs, immunomodulatory imide drugs; IP, immunoprecipitation; K_D, dissociation constant; K_i, inhibition constant; MCL1, myeloid cell leukemia 1; MELD, modeling employing limited data; MM, multiple myeloma; Mule, MCL1 ubiquitin ligase E3;

NMU, *N*-nitroso-*N*-methylurea; Pfp, pentafluorophenyl; PEG, polyethylene glycol; PPIs, protein-protein interactions; PROTAC, proteolysis targeting chimera; SAR, structure activity relationship; TBD, thalidomide binding domain; TFA, trifluoroacetic acid; UPP, ubiquitin-proteasome pathway; VHL, von Hippel-Lindau.

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