Journal of
Medicinal Chemistry

Article

Degradation Versus Inhibition: Development of Proteolysis-Targeting Chimeras for Overcoming Statin-Induced Compensatory Upregulation of 3-Hydroxy-3-methylglutaryl Coenzyme A Reductase

Mei-Xin Li, Yiqing Yang, Qiuye Zhao, Yue Wu, Lei Song, Haiyan Yang, Ming He, Hongying Gao, Baoliang Song, Jie Luo, and Yu Rao

J. Med. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jmedchem.0c00339 • Publication Date (Web): 22 Apr 2020

Just Accepted

is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Degradation Versus Inhibition: Development of Proteolysis-Targeting Chimeras for Overcoming Statin-Induced Compensatory Upregulation of 3-Hydroxy-3-methylglutaryl Coenzyme A Reductase

*Mei-Xin Li[†, #], Yiqing Yang[‡, #], Qiuye Zhao[‡], Yue Wu[‡], Lei Song[⊥], Haiyan Yang[§], Ming He[‡], Hongying Gao[‡], Bao-Liang Song[†], Jie Luo[†, *], Yu Rao[‡, *]*

[†]Hubei Key Laboratory of Cell Homeostasis, College of Life Sciences, Wuhan University, Wuhan 430072, P.R. China.

[‡]MOE Key Laboratory of Protein Sciences, School of Pharmaceutical Sciences, MOE Key Laboratory of Bioorganic Phosphorus Chemistry & Chemical Biology, Tsinghua University, Beijing 100084, P.R. China.

[⊥]State Key Laboratory of Proteomics, Beijing Proteome Research Center, National Center for Protein Sciences PHOENIX Center, Beijing Institute of LifeOmics, Beijing, 102206, P.R. China

[§]School of Life Sciences, Tsinghua University, Beijing 100084, P.R. China.

ABSTRACT

3-Hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR) is an eight-pass transmembrane protein in the endoplasmic reticulum (ER) and a classical drug target to treat dyslipidemia. Statins including the well-known atorvastatin (Lipitor®; Pfizer) have been widely used for the prevention and treatment of cardiovascular disease for decades. However, statins can elicit a compensatory upregulation of HMGCR protein and cause adverse effects including skeletal muscle damage. They are ineffective for patients with statin intolerance. Inspired by the recently emerging proteolysis-targeting chimeras (PROTACs), we set out to eliminate HMGCR protein using PROTAC-mediated degradation. One PROTAC designated as P22A was found to reduce HMGCR protein level and block cholesterol biosynthesis potently with less compensatory upregulation of HMGCR. To the best of our knowledge, HMGCR is the first ER-localized, polytopic transmembrane protein successfully degraded by the PROTAC technique. This finding may provide a new strategy to lower cholesterol levels and treat the associated diseases.

INTRODUCTION

3-Hydroxy-3-methylglutaryl (HMG) coenzyme A (HMG-CoA) reductase (HMGCR) is the rate-limiting enzyme catalyzing the conversion of HMG-CoA to mevalonate in the cholesterol biosynthetic pathway. It is localized in the endoplasmic reticulum (ER) and composed of a membrane-spanning domain and a catalytic domain protruding into the cytosol. HMGCR serves as the target of statins, a class of cholesterol-lowering drugs most commonly prescribed for preventing and treating cardiovascular disease (CVD)[1-5]. By possessing the HMG-like moieties, statins bind competitively to the active site of the enzyme, blocking generation of mevalonate and downstream derivatives including cholesterol. Among all types of statins on the market, atorvastatin (Lipitor®; Pfizer) has demonstrated superior efficacy in lowering plasma low-density lipoprotein (LDL) cholesterol levels and reducing adverse cardiovascular outcomes[6-9].

Although statins can blunt cholesterol biosynthesis via inhibiting HMGCR activity, a compensatory increase in protein expression has often been encountered in cultured cells[10-13], as well as in the livers of several animal species including humans[14-22]. This statin-induced HMGCR increment is not surprising, as the reductions in cholesterol and other products of the mevalonate pathway can mitigate normal feedback inhibition of the enzyme in a multivalent manner[23-24]. It, however, does limit the maximal effectiveness of the drug, provoking more intensive therapies associated with higher risks of side effects[25-29]. Skeletal muscle damage is the most alarming adverse effect of statins. In fact, about 1%-5% of patients receiving statin treatments develop myopathy[27]. Hence, strategies that can ablate both activity and abundance of HMGCR protein are urgently needed.

In the native process of ER-associated degradation (ERAD)[30], HMGCR binds to the ER membrane

proteins Insigs via its membrane domain. The E3 ubiquitin ligases gp78, TRC8 and RNF145 that associate with Insigs then catalyze ubiquitination of two lysine residues in the HMGCR membrane domain[31-33]. Ubiquitinated HMGCR is recognized by the AAA-ATPase, extracted across the ER membrane, and degraded by proteasome. Chemical knockdown by small-molecule proteolysis targeting chimeras (PROTACs, Figure 1A), heterobifunctional compounds consisting of a binding moiety for the protein of interest, a linker and another moiety for a specific E3 ubiquitin ligase, has gained widespread attention as a promising therapeutic approach to induce degradation of disease-causing proteins via the ubiquitin–proteasome system (UPS)[34-41]. Moreover, PROTACs containing small molecule inhibitors coupled to cereblon (CRBN)-recruiting immunomodulatory drugs might elicit more pronounced downstream biological effects than conventional inhibitors alone that last even after washout. Such encouraging results have spurred our interests in investigating whether PROTACs can be effectively used to eliminate HMGCR protein and thus, inhibit cholesterol production. In particular, we are very curious whether PROTACs can overcome statin-induced compensatory upregulation of HMGCR.

In this study, we designed and synthesized a battery of PROTAC molecules where atorvastatin was conjugated to ligands of the E3 ligase CRBN (Figure 1). We found that compound P22A was most potent in promoting degradation of endogenous HMGCR protein in Chinese hamster ovary (CHO) cells deficient in Insig-1 and Insig-2 (referred as SRD15 cells). P22A induced ubiquitination of HMGCR in a CRBN-dependent manner, and P22A-mediated HMGCR degradation required formation of the HMGCR/PROTAC/CRBN complex. Importantly, the increase in HMGCR protein induced by P22A was much less than that by atorvastatin in human hepatocarcinomatous Huh7 cells. P22A was also able to activate the sterol regulatory element-binding protein (SREBP) pathway and block

cholesterol biosynthesis. To our knowledge, HMGCR represents the first ER-localized, polytopic transmembrane protein successfully degraded by the PROTAC technique. Our study provides a proof-of-concept for PROTAC-induced HMGCR degradation as a new and potential strategy for treating hypercholesterolemia and CVD.

RESULTS AND DISCUSSION

Design and synthesis of a HMGCR-PROTAC library. We first sought to construct a HMGCR-targeting PROTAC library (Figure 1B). Atorvastatin, which recognizes the binding pocket of HMGCR, was used as the moiety for HMGCR recruitment (Figure 1B). The ligands of CRBN successfully used in previous PROTACs[34] were examined in this work. We installed the azide-tagged polyethylene glycol (PEG) on the amine group of pomalidomide, a ligand of CRBN, and the alkyne tags on a position at the benzene ring of atorvastatin that is exposed to solvent (indicated by the red arrow in Figure 1C). The azide- and alkyne- tagged intermediates were conjugated via click chemistry to synthesize HMGCR-PROTACs (Figure 1B). Immunoblotting was conducted to evaluate the degradative potency of each PROTAC. We found that the CRBN-based PROTACs could efficiently induce the degradation of HMGCR, which encouraged us to prepare more CRBN-based PROTACs and evaluate their biological activity carefully in the following study.

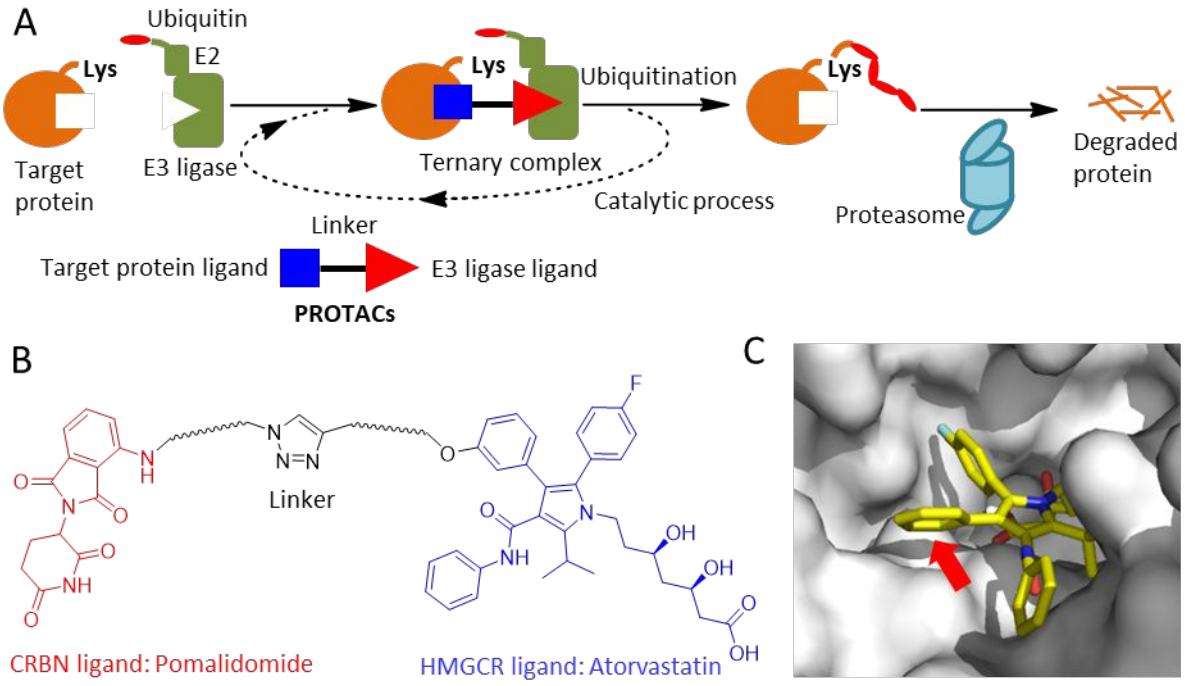
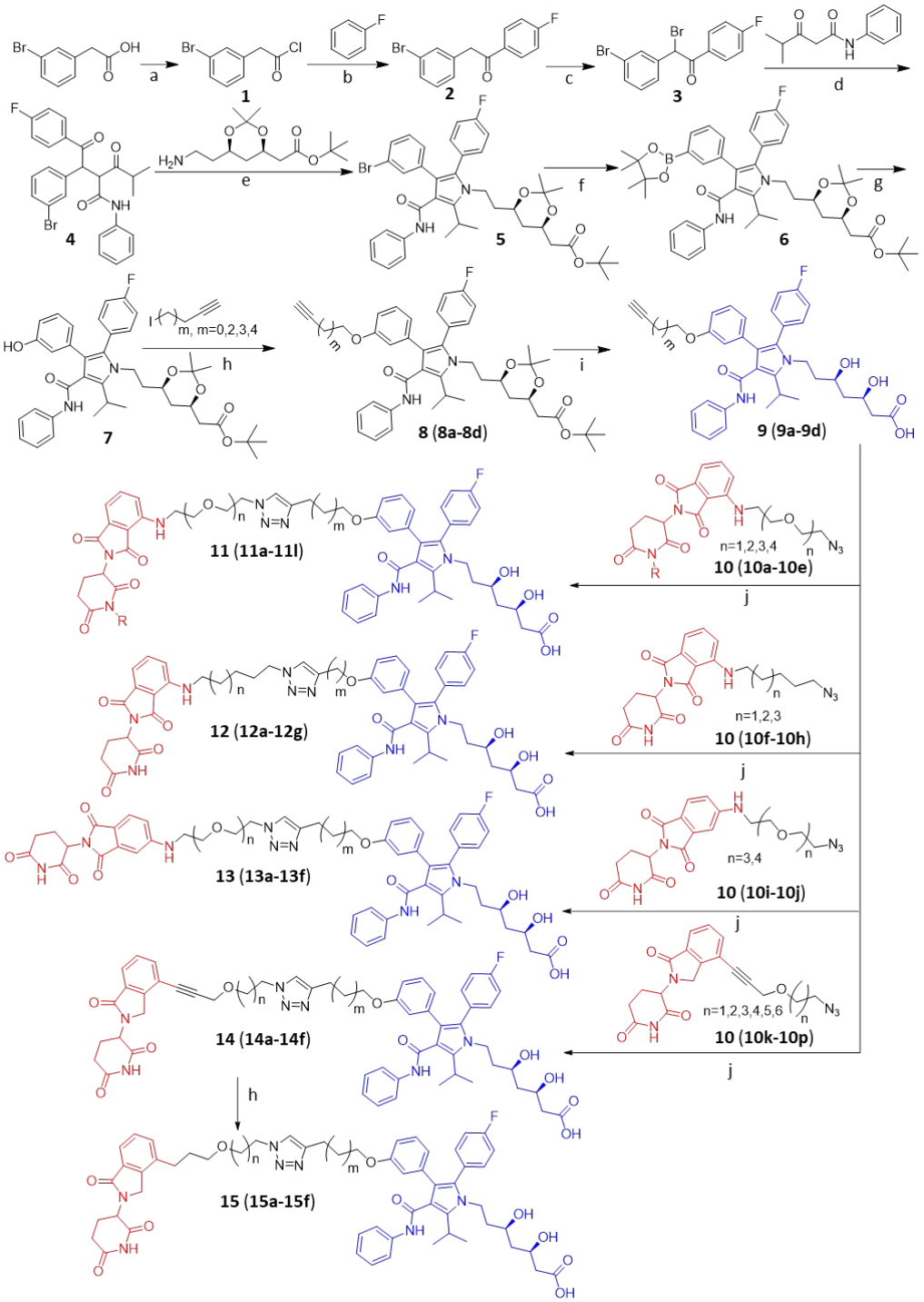


Figure 1. Design of a HMGCR-PROTACs library. (A) Schematic presentation of PROTAC-mediated protein degradation. (B) Construction of PROTACs via click chemistry. (C) The binding pocket of HMGCR in complex with atorvastatin (PDB code: IHWK).

To optimize the potency and study the structure-activity relationship, we further designed and synthesized different HMGCR-PROTACs (Scheme 1). In brief, fluorobenzene was acylated with acyl chloride (compound **1**) by Lewis acid to produce compound **2**. Compound **3** had a bromide atom installed at the α position of carbonyl group of compound **2**. After bromide atom was substituted by 4-methyl-3-oxo-N-phenylpentanamide, the important 1,4-dicarbonyl intermediate **4** was generated. Then the pyrrole ring was constructed from the condensation of **4** and primary amine via Paal-Knorr pyrrole synthesis. The C-B bond in **6** was built from C-Br bond in **5** via Miyaura borylation reaction and transformed to the C-O bond in **7** subsequently. Then the phenolic hydroxyl group in **7** was used

as a handle to attach the alkyne tags through substitution. Compounds **9** were collected after the deprotection of **8** and coupled together with different azide-tagged CRBN ligands **10** using click chemistry to afford the final HMGCR-PROTACs (Scheme 1, **11**-**14**). The alkyne groups in compounds **14** could be further reduced to compounds **15** by catalytic hydrogenation.

Scheme 1. Synthesis of HMGCR-PROTACs.



Reaction conditions: (a) $SOCl_2$, DMF, DCM. (b) $AlCl_3$. (c) Br_2 in AcOH, Br_2, $CHCl_3$. (d) K_2CO_3, Acetone. (e) *t*-BuCOOH, PhMe, THF, Cyclohexane. (f) $Pd(dppf)_2Cl_2$.DCM, KOAc, Bis(pinacoloto)diboron, DMF. (g) H_2O_2 in H_2O, NaOH, THF. (h) K_2CO_3, MeCN. (i) HCl, MeOH, NaOH. (j) $CuSO_4$, sodium ascorbate, THF, H_2O. (h) Pd/C, H_2, MeOH.

Evaluation of PROTAC-induced HMGCR degradation. It is known that high sterol levels can induce the membrane domains of HMGCR protein to bind ER-resident Insig-1 and Insig-2 proteins, resulting in rapid degradation of the enzyme via the UPS. To exclude any interference of sterol-regulated, Insig-mediated HMGCR degradation when screening for PROTACs, we utilized SRD15 cells that lack both Insig-1 and Insig-2[11]. Compared with the parental CHO cells, SRD15 cells exhibited much higher levels of endogenous HMGCR protein when grown in normal culture medium containing 5% FBS (Figure 2A, compare *lanes 1* and *4*), which, however, remained relatively unchanged regardless of sterol depletion or oxysterol repletion (Figure 2A, *lanes 5-6*). In addition, atorvastatin elicited a dose-dependent increase of HMGCR protein in CHO cells but not in SRD15 cells (Figure 2B), further supporting the notion that decreased protein degradation primarily accounts for HMGCR accumulation induced by statins. Together, these results suggest that SRD15 cells are suitable to evaluate PROTAC-mediated HMGCR degradation *in vitro*.

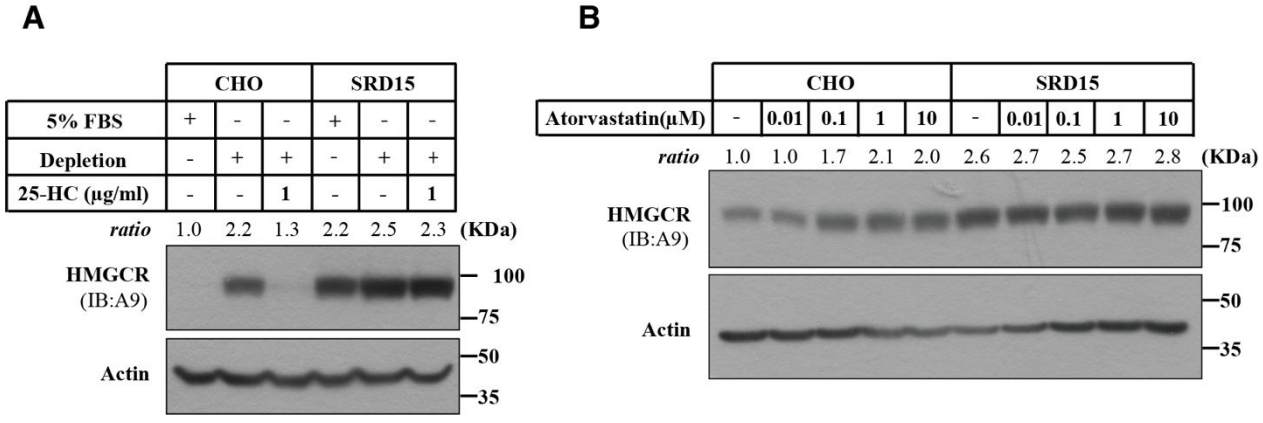
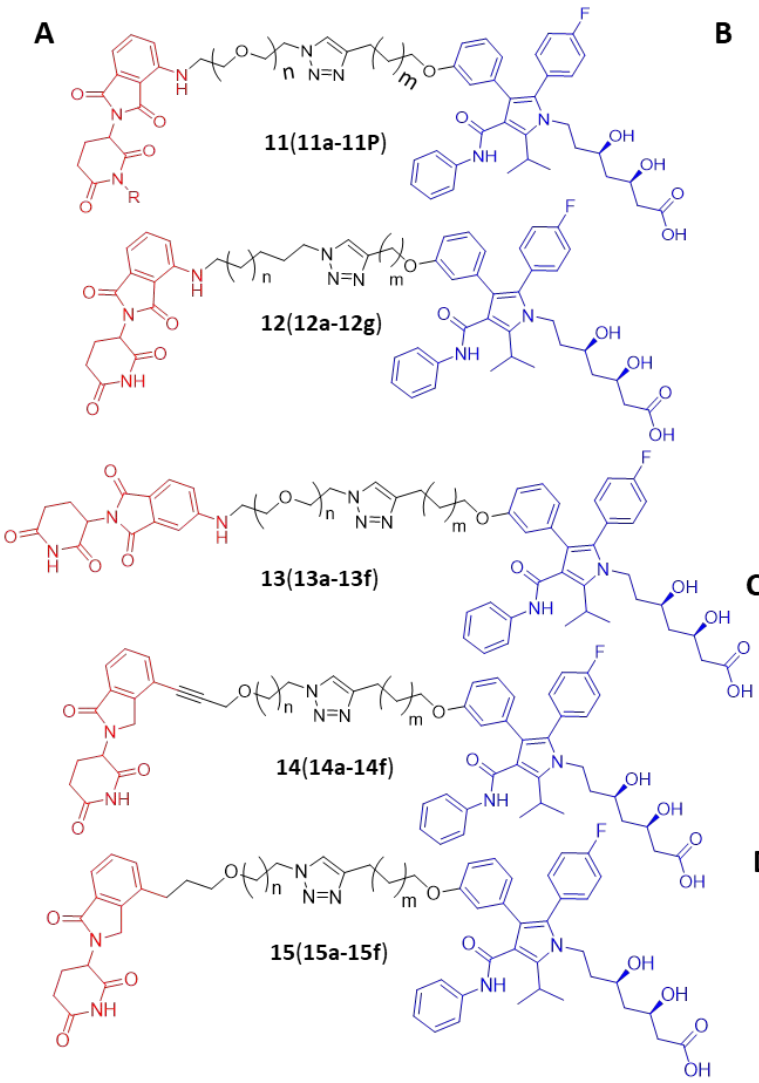


Figure 2. Selection of SRD15 cells to examine PROTAC-mediated HMGCR degradation. (A) CHO and SRD15 cells were either maintained in normal culture medium containing 5% FBS (*lanes 1* and *4*), or depleted of lipids by incubating in the medium supplemented with 5% lipoprotein-deficient serum, 1 μM atorvastatin and 50 μM

mevalonate for 16 h (*lanes 2*, *3*, *5* and *6*). Cells were then treated without or with 25-hydroxycholesterol (25-HC) for 16 h and harvested for immunoblotting. (B) CHO and SRD15 cells were depleted of lipids for 16 h, incubated with atorvastatin at the indicated concentrations for 16 h and harvested for immunoblotting. The densitometry of HMGCR was normalized to that of actin. The normalized value in DMSO-treated group is defined as 1.

We then tested the degradative potency of HMGCR-PROTACs on SRD15 cells. Firstly, varying concentrations of pomalidomide-based PROTACs modified with the PEG linkers of different lengths were evaluated by immunoblotting (Figure 3A-3B and Figure S1 and S2). In a series of PROTACs that covered a wide range of the linker length (Figure 3B), those with short linkers (compounds **11a-11b**, also named P13A and P14A) failed to induce the degradation of HMGCR (Figure S1). Extending the linker of compound **11b** with just one more atom (compound **11c**, also named P15A**)** caused moderate HMGCR degradation half degraded concentration (DC_{50} = 3 μM) (Figure 3B and Figure S1). Further increasing the linker length (compounds **11d-11i**, also named P16A-P21A) did not enhance degradation potency considerably (Figure 3B and Figure S1 and S2), probably due to improper folding or stretched conformations of the ternary complex. When the linker length reached 22 atoms (compound **11j**, also named P22A) and above (compounds **11l-11n**, also named P23A-P25A), PROTACs could induce obvious degradation of HMGCR (Figure 3B and Figure S2), suggesting that the increased rotatable bonds might contribute to more degree of freedom and suitable conformations. Compound **11j** (P22A) with a DC_{50} value of 0.1 μM was most potent in promoting degradation of endogenous HMGCR. However, longer linkers as in compounds **11o** (also named P26A) and **11p** (also named P27A) seemed to lessen the potency in HMGCR degradation (Figure 3B and Figure S2).

A



B

Compound	m	n	R	DC_{50} (μM)
11a (P13A)	1	1	-H	> 3
11b (P14A)	2	1	-H	> 3
11c (P15A)	3	1	-H	3*
11d (P16A)	1	2	-H	> 3*
11e (P17A)	2	2	-H	> 3*
11f (P18A)	3	2	-H	> 3*
11g (P19A)	1	3	-H	> 3*
11h (P20A)	2	3	-H	> 3*
11i (P21A)	3	3	-H	1.8*
11j (P22A)	1	4	-H	0.1*
11k (P22A-Et)	1	4	-Et	> 3
11l (P23A)	2	4	-H	0.4
11m (P24A)	3	4	-H	0.5
11n (P25A)	1	5	-H	0.2
11o (P26A)	2	5	-H	> 3
11p (P27A)	3	5	-H	0.6

C

Compound	m	n	DC_{50} (μM)
12a (P13A1)	1	7	n.d.
12b (P14A1)	3	6	0.4
12c (P15A1)	3	7	0.2
12d (P16A1)	4	7	0.3
12e (P17A1)	5	7	0.4
12f (P18A1)	6	7	0.3
12g (P19A1)	7	7	0.5

D

Compound	m	n	DC_{50} (μM)
13a (P19AM)	1	7	> 3
13b (P20AM)	3	6	> 3
13c (P21AM)	3	7	> 3
13d (P22AM)	4	7	0.3
13e (P23AM)	5	7	> 3
13f (P24AM)	7	7	> 3

E

Compound	m	n	DC_{50} (μM)
14a (L13A)	5	1	> 3
14b (L14A)	5	2	> 3
14c (L15A)	5	3	> 3
14d (L16A)	5	4	0.3
14e (L17A)	5	5	0.2
14f (L18A)	5	6	0.7

F

Compound	m	n	DC_{50} (μM)
15a (L13AR)	5	1	> 3
15b (L14AR)	5	2	> 3
15c (L15AR)	5	3	> 3*
15d (L16AR)	5	4	2.3*
15e (L17AR)	5	5	0.5*
15f (L18AR)	5	6	0.6

Figure 3. Potency of HMGCR-PROTACs. (A) The structures of HMGCR-PROTACs. (B-F) The degradative activity of HMGCR-PROTACs. DC_{50} values, the compound concentration leading to 50% protein loss relative to DMSO as determined by the semi-quantitative immunoblotting, were calculated by fitting the data to a dose-response curve using Graphpad Prism. The DC_{50} values as the means of 3 independent experiments are indicated by asterisks. n.d., not determined. Corresponding blots are shown in the Supporting Information: Figure S1 and S2 for (B), Figure S3 for (C), Figure S4 for (D), Figure S5 for (E), Figure S6 for (F).

Given the heteroatomic property of the oxygen atom in the PEG linker could change its conformation, we next replaced it with the carbon atom and evaluated the degradative potency of this new series (compounds **12a**-**12g**, also named P13A1-P19A1) (Figure 3A and C). The carbon substitution also confers hydrophobicity that might facilitate the membrane permeability of PROTACs. As shown in Figure 3C and Figure S3, the homogeneous linkers markedly promoted HMGCR degradation (compare compounds **11a**-**11g** with **12a**-**12g**). However, the DC_{50} values of this carbon-substituted series showed no improvement compared to compound P22A. Other CRBN ligands including *meta*-substituted pomalidomide or alkyne-substituted lenalidomide derivatives have been found to significantly affect the potency of PROTACs in degrading BET[38-40]. In our study, compounds that harbor *meta*-substituted pomalidomide (compounds **13a**-**13f**, also named P19AM-P24AM) were less capable of inducing HMGCR degradation than P22A (Figure 3D and Figure S4). Among these, the compound that also contains a linker of 22 atoms in length (compound **13d**, also named P22AM) showed strongest degradative potency. A possible hook effect was detected at the concentration of 3 μM (Figure S4). For the PROTACs with alkyne-substituted lenalidomide (compounds **14a**-**14f**, also named L13A-L18A), which is a more rigid CRBN ligand, those encompassing long linkers (compounds **14d**-**14f**) were superior to those with short linkers (compounds **14a**-**14c**) in degrading HMGCR (Figure 3E and Figure S5), probably because the rigidity of CRBN ligands confers the linkers less flexibility so that compounds in this series are more comparable to compounds containing shorter linkers in other series. Alkyne reduction (compounds **15a**-**15f**, also named L13AR-L18AR) slightly lessened the degradative potency (Figure 3F and Figure S6). We believed that, in contrast to compounds **12a**-**12f**, the oxygen atoms in the PEG linkers of **11a** -**11f** and **15a**-**15f** cause very different

conformations. It is noteworthy that, while the linkers apparently influence the potency of PROTACs, the precise effects are difficult to predict owing to their large degree of freedom.

P22A promotes HMGCR degradation by hijacking the CRBN-E3 ubiquitin ligase complex. P22A induced degradation of HMGCR in a dose-dependent manner, and as high as 70% of HMGCR protein was degraded by P22A at the concentration of 1 μM (Figure 4A and Figure S2). No further degradation was detected when higher concentrations of P22A were used (Figure S7A). To determine whether P22A-induced HMGCR degradation occurs via the UPS, we incubated SRD15 cells with P22A, the proteasome inhibitor MG132 and the neddylation inhibitor MLN-4924 in various combinations. Addition of MG132 or MLN-4924 completely blocked P22A-induced HMGCR degradation (Figure 4B-4C). HMGCR degradation was also completely reversed by atorvastatin at 0.3 μM (Figure 4D) or pomalidomide at 1 μM (Figure 4E) via competition with P22A. Notably, atorvastatin tagged with alkyne (compounds **9a-9d,** Scheme 1) failed to elicit HMGCR degradation (Figure S7B), indicating that the E3 ligand was required for degradation. Attachment of an ethyl group to the piperidine-2,6-dione ring of pomalidomide (compound **11k**, also named P22A-Et, Figure 3B)[41] impaired the pomalidomide-CRBN interaction and abolished HMGCR degradation by P22A (Figure 4F).

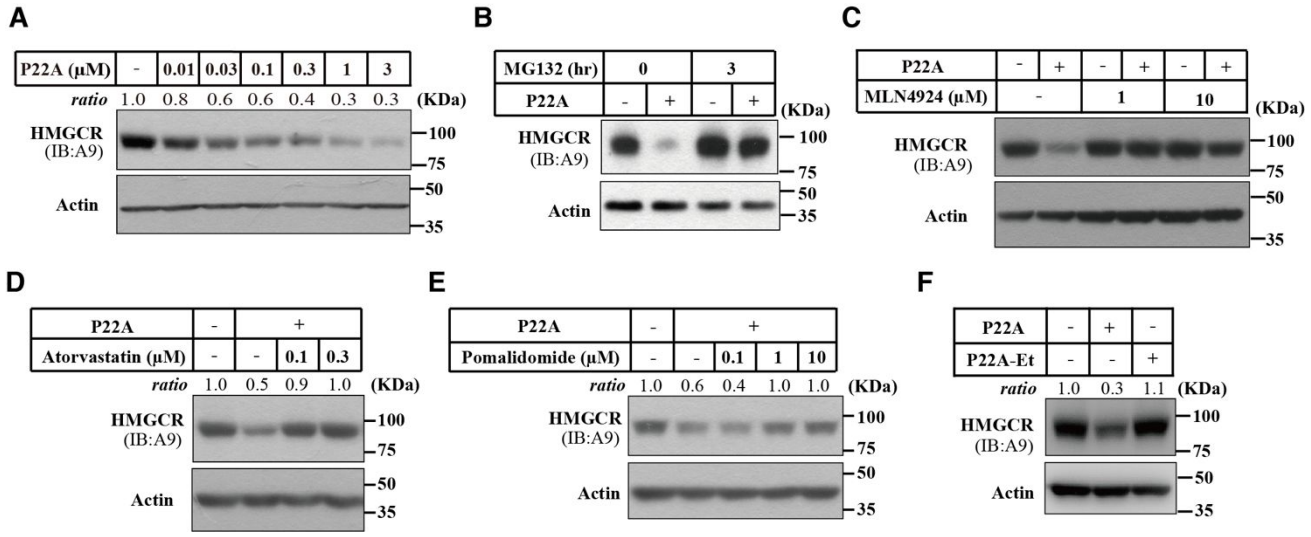


Figure 4. P22A is an effective HMGCR degrader. (A) SRD15 cell were treated with the indicated concentrations of P22A for 16 h and harvested for immunoblotting. Blots shown here are one of the three repeats in Figure S2. The densitometry of HMGCR was normalized to that of actin. The normalized value in DMSO-treated group is defined as 1. (B-C) SRD15 cell were treated with 1 μM P22A for 16 h, and then with 10 μM MG132 (B) or the indicated concentrations of MLN-4924 (C) for 3 h. (D-F) SRD15 cell were depleted of lipids for 16 h and treated with 1 μM P22A and atorvastatin (D) or pomalidomide (E) at the indicated concentrations, or with 1 μM P22A-Et for 16 h (F).

We next transfected SRD15 cells with T7-tagged HMGCR together with Flag-tagged CRBN or Flag-tagged $CRBN^{YW/AA}$, a mutant defective for pomalidomide binding[42-43]. Overexpression of wild-type CRBN drastically accelerated P22A-induced degradation of transfected HMGCR protein (Figure 5A, compare *lanes 2* and *4*), whereas of $CRBN^{YW/AA}$ failed to do so (Figure 5A, compare *lanes 2* and *6*). Importantly, P22A did not alter *Hmgcr* mRNA levels at any of the concentrations tested (Figure 5B), suggestive of a post-transcriptional modulation of HMGCR protein by P22A. Depletion of *Crbn* using siRNA-mediated knockdown greatly diminished the potency of P22A in eliminating endogenous

HMGCR protein (Figure 5C and 5D). We next evaluated the ubiquitination level of HMGCR immunoprecipitated from cells treated with or without P22A. High-molecular-weight smears were detected upon addition of P22A and MG132 (Figure 5E). To directly demonstrate P22A mediates the interaction between HMGCR and CRBN, we performed an immunoprecipitation experiment in which HEK293T cells were co-transfected with Myc-tagged HMGCR and Flag-tagged CRBN followed by treatments with or without P22A for 16 h. The overexpressed HMGCR protein was only co-immunoprecipitated with CRBN in the presence of P22A (Figure 5F). Addition of atorvastatin (Figure 5G) or pomalidomide (Figure 5H) blocked HMGCR immunoprecipitation by CRBN. To further demonstrate that CRBN participates in HMGCR ubiquitination by P22A, we transfected SRD15 cells with plasmids encoding T7-tagged HMGCR, Flag-tagged CRBN and HA-tagged ubiquitin. Cells were then incubated with or without P22A for 16 h, and the transfected HMGCR protein was immunoprecipitated and subjected to immunoblotting with the anti-ubiquitin antibody. P22A markedly increased ubiquitination of the transfected HMGCR protein (Figure 5I), which, however, was competitively inhibited by atorvastatin (Figure 5J) and pomalidomide (Figure 5K). Taken together, these results suggest that P22A induces degradation of HMGCR protein via UPS.

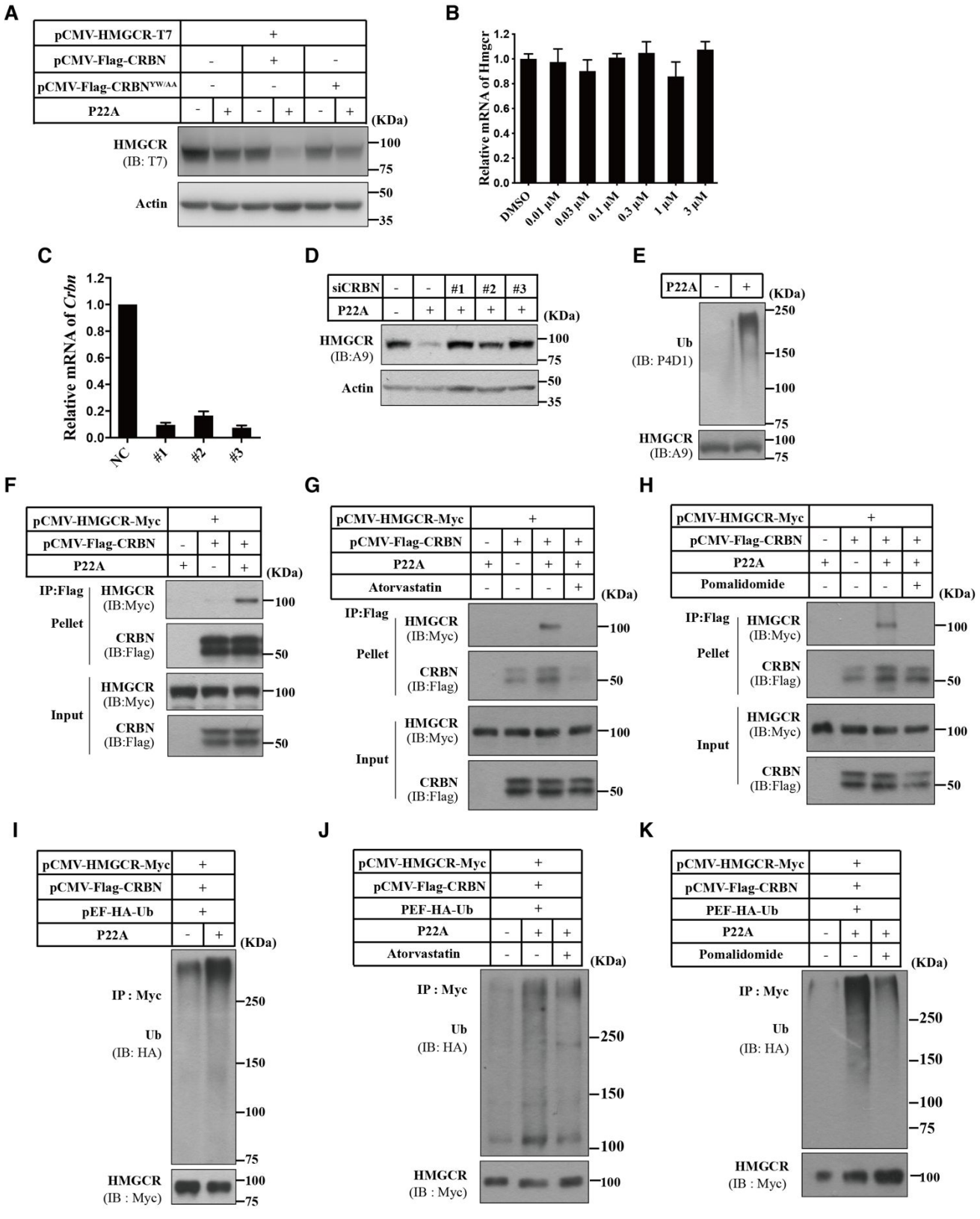


Figure 5. P22A induces degradation of HMGCR via UPS. (A) SRD15 cells were transfected with the indicated plasmids and treated with DMSO or 1 μM P22A for 16 h. Cells were harvested for immunoblotting. (B) SRD15 cells

were treated with 1 μM P22A at the indicated concentrations for 16 h and harvested for quantitative real-time PCR. Data are presented as mean ± s.d. (n = 3 independent experiments). (C-D) SRD15 cells were transfected with the indicated siRNAs for 48 h and treated with 1 μM P22A for 16 h. Cells were harvested for quantitative real-time PCR (C) to measure the knockdown efficiency of siRNA duplexes against *Crbn* and immunoblotting (D). NC, negative control. Data are normalized to control cells and presented as mean ± s.d. (n = 3 independent experiments). (E) SRD15 cells were treated with DMSO or 1 μM P22A together with 10 μM MG132 for 4 h. Cells were then harvested and probed with P4D1 (anti-ubiquitin) antibodies. (F-H) SRD15 cells were transfected with the indicated plasmids and treated with 1 μM P22A, 1 μM atorvastatin or 10 μM pomalidomide together with10 μM MG132 as indicated for 5 h. Cell lysates were immunoprecipitated with the anti-Flag coupled agarose. (I-K) SRD15 cells were transfected with the indicated plasmids and treated with 1 μM P22A, 1 μM atorvastatin or 10 μM pomalidomide together with 10μM MG132 as indicated for 5 h. Cell lysates were immunoprecipitated with the anti-Myc coupled agarose.

P22A is capable of blocking cholesterol biosynthesis. We next sought to monitor the real-time degradation of endogenous HMGCR in live cells. A construct encoding HMGCR tagged with GFP was prepared and its ER targeting was confirmed by the colocalization with RFP-tagged KDEL (Lys-Asp-Glu-Leu), a well-established ER retention motif (Figure 6A). We then established a HMGCR-GFP stable cell line by infecting SRD15 cells with lentivirus expressing HMGCR-GFP followed by fluorescence-activated cell sorting of GFP positive cells. The HMGCR-GFP stable cells were incubated with either DMSO or P22A for 16 h and examined under a confocal microscope. Consistent with the earlier immunoblotting results (Figure 4A and Figure S2), P22A completely eliminated GFP intensity that was normally visible in DMSO-treated cells (Figure 6B). It is known that statins by

inhibiting HMGCR-mediated cholesterol biosynthesis promote proteolytic processing of SREBP[44-48], generating the cleaved N terminus that translocates into the nucleus to activate the transcription of target gene including *HMGCR* and LDL receptor (*LDLR*). We reasoned P22A, by inhibiting HMGCR activity via the atorvastatin moiety while inducing HMGCR degradation via the pomalidomide moiety, would further decrease sterol levels in the cell and thus activate the SREBP pathway in a greater extent than atorvastatin alone did. Indeed, the compensatory increases in HMGCR protein were markedly attenuated in Huh7 cells treated with P22A relative to those with atorvastatin (Figure 6C). We next examined the expression of *Hmgcr* and *Ldlr*, two SREBP2 targets, in mouse primary hepatocytes receiving side-by-side treatments of atorvastatin and P22A. P22A increased slightly, if not significantly, the mRNA abundance of *Hmgcr* and *Ldlr* relative to atorvastatin at both 0.3 and 1 μM (Figure 6D). In addition to P22A, we also examined the protein levels of HMGCR, SREBP2 and LDLR in Huh7 cells treated with compounds **12b**-**12e**, and found these PROTAC molecules showed similar activities as P22A (Figure S8). To directly compare the effects of atorvastatin and P22A on cholesterol biosynthesis, we pulse-labeled Huh7 cells pre-treated with P22A or atorvastatin with ^{14}C-acetate. The radiolabeled lipids were fractionated by thin-layer chromatography (TLC) and visualized using a phosphorimager. The radioactivity of ^{14}C-labeled cholesterol in P22A-treated cells was dose-dependently decreased in an extent similar to that in atorvastatin-treated cells (Figure 6E). As excess cholesterol can be converted to cholesteryl esters that store with triglycerides in the lipid droplets, we also examined the amounts of lipid droplets in Huh7 cells treated with P22A and found no significant changes in Oil Red O staining relative to cells exposed to DMSO (Figure S9). These results suggest that, although de novo cholesterol synthesis is decreased by P22A, the total neutral lipids (cholesteryl ester and triglyceride) are not altered under these conditions.

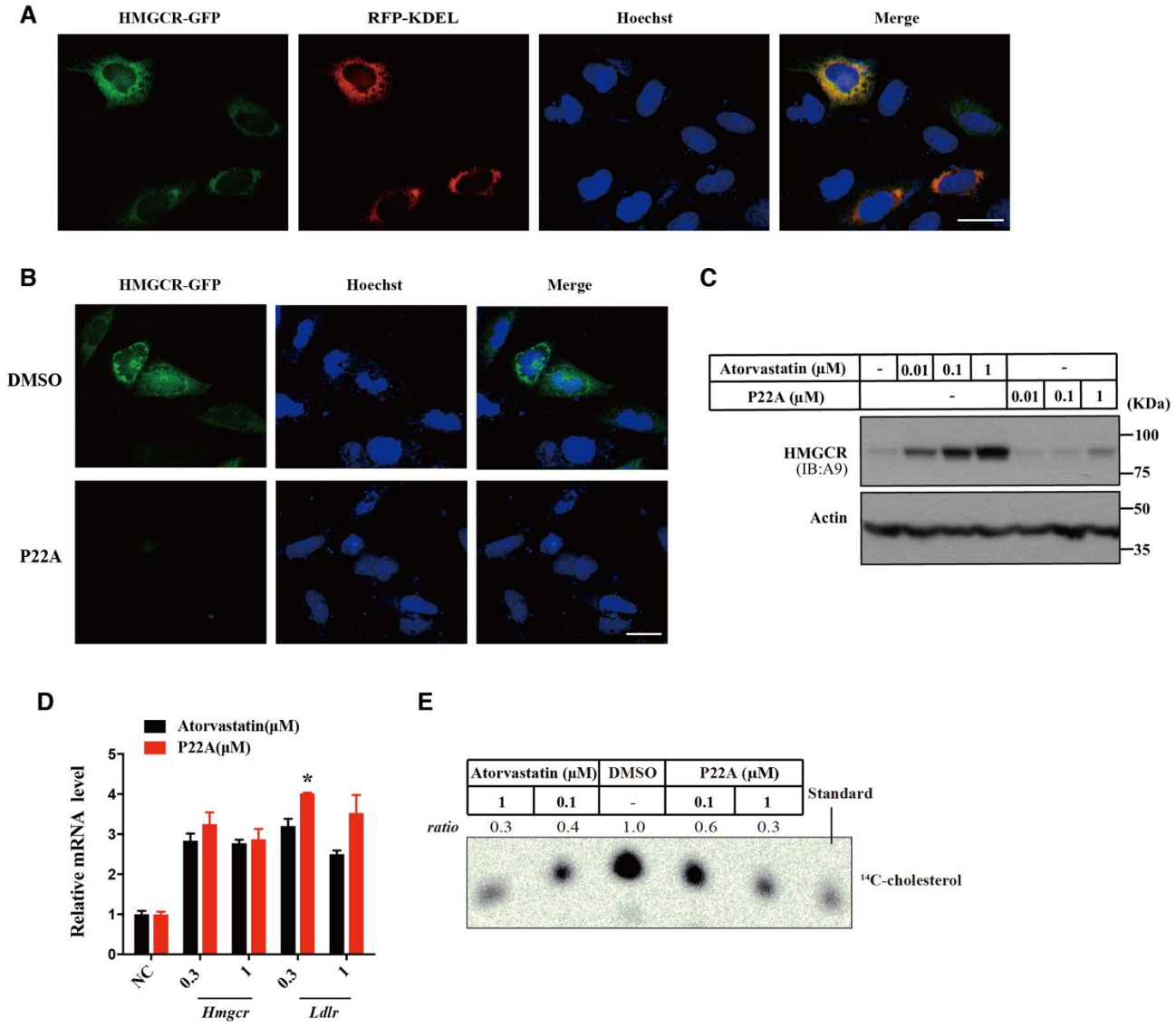


Figure 6. P22A can inhibit cholesterol biosynthesis. (A) SRD15 cells were transfected with HMGCR-GFP (green) and RFP-KDEL (red). After 48 h, cells were fixed and counterstained with Hoechst (blue). Scale bar, 50 μm. (B) SRD15 cells stably expressing HMGCR-GFP were treated with DMSO or 1 μM P22A for 16 h. Cells were fixed and counterstained with Hoechst (blue). Scale bar, 50 μm. (C) Huh7 cells were treated with atorvastatin or P22A as indicated for 16 h and harvested for immunoblotting. (D) Mouse primary hepatocytes were treated with atorvastatin or P22A as indicated for 16 h and harvested for quantitative real-time PCR. Data are presented as mean ± s.d. (n = 3 independent experiments). (E) Huh7 cells were depleted of sterols and incubated with varying concentrations of

atorvastatin or P22A for 14 h. Cells were then pulse-labeled with 1 μCi/μL ^{14}C-acetate for 2 h and the radiolabeled lipids were fractionated by TLC and visualized using a phosphorimager. The value in DMSO-treated group is defined as 1.

P22A and atorvastatin intervene differently on the proteomic level. Then we performed a proteomic study to compare the expressional differences in a broad spectrum of proteins between inhibition by atorvastatin and degradation by P22A. SRD15 cells were treated with DMSO, atorvastatin or P22A and whole proteome was analyzed quantitatively. The quality of acquired data was validated by their normal distribution pattern and Pearson correlation coefficient (Figure S10A and S10B). The triplicates of DMSO, atorvastatin and P22A groups were clustered within very different regions in the plot of principal component analysis (PCA, Figure 7A). The ANOVA analysis of the whole proteome revealed a total of 122 proteins with distinct expression levels that could be further divided into 4 clusters based on their normalized abundance (Figure 7B, Cluster 1-4 and Table S1). The HMGCR abundance was approximately doubled in the inhibition group but reduced to half in the degradation group, consistent with the immunoblotting results (Figure 4A and Figure S11). Another key player in cholesterol homeostasis, Niemann-Pick type C2 protein, was found to increase following both atorvastatin and P22A treatments by proteomic and immunoblotting analysis (Figure S11 and Table S2), further confirming the legitimacy of our proteomic data. Intriguingly, we observed that TXNDC11, an uncharacterized disulphide reductase involved in the ERAD pathway[49], was increased by 108 fold (Table S2, $p = 0.17$) in the inhibition group and 7 fold (Table S2, $p < 0.01$) in the degradation group, suggesting it could be a factor that responds to atorvastatin stimulation. As the

degradation mechanism of HMGCR via the Insig-mediated ERAD pathway is very different from the PROTAC-induced process[50-52], it would be interesting to investigate the relationship between statins and TXNDC11 in the future.

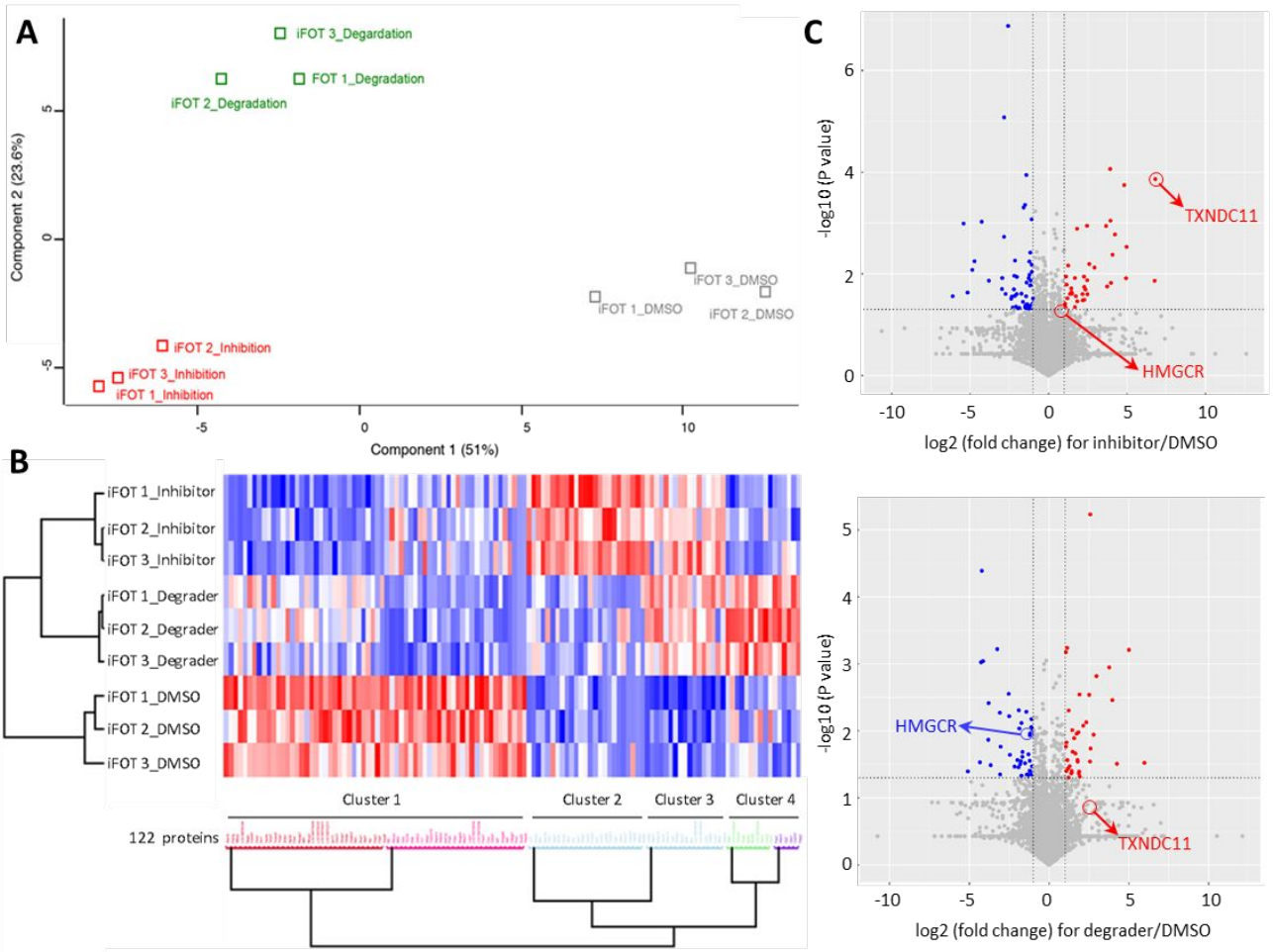


Figure 7. P22A and atorvastatin affect proteomic level differently. (A) The PCA plot of DMSO, atorvastatin (inhibition) and P22A (degradation). (B) The heatmap from ANOVA for whole proteomic data. Blue means low abundance and red means high abundance after normalization. (C) The volcano plots of fold change for atorvastatin (inhibitor, *upper panel*) and P22A (degrader, *lower panel*) compared with DMSO.

CONCLUSIONS

In the process of traditional drug discovery, small molecules have been designed to affect protein activity by binding to the active and allosteric sites of target proteins, or disrupting their protein-protein interactions. Instead of protein activity, modulators of protein abundance could function as promising therapeutic tools for complementing the deficiency of traditional drug discovery[53-54]. RNA interference (RNAi)[55-56] and CRISPR-Cas9[57] are two representative techniques in this respect. However, RNA-based therapeutics are limited by RNA instability, immunogenicity, and the requirement for a vehicle for efficient transport into the target cells, whereas CRISPR-Cas9 remains untested in clinic yet[56-57]. By contrast, PROTACs that combine the pharmacokinetic properties of small molecules and the advantages of genetic knockdown techniques could have more promising opportunities in the future, especially for the anti-cancer field[36,58].

Dyslipidemia is a well-established risk factor for CVD characterized by high levels of cholesterol[2,59], and is prevalent in both developed and developing countries. Atorvastatin under the trade name Lipitor was the world's best-selling drug with 130 billion dollars in sales from 1996 to 2012[60]. However, few options are available for patients with statin intolerance, CVD after maximal dosage of statin, or severe dyslipidemia[61]. In particular, statin-induced compensatory upregulation of HMGCR unavoidably limits their utilization, urging for the development of additional therapy strategies. The proprotein convertase subtilisin-kexin type 9 (PCSK9) monoclonal antibodies are a milestone in the field[62]. Small interfering RNAs targeting PCSK9 have also been developed[63]. Antibody drugs may always be limited by their price, immunogenicity and route of administration, while the disadvantages of RNA-based methods are discussed above. Several ubiquitin ligases have been reported to mediate degradation of HMGCR via the UPS under physiological conditions, and this native process is valuable for developing new therapies for diseases caused by high cholesterol[31,45,64]. Therefore, the artificial

atorvastatin-pomalidomide conjugates such as HMGCR-PROTACs may be applicable for the treatment of dyslipidemia treatment, CVD prevention and other diseases.

In comparison with the native Insig-mediated HMGCR degradation pathway, PROTACs utilize different E3 ligases to degrade HMGCR and are able to induce degradation of both endogenous and ectopically expressed HMGCR protein. Particularly, the P22A compound has an atorvastatin moiety and a pomalidomide moiety. On one hand, the statin group of P22A inhibits HMGCR activity, decreases the downstream sterols and then activates the SREBP pathway, thereby causing compensatory increases in HMGCR protein in the cells with Insigs. On the other hand, the pomalidomide moiety recruits the E3 ubiquitin ligase CRBN to degrade HMGCR. In SRD15 cells that lack Insig-1 and -2, the SREBP pathway has been constitutively activated, and statins cannot further increase HMGCR expression. The basal level of HMGCR is very high. Therefore, P22A-induced HMGCR degradation through CRBN is very dramatic. In Huh7 cells, the two effects of P22A exist and atorvastatin only increases HMGCR. Hence, the HMGCR level in P22A-treated cells were much less than that in atorvastatin-treated cells (Figure 6C), indicating that P22A does promote HMGCR degradation via CRBN. Notably, P22A inhibits cholesterol biosynthesis with the similar potency to atorvastatin (Figure 6E).

Together, our study establishes a proof-of-concept for HMGCR degradation by PROTACs and suggests a new potential strategy for treating hypercholesterolemia and CVD. Our work also reveals that, in contrast to conventional inhibition by small molecule inhibitors (such as statins), PROTACs may potentially be used as a general way to solve compensatory upregulation of proteins that contributes to illness, adverse effects and drug resistance[65-67].

EXPERIMENTAL SECTION

Chemistry. All commercial chemical materials (Energy, Ouhe, Aladdin, J&K Chemical Co. Ltd.) were used without further purification. All solvents were analytical grade. The ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker AVANCE III 400 MHz spectrometer in $CDCl_3$, CD_3OD, using tetramethylsilane (TMS) or solvent peak as a standard. All ^{13}C NMR spectra were recorded with complete proton decoupling. Low-resolution mass spectral analyses were performed with a Waters ACQUITY UPLC/MS. High-resolution mass spectral analyses were performed with a Thermo Scientific OrbiTrap Q-Exactive. Analytical thin-layer chromatography (TLC) was performed on Yantai Chemical Industry Research Institute silica gel 60 F254 plates, and flash column chromatography was performed on Qingdao Haiyang Chemical Co. Ltd. silica gel 60 (200−300 mesh). A BUCHI Rotavapor R-3 was used to remove solvents by evaporation. The purity of all the final tested compounds was more than 95% as confirmed by NMR and UPLC. Atorvastatin was from Energy Chemical (E1201400010), Pomalidomide was from Bide Pharmatech Ltd (BD235626). Compounds P13A-P23A were synthesized and dissolved in DMSO at a concentration of 10 mM and stored at -20°C. For cell culture experiments drugs were diluted at least by 1:1,000 so that the final DMSO concentration was 0.1% or lower. Sterols were obtained from Steraloids. MG-132 was obtained from Sigma. ^{14}C-acetic acid sodium salt was obtained from Perkin Elmer.

2-(3-Bromophenyl)acetyl chloride (1). To a 100 ml round-bottom flask was added 2-(3-bromophenyl)acetic acid (4.2 g, 20 mmol), thionyl chloride (4 ml, 60 mmol), DMF (1 drop) and DCM (30 ml) as solvent. The mixture was stirred at room temperature overnight. Then the solvent was

removed under reduced pressure. The residual was used directly in the next step without purification.

2-(3-Bromophenyl)-1-(4-fluorophenyl)ethan-1-one (2). To a 100 ml round-bottom flask containing $AlCl_3$ (3.2 g, 24 mmol) and fluorobenzene (9 ml, 100 mmol) at ice bath was added **1** dropwise. After stirring at ice bath for 20 min, it was stirred at 50 ^oC for another 7 h. The mixture was diluted by 2N HCl solution and extracted by EtOAc. The organic layer was purified by silica gel column with hexane: EtOAc = 20: 1 as eluent to get **2** (Isolated yield = 93 %). ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 8.02 – 7.99 (m, 2H), 7.40 – 7.38 (m, 2H), 7.21 – 7.11 (m, 4H), 4.42 (s, 2H). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 195.3, 167.3, 164.7, 136.6, 132.9, 132.9, 132.6, 131.4, 131.3, 130.3, 128.3, 122.8, 116.1, 115.9, 44.9. LR−MS: calculated for $C_{14}H_{11}BrFO$ $[M+H]^+$, 292.99; found, 293.15.

2-Bromo-2-(3-bromophenyl)-1-(4-fluorophenyl)ethan-1-one (3). Compound **2** (2.9 g, 10 mmol) was dissolved in $CHCl_3$ to which 30 % HBr in AcOH (1 ml) was added at room temperature. A solution of bromine (about 600 uL) in $CHCl_3$ was added dropwise. The addition continued until slight bromine coloration remined. Then a saturated Na_2SO_3 solution was added and extract with EtOAc. The organic layer was purified by silica gel column with hexane: EtOAc = 20: 1 as eluent to get **3** (Isolated yield = 70 %). ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 8.03 (dd, J = 8.88 Hz, J = 5.24 Hz, 2H), 7.69 (t, J = 1.72 Hz, 1H), 7.46 (t, J = 7.08 Hz, 2H), 7.25 (t, J = 7.88 Hz, 1H), 7.14 (t, J = 8.48 Hz, 2H), 6.22 (s, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 189.2, 167.5, 164.9, 137.8, 132.5, 132.3, 132.1, 132.0, 130.6, 130.4, 130.4, 128.0, 122.9, 116.4, 116.2, 48.8. LR−MS: calculated for $C_{14}H_{10}Br_2FO$ $[M+H]^+$, 370.90; found, 371.34.

2-(1-(3-Bromophenyl)-2-(4-fluorophenyl)-2-oxoethyl)-4-methyl-3-oxo-N-phenylpentanamid (4). A mixture of compound **3** (2.2 g, 5.9 mmol), 4-Methyl-3-oxo-N-phenylpentanamide (1.2 g, 5.9 mmol)

and K_2CO_3 (1.2 g, 8.9 mmol) in 30 ml acetone was refluxed at 60 °C overnight. Then dilute with brine solution and extract with EtOAc. The organic layer was purified by silica gel column with hexane: EtOAc = 7: 1 as eluent to get **4** (Isolated yield = 55 %). ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 7.98 (dd, *J* = 8.84 Hz, *J* = 5.36 Hz, 2H), 7.45 (t, *J* = 1.56 Hz, 1H), 7.37 – 7.04 (m, 12H), 5.36 (d, *J* = 9.52 Hz, 1H), 4.51 (d, *J* = 9.56 Hz, 1H), 3.01 – 2.95 (m, 1H), 1.24 (d, *J* = 5.92 Hz, 3H), 1.15 (d, *J* = 5.84 Hz, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 209.6, 196.0, 167.2, 164.9, 164.8, 137.6, 136.7, 132.0, 132.0, 131.9, 131.8, 131.6, 131.2, 131.1, 129.2, 127.5, 125.3, 123.5, 120.6, 116.1, 115.9, 64.30, 54.0, 41.3, 18.7, 18.2. LR−MS: calculated for $C_{26}H_{24}BrFNO_3$ $[M+H]^+$, 496.08; found, 496.27.

Tert-butyl 2-((4R,6R)-6-(2-(3-(3-bromophenyl)-2-(4-fluorophenyl)-5-isopropyl-4-(phenylcarbamoyl)-1H-pyrrol-1-yl)ethyl)-2,2-dimethyl-1,3-dioxan-4-yl)acetate (5). To a 50 ml round-bottom flask containing 5 ml toluene, 5 ml THF and 10 ml cyclohexane was added **4** (2.3 g, 4.6 mmol), *tert*-butyl 2-((4R,6R)-6-(2-aminoethyl)-2,2-dimethyl-1,3-dioxan-4-yl)acetate (2.5g, 9.2 mmol) and *t*-BuCOOH (470mg, 4.6mmol). The mixture was refluxed at 115 °C for 40 h and extracted with EtOAc. The organic layer was purified by silica gel column with hexane: EtOAc = 15: 1 as eluent to get **5** (Isolated yield = 82%). ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 7.30 (s, 1H), 7.26 – 7.16 (m, 7H), 7.05 – 7.01 (m, 5H), 6.86 (s, 1H), 4.17 – 4.13 (m, 1H), 4.09 – 4.02 (m, 1H), 3.84 – 3.77 (m, 1H), 3.72 – 3.65 (m, 1H), 3.51 – 3.44 (m, 1H), 2.39 (dd, *J* = 15.24 Hz, *J* = 7.00 Hz, 1H), 2.24 (dd, *J* = 15.24 Hz, *J* = 6.12 Hz, 1H), 1.69 – 1.62 (m, 2H), 1.52 (s, 3H), 1.50 (s, 3H), 1.43 (s, 9H), 1.36 (s, 4H), 1.30 (s, 3H), 1.08 – 1.03 (m, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 170.30, 165.0, 163.8, 161.3, 141.1, 138.2, 136.9, 133.3, 133.2, 133.1, 129.8, 129.4, 129.1, 129.0, 128.9, 127.9, 127.9, 124.0, 122.2, 120.3, 120.0, 115.8, 115.6, 98.8, 80.8, 66.4, 66.0, 42.5, 40.9, 38.2, 36.0, 30.0, 28.2, 26.2, 22.0, 21.8, 19.8. LR−MS: calculated for $C_{40}H_{47}BrFN_2O_5$ $[M+H]^+$, 733.26; found, 733.36.

Tert-butyl 2-((4R,6R)-6-(2-(2-(4-fluorophenyl)-5-isopropyl-4-(phenylcarbamoyl)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-1H-pyrrol-1-yl)ethyl)-2,2-dimethyl-1,3-dioxan-4-yl)acetate (6). To a 50 ml round-bottom flask was added **5** (2.5 g, 3.4 mmol), $Pd(dppf)_2Cl_2$.DCM (90 mg, 0.1 mmol), KOAc (700 mg, 6.8 mmol), Bis(pinacoloto)diboron (1 g, 3.75 mmol) and 30 ml DMF as solvent under the protection of argon. The mixture was stirred at 80 ^oC overnight and extracted with EtOAc. The organic layer was purified by silica gel column with hexane: EtOAc = 10: 1 as eluent to get **6** (Isolated yield = 68%). ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 7.68 (s, 1H), 7.68 (d, J = 5.12 Hz, 1H), 7.20 – 7.14 (m, 6H), 7.07 (d, J = 7.84 Hz, 2H), 7.00 – 6.94 (m, 3H), 6.88 (s, 1H), 4.17 – 4.13 (m, 1H), 4.09 – 4.02 (m, 1H), 3.87 – 3.81 (m, 1H), 3.72 – 3.65 (m, 1H), 3.60 – 3.57 (m, 1H), 2.39 (dd, J = 15.24 Hz, J = 6.96 Hz, 1H), 2.24 (dd, J = 15.48 Hz, J = 6.24 Hz, 1H), 1.68 – 1.65 (m, 2H), 1.53 (s, 3H), 1.52 (s, 3H), 1.43 (s, 9H), 1.36 (s, 4H), 1.30 (s, 3H), 1.23 (s, 12H), 1.07 – 1.02 (m, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 170.3, 164.91, 163.6, 161.1, 141.7, 138.5, 137.4, 134.0, 133.4, 133.4, 133.3, 132.7, 129.0, 128.9, 128.6, 128.4, 128.4, 127.8, 123.6, 121.9, 120.0, 115.4, 115.2, 115.2, 99.7, 83.7, 80.7, 66.5, 66.0, 42.5, 40.9, 38.1, 36.0, 29.9, 28.2, 26.13, 24.8, 24.8, 21.8, 21.6, 19.7. LR−MS: calculated for $C_{46}H_{59}BFN_2O_7$ $[M+H]^+$, 781.43; found, 781.45.

Tert-butyl 2-((4R,6R)-6-(2-(2-(4-fluorophenyl)-3-(3-hydroxyphenyl)-5-isopropyl-4-(phenylcarbamoyl)-1H-pyrrol-1-yl)ethyl)-2,2-dimethyl-1,3-dioxan-4-yl)acetate (7). To a 50 ml round-bottom flask containing 20ml THF was added **6** (1.8 g, 2.3mmol), 30% H_2O_2 in H_2O (2ml) and NaOH (200 mg, 4.6 mmol) at ice bath. After stirring at ice bath for 15 min, it was warmed up to room temperature for another 15 min. Extract with EtOAc and wash by brine. The organic layer was purified by silica gel column with hexane: EtOAc = 5: 1 as eluent to get **7** (Isolated yield = 84%). ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 7.26 – 7.08 (m, 6H), 7.03 – 6.94 (m, 5H), 6.65 – 6.63 (m, 3H), 4.19 – 4.14

(m, 1H), 4.08 – 4.02 (m, 1H), 3.85 – 3.81 (m, 1H), 3.79 – 3.67 (m, 1H), 3.60 – 3.56 (m, 1H), 2.38 (dd, *J* = 15.28 Hz, *J* = 7.08 Hz, 1H), 2.23 (dd, *J* = 15.28 Hz, *J* = 6.96 Hz, 1H), 1.66 – 1.63 (m, 2H), 1.50 (s, 3H), 1.49 (s, 3H), 1.43 (s, 3H), 1.35 (s, 3H), 1.29 – 1.20 (m, 4H), 1.07 – 1.02 (m, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 170.5, 165.3, 163.6, 161.1, 156.4, 141.6, 138.2, 136.1, 133.2, 133.2, 129.6, 128.9, 128.8, 128.3, 128.2, 123.9, 122.5, 121.7, 120.3, 117.5, 115.5, 115.3, 115.1, 114.0, 98.8, 80.9, 66.5, 66.0, 42.5, 40.9, 38.1, 36.0, 30.0, 28.2, 26.1, 24.8, 21.8, 21.6, 19.7. LR−MS: calculated for $C_{40}H_{48}FN_2O_6$ $[M+H]^+$, 671.34; found, 671.32.

Tert-butyl 2-((4R,6R)-6-(2-(2-(4-fluorophenyl)-5-isopropyl-4-(phenylcarbamoyl)-3-(3-(prop-2-yn-1-yloxy)phenyl)-1H-pyrrol-1-yl)ethyl)-2,2-dimethyl-1,3-dioxan-4-yl)acetate (8a). To a 10 ml sealed tube containing 3ml MeCN was added **7** (670 mg, 1 mmol), K_2CO_3 (540mg, 4 mmol) and 3-bromopropyne (4 mmol). The mixture was stirred at 70 ^oC for 20 h and extracted with EtOAc. The organic layer was purified by silica gel column with hexane: EtOAc = 10: 1 as eluent to get **8a** (Isolated yield = 88%). ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 7.20 – 7.16 (m, 4H), 7.13 – 7.06 (m, 3H), 7.05 – 6.96 (m, 4H), 6.73 – 6.69 (m, 3H), 4.44 (s, 2H), 4.18 – 4.13 (m, 1H), 4.09 – 4.03 (m, 1H), 3.86 – 3.77 (m, 1H), 3.69 – 3.68 (m, 1H), 3.57 – 3.53 (m, 1H), 2.38 (dd, *J* = 15.20 Hz, *J* = 6.80 Hz, 1H), 2.26 – 2.21 (m, 3H), 1.68 – 1.65 (m, 2H), 1.54 (s, 3H), 1.52 (s, 3H), 1.43 (s, 9H), 1.37 – 1.32 (m, 4H), 1.30 (m, 3H), 1.09 – 1.01 (m, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 170.3, 164.8, 163.6, 161.2, 157.5, 141.6, 138.6, 136.9, 133.3, 133.2, 129.5, 128.9, 128.8, 128.3, 124.0, 123.6, 121.6, 119.7, 116.4, 115.6, 115.4, 115.4, 114.2, 98.8, 80.8, 78.4, 75.5, 66.5, 66.0, 55.8, 42.6, 40.9, 38.2, 36.1, 30.0, 28.2, 26.2, 21.8, 21.7, 19.8. LR−MS: calculated for $C_{43}H_{50}FN_2O_6$ $[M+H]^+$, 709.36; found, 709.46.

Tert-butyl 2-((4R,6R)-6-(2-(2-(4-fluorophenyl)-5-isopropyl-3-(3-(pent-4-yn-1-yloxy)phenyl)-4-

(phenylcarbamoyl)-1H-pyrrol-1-yl)ethyl)-2,2-dimethyl-1,3-dioxan-4-yl)acetate (8b). It was synthesized as compound **8a**. ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 7.19 – 7.16 (m, 4H), 7.13 – 7.06 (m, 3H), 7.00 – 6.96 (m, 4H), 6.73 – 6.69 (m, 3H), 4.18 – 4.13 (m, 1H), 4.09 – 4.03 (m, 1H), 3.86 – 3.77 (m, 3H), 3.69 – 3.68 (m, 1H), 3.57 – 3.53 (m, 1H), 2.38 (dd, *J* = 15.20 Hz, *J* = 6.80 Hz, 1H), 2.26 – 2.21 (m, 3H), 1.91 (t, *J* = 1.04 Hz, 1H), 1.81 – 1.78 (m, 2H), 1.68 – 1.65 (m, 2H), 1.54 (s, 3H), 1.52 (s, 3H), 1.43 (s, 9H), 1.37 – 1.32 (m, 4H), 1.30 (m, 3H), 1.09 – 1.01 (m, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 170.3, 165.0, 163.6, 161.1, 158.8, 141.5, 138.6, 136.0, 133.3, 133.2, 129.4, 128.8, 128.8, 128.4, 128.4, 123.6, 123.0, 121.8, 119.7, 115.9, 115.6, 115.4, 114.0, 98.8, 83.5, 80.8, 68.9, 66.5, 66.1, 66.0, 53.5, 42.5, 40.9, 38.2, 36.1, 30.0, 28.2, 28.0, 26.2, 21.9, 21.7, 19.8, 15.1. LR−MS: calculated for $C_{45}H_{54}FN_2O_6$ $[M+H]^+$, 737.39; found, 737.36.

Tert-butyl 2-((4R,6R)-6-(2-(2-(4-fluorophenyl)-3-(3-(hex-5-yn-1-yloxy)phenyl)-5-isopropyl-4-(phenylcarbamoyl)-1H-pyrrol-1-yl)ethyl)-2,2-dimethyl-1,3-dioxan-4-yl)acetate (8c). It was synthesized as compound **8a**. ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 7.20 – 7.17 (m, 4H), 7.12 – 7.06 (m, 3H), 7.03 – 6.97 (m, 3H), 6.94 (s, 1H), 6.71 – 6.69 (m, 3H), 4.18 – 4.03 (m, 2H), 3.86 – 3.78 (m, 1H), 3.71 – 3.64 (m, 3H), 3.57 – 3.51 (m, 1H), 2.38 (dd, *J* = 15.28 Hz, *J* = 6.80 Hz, 1H), 2.23 (dd, *J* = 15.28 Hz, *J* = 7.06 Hz, 1H), 2.16 – 2.12 (m, 2H), 1.93 (t, *J* = 1.04 Hz, 1H), 1.73 – 1.63 (m, 4H), 1.58 – 1.49 (m, 8H), 1.44 (s, 9H), 1.36 (s, 3H), 1.31 – 1.27 (m, 4H), 1.09 – 1.01 (m, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 170.3, 165.0, 163.6, 161.2, 158.9, 141.5, 138.6, 136.0, 133.3, 133.2, 129.4, 128.8, 128.8, 128.4, 128.4, 123.7, 122.9, 121.8, 119.6, 115.9, 115.6, 115.5, 115.4, 114.0, 98.8, 84.2, 80.8, 68.7, 67.2, 66.5, 66.0, 42.6, 40.9, 38.2, 36.1, 30.0, 28.2, 28.1, 26.2, 25.0, 21.9, 21.7, 19.8, 18.1. LR−MS: calculated for $C_{46}H_{56}FN_2O_6$ $[M+H]^+$, 751.40; found, 751.37.

Tert-butyl 2-((4R,6R)-6-(2-(2-(4-fluorophenyl)-3-(3-(hept-6-yn-1-yloxy)phenyl)-5-isopropyl-4-(phenylcarbamoyl)-1H-pyrrol-1-yl)ethyl)-2,2-dimethyl-1,3-dioxan-4-yl)acetate (8d). It was synthesized as compound **8a**. ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 7.20 – 7.17 (m, 4H), 7.12 – 7.06 (m, 3H), 7.03 – 6.97 (m, 3H), 6.95 (s, 1H), 6.71 – 6.68 (m, 3H), 4.19 – 4.03 (m, 2H), 3.87 – 3.78 (m, 1H), 3.71 – 3.63 (m, 3H), 3.56 – 3.51 (m, 1H), 2.39 (dd, J = 15.28 Hz, J = 6.80 Hz, 1H), 2.23 (dd, J = 15.28 Hz, J = 7.06 Hz, 1H), 2.16 – 2.13 (m, 2H), 1.94 (t, J = 1.04 Hz, 1H), 1.69 – 1.61 (m, 4H), 1.56 – 1.45 (m, 8H), 1.44 (s, 11H), 1.37 (s, 3H), 1.30 – 1.26 (m, 4H), 1.09 – 1.01 (m, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 170.2, 165.0, 164.6, 161.1, 159.0, 141.5, 138.6, 135.9, 133.3, 133.2, 129.3, 128.8, 128.8, 128.4, 128.4, 123.6, 122.8, 121.8, 199.6, 115.9, 115.5, 115.3, 114.0, 98.7, 84.5, 80.8, 68.5, 67.7, 66.5, 66.0, 53.5, 42.5, 40.9, 38.1, 36.0, 30.0, 29.8, 28.6, 28.2, 28.1, 26.2, 25.2, 21.8, 21.7, 19.7, 18.4. LR−MS: calculated for $C_{47}H_{58}FN_2O_6$ $[M+H]^+$, 765.42; found, 765.45.

(3R,5R)-7-(2-(4-fluorophenyl)-5-isopropyl-4-(phenylcarbamoyl)-3-(3-(prop-2-yn-1-yloxy)phenyl)-1H-pyrrol-1-yl)-3,5-dihydroxyheptanoic acid (9a). To a 25 ml round-bottom flask containing 4 ml MeOH was added **8a** (600 mg, 0.82 mmol) and 6N HCl (200 μL). After stirring at room temperature for 4 h, NaOH (400 mg, 10 mmol) and H_2O (100 μL) were added. The mixture was stirred for another 5 h and acidified by HCl solution. Extract with EtOAc and purify by silica gel column with EtOAc as eluent to get **9a** (Isolated yield = 75%)^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 7.26 – 6.93 (m, 11H), 6.70 – 6.65 (m, 3H), 5.81 (b, 2H), 4.44 (s, 2H), 4.09 – 4.03 (m, 2H), 3.96 – 3.86 (m, 1H), 3.77 (t, J = 5.52 Hz, 2H), 3.66 – 3.62 (m, 1H), 3.53 – 3.48 (m, 1H), 2.48 – 2.35 (m, 2H), 2.31 (s, 1H), 1.65 – 1.55 (m, 2H), 1.49 – 1.42 (m, 8H). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 165.4, 163.6, 161.1, 157.5, 141.3, 138.2, 136.0, 133.3, 133.2, 129.5, 129.0, 128.8, 128.3, 124.1, 123.9, 121.7, 120.1, 116.4, 115.8, 115.5, 114.2, 78.5, 75.6, 69.8, 68.8, 55.8, 41.6, 41.3, 39.0, 29.8, 26.3, 22.0, 21.9. LR−MS:

calculated for $C_{36}H_{38}FN_2O_6$ $[M+H]^+$, 613.26; found, 613.44.

(3R,5R)-7-(2-(4-fluorophenyl)-5-isopropyl-3-(3-(pent-4-yn-1-yloxy)phenyl)-4-(phenylcarbamoyl)-1H-pyrrol-1-yl)-3,5-dihydroxyheptanoic acid (9b). It was synthesized as compound **9a**.^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 7.25 – 6.93 (m, 11H), 6.70 – 6.65 (m, 3H), 5.81 (b, 2H), 4.09 – 4.03 (m, 2H), 3.96 – 3.86 (m, 1H), 3.77 (t, J = 5.52 Hz, 2H), 3.66 – 3.62 (m, 1H), 3.53 – 3.47 (m, 1H), 2.24 – 2.16 (m, 4H), 1.89 (s, 1H), 1.80 – 1.73 (m, 2H), 1.65 – 1.55 (m, 2H), 1.49 – 1.42 (m, 8H). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 169.5, 165.0, 162.6, 159.8, 139.8, 139.0, 137.5, 134.7, 134.6, 130.3, 130.2, 129.8, 129.7, 129.6, 125.2, 123.3, 123.0, 121.4, 118.0, 116.5, 116.3, 114.0, 84.1, 70.0, 68.6, 68.1, 67.1, 44.0, 42.1, 40.1, 29.3, 27.6, 22.8, 22.8, 15.7. LR−MS: calculated for $C_{38}H_{42}FN_2O_6$ $[M+H]^+$, 641.29; found, 641.32.

(3R,5R)-7-(2-(4-fluorophenyl)-3-(3-(hex-5-yn-1-yloxy)phenyl)-5-isopropyl-4-(phenylcarbamoyl)-1H-pyrrol-1-yl)-3,5-dihydroxyheptanoic acid (9c). It was synthesized as compound **9a**. ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 7.22 – 7.14 (m, 4H), 7.12 – 6.98 (m, 6H), 6.95 (s, 1H), 6.71 – 6.62 (m, 3H), 4.17 – 4.09 (m, 2H), 4.02 – 3.90 (m, 1H), 3.78 – 3.67 (m, 3H), 3.57 – 3.49 (m. 1H), 2.45 (d, J = 5.80 Hz, 2H), 2.18 – 2.12 (m, 2H), 1.93 (t, J = 1.40 Hz, 1H), 1.76 – 1.69 (m, 2H), 1.65 – 1.49 (m, 12H). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 165.6, 163.6, 161.1, 158.9, 141.3, 138.2, 135.8, 133.3, 133.2, 129.4, 128.8, 128.4, 124.1, 122.8, 122.0, 120.0, 115.9, 115.7, 115.5, 115.4, 115.9, 113.9, 84.2, 69.8, 68.8, 68.7, 67.3, 41.6, 41.3, 38.9, 29.8, 28.1, 26.3, 25.0, 22.0, 21.9, 18.1. LR−MS: calculated for $C_{39}H_{44}FN_2O_6$ $[M+H]^+$, 655.31; found, 641.26.

(3R,5R)-7-(2-(4-fluorophenyl)-3-(3-(hept-6-yn-1-yloxy)phenyl)-5-isopropyl-4-(phenylcarbamoyl)-1H-pyrrol-1-yl)-3,5-dihydroxyheptanoic acid (9d). It was synthesized as compound **9a**. ^{1}H-NMR

(400 MHz, $CDCl_3$, ppm): 7.21 – 6.96 (m, 11H), 6.70 – 6.68 (m, 3H), 4.15 – 4.13 (m, 2H), 4.11 – 3.93 (m, 1H), 3.78 – 3.62 (m, 3H), 3.57 – 3.49 (m. 1H), 2.45 (d, J = 5.82 Hz, 2H), 2.18 – 2.12 (m, 2H), 1.94 (t, J = 1.46 Hz, 1H), 1.65 – 1.56 (m, 4H), 1.55 – 1.39 (m, 12H). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 165.6, 163.6, 161.2, 159.0, 141.4, 138.3, 135.8, 133.3, 133.2, 129.4, 128.9, 128.5, 128.4, 124.0, 122.8, 122.0, 120.0, 115.9, 115.7, 115.5, 115.4, 114.0, 84.5, 73.2, 69.8, 68.9, 68.5, 67.8, 41.6, 41.3, 38.9, 37.2, 32.0, 29.8, 29.4, 28.6, 28.2, 26.3, 25.2, 22.0, 21.9, 18.4. LR−MS: calculated for $C_{40}H_{46}FN_2O_6$ $[M+H]^+$, 669.33; found, 669.27.

4-((2-(2-Azidoethoxy)ethyl)amino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (10a). To a 10 ml sealed tube charged with stirring bar was added 2-(2-azidoethoxy)ethanamine (150 mg, 1.1 mmol), 2-(2,6-dioxopiperidin-3-yl)-4-fluoroisoindoline-1,3-dione (140 mg, 1.08 mmol) and *N,N*-Diisopropylethylamine (280 mg, 2.16 mmol) with 2 ml DMF as solvent. The mixture was stirred at 90 ^{o}C for 12 h. After extracted with EtOAc and washed by brine, the organic layer was purified by silica gel column with DCM: EtOAc = 10: 1 as eluent to get **10a** (Isolated yield = 39%). ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 8.34 (s, 1H), 7.49 (t, J = 7.72 Hz, 1H), 7.10 (d, J = 7.12 Hz, 1H), 6.93 (d, J = 8.52 Hz, 1H), 6.49 (t, J = 5.56 Hz, 1H), 4.91 (dd, J = 12 Hz, J = 5.44 Hz, 1H), 3.72 (t, J = 5.28 Hz, 2H), 3.67 (t, J = 4.72 Hz, 2H), 3.50 (t, J = 5.36 Hz, 2H), 3.40 (t, J = 4.68 Hz, 2H), 2.95 – 2.69 (m, 3H), 2.13 – 2.09 (m, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 171.3, 169.4, 168.6, 167.7, 146.9, 136.2, 132.6, 116.9, 111.9, 110.5, 70.2, 69.9, 50.8, 49.2, 49.0, 42.5, 31.5, 22.9. LR−MS: calculated for $C_{17}H_{19}N_6O_5$ $[M+H]^+$, 387.13; found, 387.28.

4-((2-(2-(2-Azidoethoxy)ethoxy)ethyl)amino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (10b). Following the procedure to synthesize **10a**, 2-(2-azidoethoxy)ethanamine was replaced by 2-

(2-(2-azidoethoxy)ethoxy)ethanamine to get **10b** (Isolated yield = 33%). ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 8.37 (s, 1H), 7.49 (t, *J* = 7.72 Hz, 1H), 7.09 (d, *J* = 7.08 Hz, 1H), 6.92 (d, *J* = 8.52 Hz, 1H), 6.49 (t, *J* = 5.44 Hz, 1H), 4.91 (dd, *J* = 12.24 Hz, *J* = 5.60 Hz, 1H), 3.74 – 3.66 (m, 8H), 3.48 – 3.46 (m, 2H), 3.37 (t, *J* = 5.08 Hz, 2H), 2.85 – 2.67 (m, 3H), 2.09 – 2.07 (m, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 171.5, 170.4, 169.3, 168.6, 167.7, 146.9, 136.1, 132.6, 116.9, 111.6, 110.3, 70.7, 70.3, 69.7, 68.8, 51.0, 48.9, 42.5, 31.5, 22.8. LR−MS: calculated for $C_{19}H_{23}N_6O_6$ $[M+H]^+$, 431.16; found, 431.34.

4-((2-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)ethyl)amino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (10c). Following the procedure to synthesize **10a**, 2-(2-azidoethoxy)ethanamine was replaced by 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethanamine to get **10c** (Isolated yield = 45%). ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 8.66 (s, 1H), 7.45 (t, *J* = 7.20 Hz, 1H), 7.05 (d, *J* = 7.12 Hz, 1H), 6.88 (d, *J* = 8.32 Hz, 1H), 6.45 (s, 1H), 4.91 (m, 1H), 3.78 – 3.58 (m, 12H), 3.50 – 3.48 (m, 2H), 3.36 – 3.34 (m, 2H), 2.89-2.71 (m, 3H), 2.13 – 2.09 (m, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 171.3, 169.4, 168.6, 167.7, 146.9, 136.2, 132.6, 116.9, 111.9, 110.5, 70.2, 69.9, 50.8, 49.2, 49.0, 42.5, 31.5, 22.9. LR−MS: calculated for $C_{21}H_{27}N_6O_7$ $[M+H]^+$, 475.19; found, 475.21.

4-((14-Azido-3,6,9,12-tetraoxatetradecyl)amino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (10d). Following the procedure to synthesize **10a**, 2-(2-azidoethoxy)ethanamine was replaced by 14-azido-3,6,9,12-tetraoxatetradecan-1-amine to get **10d** (Isolated yield = 30%). ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 8.08 (s, 1H), 7.49 (t, *J* = 7.60 Hz, 1H), 7.10 (d, *J* = 7.08 Hz, 1H), 6.92 (d, *J* = 8.52 Hz, 1H), 6.49 (t, *J* = 5.64 Hz, 1H), 4.91 (dd, *J* = 12.24 Hz, *J* = 5.52 Hz, 1H), 3.67 – 3.49 (m, 16H), 3.48-3.45 (m, 2H), 3.39 (t, *J* = 5.16 Hz, 2H), 2.91 – 2.71 (m, 3H), 2.15-2.10 (m, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 171.6, 169.3, 168.8, 167.7, 146.8, 136.0, 132.5, 116.8, 111.6, 110.3, 71.3, 70.7,

70.6, 70.5, 69.7, 69.5, 50.7, 42.8, 42.4, 31.4, 22.8. LR−MS: calculated for $C_{23}H_{31}N_6O_8$ $[M+H]^+$, 519.21; found, 519.13.

4-((14-azido-3,6,9,12-tetraoxatetradecyl)amino)-2-(1-ethyl-2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (10e). It was synthesized as compound **10a**. ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 7.50 (t, *J* = 7.60 Hz, 1H), 7.10 (d, *J* = 7.08 Hz, 1H), 6.92 (d, *J* = 8.52 Hz, 1H), 6.49 (t, *J* = 5.64 Hz, 1H), 4.91 (m, 1H), 3.67 – 3.49 (m, 18H), 3.48-3.45 (q, *J* = 6.80 Hz, 2H), 3.39 (t, *J* = 5.16 Hz, 2H), 2.91 – 2.71 (m, 3H), 2.15-2.10 (m, 1H), 1.15 (t, *J* = 6.80 Hz, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 171.6, 169.3, 168.7, 167.7, 146.8, 136.0, 132.5, 116.8, 111.6, 110.3, 71.3, 70.7, 70.6, 70.5, 69.7, 69.5, 50.7, 42.8, 42.4, 31.4, 22.7. LR−MS: calculated for $C_{25}H_{35}N_6O_8$ $[M+H]^+$, 547.24; found, 547.56.

4-((5-azidopentyl)amino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (10f). Following the procedure to synthesize **10a**, 2-(2-azidoethoxy)ethanamine was replaced by 5-azidopentan-1-amine to get **10f** (Isolated yield = 34%). ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 8.43 (s, 1H), 7.48 (t, *J* = 7.60 Hz, 1H), 7.07 (d, *J* = 7.08 Hz, 1H), 6.86 (d, *J* = 8.52 Hz, 1H), 6.23 (t, *J* = 5.64 Hz, 1H), 4.91 (dd, *J* = 12.24 Hz, *J* = 5.52 Hz, 1H), 3.28 – 3.25 (m, 4H), 2.91 – 2.71 (m, 3H), 2.15-2.10 (m, 1H), 1.75 – 1.52 (m, 4H), 1.50 – 1.35 (m, 2H). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 171.4, 169.6, 168.6, 167.7, 147.0, 136.2, 132.6, 116.7, 111.5, 110.0, 51.4, 49.0, 42.6, 31.5, 29.2, 28.8, 26.6, 26.5, 22.9. LR−MS: calculated for $C_{18}H_{21}N_6O_4$ $[M+H]^+$, 385.15; found, 385.23.

4-((6-azidohexyl)amino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (10g). Following the procedure to synthesize **10a**, 2-(2-azidoethoxy)ethanamine was replaced by 5-azidopentan-1-amine to get **10g** (Isolated yield = 34%). ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 8.42 (s, 1H), 7.49 (t, *J* = 7.60 Hz, 1H), 7.07 (d, *J* = 7.08 Hz, 1H), 6.86 (d, *J* = 8.52 Hz, 1H), 6.24 (t, *J* = 5.64 Hz, 1H), 4.91 (dd, *J* = 12.24

Hz, *J* = 5.52 Hz, 1H), 3.28 – 3.25 (m, 4H), 2.91 – 2.72 (m, 3H), 2.15-2.10 (m, 1H), 1.75 – 1.52 (m, 4H), 1.50 – 1.35 (m, 4H). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 171.3, 169.6, 168.7, 167.7, 147.1, 136.3, 132.6, 116.7, 111.5, 110.0, 51.4, 49.0, 42.6, 31.5, 29.2, 28.8, 26.6, 22.9. LR−MS: calculated for $C_{19}H_{23}N_6O_4$ $[M+H]^+$, 399.17; found, 399.35.

4-((7-azidoheptyl)amino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (10h). Following the procedure to synthesize **10a**, 2-(2-azidoethoxy)ethanamine was replaced by 7-azidoheptan-1-amine to get **10h** (Isolated yield = 33%). ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 8.43 (s, 1H), 7.48 (t, *J* = 7.60 Hz, 1H), 7.07 (d, *J* = 7.08 Hz, 1H), 6.86 (d, *J* = 8.52 Hz, 1H), 6.23 (t, *J* = 5.64 Hz, 1H), 4.91 (dd, *J* = 12.24 Hz, *J* = 5.52 Hz, 1H), 3.28 – 3.25 (m, 4H), 2.91 – 2.71 (m, 3H), 2.15-2.10 (m, 1H), 1.75 – 1.52 (m, 4H), 1.50 – 1.35 (m, 6H). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 171.2, 169.6, 168.5, 167.8, 147.1, 136.3, 132.6, 116.7, 111.5, 110.0, 51.5, 49.0, 42.7, 31.5, 29.3, 29.0, 28.9, 26.9, 26.7, 22.9. LR−MS: calculated for $C_{20}H_{25}N_6O_4$ $[M+H]^+$, 413.16; found, 413.17.

5-((2-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)ethyl)amino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (10i). Following the procedure to synthesize **10c**, 2-(2,6-dioxopiperidin-3-yl)-4-fluoroisoindoline-1,3-dione was replaced by 2-(2,6-dioxopiperidin-3-yl)-5-fluoroisoindoline-1,3-dione to get **10i** (Isolated yield = 13%). ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 8.42 (s, 1H), 7.55 (d, *J* = 7.60 Hz, 1H), 6.95 (s, 1H), 6.76 (d, *J* = 8.52 Hz, 1H), 5.24 (m, 1H), 4.91 (dd, *J* = 12.24 Hz, *J* = 5.52 Hz, 1H), 3.71 – 3.60 (m, 12H), 3.38 – 3.31 (m, 4H), 2.90-2.60 (m, 3H), 2.15 – 2.02 (m, 1H). LR−MS: calculated for $C_{21}H_{27}N_6O_7$ $[M+H]^+$, 475.19; found, 475.21.

5-((14-Azido-3,6,9,12-tetraoxatetradecyl)amino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (10j). It was synthesized as compound **10i**. ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 8.33 (s, 1H),

7.57 (d, J = 7.60 Hz, 1H), 6.96 (s, 1H), 6.76 (d, J = 8.52 Hz, 1H), 5.32 (m, 1H), 4.91 (dd, J = 12.24 Hz, J = 5.52 Hz, 1H), 3.71 – 3.60 (m, 16H), 3.38 – 3.31 (m, 4H), 2.90-2.60 (m, 3H), 2.15 – 2.02 (m, 1H). LR−MS: calculated for $C_{23}H_{31}N_6O_8$ $[M+H]^+$, 519.21; found, 519.23.

3-(4-(3-(2-azidoethoxy)prop-1-yn-1-yl)-1-oxoisoindolin-2-yl)piperidine-2,6-dione (10k). To a 100 ml round bottom flask charged with stirring bar was added 3-(4-bromo-1-oxoisoindolin-2-yl)piperidine-2,6-dione (1.6 g, 5 mmol), 2-(prop-2-yn-1-yloxy)ethan-1-ol (1.0 g, 10 mmol), CuI (190 mg, 1 mmol), $Pd(PPh_3)_2Cl_2$(140 mg, 0.02 mmol), Et_3N 20 ml and DMF 2 ml as solvent. The mixture was stirred at 90 ^oC for 12 h under argon. After extracted with EtOAc and washed by brine, the solvent was removed under vacuum and the product was transferred to a 50 ml round bottom flask charged with stirring bar. Add methanesulfonyl chloride 1.5 ml, Et_3N 3 ml and DCM as the solvent. The mixture was stirred at room temperature overnight. After extracted with EtOAc and washed by brine, the solvent was removed under vacuum and the product was transferred to a 50 ml round bottom flask charged with stirring bar. Add NaN_3 1.2 g and DMF 20 ml to the flask. The mixture was stirred at 80 ^oC overnight. After extracted with EtOAc and washed by brine, the solvent was removed under vacuum and the product was purified by silica gel column with to get **10k** (Isolated yield = 15%). ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 8.22 (s, 1H), 7.85 (d, J = 7.60 Hz, 1H), 7.63 (d, J = 7.56 Hz, 1H), 7.48 (t, J = 7.60 Hz, 1H), 5.25 (m, 1H), 4.51 – 4.30 (m, 4H), 3.71 (t, J = 5.00 Hz, 2H), 3.24 (t, J = 5.00 Hz, 2H), 2.90-2.60 (m, 2H), 2.47 – 2.31 (m, 1H), 2.25 – 2.20 (m, 1H). LR−MS: calculated for $C_{18}H_{18}N_5O_4$ $[M+H]^+$, 368.13; found, 368.23.

3-(4-(3-(3-azidopropoxy)prop-1-yn-1-yl)-1-oxoisoindolin-2-yl)piperidine-2,6-dione (10l). It was synthesized as compound **10k**. ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 8.37 (s, 1H), 7.80 (d, J = 7.60 Hz,

1H), 7.59 (d, *J* = 7.56 Hz, 1H), 7.46 (t, *J* = 7.60 Hz, 1H), 5.19 (m, 1H), 4.53 – 4.35 (m, 4H), 3.64 (t, *J* = 5.00 Hz, 2H), 3.34 (t, *J* = 5.00 Hz, 2H), 2.90-2.80 (m, 2H), 2.39 – 2.31 (m, 1H), 2.24 – 2.20 (m, 1H), 1.67 – 1.51 (m, 2H). LR−MS: calculated for $C_{19}H_{20}N_5O_4$ $[M+H]^+$, 382.14; found, 382.16.

3-(4-(3-(4-azidobutoxy)prop-1-yn-1-yl)-1-oxoisoindolin-2-yl)piperidine-2,6-dione (10m). It was synthesized as compound **10k**. ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 8.38 (s, 1H), 7.81 (d, *J* = 7.60 Hz, 1H), 7.60 (d, *J* = 7.56 Hz, 1H), 7.45 (t, *J* = 7.60 Hz, 1H), 5.21 (m, 1H), 4.53 – 4.35 (m, 4H), 3.54 (t, *J* = 5.00 Hz, 2H), 3.44 (t, *J* = 5.00 Hz, 2H), 2.90-2.80 (m, 2H), 2.39 – 2.31 (m, 1H), 2.24 – 2.20 (m, 1H), 1.67 – 1.51 (m, 4H). LR−MS: calculated for $C_{20}H_{22}N_5O_4$ $[M+H]^+$, 396.16; found, 396.18.

3-(4-(3-((5-azidopentyl)oxy)prop-1-yn-1-yl)-1-oxoisoindolin-2-yl)piperidine-2,6-dione (10n). It was synthesized as compound **10k**. ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 8.38 (s, 1H), 7.80 (d, *J* = 7.60 Hz, 1H), 7.60 (d, *J* = 7.56 Hz, 1H), 7.44 (t, *J* = 7.60 Hz, 1H), 5.20 (m, 1H), 4.52 – 4.33 (m, 4H), 3.54 (t, *J* = 5.00 Hz, 2H), 3.44 (t, *J* = 5.00 Hz, 2H), 2.90-2.80 (m, 2H), 2.39 – 2.31 (m, 1H), 2.24 – 2.20 (m, 1H), 1.65 – 1.30 (m, 6H). LR−MS: calculated for $C_{21}H_{24}N_5O_4$ $[M+H]^+$, 410.18; found, 410.20.

3-(4-(3-((6-azidohexyl)oxy)prop-1-yn-1-yl)-1-oxoisoindolin-2-yl)piperidine-2,6-dione (10o). It was synthesized as compound **10k**. ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 8.40 (s, 1H), 7.82 (d, *J* = 7.60 Hz, 1H), 7.60 (d, *J* = 7.56 Hz, 1H), 7.45 (t, *J* = 7.60 Hz, 1H), 5.22 (m, 1H), 4.54 – 4.34 (m, 4H), 3.55 (t, *J* = 5.00 Hz, 2H), 3.45 (t, *J* = 5.00 Hz, 2H), 2.90-2.80 (m, 2H), 2.39 – 2.31 (m, 1H), 2.24 – 2.20 (m, 1H), 1.65 – 1.30 (m, 8H). LR−MS: calculated for $C_{22}H_{26}N_5O_4$ $[M+H]^+$, 424.19; found, 424.23.

3-(4-(3-((7-azidoheptyl)oxy)prop-1-yn-1-yl)-1-oxoisoindolin-2-yl)piperidine-2,6-dione (10p). It was synthesized as compound **10k**. ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 8.42 (s, 1H), 7.84 (d, *J* = 7.60 Hz, 1H), 7.62 (d, *J* = 7.56 Hz, 1H), 7.46 (t, *J* = 7.60 Hz, 1H), 5.23 (m, 1H), 4.54 – 4.34 (m, 4H), 3.56

(t, J = 5.00 Hz, 2H), 3.46 (t, J = 5.00 Hz, 2H), 2.90-2.80 (m, 2H), 2.39 – 2.31 (m, 1H), 2.24 – 2.20 (m, 1H), 1.65 – 1.30 (m, 10H). LR−MS: calculated for $C_{23}H_{28}N_5O_4$ $[M+H]^+$, 438.21; found, 438.35.

(3R,5R)-7-(3-(3-(3-(1-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)propoxy)phenyl)-2-(4-fluorophenyl)-5-isopropyl-4-(phenylcarbamoyl)-1H-pyrrol-1-yl)-3,5-dihydroxyheptanoic acid (11a, P13A). To a round-bottom flask containing 1 ml THF was added **10a** (40 mg, 0.1 mmol), **9b** (50 mg, 0.09 mmol), $CuSO_4$ (6 mg, 0.036 mmol), sodium ascorbate (40 mg, 0.18 mmol) and H_2O (0.5 ml). The mixture was stirred at room temperature overnight and extracted with EtOAc. The organic layer was purified by silica gel to get **11a** (Isolated yield = 75%). The eluent was EtOAc at first and DCM:MeOH=100:6 subsequently. ^{1}H-NMR (400 MHz, CD_3OD, ppm): 7.62 (s, 1H), 7.52 – 7.47 (m, 1H), 7.33 (d, J = 8.00 Hz, 2H), 7.26 – 7.18 (m, 4H), 7.08 (t, J = 8.52 Hz, 2H), 7.03 – 6.95 (m, 4H), 6.71 (s, 1H), 6.68 (d, J = 7.88 Hz, 1H), 6.57 (d, J = 7.68 Hz, 1H), 5.02 – 4.98 (m, 1H), 4.52 (s, 2H), 4.07 – 3.96 (m, 2H), 3.91 – 3.82 (m. 3H), 3.70 – 3.57 (m, 5H), 3.42 – 3.38 (m, 3H), 2.69 – 2.60 (m, 5H), 2.40 – 2.27 (m, 2H), 2.08 – 1.98 (m, 1H), 1.89 – 1.82 (m, 2H), 1.72 – 1.51 (m, 3H), 1.55 – 1.40 (m, 7H). ^{13}C-NMR (100 MHz, $CDCl_3$:CD_3OD = 4:1, ppm): 172.3, 169.4, 169.1, 167.8, 161.1, 158.7, 146.6, 136.2, 135.9, 133.2, 132.4, 129.3, 128.7, 123.9, 122.7, 121.8, 119.9, 116.9, 116.0, 113.5, 111.8, 110.2, 69.6, 69.5, 66.9, 50.0, 42.1, 41.2, 39.9, 31.3, 29.7, 26.2, 22.7, 21.8. LR−MS: calculated for $C_{55}H_{60}FN_8O_{11}$ $[M+H]^+$, 1027.43; found, 1027.53.

(3R,5R)-7-(3-(3-(4-(1-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)butoxy)phenyl)-2-(4-fluorophenyl)-5-isopropyl-4-(phenylcarbamoyl)-1H-pyrrol-1-yl)-3,5-dihydroxyheptanoic acid 11b, P14A). It was synthesized

as compound **11a**. ^{1}H-NMR (400 MHz, CD_3OD, ppm): 7.63 (s, 1H), 7.48 – 7.43 (m, 1H), 7.33 (d, *J* = 8.00 Hz, 2H), 7.25 – 7.18 (m, 4H), 7.07 (t, *J* = 8.60 Hz, 2H), 7.03 – 6.95 (m, 4H), 6.69 (s, 1H), 6.67 (d, *J* = 7.56 Hz, 1H), 6.55 (d, *J* = 7.96 Hz, 1H), 5.03 – 4.98 (m, 1H), 4.52 (s, 2H), 4.06 – 4.03 (m, 2H), 3.92 – 3.82 (m. 3H), 3.68 – 3.57 (m, 5H), 3.45 – 3.39 (m, 3H), 2.75 – 2.55 (m, 5H), 2.42 – 2.29 (m, 2H), 2.08 – 1.99 (m, 1H), 1.79 – 1.42 (m, 14H). ^{13}C-NMR (100 MHz, CD_3OD, ppm): 174.7, 171.5, 170.7, 169.7, 167.8, 162.6, 159.9, 148.2, 139.9, 138.9, 137.6, 137.3, 134.8, 134.7, 133.7, 130.3, 129.8, 129.7, 129.6, 125.2, 123.3, 123.0, 121.4, 118.3, 118.0, 116.5, 116.3, 113.9, 112.2. 70.9, 70.4, 68.5, 68.4, 54.8, 51.5, 50.2, 43.1, 42.1, 40.1, 32.2, 29.5, 27.6, 26.9, 25.9, 23.8, 22.8. LR−MS: calculated for $C_{56}H_{62}FN_8O_{11}$ $[M+H]^+$, 1041.44; found, 1041.45.

(3R,5R)-7-(3-(3-((5-(1-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)pentyl)oxy)phenyl)-2-(4-fluorophenyl)-5-isopropyl-4-(phenylcarbamoyl)-1H-pyrrol-1-yl)-3,5-dihydroxyheptanoic acid (11c, P15A). It was synthesized as compound **11a**. ^{1}H-NMR (400 MHz, CD_3OD, ppm): 7.66 (s, 1H), 7.48 – 7.43 (m, 1H), 7.33 (d, *J* = 7.96 Hz, 2H), 7.26 – 7.16 (m, 4H), 7.06 (t, *J* = 8.48 Hz, 2H), 7.02 – 6.94 (m, 4H), 6.70 (s, 1H), 6.68 (d, *J* = 7.56 Hz, 1H), 6.55 (d, *J* = 8.04 Hz, 1H), 5.04 – 4.99 (m, 1H), 4.53 (s, 2H), 4.09 – 4.03 (m, 2H), 3.95 – 3.82 (m. 3H), 3.68 – 3.57 (m, 5H), 3.45 – 3.35 (m, 3H), 2.85 – 2.54 (m, 5H), 2.46 – 2.31 (m, 2H), 2.09 – 2.01 (m, 1H), 1.75 – 1.41 (m, 16H). ^{13}C-NMR (100 MHz, CD_3OD, ppm): 174.6, 171.5, 170.8, 169.3, 160.0, 148.2, 140.0, 139.0, 137.6, 137.3, 134.8, 134.7, 133.8, 129.8, 129.7, 125.2, 123.3, 123.1, 121.4, 118.3, 116.5, 116.4, 116.3, 114.1, 112.2, 111.3, 71.0, 70.1, 68.6, 51.4, 50.2, 43.2, 42.2, 40.1, 32.3, 30.1, 29.8, 27.7, 26.5, 26.2, 23.9, 22.8. LR−MS: calculated for $C_{57}H_{64}FN_8O_{11}$ $[M+H]^+$, 1055.46; found, 1055.45.

(3R,5R)-7-(3-(3-(3-(1-(2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)propoxy)phenyl)-2-(4-fluorophenyl)-5-isopropyl-4-(phenylcarbamoyl)-1H-pyrrol-1-yl)-3,5-dihydroxyheptanoic acid (11d, P16A). It was synthesized as compound **11a**. ^{1}H-NMR (400 MHz, CD_3OD, ppm): 7.67 (s, 1H), 7.46 (t, *J* = 7.28 Hz, 1H), 7.34 (d, *J* = 7.96 Hz, 2H), 7.26 – 7.18 (m, 4H), 7.08 (t, *J* = 8.48 Hz, 2H), 7.03 – 6.94 (m, 4H), 6.71 (s, 1H), 6.68 (d, *J* = 7.68 Hz, 1H), 6.56 (d, *J* = 7.96 Hz, 1H), 5.03 – 4.99 (m, 1H), 4.49 (s, 2H), 4.09 – 4.03 (m, 2H), 3.95 – 3.82 (m. 3H), 3.68 – 3.57 (m, 9H), 3.44 – 3.35 (m, 3H), 2.81 – 2.58 (m, 5H), 2.41 – 2.31 (m, 2H), 2.07 – 2.01 (m, 1H), 1.86 – 1.82 (m, 2H), 1.75 – 1.43 (m, 10H). ^{13}C-NMR (100 MHz, CD_3OD, ppm): 174.6, 171.5, 170.7, 169.6, 169.2, 166.8, 165.1, 162.1, 159.9, 148.1, 139.9, 138.0, 137.6, 137.3, 134.8, 134.7, 133.8, 130.8, 130.3, 129.8, 129.7, 129.6, 125.2, 124.2, 123.4, 123.0, 121.4, 118.3, 118.1, 116.6, 116.5, 116.3, 113.8, 112.1, 112.2, 71.5, 70.5, 70.4, 68.6, 67.7, 54.8, 51.4, 44.1, 43.2, 42.1, 40.2, 32.2, 29.9, 27.6, 23.8, 22.8. LR−MS: calculated for $C_{57}H_{64}FN_8O_{12}$ $[M+H]^+$, 1070.45; found, 1070.34.

(3R,5R)-7-(3-(3-(4-(1-(2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)butoxy)phenyl)-2-(4-fluorophenyl)-5-isopropyl-4-(phenylcarbamoyl)-1H-pyrrol-1-yl)-3,5-dihydroxyheptanoic acid (11e, P17A). It was synthesized as compound **11a**. ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 8.87 (s, 1H), 7.52 – 7.42 (m, 2H), 7.17 – 6.95 (m, 10H), 6.86 (d, *J* = 7.96 Hz, 1H), 7.71 – 7.62 (m, 3H), 6.48 (s, 1H), 4.89 (m, 1H), 4.51 – 4.42 (m, 2H), 4.15 – 4.02 (m, 2H), 3.97 – 3.81 (m, 3H), 3.72 – 3.55 (m, 9H), 3.49 – 3.30 (m, 3H), 2.72 – 2.55 (m, 5H), 2.45 – 2.38 (m, 2H), 2.11 – 2.00 (m, 1H), 1.72 – 1.47 (m, 14H). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 171.8, 169.5, 168.9, 167.7, 165.4, 158.9, 146.8, 138.6, 136.2, 135.9, 133.3, 132.6, 129.4, 128.8, 128.4, 123.8, 122.8, 121.9, 119.8, 116.9, 116.0, 115.7, 113.9, 111.8, 110.4, 70.7,

70.5, 69.7, 69.4, 50.3, 67.5, 53.5, 49.0, 42.4, 31.4, 29.8, 28.6, 26.3, 25.9, 22.8, 22.0, 21.9. LR−MS: calculated for $C_{58}H_{66}FN_8O_{12}$ $[M+H]^+$, 1085.47; found, 1085.36.

(3R,5R)-7-(3-(3-((5-(1-(2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)pentyl)oxy)phenyl)-2-(4-fluorophenyl)-5-isopropyl-4-(phenylcarbamoyl)-1H-pyrrol-1-yl)-3,5-dihydroxyheptanoic acid (11f, P18A). It was synthesized as compound **11a**. ^{1}H-NMR (400 MHz, CD_3OD, ppm): 7.71 (s, 1H), 7.50 (t, J = 7.80 Hz, 1H), 7.33 (d, J = 7.72 Hz, 2H), 7.24 – 7.17 (m, 4H), 7.08 (t, J = 7.96 Hz, 2H), 7.02 – 6.94 (m, 4H), 6.70 (s, 1H), 6.67 (d, J = 7.36 Hz, 1H), 5.14 – 5.97 (m, 1H), 4.51 (s, 2H), 4.16 – 4.02 (m, 2H), 3.97 – 3.82 (m, 3H), 3.69 – 3.53 (m, 9H), 3.45 – 3.30 (m, 3H), 2.76 – 2.52 (m, 5H), 2.45 – 2.28 (m, 2H), 2.09 – 2.01 (m, 1H), 1.76 – 1.42 (m, 16H). ^{13}C-NMR (100 MHz, CD_3OD, ppm): 174.6, 171.5, 170.7, 169.6, 169.2, 165.0, 162.6, 160.0, 148.1, 139.9, 138.9, 137.5, 137.3, 134.8, 134.7, 133.8, 130.3, 129.8, 129.7, 129.6, 125.2, 123.2, 123.1, 121.4, 118.3, 118.0, 116.5, 116.3, 114.0, 112.1, 111.2, 71.5, 70.5, 70.4, 68.6, 51.4, 50.2, 43.2, 42.1, 40.1, 32.2, 30.8, 30.0, 29.8, 27.6, 26.5, 26.2, 23.8, 22.8, 22.8. LR−MS: calculated for $C_{59}H_{67}FN_8O_{12}$ $[M+H]^+$, 1099.49; found, 1099.45.

(3R,5R)-7-(3-(3-(3-(1-(2-(2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethoxy)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)propoxy)phenyl)-2-(4-fluorophenyl)-5-isopropyl-4-(phenylcarbamoyl)-1H-pyrrol-1-yl)-3,5-dihydroxyheptanoic acid (11g, P19A). It was synthesized as compound **11a**. ^{1}H-NMR (400 MHz, CD_3OD, ppm): 7.72 (s, 1H), 7.50 (t, J = 8.16 Hz, 1H), 7.36 (d, J = 7.64 Hz, 2H), 7.27 – 7.20 (m, 4H), 7.10 (t, J = 8.16 Hz, 2H), 7.03 – 6.97 (m, 4H), 6.73 (s, 1H), 6.69 (d, J = 7.40 Hz, 1H), 6.59 (d, J = 7.88 Hz, 1H), 5.06 – 5.01 (m, 1H), 4.49 (s, 2H), 4.13 – 4.02 (m, 2H), 3.97 – 3.82 (m, 3H), 3.73 – 3.53 (m, 13H), 3.45 – 3.30 (m, 3H), 2.80 – 2.61 (m,

5H), 2.42 – 2.28 (m, 2H), 2.09 – 2.02 (m, 1H), 1.96 – 1.85 (m, 2H), 1.75 – 1.45 (m, 10H). ^{13}C-NMR (100 MHz, CD_3OD, ppm): 173.9, 170.6, 170.1, 168.8, 164.4, 161.9, 159.3, 147.5, 139.7, 139.0, 136.8, 134.1, 134.0, 133.2, 129.7, 129.3, 129.2, 124.7, 123.6, 123.1, 122.5, 120.9, 117.8, 116.9, 116.3, 116.2, 115.9, 113,7, 112.0, 110.7, 71.2, 71.1, 71.1, 71.0, 70.1, 69.9, 69.0, 67.4, 50.8, 42.8, 41.8, 39.9, 31.9, 30.3, 29.4, 27.0, 23.4, 22.5. LR−MS: calculated for $C_{59}H_{68}FN_8O_{13}$ $[M+H]^+$, 1115.48; found, 1115.45.

(3R,5R)-7-(3-(3-(4-(1-(2-(2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethoxy)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)butoxy)phenyl)-2-(4-fluorophenyl)-5-isopropyl-4-(phenylcarbamoyl)-1H-pyrrol-1-yl)-3,5-dihydroxyheptanoic acid (11h, P20A). It was synthesized as compound **11a**. ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 8.79 (s, 1H), 7.49 - 7.42 (m, 2H), 7.28 – 6.95 (m, 10H), 6.88 (d, J = 8.80 Hz, 1H), 6.70 – 6.63 (m, 3H), 6.49 – 6.42 (m, 1H), 4.91 – 4.85 (m, 1H), 4.49 – 4.46 (m, 2H), 4.16 – 4.03 (m, 2H), 3.98 -3.82 (m, 3H), 3.71 – 3.59 (m, 13H), 3.54 – 3.47 (m, 3H), 2.85 – 2.61 (m, 5H), 2.55 – 2.45 (m, 2H), 2.11 – 2.06 (m, 1H), 1.73 – 1.42 (m, 14H). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 171.8, 169.4, 168.9, 167.8, 161.1, 158.9, 146.9, 141.4, 138.5, 136.2, 136.0, 133.3, 132.6, 129.4, 128.9, 128.8, 128.5, 123.8, 122.8, 121.9, 119.9, 116.9, 116.0, 115.7, 115.5, 113.9, 111.8, 110.3, 70.8, 70.7, 70.6, 69.6, 67.5, 50.4, 49.0, 42.5, 41.4, 31.5, 29.8, 29.4, 28.6, 26.3, 25.9, 25.2, 22.9, 22.8, 22.0, 21.9. LR−MS: calculated for $C_{60}H_{70}FN_8O_{13}$ $[M+H]^+$, 1129.50; found, 1129.24.

(3R,5R)-7-(3-(3-((5-(1-(2-(2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethoxy)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)pentyl)oxy)phenyl)-2-(4-fluorophenyl)-5-isopropyl-4-(phenylcarbamoyl)-1H-pyrrol-1-yl)-3,5-dihydroxyheptanoic acid (11i, P21A). It was synthesized as compound **11a**. ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 8.64 (s, 1H),

7.47 (t, *J* = 7.60 Hz, 2H), 7.17 – 6.95 (m, 10H), 6.89 (d, *J* = 8.56 Hz, 1H), 6.68 – 6.67 (m, 3H), 6.47 (m, 1H), 4.91 – 4.85 (m, 1H), 4.55 – 4.46 (m, 2H), 4.16 – 4.03 (m, 2H), 3.98 -3.81 (m, 3H), 3.71 – 3.59 (m, 13H), 3.54 – 3.47 (m, 3H), 2.85 – 2.61 (m, 5H), 2.55 – 2.45 (m, 2H), 2.11 – 2.06 (m, 1H), 1.73 – 1.42 (m, 16H). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 171.9, 169.4, 169.0, 167.7, 165.4, 161.1, 158.9, 146.9, 141.4, 138.4, 136.2, 135.9, 133.3, 133.3, 132.6, 129.4, 128.8, 123.8, 123.8, 122.8, 121.9, 119.9, 116.9, 115.9, 115.7, 115.4, 114.0, 111.8,110.3, 70.8, 70.7, 70.6, 69.6, 67.7, 50.3, 49.9, 42.5, 41.4, 31.5, 29.8, 29.4, 29.1, 28.8, 26.3, 26.5, 22.8, 22.0, 21.9. LR−MS: calculated for $C_{61}H_{72}FN_8O_{13}$ $[M+H]^+$, 1143.51; found, 1143.56.

(3R,5R)-7-(3-(3-(3-(1-(14-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-3,6,9,12-tetraoxatetradecyl)-1H-1,2,3-triazol-4-yl)propoxy)phenyl)-2-(4-fluorophenyl)-5-isopropyl-4-(phenylcarbamoyl)-1H-pyrrol-1-yl)-3,5-dihydroxyheptanoic acid (11j, P22A). To a round-bottom flask containing 1 ml THF was added **10e** (53 mg, 0.1 mmol), **9b** (50 mg, 0.09 mmol), $CuSO_4$ (3 mg, 0.018 mmol), sodium ascorbate (20 mg, 0.09 mmol) and H_2O (0.5 ml). The mixture was stirred at room temperature overnight and extracted with EtOAc. The organic layer was purified by silica gel to get **11j** (Isolated yield = 80 %). The eluent was EtOAc at first and DCM:MeOH=100:15 in the following. ^{1}H-NMR (400 MHz, CD_3OD, ppm): 7.69 (s, 1H), 7.49 (t, *J* = 8.20 Hz, 1H), 7.34 (d, *J* = 8.16 Hz, 2H), 7.28 – 7.17 (m, 4H), 7.08 (t, *J* = 8.20 Hz, 2H), 7.02 – 6.95 (m, 4H), 6.73 (s, 1H), 6.68 (d, *J* = 7.60 Hz, 1H), 6.58 (d, *J* = 7.76 Hz, 1H), 5.04 – 5.00 (m, 1H), 4.48 (t, *J* = 4.80 Hz, 2H), 4.11 – 3.95 (m, 2H), 3.94 – 3.47 (m, 3H), 3.72 – 3.49 (m, 17H), 3.45 -3.35 (m, 3H), 2.82 – 2.62 (m, 5H), 2.38 – 2.31 (m, 2H), 2.08 – 2.02 (m, 1H), 1.95 – 1.85 (m, 2H), 1.71 – 1.61 (m, 3H), 1.55 – 1.41 (m, 7H). ^{13}C-NMR (100 MHz, CD_3OD, ppm): 174.6, 171.5, 170.7, 169.3, 168.7, 159.9, 148.2, 140.0, 139.0, 137.7, 137.2, 134.8, 134.7, 133.8, 130.4, 129.8, 129.7, 129.6, 125.2, 124.2, 123.4, 121.4, 118.3, 116.6,

116.5, 116.3, 113.9, 112.1, 111.2, 71.6, 71.5, 71.5, 71.4, 70.6, 70.4, 68.6, 67.8, 51.3, 50.2, 43.2, 42.2, 40.2, 32.2, 30.0, 27.6, 23.8, 22.9. HR−MS: calculated for $C_{61}H_{72}FN_8O_{14}$ $[M+H]^+$, 1159.5152; found, 1159.5101.

(3R,5R)-7-(3-(3-(3-(1-(14-((2-(1-ethyl-2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-3,6,9,12-tetraoxatetradecyl)-1H-1,2,3-triazol-4-yl)propoxy)phenyl)-2-(4-fluorophenyl)-5-isopropyl-4-(phenylcarbamoyl)-1H-pyrrol-1-yl)-3,5-dihydroxyheptanoic acid (11k, P22A-Et). It was synthesized as compound **11a**. ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 7.44 – 7.39 (m, 2H), 7.16 – 6.94 (m, 10H), 6.86 (d, *J* = 8.16 Hz, 1H), 6.67 – 6.62 (m, 3H), 6.46 (s, 1H), 4.89 – 4.82 (m, 1H), 4.48 – 4.42 (m, 2H), 4.06 – 4.02 (m, 2H), 3.87 – 3.78 (m, 5H), 3.71 – 3.55 (m, 17H), 3.45 – 3.37 (m, 5H), 2.91 – 2.61 (m, 5H), 2.32 – 2.15 (m, 2H), 2.09 – 2.02 (m, 1H), 1.96 – 1.85 (m, 2H), 1.75 – 1.45 (m, 12H). 1.11 (t, *J* = 7.16 Hz, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 171.0, 169.5, 168.9, 167.8, 161.0, 158.9, 147.1, 146.8, 138.5, 136.1, 136.0, 133.3, 132.6, 129.4, 128.8, 128.5, 123.8, 122.8, 122.2, 121.8, 119.8, 116.8, 116.0, 115.6, 111.7, 110.5, 70.6, 70.6, 69.7, 69.5, 67.0, 53.5, 50.2, 49.8, 42.4, 39.5, 37.5, 35.8, 32.8, 32.1, 32.0, 29.8, 29.7, 29.4, 28.9, 28.1, 22.8, 22.2, 22.1, 22.0, 19.8, 14.2, 13.2. LR−MS: calculated for $C_{63}H_{76}FN_8O_{14}$ $[M+H]^+$, 1187.54; found, 1187.67.

(3R,5R)-7-(3-(3-(4-(1-(14-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-3,6,9,12-tetraoxatetradecyl)-1H-1,2,3-triazol-4-yl)butoxy)phenyl)-2-(4-fluorophenyl)-5-isopropyl-4-(phenylcarbamoyl)-1H-pyrrol-1-yl)-3,5-dihydroxyheptanoic acid (11l, P23A). It was synthesized as compound **11a**. ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 8.61 (s, 1H), 7.51 – 7.42 (m, 2H), 7.20 – 6.93 (m, 10H), 6.90 (d, *J* = 8.62 Hz, 1H), 6.69 – 6.61 (m, 3H), 6.48 (s, 1H), 4.91 – 4.87 (m, 1H), 4.52 – 4.42 (m, 2H), 4.21 – 4.05 (m, 2H), 4.05 – 3.81 (m, 3H), 3.75 – 3.55 (m, 17H), 3.52 – 3.43 (m, 3H),

2.85 – 2.58 (m, 5H), 2.47 – 2.41 (m, 2H), 2.13 – 2.08 (m, 1H), 1.80 – 1.47 (m, 14H). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 171.4, 170.3, 169.4, 168.7, 167.6, 165.1, 163.6, 161.1, 158.8, 146.8, 141.3, 138.5, 136.2, 136.0, 133.3, 133.2, 132.6, 132.5, 132.4, 132.0, 131.0, 129.3, 128.9, 128.9, 128.8, 128.4, 128.4, 123.6, 122.9, 122.1, 121.7, 119.7, 116.8, 116.1, 115.5, 115.4, 115.3, 113.7, 111.8, 110.4, 71.6, 71.5, 71.5, 71.4, 70.6, 70.4, 68.6, 67.8, 51.3, 50.2, 43.2, 42.2, 40.2, 32.2, 30.0, 27.6, 23.8, 22.9. LR−MS: calculated for $C_{62}H_{74}FN_8O_{14}$ $[M+H]^+$, 1173.52; found, 1173.52.

(3R,5R)-7-(3-(3-((5-(1-(14-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-3,6,9,12-tetraoxatetradecyl)-1H-1,2,3-triazol-4-yl)pentyl)oxy)phenyl)-2-(4-fluorophenyl)-5-isopropyl-4-(phenylcarbamoyl)-1H-pyrrol-1-yl)-3,5-dihydroxyheptanoic acid (11m, P24A). It was synthesized as compound **11a**. ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 8.60 (s, 1H), 7.51 – 7.45 (m, 3H), 7.21 – 6.96 (m, 10H), 6.91 (d, J = 8.62 Hz, 1H), 6.69 – 6.61 (m, 3H), 6.48 (s, 1H), 4.91 – 4.87 (m, 1H), 4.52 – 4.42 (m, 2H), 4.21 – 4.05 (m, 2H), 4.05 – 3.81 (m, 3H), 3.75 – 3.55 (m, 17H), 3.52 – 3.43 (m, 3H), 2.85 – 2.58 (m, 5H), 2.47 – 2.41 (m, 2H), 2.13 – 2.08 (m, 1H), 1.80 – 1.47 (m, 16H). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 171.4, 170.2, 169.3, 168.6, 167.6, 165.0, 163.5, 161.1, 158.8, 146.8, 141.3, 138.5, 136.1, 136.1, 133.2, 133.1, 132.1, 132.2, 132.1, 131.0, 130.9, 129.3, 128.9, 128.8, 128.7, 128.40, 123.6, 122.9, 122.1, 121.7, 119.7, 116.8, 116.1, 115.5, 115.3, 113.7, 111.7, 110.3, 71.6, 71.51, 71.45, 71.4, 70.6, 70.3, 68.6, 67.8, 51.3, 43.2, 42.2, 40.2, 32.2, 30.0, 27.5, 23.8, 22.7. LR−MS: calculated for $C_{63}H_{76}FN_8O_{14}$ $[M+H]^+$, 1186.55; found, 1186.72.

(3R,5R)-7-(3-(3-(3-(1-(17-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-3,6,9,12,15-pentaoxaheptadecyl)-1H-1,2,3-triazol-4-yl)propoxy)phenyl)-2-(4-fluorophenyl)-5-isopropyl-4-(phenylcarbamoyl)-1H-pyrrol-1-yl)-3,5-dihydroxyheptanoic acid (11n, P25A). It

was synthesized as compound **11a**. ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 9.10 (s, 1H), 7.49 (t, *J* = 8.20 Hz, 1H), 7.34 (d, *J* = 8.16 Hz, 2H), 7.28 – 6.85 (m, 10H), 6.73 – 6.68 (m, 3H), 6.48 (s, 1H), 4.94 – 4.85 (m, 1H), 4.48 (t, *J* = 4.80 Hz, 2H), 4.11 – 3.95 (m, 2H), 3.94 – 3.47 (m, 3H), 3.72 – 3.49 (m, 21H), 3.45 -3.35 (m, 3H), 2.82 – 2.62 (m, 5H), 2.38 – 2.31 (m, 2H), 2.08 – 2.02 (m, 1H), 1.95 – 1.85 (m, 2H), 1.71 – 1.61 (m, 3H), 1.55 – 1.41 (m, 7H). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 174.6, 171.5, 170.6, 169.2, 168.6, 159.9, 148.2, 140.0, 139.0, 137.7, 137.2, 134.8, 134.7, 133.8, 130.4, 129.8, 129.7, 128.9, 125.1, 124.1, 123.4, 121.4, 118.3, 116.6, 116.5, 116.3, 113.9, 112.1, 111.3, 71.59, 71.55, 71.41, 71.40, 70.5, 70.4, 68.6, 67.6, 51.3, 43.2, 42.2, 40.1, 32.1, 29.8, 27.6, 23.8, 22.3. LR−MS: calculated for $C_{63}H_{76}FN_8O_{15}$ $[M+H]^+$, 1203.54; found, 1203.75.

(3R,5R)-7-(3-(3-(4-(1-(17-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-3,6,9,12,15-pentaoxaheptadecyl)-1H-1,2,3-triazol-4-yl)butoxy)phenyl)-2-(4-fluorophenyl)-5-isopropyl-4-(phenylcarbamoyl)-1H-pyrrol-1-yl)-3,5-dihydroxyheptanoic acid (11o, P26A). It was synthesized as compound **11a**. ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 9.07 (s, 1H), 7.49 (t, *J* = 8.20 Hz, 1H), 7.34 (d, *J* = 8.16 Hz, 2H), 7.30 – 6.85 (m, 10H), 6.77 – 6.60 (m, 3H), 6.48 (s, 1H), 4.94 – 4.85 (m, 1H), 4.49 (t, *J* = 4.80 Hz, 2H), 4.11 – 3.94 (m, 2H), 3.94 – 3.47 (m, 3H), 3.72 – 3.49 (m, 21H), 3.45 -3.35 (m, 3H), 2.87 – 2.62 (m, 5H), 2.38 – 2.31 (m, 2H), 2.12 – 2.04 (m, 1H), 1.95 – 1.85 (m, 2H), 1.71 – 1.61 (m, 3H), 1.55 – 1.41 (m, 9H). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 174.6, 171.5, 170.6, 169.5, 167.8, 159.9, 147.0, 143.3, 141.3, 140.0, 139.0, 137.7, 136.2, 134.8, 134.7, 132.6, 130.4, 129.8, 129.7, 128.8, 125.1, 124.1, 123.4, 121.4, 119.9, 117.0, 116.5, 115.7, 113.9, 112.1, 111.7, 73.0, 70.6, 69.6, 67.5, 63.5, 60.2, 53.6, 49.0. 42.5, 32.1, 29.1, 28.7, 26.4, 26.0, 25.4, 22.8, 21.0. LR−MS: calculated for $C_{64}H_{78}FN_8O_{15}$ $[M+H]^+$, 1217.55; found, 1217.78.

(3R,5R)-7-(3-(3-((5-(1-(17-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-3,6,9,12,15-pentaoxaheptadecyl)-1H-1,2,3-triazol-4-yl)pentyl)oxy)phenyl)-2-(4-fluorophenyl)-5-isopropyl-4-(phenylcarbamoyl)-1H-pyrrol-1-yl)-3,5-dihydroxyheptanoic acid (11p, P27A). It was synthesized as compound **11a**. ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 9.07 (s, 1H), 7.44 (t, *J* = 8.20 Hz, 1H), 7.34 (d, *J* = 8.16 Hz, 2H), 7.30 – 6.85 (m, 10H), 6.77 – 6.60 (m, 3H), 6.47 (s, 1H), 4.90 – 4.85 (m, 1H), 4.49 (t, *J* = 4.80 Hz, 2H), 4.11 – 3.94 (m, 2H), 3.94 – 3.47 (m, 3H), 3.72 – 3.49 (m, 21H), 3.45 -3.35 (m, 3H), 2.87 – 2.62 (m, 5H), 2.38 – 2.31 (m, 2H), 2.12 – 2.04 (m, 1H), 1.95 – 1.85 (m, 2H), 1.71 – 1.61 (m, 3H), 1.55 – 1.41 (m, 11H). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 174.6, 171.5, 170.6, 169.5, 167.9, 159.9, 147.0, 143.3, 141.3, 140.0, 139.0, 137.7, 136.2, 133.4, 134.7, 132.6, 130.4, 129.8, 129.7, 128.8, 125.1, 124.1, 122.8, 121.4, 119.9, 117.1, 116.5, 115.7, 113.9, 112.1, 111.9, 109.6, 73.0, 70.7, 69.6, 67.5, 63.5, 60.2, 53.5, 49.0, 42.5, 32.1, 29.8, 29.0, 26.4, 25.8, 23.0, 22.1. LR−MS: calculated for $C_{65}H_{80}FN_8O_{15}$ $[M+H]^+$, 1231.56; found, 1231.88.

(3R,5R)-7-(3-(3-((1-(7-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)heptyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-2-(4-fluorophenyl)-5-isopropyl-4-(phenylcarbamoyl)-1H-pyrrol-1-yl)-3,5-dihydroxyheptanoic acid (12a). It was synthesized as compound **11a**. ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 8.82 (s, 1H), 7.47 – 7.40 (m, 2H), 7.27 – 7.00 (m, 12H), 6.86 – 6.76 (m, 3H), 6.22 (s, 1H), 5.05 – 4.82 (m, 3H), 4.35 – 4.00 (m, 3H), 3.93 – 3.82 (m, 1H), 3.78 – 3.61 (m, 1H), 3.57 – 3.30 (m, 2H), 3.27 – 3.18 (m, 2H), 2.90 – 3.65 (m, 3H), 2.45 – 2.27 (m, 2H), 2.11 – 2.01 (m, 1H), 1.92 – 1.75 (m, 2H), 1.69 – 1.12 (m, 14H). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 171.8, 169.6, 169.0, 167.8, 165.5, 163.6, 158.3, 147.1, 144.1, 141.2, 138.5, 136.3, 133.2, 132.6, 129.6, 128.8, 128.4, 123.9, 123.6, 122.7, 121.8, 120.0, 116.8, 116.3, 115.7, 115.4, 114.0, 111.5, 109.9, 69.6, 68.8, 61.9, 50.4, 49.7, 48.9, 42.6, 41.8, 41.4, 38.9, 31.5, 30.7, 30.1, 29.8, 29.1, 28.7, 26.8, 26.4, 26.3, 22.9, 22.0, 21.9, 17.7.

LR−MS: calculated for $C_{56}H_{62}FN_8O_{10}$ $[M+H]^+$, 1025.45; found, 1025.66.

(3R,5R)-7-(3-(3-(3-(1-(6-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)hexyl)-1H-1,2,3-triazol-4-yl)propoxy)phenyl)-2-(4-fluorophenyl)-5-isopropyl-4-(phenylcarbamoyl)-1H-pyrrol-1-yl)-3,5-dihydroxyheptanoic acid (12b). It was synthesized as compound **11a**. ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 8.70 (s, 1H), 7.47 (t, *J* = 7.24 Hz, 1H), 7.27 – 7.06 (m, 12H), 6.86 (d, *J* = 8.48 Hz, 1H), 6.70 – 6.66 (m, 3H), 6.22 (s, 1H), 4.95 – 4.82 (m, 1H), 4.55 – 3.20 (m, 11H), 2.93 – 2.12 (m, 8H), 1.92 – 1.15 (m, 16H). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 169.6, 168.9, 168.1, 167.8, 159.8, 159.9, 147.3, 147.2, 136.3, 133.3, 132.6, 128.8, 123.1, 121.3, 120.0, 116.8, 116.20, 115.7, 111.6, 66.9, 62.9, 50.2, 49.2, 49.0, 42.5, 41.8, 41.5, 41.0, 39.0, 31.5, 30.2, 29.8, 29.1, 28.8, 26.5, 26.3, 22.9, 22.0. HR−MS: calculated for $C_{57}H_{62}FN_8O_{10}$ $[M-H]^-$, 1037.4572; found, 1037.4559.

(3R,5R)-7-(3-(3-(3-(1-(7-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)heptyl)-1H-1,2,3-triazol-4-yl)propoxy)phenyl)-2-(4-fluorophenyl)-5-isopropyl-4-(phenylcarbamoyl)-1H-pyrrol-1-yl)-3,5-dihydroxyheptanoic acid (12c). It was synthesized as compound **11a**. ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 8.66 (s, 1H), 7.48 (t, *J* = 7.24 Hz, 1H), 7.27 – 7.06 (m, 12H), 6.85 (d, *J* = 8.48 Hz, 1H), 6.70 – 6.66 (m, 3H), 6.21 (s, 1H), 4.95 – 4.82 (m, 1H), 4.35 – 3.18 (m, 11H), 2.83 – 2.12 (m, 8H), 1.92 – 1.15 (m, 18H). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 175.5, 171.6, 169.6, 168.8, 167.8, 161.1, 159.0, 148.1, 147.1, 138.5, 136.3, 136.0, 133.3, 133.3, 132.6, 129.4, 128.8, 123.8, 122.8, 121.9, 120.9, 119.9, 116.8, 115.9, 115.7, 115.4, 114.1, 111.5, 114.1, 111.5, 110.0, 69.7, 67.7, 50.3, 49.7, 49.0, 42.3, 41.8, 41.4, 39.0, 31.5, 30.8, 30.3, 29.8, 29.4, 29.2, 29.0, 28.8, 28.8, 26.8, 26.5, 26.3, 25.6, 25.5, 22.9, 22.0, 21.9, 17.7. HR−MS: calculated for $C_{58}H_{66}FN_8O_{10}$ $[M+H]^+$, 1053.4886; found, 1053.4875.

(3R,5R)-7-(3-(3-(4-(1-(7-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)heptyl)-

1H-1,2,3-triazol-4-yl)butoxy)phenyl)-2-(4-fluorophenyl)-5-isopropyl-4-(phenylcarbamoyl)-1H-pyrrol-1-yl)-3,5-dihydroxyheptanoic acid (12d). It was synthesized as compound **11a**. ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 8.88 (s, 1H), 7.47 (t, *J* = 7.24 Hz, 1H), 7.19 – 7.06 (m, 12H), 6.85 (d, *J* = 8.48 Hz, 1H), 6.70 – 6.66 (m, 3H), 6.22 (s, 1H), 4.95 – 4.82 (m, 1H), 4.35 – 3.08 (m, 11H), 2.83 – 2.12 (m, 8H), 1.92 – 1.15 (m, 20H). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 175.8, 171.8, 169.6, 168.9, 167.7, 165.3, 161.1, 158.8, 147.0, 141.3, 138.4, 136.2, 136.0, 133.3, 133.2, 132.5, 129.30, 128.7, 128.4, 123.7, 122.8, 121.8, 119.8, 116.7, 116.0, 115.6, 115.4, 113.8, 111.4, 109.9, 69.6, 67.4, 50.3, 49.7, 48.9, 42.6, 41.4, 39.0, 31.4, 30.7, 30.2, 29.8, 29.1, 28.7, 28.5, 26.8, 26.4, 26.2, 25.8, 25.1, 22.8, 21.9, 21.8, 17.7. HR−MS: calculated for $C_{59}H_{66}FN_8O_{10}$ $[M-H]^-$, 1065.4886; found, 1065.4863.

(3R,5R)-7-(3-(3-((5-(1-(7-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)heptyl)-1H-1,2,3-triazol-4-yl)pentyl)oxy)phenyl)-2-(4-fluorophenyl)-5-isopropyl-4-(phenylcarbamoyl)-1H-pyrrol-1-yl)-3,5-dihydroxyheptanoic acid (12e). It was synthesized as compound **11a**. ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 8.60 (s, 1H), 7.48 (t, *J* = 7.24 Hz, 1H), 7.19 – 7.06 (m, 12H), 6.86 (d, *J* = 8.48 Hz, 1H), 6.71 – 6.66 (m, 3H), 6.21 (s, 1H), 4.95 – 4.82 (m, 1H), 4.35 – 3.08 (m, 11H), 2.83 – 2.12 (m, 8H), 1.93 – 1.21 (m, 22H). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 171.4, 169.9, 169.6, 168.7, 167.8, 158.8, 147.1, 141.3, 138.5, 136.3, 135.9, 133.3, 132.6, 129.5, 128.8, 128.2, 123.8, 122.9, 122.1, 119.9, 116.8, 116.0, 115.8, 115.6, 114.0, 111.6, 110.0, 73.2, 66.9, 62.4, 53.6, 50.3, 49.6, 49.0, 42.6, 40.9, 38.6, 37.3, 35.8, 31.5, 30.8, 30.3, 29.8, 29.2, 28.8, 26.8, 26.5, 26.3, 22.9, 22.1, 21.9, 17.7. HR−MS: calculated for $C_{60}H_{70}FN_8O_{10}$ $[M+H]^+$, 1081.5199; found, 1081.5172.

(3R,5R)-7-(3-(3-((6-(1-(7-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)heptyl)-1H-1,2,3-triazol-4-yl)hexyl)oxy)phenyl)-2-(4-fluorophenyl)-5-isopropyl-4-(phenylcarbamoyl)-

1H-pyrrol-1-yl)-3,5-dihydroxyheptanoic acid (12f). It was synthesized as compound **11a**. ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 8.61 (s, 1H), 7.47 (t, *J* = 7.24 Hz, 1H), 7.19 – 7.06 (m, 12H), 6.85 (d, *J* = 8.48 Hz, 1H), 6.71 – 6.66 (m, 3H), 6.21 (s, 1H), 4.97 – 4.82 (m, 1H), 4.35 – 3.08 (m, 11H), 2.83 – 2.12 (m, 8H), 1.93 – 1.22 (m, 24H). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 171.3, 169.8, 169.7, 168.6, 167.7, 158.8, 147.1, 141.2, 138.3, 136.3, 135.8, 133.2, 132.6, 129.6, 128.8, 123.7, 122.2, 122.0, 120.0, 116.1, 116.0, 115.9, 115.6, 114.6, 111.6, 110.0, 73.3, 67.0, 62.5, 53.4, 50.3, 49.7, 48.0, 41.6, 40.8, 37.6, 37.3, 32.8, 31.5, 30.2, 30.1, 29.3, 29.2, 28.9, 26.6, 26.2, 26.0, 22.9, 22.0, 21.9, 17.7. LR−MS: calculated for $C_{61}H_{72}FN_8O_{10}$ $[M+H]^+$, 1095.53; found, 1095.86.

(3R,5R)-7-(3-(3-((7-(1-(7-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)heptyl)-1H-1,2,3-triazol-4-yl)heptyl)oxy)phenyl)-2-(4-fluorophenyl)-5-isopropyl-4-(phenylcarbamoyl)-1H-pyrrol-1-yl)-3,5-dihydroxyheptanoic acid (12g). It was synthesized as compound **11a**. ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 8.65 (s, 1H), 7.47 (t, *J* = 7.24 Hz, 1H), 7.19 – 7.06 (m, 12H), 6.86 (d, *J* = 8.48 Hz, 1H), 6.71 – 6.66 (m, 3H), 6.21 (s, 1H), 4.97 – 4.82 (m, 1H), 4.35 – 3.10 (m, 11H), 2.83 – 2.12 (m, 8H), 1.93 – 1.22 (m, 26H). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 171.6, 171.5, 170.0, 169.6, 168.8, 168.7, 167.7, 159.0, 147.0, 141.3, 138.5, 138.4, 136.3, 135.9, 135.8, 133.3, 133.2, 133.2, 132.6, 129.4, 128.8, 128.2, 123.8, 122.7, 122.1, 121.9, 120.7, 119.8, 116.8, 115.9, 115.8, 115.7, 115.6, 115.4, 114.0, 111.5, 109.9, 73.2, 67.9, 62.2, 49.6, 48.9, 42.6, 40.8, 38.6, 37.3, 35.7, 31.5, 30.8, 30.3, 29.7, 29.4, 29.2, 29.0, 28.8, 26.8, 26.5, 26.3, 25.9, 25.6, 22.9, 22.1, 21.9, 21.8, 17.7. LR−MS: calculated for $C_{62}H_{74}FN_8O_{10}$ $[M+H]^+$, 1109.54; found, 1109.76.

(3R,5R)-7-(3-(3-(3-(1-(2-(2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-5-yl)amino)ethoxy)ethoxy)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)propoxy)phenyl)-2-(4-fluorophenyl)-

5-isopropyl-4-(phenylcarbamoyl)-1H-pyrrol-1-yl)-3,5-dihydroxyheptanoic acid (13a). It was synthesized as compound **11a**. ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 7.41 (s, 1H), 7.19 – 6.80 (m, 12H), 6.78 – 6.50 (m, 4H), 5.51 (s, 1H), 4.90 (m, 1H), 4.51 – 4.40 (m, 2H), 4.11 – 3.27 (m, 20H), 2.87 – 2.51 (m, 5H), 2.37 – 2.10 (m, 1H), 1.93 – 1.32 (m, 12H). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 170.6, 170.4, 170.0, 168.1, 167.6, 161.3, 158.7, 147.0, 141.3, 138.5, 133.5, 133.3, 128.9, 128.2, 125.7, 123.9, 122.32 120.0, 116.2, 115.6, 106.6, 70.6, 69.6, 69.0, 67.0, 60.5, 53.6, 50.2, 43.1, 29.8, 28.9, 22.8, 22.2, 14.3. LR−MS: calculated for $C_{59}H_{68}FN_8O_{13}$ $[M+H]^+$, 1115.48; found, 1115.56.

(3R,5R)-7-(3-(3-(4-(1-(2-(2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-5-yl)amino)ethoxy)ethoxy)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)butoxy)phenyl)-2-(4-fluorophenyl)-5-isopropyl-4-(phenylcarbamoyl)-1H-pyrrol-1-yl)-3,5-dihydroxyheptanoic acid (13b). It was synthesized as compound **11a**. ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 7.52 (s, 1H), 7.20 – 6.83 (m, 12H), 6.78 – 6.55 (m, 4H), 4.91 (m, 1H), 4.50 – 4.40 (m, 2H), 4.17 – 3.21 (m, 20H), 2.87 – 2.10 (m, 6H), 1.83 – 1.32 (m, 14H). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 170.6, 170.3, 170.1, 168.1, 161.6, 159.0, 147.8, 141.3, 138.4, 133.4, 129.4, 128.9, 123.9, 122.9, 122.0, 119.9, 115.9, 115.6, 113.9, 70.6, 69.0, 68.7, 67.3, 63.3, 55.6, 50.4, 49.1, 43.3, 41.7, 41.3, 39.3, 29.8, 29.5, 28.2, 27.4, 25.1, 24.6, 22.8, 22.0, 18.2, 14.2. LR−MS: calculated for $C_{60}H_{70}FN_8O_{13}$ $[M+H]^+$, 1129.50; found, 1129.76.

(3R,5R)-7-(3-(3-((5-(1-(2-(2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-5-yl)amino)ethoxy)ethoxy)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)pentyl)oxy)phenyl)-2-(4-fluorophenyl)-5-isopropyl-4-(phenylcarbamoyl)-1H-pyrrol-1-yl)-3,5-dihydroxyheptanoic acid (13c). It was synthesized as compound **11a**. ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 7.42 (s, 1H), 7.20 – 6.83 (m, 12H), 6.78 – 6.55 (m, 4H), 4.92 (m, 1H), 4.52 – 4.41 (m, 2H), 4.17 – 3.24 (m, 20H), 2.87 –

2.10 (m, 6H), 1.83 – 1.32 (m, 16H). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 172.6, 169.3, 168.1, 167.1, 165.6, 161.9, 159.0, 147.8, 141.3, 138.4, 135.9, 133.3, 129.4, 128.8, 125.6, 123.8, 122.8, 122.0, 119.9, 115.9, 115.7, 70.6, 69.4, 68.5, 67.7, 65.3, 53.6, 44.7, 43.2, 41.7, 38.7, 35.7, 31.9, 29.8, 29.3, 28.6, 28.2, 26.5, 25.2, 23.0, 22.2, 18.5, 14.0. LR−MS: calculated for $C_{61}H_{72}FN_8O_{13}$ $[M+H]^+$, 1143.51; found, 1143.55.

(3R,5R)-7-(3-(3-(3-(1-(14-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-5-yl)amino)-3,6,9,12-tetraoxatetradecyl)-1H-1,2,3-triazol-4-yl)propoxy)phenyl)-2-(4-fluorophenyl)-5-isopropyl-4-(phenylcarbamoyl)-1H-pyrrol-1-yl)-3,5-dihydroxyheptanoic acid (13d). It was synthesized as compound **11a**. ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 7.48 (d, J = 6.72 Hz, 1H), 7.40 (s, 1H), 7.19 – 6.80 (m, 11H), 6.76 – 6.52 (m, 4H), 5.62 (s, 1H), 4.89 (m, 1H), 4.51 – 4.37 (m, 2H), 4.11 – 3.27 (m, 24H), 2.87 – 2.51 (m, 5H), 2.37 – 2.00 (m, 1H), 1.93 – 1.32 (m, 12H). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 170.1, 169.6, 168.1, 167.6, 165.4, 159.0, 154.1, 149.4, 147.1, 144.7, 141.1, 138.5, 136.1, 134.7, 133.4, 129.4, 128.8, 125.7, 122.8, 122.3, 121.9, 119.9, 116.3, 115.6, 70.6, 70.5, 70.4, 69.6, 69.0, 67.1, 50.2, 49.1, 43.1, 36.6, 31.6, 29.8, 28.9, 22.8, 22.2, 22.0. LR−MS: calculated for $C_{61}H_{72}FN_8O_{14}$ $[M+H]^+$, 1159.51; found, 1159.64.

(3R,5R)-7-(3-(3-(4-(1-(14-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-5-yl)amino)-3,6,9,12-tetraoxatetradecyl)-1H-1,2,3-triazol-4-yl)butoxy)phenyl)-2-(4-fluorophenyl)-5-isopropyl-4-(phenylcarbamoyl)-1H-pyrrol-1-yl)-3,5-dihydroxyheptanoic acid (13e). It was synthesized as compound **11a**. ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 7.50 (s, 1H), 7.20 – 6.82 (m, 12H), 6.76 – 6.52 (m, 4H), 4.89 (m, 1H), 4.51 – 4.37 (m, 2H), 4.11 – 3.27 (m, 24H), 2.87 – 2.51 (m, 5H), 2.37 – 2.00 (m, 1H), 1.93 – 1.32 (m, 14H). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 171.1, 170.1, 165.4, 162.9, 161.3,

160.6, 158.9, 138.4, 135.9, 134.6, 133.4, 129.4, 128.9, 128.5, 123.9, 122.9, 120.0, 119.9, 115.9, 115.7, 115.5, 113.8, 70.6, 69.7, 69.0, 68.7, 67.3, 53.6, 49.2, 43.3, 41.3, 36.7, 31.7, 29.8, 29.5, 29.4, 28.2, 26.3, 25.1, 22.8, 22.0, 18.2. LR−MS: calculated for $C_{62}H_{74}FN_8O_{14}$ $[M+H]^+$, 1173.52; found, 1173.67.

(3R,5R)-7-(3-(3-((5-(1-(14-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-5-yl)amino)-3,6,9,12-tetraoxatetradecyl)-1H-1,2,3-triazol-4-yl)pentyl)oxy)phenyl)-2-(4-fluorophenyl)-5-isopropyl-4-(phenylcarbamoyl)-1H-pyrrol-1-yl)-3,5-dihydroxyheptanoic acid (13f). It was synthesized as compound **11a**. ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 7.46 (s, 1H), 7.22 – 6.82 (m, 12H), 6.80 – 6.60 (m, 4H), 4.92 (m, 1H), 4.51 – 4.40 (m, 2H), 4.11 – 3.27 (m, 24H), 2.87 – 2.51 (m, 5H), 2.37 – 2.00 (m, 1H), 1.83 – 1.22 (m, 16H). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 171.0, 170.0, 168.4, 167.8, 166.8, 160.6, 159.0, 138.5, 136.0, 135.0, 133.2, 129.4, 128.8, 128.5, 123.8, 122.8, 121.9, 119.8, 115.9, 115.5, 115.4, 113.8, 70.6, 68.6, 68.3, 67.7, 66.5, 56.9, 53.4, 49.9, 43.2, 41.3, 36.7, 32.1, 31.2, 29.8, 29.5, 28.7, 28.2, 26.5, 25.5, 22.8, 21.9, 18.5. LR−MS: calculated for $C_{63}H_{76}FN_8O_{14}$ $[M+H]^+$, 1187.54; found, 1187.88.

(3R,5R)-7-(3-(3-(3-(1-(2-((3-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)prop-2-yn-1-yl)oxy)ethyl)-1H-1,2,3-triazol-4-yl)propoxy)phenyl)-2-(4-fluorophenyl)-5-isopropyl-4-(phenylcarbamoyl)-1H-pyrrol-1-yl)-3,5-dihydroxyheptanoic acid (14a). It was synthesized as compound **11a**. ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 9.10 (s, 1H), 7.78 (d. J = 6.80 Hz, 1H), 7.54 (d, J = 7.16 Hz, 1H), 7.41 (t, J = 7.40 Hz, 1H), 7.21 – 6.80 (m, 12H), 6.70 – 6.50 (m, 3H), 5.19 (m, 1H), 4.55 – 3.30 (m, 15H), 2.85 – 2.61 (m, 5H), 2.35 – 2.12 (m, 3H), 1.91 – 1.27 (m, 12H). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 170.0, 169.3, 165.4, 161.2, 158.8, 143.9, 141.3, 139.4, 138.4, 135.9, 133.3, 129.4, 128.8, 128.6, 124.6, 123.9, 122.9, 121.8, 120.0, 119.9, 117.9, 115.9, 115.7, 115.5, 114.2, 91.1, 81.2,

69.0, 68.3, 67.1, 66.1, 62.4, 59.4, 33.9, 32.0, 31.5, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 29.1, 28.0, 26.3, 22.8, 22.0, 15.1, 14.3. LR−MS: calculated for $C_{56}H_{59}FN_7O_{10}$ $[M+H]^+$, 1008.42; found, 1008.56.

(3R,5R)-7-(3-(3-(3-(1-(3-((3-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)prop-2-yn-1-yl)oxy)propyl)-1H-1,2,3-triazol-4-yl)propoxy)phenyl)-2-(4-fluorophenyl)-5-isopropyl-4-(phenylcarbamoyl)-1H-pyrrol-1-yl)-3,5-dihydroxyheptanoic acid (14b). It was synthesized as compound **11a**. ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 9.05 (s, 1H), 7.76 (d. *J* = 6.80 Hz, 1H), 7.52 (d, *J* = 7.16 Hz, 1H), 7.40 (t, *J* = 7.40 Hz, 1H), 7.21 – 6.80 (m, 12H), 6.72 – 6.60 (m, 3H), 5.15 (m, 1H), 4.55 – 3.30 (m, 15H), 2.84 – 2.60 (m, 5H), 2.33 – 2.10 (m, 3H), 1.91 – 1.27 (m, 14H). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 170.0, 169.5, 165.6, 163.6, 161.2, 158.8, 141.5, 139.5, 138.4, 135.8, 133.3, 130.2, 129.5, 128.9, 128.5, 123.9, 122.9, 121.9, 119.9, 115.9, 115.7, 115.5, 114.7, 114.2, 113.9, 91.1, 81.6, 69.0, 68.3, 67.1, 66.1, 32.1, 29.8, 29.5, 29.5, 29.3, 29.1, 28.0, 27.3, 26.0, 22.8, 22.1, 15.2, 14.3. LR−MS: calculated for $C_{57}H_{61}FN_7O_{10}$ $[M+H]^+$, 1021.44; found, 1021.66.

(3R,5R)-7-(3-(3-(3-(1-(4-((3-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)prop-2-yn-1-yl)oxy)butyl)-1H-1,2,3-triazol-4-yl)propoxy)phenyl)-2-(4-fluorophenyl)-5-isopropyl-4-(phenylcarbamoyl)-1H-pyrrol-1-yl)-3,5-dihydroxyheptanoic acid (14c). It was synthesized as compound **11a**. ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 9.15 (s, 1H), 7.78 (d. *J* = 6.80 Hz, 1H), 7.58 (d, *J* = 7.16 Hz, 1H), 7.42 (t, *J* = 7.40 Hz, 1H), 7.27 – 6.80 (m, 12H), 6.72 – 6.50 (m, 3H), 5.17 (m, 1H), 4.55 – 3.30 (m, 15H), 2.84 – 2.60 (m, 5H), 2.43 – 2.10 (m, 3H), 1.91 – 1.27 (m, 16H). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 170.0, 169.1, 165.6, 163.8, 161.0, 158.7, 143.9, 141.2, 138.4, 136.0, 135.1, 133.3, 131.7, 129.4, 128.8, 128.6, 124.2, 123.9, 122.9, 121.9, 121.8, 120.0, 119.8, 118.2, 115.9, 115.5, 113.8, 90.9, 81.8, 69.3, 69.0, 66.8, 66.1, 66.0, 58.8, 52.2, 32.0, 31.5, 29.8, 29.5, 29.4, 28.6, 28.0, 27.3, 26.4,

22.8, 22.0, 15.4. LR−MS: calculated for $C_{58}H_{63}FN_7O_{10}$ $[M+H]^+$, 1036.45; found, 1036.86.

(3R,5R)-7-(3-(3-(3-(1-(5-((3-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)prop-2-yn-1-yl)oxy)pentyl)-1H-1,2,3-triazol-4-yl)propoxy)phenyl)-2-(4-fluorophenyl)-5-isopropyl-4-(phenylcarbamoyl)-1H-pyrrol-1-yl)-3,5-dihydroxyheptanoic acid (14d). It was synthesized as compound **11a**. ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 9.11 (s, 1H), 7.82 (d. *J* = 6.80 Hz, 1H), 7.62 (d, *J* = 7.16 Hz, 1H), 7.45 (t, *J* = 7.40 Hz, 1H), 7.20 – 6.90 (m, 12H), 6.72 – 6.60 (m, 3H), 5.20 (m, 1H), 4.50 – 3.47 (m, 15H), 2.84 – 2.60 (m, 5H), 2.40 – 2.10 (m, 3H), 1.91 – 1.27 (m, 18H). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 170.1, 169.2, 165.4, 163.6, 160.9, 158.8, 143.9, 141.2, 138.4, 135.8, 133.3, 130.0, 129.4, 128.9, 128.5, 123.9, 122.9, 121.9, 119.9, 115.9, 115.7, 115.5, 113.9, 90.9, 81.7, 70.0, 69.0, 66.8, 66.1, 66.0, 58.8, 32.0, 29.8, 29.4, 28.0, 27.3, 26.3, 22.8, 22.0, 15.2, 14.3. LR−MS: calculated for $C_{59}H_{65}FN_7O_{10}$ $[M+H]^+$, 1050.47; found, 1050.67.

(3R,5R)-7-(3-(3-(3-(1-(6-((3-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)prop-2-yn-1-yl)oxy)hexyl)-1H-1,2,3-triazol-4-yl)propoxy)phenyl)-2-(4-fluorophenyl)-5-isopropyl-4-(phenylcarbamoyl)-1H-pyrrol-1-yl)-3,5-dihydroxyheptanoic acid (14e). It was synthesized as compound **11a**. ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 9.08 (s, 1H), 7.76 (d. *J* = 6.80 Hz, 1H), 7.56 (d, *J* = 7.16 Hz, 1H), 7.39 (t, *J* = 7.40 Hz, 1H), 7.22 – 6.82 (m, 12H), 6.65 – 6.55 (m, 3H), 5.17 (m, 1H), 4.51 – 3.40 (m, 15H), 2.82 – 2.61 (m, 5H), 2.40 – 2.02 (m, 3H), 1.97 – 1.27 (m, 20H). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 170.0, 169.2, 165.4, 161.0, 158.8, 143.9, 141.1, 138.5, 136.0, 135.1, 133.3, 131.7, 129.4, 128.8, 124.3, 123.8, 122.9, 121.8, 121.2, 119.9, 118.4, 116.0, 115.6, 113.9, 91.1, 81.6, 70.3, 66.8, 58.8, 52.1, 50.2, 47.4, 32.0, 31.6, 30.3, 29.8, 29.5, 29.4, 29.3, 28.7, 26.3, 25.7, 23.4, 22.8, 22.0. LR−MS: calculated for $C_{60}H_{67}FN_7O_{10}$ $[M+H]^+$, 1064.49; found, 1064.80.

(3R,5R)-7-(3-(3-(3-(1-(7-((3-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)prop-2-yn-1-yl)oxy)heptyl)-1H-1,2,3-triazol-4-yl)propoxy)phenyl)-2-(4-fluorophenyl)-5-isopropyl-4-(phenylcarbamoyl)-1H-pyrrol-1-yl)-3,5-dihydroxyheptanoic acid (14f). It was synthesized as compound **11a**. ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 9.13 (s, 1H), 7.80 (d. *J* = 6.80 Hz, 1H), 7.60 (d, *J* = 7.16 Hz, 1H), 7.44 (t, *J* = 7.40 Hz, 1H), 7.22 – 6.84 (m, 12H), 6.75 – 6.60 (m, 3H), 5.20 (m, 1H), 4.53 – 3.40 (m, 15H), 2.88 – 2.65 (m, 5H), 2.48 – 2.12 (m, 3H), 1.97 – 1.27 (m, 22H). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 170.4, 169.1, 165.4, 163.4, 161.0, 158.8, 143.9, 141.2, 138.5, 136.0, 135.1, 133.3, 131.7, 129.4, 128.8, 124.2, 123.8, 122.9, 121.8, 119.9, 118.4, 116.0, 115.6, 113.8, 91.2, 81.5, 70.5, 66.8, 66.0, 58.8, 52.0, 50.3, 47.3, 32.0, 31.6, 30.2, 29.8, 29.5, 28.8, 27.3, 26.4, 26.3, 26.0, 23.4, 22.8, 22.0. LR−MS: calculated for $C_{61}H_{69}FN_7O_{10}$ $[M+H]^+$, 1078.50; found, 1078.67.

(3R,5R)-7-(3-(3-(3-(1-(2-(3-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)propoxy)ethyl)-1H-1,2,3-triazol-4-yl)propoxy)phenyl)-2-(4-fluorophenyl)-5-isopropyl-4-(phenylcarbamoyl)-1H-pyrrol-1-yl)-3,5-dihydroxyheptanoic acid (15a). It was synthesized as compound **11a**. ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 8.47 (s, 1H), 7.74 (d. *J* = 6.80 Hz, 1H), 7.40 (m, 2H), 7.22 – 6.84 (m, 12H), 6.71 – 6.68 (m, 3H), 5.24 (m, 1H), 4.43 – 3.30 (m, 15H), 2.88 – 2.15 (m, 9H), 1.97 – 1.27 (m, 14H). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 173.1, 169.8, 166.3, 161.4, 157.9, 147.3, 143.0, 141.7, 138.6, 136.0, 135.8, 133.4, 133.3, 129.5, 128.8, 128.3, 125.5, 123.6, 122.9, 121.8, 119.7, 115.9, 115.6, 115.1, 114.0, 70.1, 69.7, 69.1, 68.0, 41.4, 41.3, 39.2, 34.3, 32.0, 30.2, 29.8, 26.2, 22.7. LR−MS: calculated for $C_{56}H_{63}FN_7O_{10}$ $[M+H]^+$, 1012.45; found, 1012.55.

(3R,5R)-7-(3-(3-(3-(1-(3-(3-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)propoxy)propyl)-1H-1,2,3-triazol-4-yl)propoxy)phenyl)-2-(4-fluorophenyl)-5-isopropyl-4-(phenylcarbamoyl)-

1H-pyrrol-1-yl)-3,5-dihydroxyheptanoic acid (15b). It was synthesized as compound **11a**. ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 8.46 (s, 1H), 7.73 (d. *J* = 6.80 Hz, 1H), 7.41 (m, 2H), 7.22 – 6.84 (m, 12H), 6.71 – 6.68 (m, 3H), 5.24 (m, 1H), 4.43 – 3.30 (m, 15H), 2.88 – 2.15 (m, 9H), 1.97 – 1.27 (m, 16H). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 173.3, 169.9, 166.2, 161.2, 157.9, 147.3, 143.0, 141.7, 138.6, 135.9, 135.8, 133.4, 133.3, 129.4, 128.8, 128.3, 125.7, 123.6, 122.9, 122.0 119.7, 115.9, 115.6, 115.1, 114.0, 70.1, 69.7, 69.1, 68.0, 41.4, 41.0, 39.2, 34.3, 32.0, 30.4, 29.8, 29.5, 28.1, 26.2, 22.9, 22.6, 21.1. LR−MS: calculated for $C_{57}H_{65}FN_7O_{10}$ $[M+H]^+$, 1026.47; found, 1026.67.

(3R,5R)-7-(3-(3-(3-(1-(4-(3-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)propoxy)butyl)-1H-1,2,3-triazol-4-yl)propoxy)phenyl)-2-(4-fluorophenyl)-5-isopropyl-4-(phenylcarbamoyl)-1H-pyrrol-1-yl)-3,5-dihydroxyheptanoic acid (15c). It was synthesized as compound **11a**. ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 8.45 (s, 1H), 7.73 (d. *J* = 6.80 Hz, 1H), 7.41 (m, 2H), 7.22 – 6.84 (m, 12H), 6.71 – 6.68 (m, 3H), 5.24 (m, 1H), 4.43 – 3.30 (m, 15H), 2.88 – 2.15 (m, 9H), 1.97 – 1.27 (m, 18H). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 173.1, 169.8, 166.1, 161.0, 157.9, 147.3, 143.3, 141.7, 138.6, 135.9, 135.8, 133.4, 133.3, 129.4, 128.8, 128.4, 125.7, 123.7, 122.9, 122.0, 119.8, 116.0, 115.6, 115.4, 114.0, 70.2, 69.7, 69.1, 68.0, 41.4, 41.0, 39.2, 34.3, 32.0, 30.4, 29.8, 29.5, 28.1, 28.1, 26.2, 22.8, 22.4, 21.2, 21.3. LR−MS: calculated for $C_{58}H_{67}FN_7O_{10}$ $[M+H]^+$, 1040.49; found, 1040.65.

(3R,5R)-7-(3-(3-(3-(1-(5-(3-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)propoxy)pentyl)-1H-1,2,3-triazol-4-yl)propoxy)phenyl)-2-(4-fluorophenyl)-5-isopropyl-4-(phenylcarbamoyl)-1H-pyrrol-1-yl)-3,5-dihydroxyheptanoic acid (15d). It was synthesized as compound **11a**. ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 8.45 (s, 1H), 7.73 (d. *J* = 6.80 Hz, 1H), 7.41 (m, 2H), 7.22 – 6.84 (m, 12H), 6.71 – 6.68 (m, 3H), 5.24 (m, 1H), 4.43 – 3.30 (m, 15H), 2.88 – 2.15 (m, 9H), 1.97 – 1.27 (m, 20H).

^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 173.1, 169.8, 165.1, 161.3, 157.9, 147.3, 143.3, 141.7, 138.6, 135.9, 135.9, 133.4, 133.3, 129.4, 128.8, 128.4, 125.7, 123.7, 122.9, 122.0, 119.8, 115.9, 115.7, 115.4, 114.1, 70.9, 69.8, 69.1, 68.0, 41.9, 41.2, 39.2, 34.4, 32.1, 30.5, 29.8, 29.5, 28.9, 28.2, 28.1, 26.3, 22.8, 22.5, 21.9, 21.8, 21.3. LR−MS: calculated for $C_{59}H_{69}FN_7O_{10}$ $[M+H]^+$, 1054.50; found, 1054.67.

(3R,5R)-7-(3-(3-(3-(1-(6-(3-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)propoxy)hexyl)-1H-1,2,3-triazol-4-yl)propoxy)phenyl)-2-(4-fluorophenyl)-5-isopropyl-4-(phenylcarbamoyl)-1H-pyrrol-1-yl)-3,5-dihydroxyheptanoic acid (15e). It was synthesized as compound **11a**. ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 8.45 (s, 1H), 7.73 (d. *J* = 6.80 Hz, 1H), 7.41 (m, 2H), 7.22 – 6.84 (m, 12H), 6.71 – 6.68 (m, 3H), 5.24 (m, 1H), 4.43 – 3.30 (m, 15H), 2.88 – 2.15 (m, 9H), 1.97 – 1.27 (m, 22H). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 173.1, 169.8, 165.2, 161.3, 158.9, 147.3, 140.4, 138.6, 136.9, 136.3, 133.3, 132.0, 131.4, 129.4, 128.8, 123.7, 122.9, 121.9, 121.9, 119.8, 116.1, 115.6, 115.4, 113.9, 70.9, 69.7, 69.6, 69.0, 66.9, 52.1, 52.0, 50.2, 46.4, 41.9, 41.3, 39.2, 32.0, 31.7, 30.4, 30.4, 29.8, 29.6, 29.5, 28.8, 28.5, 26.4, 26.3, 25.8, 23.6, 22.8, 22.2, 21.9, 21.8. LR−MS: calculated for $C_{60}H_{71}FN_7O_{10}$ $[M+H]^+$, 1068.52; found, 1068.87.

(3R,5R)-7-(3-(3-(3-(1-(7-(3-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)propoxy)heptyl)-1H-1,2,3-triazol-4-yl)propoxy)phenyl)-2-(4-fluorophenyl)-5-isopropyl-4-(phenylcarbamoyl)-1H-pyrrol-1-yl)-3,5-dihydroxyheptanoic acid (15f). It was synthesized as compound **11a**. ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 8.49 (s, 1H), 7.72 (d. *J* = 6.80 Hz, 1H), 7.40 (m, 2H), 7.22 – 6.84 (m, 12H), 6.75 – 6.60 (m, 3H), 5.27 (m, 1H), 4.43 – 3.30 (m, 15H), 2.88 – 2.15 (m, 9H), 1.97 – 1.27 (m, 24H). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 173.1, 169.9, 165.2, 161.4, 159.0, 147.3, 141.7, 138.5, 133.4, 132.0, 131.4, 129.4, 128.8, 128.8, 123.8, 123.0, 122.0, 121.8, 119.8, 115.7, 115.4, 113.9, 71.1, 69.7,

69.0, 53.6, 41.9, 41.4, 39.2, 32.0, 30.4, 29.5, 29.0, 28.6, 27.3, 26.6, 26.3, 22.8, 22.0. LR−MS: calculated for $C_{61}H_{73}FN_7O_{10}$ $[M+H]^+$, 1082.53; found, 1082.63.

Cell culture. CHO-7 (Chinese hamster ovary cell, a clone of CHO-K1 cells) and SRD15 (an *Insig-1* and *Insig-2* deficient CHO-7 cell line), were generous gifts from Dr. Russell Debose-Boyd at UT Southwestern Medical Center, USA. HEK293T and Huh7 (a human hepatocellular carcinoma cell line) were obtained from ATCC. All cells were grown in a monolayer at 37 °C with 5% CO_2. CHO-7 and SRD15 cells were maintained Medium A (a 1:1 mixture of Ham's F-12 Medium and Dulbecco's Modified Eagle Medium (DMEM) with 100 units/mL penicillin and 100 μg/mL streptomycin sulfate) containing 5% fetal bovine serum (FBS). HEK293T and Huh7 cells were maintained in Medium B (DMEM with 100 units/mL penicillin and 100 μg/mL streptomycin sulfate) containing 10% FBS. The depletion medium was Medium A or Medium B supplemented with 5% lipoprotein-deficient serum.

Primary antibodies. Primary antibodies used for immunoblotting were as follows: mouse monoclonal anti-ubiquitin antibody (sc-8017, Santa Cruz; 1:500); mouse monoclonal anti-β-actin antibody (A1978, Sigma; 1:10,000); rabbit polyclonal anti-Myc antibody (06-549, Millipore; 1:1,000); rabbit polyclonal anti-GAPDH antibody (10494-1-AP, Proteintech; 1:10,000); mouse monoclonal anti-HA antibody (H3663, Sigma; 1:1,000); mouse monoclonal anti-Flag antibody (F3165, Sigma; 1:1,000). The mouse monoclonal anti-HMGCR antibody was prepared in our laboratory.

Plasmids. The coding sequences of *HMGCR* and *CRBN* were amplified from Huh7 cells by standard PCR. The sequence of *HMGCR* was cloned into pCDNA3-5×Myc vector and pEGFP-N1 vectors to generate pCMV-HMGCR-Myc and pCMV-HMGCR-EGFP, respectively. The sequence of *CRBN* was cloned to pCMV14-3×Flag vector. CRBN site-directed mutants (3×flag-$CRBN^{YW/AA}$) were

constructed using the QuikChange site-directed kit (Stratagene) and confirmed by sequencing. The sequence of HMGCR-EGFP gene was sub-cloned from pCMV-HMGCR-EGFP by standard PCR and inserted into the pLVX-IRES-Puro vector for lentivirus preparation.

RNA Interference. Three duplexes of siRNA were synthesized by RBOBIO (Guangzhou, China). The sequences of siRNAs targeting against *CRBN* were:

5'-CCAGAAACUGAAGAUGAAA -3',

5'-GCAAGGCAGUGUAUAUUAU -3',

5'-GGAAGUCGAAGACCAAGAU -3'.

The siRNA against *VSV-G* (5'-GGCUAUUCAAGCAGACGGUTT-3') was used as a negative control.

Western blot analysis. Cells were harvested and homogenized in 120 μL of RIPA buffer supplemented with protease inhibitors. Protein concentrations of whole cell lysates were determined according to Lowry method (Bio-Rad). Samples were mixed with 4×SDS loading buffer (150 mM Tris-HCl, pH 6.8, 12% SDS, 30% (v/v) glycerol, 0.02% (w/v) bromophenol blue, 6% (v/v) β-mercaptoethanol) and boiled for 10 min. Proteins were resolved by SDS-PAGE and transferred onto PVDF membranes. Immunoblots were blocked with 5% milk in TBS containing 0.075% Tween (TBST) and probed with primary antibodies overnight at 4 °C. After washing in TBST 3 times, blots were incubated with secondary antibodies for 1 h at room temperature.

Co-immunoprecipitation. Cells were harvested and lysed in 0.6 mL of immunoprecipitation buffer (1×PBS, 1% digitonin, 5mM EDTA, 5mM EGTA and protease inhibitors). After centrifugation at

12,000×g for 10 min at 4 °C, immunoprecipitation was carried out with indicated antibody-coupled agarose, washed with immunoprecipitation buffers followed by western blotting.

Oil Red staining. Oil Red O was dissolved in propylene glycol at a working concentration of 0.5% (w/v). Huh7 cells grown on glass coverslips were fixed with 4% paraformaldehyde in PBS at room temperature for 30 min. Cells were washed 3 times in PBS, stained with Oil Red O for 15 min at room temperature, and washed 3 times using distilled water.

Lentivirus preparation. HEK293T cells were transfected with pLVX-HMGCR-GFP-IRES-Puro or pLVX-IRES-Puro (as a control), together with envelope (pMD2.G), and packaging (psPAX2) vectors using standard calcium phosphate protocol. Forty-eight hours later, lentivirus-containing supernatants were collected and centrifuged at 3,000×g 1 h at 4°C prior to use.

Generation of SRD15 cell stably expressing HMGCR-GFP. SRD15 cells were infected by the above lentivirus-containing supernatants for 12 h. Cells were then switched to normal culture medium for 12 h, followed by fluorescence activated cell sorting so that those stably expressing HMGCR-GFP were enriched. Cells were examined and imaged under a Leica Biosystems SP8 laser-scanning microscope equipped with 405-nm, 488-nm and 561-nm lasers. Images from the same experiment were equally processed for brightness and contrast using the LAS AF software.

Quantitative real-time PCR. Total RNA was extracted using Trizol reagents (Invitrogen) and subjected to reverse transcription (RT) with oligo dTs followed by quantitative real-time PCR using target-specific primers in the Stratagene Mx30005P Q-PCR Systems. All reactions were prepared in triplicate and the relative amounts of mRNAs were calculated using the comparative CT method. Hamster *GAPDH* and mouse *Cyclophilin* were used as controls. The normalized amount of mRNA

incontrol sample was arbitrarily defined as 1. The primer sequences were as follows:

hamster *GAPDH*:

forward: 5'- GCAAGTTCAAAGGCACAGTCAA-3'

reverse: 5'- CGCTCCTGGAAGATGGTGAT-3'

hamster *CRBN:*

forward: 5'- AGCTGGTTTCCTGGGTATGC-3'

reverse: 5'- CAGAGCGAGTTAAGCCCCAA-3'

mouse *Cyclophilin*:

forward: 5'-TGGAGAGCACCAAGACAGACA-3'

reverse: 5'-TGCCGGAGTCGACAATGAT-3'

mouse *HMGCR*:

forward: 5'- CTTGTGGAATGCCTTGTGATTG-3'

reverse: 5'- AGCCGAAGCAGCACATGAT -3'

mouse *LDLR*:

forward: 5'-AGGCTGTGGGCTCCATAGG-3

reverse: 5'-TGCGGTCCAGGGTCATCT-3

***De novo* cholesterol biosynthesis in Huh7 cell.** Huh7 cells were treated with atorvastatin or P22A for 14h and refed cell with 2 mL medium containing ^{14}C-acetic acid sodium salt at a concentration of 1μCi/μL for 2 h prior to harvesting. Cells were washed by 2 mL ice-cold buffer containing 5 mM Tris HCl pH 7.4, 150 mM NaCl, 0.2% BSA. Let stand for 5 min, before aspirating, wash 2 mL ice-cold 5 mM Tris HCl pH 7.4, 150 mM NaCl. Then added 1 ml 0.1 M NaOH to dissolve cell and incubate room temperature at least 30 min. Next, we transferred cell suspension to labeled 15 cm test tube. Wash petri dish with 1 ml H_2O and combine with original cell suspension in 15 cm tube and vortexed. Added 2 ml EtOH and 3.5 ml petroleum ether and centrifuged 1000g for 10 min. After that, transferred upper petroleum ether phase to labeled 15 cm tube. Re-extract twice as described above. After evaporate organic phase to dryness, dissolved the residue in 300 μl heptane and spotted onto plastic-backed silica gel TLC plates. TLC plates was developed in 100 chloroform allowing solvent to migrate to the top of the sheet. Finally, Dried the TLC plates to excise spots and count.

Statistical analyses and reproducibility. Data were normally distributed and between-group variances were similar. All statistical analyses were performed using the GraphPad Prism software. Data were expressed as means ± S.D. and analyzed by unpaired two-tailed Student's *t* test or two-way ANOVA as indicated. Statistical tests were justified as appropriate for every figure. Statistical significance was set at $p < 0.05$. Sample sizes, statistical tests and p values for each experiment are depicted in the relevant figure legends. Specific samples sizes were chosen based on similar experiments of previous studies. No statistical method was used to predetermine the sample size.

ASSOCIATED CONTENT

Supporting Information.

The following files are available free of charge.

Evaluating the PROTACs-induced degradation of HMGCR with SRD15 cells (Figure S1-S7), Evaluating levels of HMGCR, SREBP2 and LDLR in Huh7 cells after treatment with PROTACs (Figure S8). Oil Red O staining (Figure S9). The normal distribution and Pearson correlation coefficient of proteomic data (Figure S10). Validation of proteomic data by testing the western blots of NPC2 (Figure S11). The proteomic data (Table S1 and Table S2). NMR and UPLC traces of representative compounds. Molecular Formula Strings.

AUTHOR INFORMATION

Corresponding Author

*Yu Rao, yrao@tsinghua.edu.cn

* Jie Luo, jieluo@whu.edu.cn

Author Contributions

#These authors contributed equally. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. Y.R., J.L. and B.L.S. conceived the study. Y.Y., Y.W. and M.H. synthesized the compounds. M.X.L., Q.Z., H.Y. and H.G. performed the biological evaluation. L.S. performed the proteomic study.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENT

This work was supported by the National Natural Science Foundation of China (81811530340, 81573277, 81622042 and 81773567), National Major Scientific and Technological Special Project for "Significant New Drugs Development" (SQ2017ZX095003, 2018ZX09711001), Tsinghua University-Peking University Joint Center for Life Sciences and China Postdoctoral Science Foundation (2017M620806).

ABBREVIATIONS

HMGCR, 3-Hydroxy-3-methylglutaryl coenzyme A reductase; ER, endoplasmic reticulum; PROTACs, proteolysis-targeting chimeras; CVD, cardiovascular disease; LDL, low-density lipoprotein; RNF145, Ring finger protein 145; ERAD, ER-associated degradation; UPS, ubiquitin–proteasome system; CRBN, cereblon; CHO, Chinese hamster ovary; SREBP, sterol regulatory element-binding protein; GFP, green fluorescent protein; RFP, red fluorescent protein; DMSO, dimethyl sulfoxide. LDLR, LDL receptor; TLC, thin-layer chromatography; ANOVA, analysis of variance; PCSK9, subtilisin-kexin type 9; DC_{50}, half degradation concentration;

REFERENCES

1. Baigent, C.; Keech, A.; Kearney, P. M.; Blackwell, L.; Buck, G.; Pollicino, C.; Kirby, A.; Sourjina, T.; Peto, R.; Collins, R.; Simes, R., Cholesterol Treatment Trialists, C.; Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90,056 participants in 14 randomised trials of statins. *Lancet* **2005,** *366* (9493), 1267-1278.

2. Stone, N. J.; Robinson, J. G.; Lichtenstein, A. H.; Bairey Merz, C. N.; Blum, C. B.; Eckel, R. H.; Goldberg, A. C.; Gordon, D.; Levy, D.; Lloyd-Jones, D. M.; McBride, P.; Schwartz, J. S.; Shero, S.

T.; Smith, S. C.; Watson, K.; Wilson, P. W. F., 2013 ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults. *J. Am. Coll. Cardiol.* **2014,** *63* (25), 2889-2934.

3. Group, S. S. S. S., Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the scandinavian simvastatin survival study (4S). *Lancet* **1994,** *344* (8934), 1383-1389.

4. Group, L. S., Long-term effectiveness and safety of pravastatin in 9014 patients with coronary heart disease and average cholesterol concentrations: the LIPID trial follow-up. *Lancet* **2002,** *359* (9315), 1379-1387.

5. Force, U. S. P. S. T., Statin use for the primary prevention of cardiovascular disease in adults: Us preventive services task force recommendation statement. *JAMA* **2016,** *316* (19), 1997-2007.

6. Jones, P. H.; Davidson, M. H.; Stein, E. A.; Bays, H. E.; McKenney, J. M.; Miller, E.; Cain, V. A.; Blasetto, J. W.; Group, S. S., Comparison of the efficacy and safety of rosuvastatin versus atorvastatin, simvastatin, and pravastatin across doses (STELLAR* Trial). *Am. J. Cardiol.* **2003***, 92*, 152-160.

7. Nicholls, S. J.; Brandrup-Wognsen, G.; Palmer, M.; Barter, P. J., Meta-analysis of comparative efficacy of increasing dose of atorvastatin versus rosuvastatin versus simvastatin on lowering levels of atherogenic lipids (from voyager). *Am. J. Cardiol.* **2010**, *105*, 69-76.

8. Nicholls, S. J.; Ballantyne, C. M.; Barter, P. J.; Chapman, M. J.; Erbel, R. M.; Libby, P.; Raichlen, J. S.; Uno, K.; Borgman, M.; Wolski, K.; Nissen, S. E., Effect of two intensive statin regimens on progression of coronary disease. *N. Engl. J. Med.* **2011,** *365*, 2078-2087.

9. LaRosa, J. C.; Grundy, S. M.; Waters, D. D.; Shear, C.; Barter, P.; Fruchart, J. C.; Gotto, A. M.; Greten, H.; Kastelein, J. J.; Shepherd, J.; Wenger, N. K.; Treating to New Targets, I., Intensive lipid lowering with atorvastatin in patients with stable coronary disease. *N. Engl. J. Med.* **2005***, 352*, 1425-1435.

10. Brown, M. S.; Faust, J. R.; Goldstein, J. L.; Kaneko, I.; Endo, A., Induction of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in human fibroblasts incubated with compactin (ML-236B). *J. Biol. Chem.* **1978**, *253*, 1121-1128.

11. Lee, P. C.; Sever, N.; Debose-Boyd, R. A., Isolation of sterol-resistant Chinese hamster ovary cells with genetic deficiencies in both insig-1 and insig-2. *J. Biol. Chem.* **2005**, *280*, 25242-25249.

12. Chin, D. J.; Luskey, K. L.; Anderson, R. G.; Faust, J. R.; Goldstein, J. L.; Brown, M. S., Appearance of crystalloid endoplasmic reticulum in compactin-resistant Chinese hamster cells with a 500-fold increase in 3-hydroxy-3-methylglutaryl-coenzyme A reductase. *Proc. Natl. Acad. Sci. U. S. A.* **1982**, *79*, 1185-1189.

13. Bergstrom, J. D.; Bostedor, R. G.; Rew, D. J.; Geissler, W. M.; Wright, S. D.; Chao, Y. S., Hepatic responses to inhibition of 3-hydroxy-3-methylglutaryl-CoA reductase: a comparison of atorvastatin and simvastatin. *Biochim. Biophys. Acta.* **1988***, 1389*, 213-221.

14. Kita, T.; Brown, M. S.; Goldstein, J. L., Feedback regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase in livers of mice treated with mevinolin, a competitive inhibitor of the reductase. *J. Clin. Invest.* **1980***, 66*, 1094-1100.

15. Schonewille, M.; de Boer, J. F.; Mele, L.; Wolters, H.; Bloks, V. W.; Wolters, J. C.; Kuivenhoven,

J. A.; Tietge, U. J.; Brufau, G.; Groen, A. K., Statins increase hepatic cholesterol synthesis and stimulate fecal cholesterol elimination in mice. *J. Lipid Res.* **2016***, 57*, 1455-1464.

16. Hwang, S.; Hartman, I. Z.; Calhoun, L. N.; Garland, K.; Young, G. A.; Mitsche, M. A.; McDonald, J.; Xu, F.; Engelking, L.; DeBose-Boyd, R. A., Contribution of accelerated degradation to feedback regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase and cholesterol metabolism in the liver. *J. Biol. Chem.* **2016***, 291*, 13479-13494.

17. Ness, G. C.; Chambers, C. M.; Lopez, D., Atorvastatin action involves diminished recovery of hepatic HMG-CoA reductase activity. *J. Lipid Res.* **1988**, *39*, 75-84.

18. Bilhartz, L. E.; Spady, D. K.; Dietschy, J. M., Inappropriate hepatic cholesterol synthesis expands the cellular pool of sterol available for recruitment by bile acids in the rat. *J. Clin. Invest.***1989***, 84*, 1181-1187.

19. Burnett, J. R.; Wilcox, L. J.; Telford, D. E.; Kleinstiver, S. J.; Barrett, P. H.; Newton, R. S.; Huff, M. W., The magnitude of decrease in hepatic very low density lipoprotein apolipoprotein B secretion is determined by the extent of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibition in miniature pigs. *Endocrinology* **1999***, 140*, 5293-5302.

20. Reihner, E.; Rudling, M.; Stahlberg, D.; Berglund, L.; Ewerth, S.; Bjorkhem, I.; Einarsson, K.; Angelin, B., Influence of pravastatin, a specific inhibitor of HMG-CoA reductase, on hepatic metabolism of cholesterol. *N. Engl. J. Med.* **1990**, *323*, 224-228.

21. Conde, K.; Roy, S.; Freake, H. C.; Newton, R. S.; Fernandez, M. L., Atorvastatin and simvastatin have distinct effects on hydroxy methylglutaryl-CoA reductase activity and mRNA abundance in the

guinea pig. *Lipids* **1999**, *34*, 1327-1332.

22. Liscum, L.; Luskey, K. L.; Chin, D. J.; Ho, Y. K.; Goldstein, J. L.; Brown, M. S., Regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase and its mRNA in rat liver as studied with a monoclonal antibody and a cDNA probe. *J. Biol. Chem.* **1983**, *258*, 8450-8455.

23. Brown, M. S.; Goldstein, J. L., Multivalent feedback regulation of HMG CoA reductase, a control mechanism coordinating isoprenoid synthesis and cell growth. *J. Lipid Res.* **1980**, *21*, 505-517.

24. Goldstein, J. L.; Brown, M. S., Regulation of the mevalonate pathway. *Nature* **1990**, *343*, 425-430.

25. Preiss, D.; Seshasai, S. R.; Welsh, P.; Murphy, S. A.; Ho, J. E.; Waters, D. D.; DeMicco, D. A.; Barter, P.; Cannon, C. P.; Sabatine, M. S.; Braunwald, E.; Kastelein, J. J.; de Lemos, J. A.; Blazing, M. A.; Pedersen, T. R.; Tikkanen, M. J.; Sattar, N.; Ray, K. K., Risk of incident diabetes with intensive-dose compared with moderate-dose statin therapy: a meta-analysis. *JAMA* **2011**, *305*, 2556-2564.

26. Maki, K. C.; Ridker, P. M.; Brown, W. V.; Grundy, S. M.; Sattar, N., The diabetes subpanel of the national lipid association expert, p.; An assessment by the statin diabetes safety task force: 2014 update. *J. Clin. Lipidol*. **2014**, *8*, S17-29.

27. Rosenson, R. S.; Baker, S. K.; Jacobson, T. A.; Kopecky, S. L.; Parker, B. A., The national lipid association's muscle safety expert, p.; an assessment by the statin muscle safety task force: 2014 update. *J. Clin. Lipidol*. **2104**, *8*, S58-71.

28. Armitage, J., The safety of statins in clinical practice. *Lancet* **2007**, *370*, 1781-1790.

29. Buettner, C.; Rippberger, M. J.; Smith, J. K.; Leveille, S. G.; Davis, R. B.; Mittleman, M. A., Statin

use and musculoskeletal pain among adults with and without arthritis. *Am. J. Med.* **2012**, *125*, 176-182.

30. Morris, L.L.; Hartman, I.Z.; Jun, D.-J.; Seemann, J.; DeBose-Boyd, R.A., Sequential actions of the AAA-ATPase valosin-containing protein (VCP)/p97 and the proteasome 19 S regulatory particle in Sterol-accelerated, endoplasmic reticulum (ER)-associated degradation of 3-hydroxy-3-methylglutaryl-coenzyme A reductase. *J. Biol. Chem.* **2014**, 289, 19053-19066.

31. Song, B. L.; Sever, N.; DeBose-Boyd, R. A., Gp78, a membrane-anchored ubiquitin ligase, associates with Insig-1 and couples sterol-regulated ubiquitination to degradation of HMG CoA reductase. *Mol. Cell* **2005,** *19* (6), 829-840.

32. Jo, Y.; Lee, P. C.; Sguigna, P. V.; DeBose-Boyd, R. A., Sterol-induced degradation of HMG CoA reductase depends on interplay of two Insigs and two ubiquitin ligases, gp78 and Trc8. *Proc. Natl. Acad. Sci. U. S. A.* **2011,** *108* (51), 20503-20508.

33. Jiang, L. Y.; Jiang, W.; Tian, N.; Xiong, Y. N.; Liu, J.; Wei, J.; Wu, K. Y.; Luo, J.; Shi, X. J.; Song, B. L., Ring finger protein 145 (RNF145) is a ubiquitin ligase for sterol-induced degradation of HMG-CoA reductase. *J. Biol. Chem.* **2018,** *293* (11), 4047-4055.

34. Toure, M.; Crews, C. M., Small-molecule PROTACS: new approaches to protein degradation. *Angew. Chem. Int. Ed.* **2016***, 55*, 1966-1973.

35. Churcher, I., Protac-induced protein degradation in drug discovery: breaking the rules or just making new ones? *J. Med. Chem.* **2018***, 61*, 444-452.

36. Neklesa, T. K.; Winkler, J. D.; Crews, C. M., Targeted protein degradation by PROTACs. *Pharmacol. Ther.* **2017***, 174*, 138-144.

37. Salami, J.; Crews, C. M., Waste disposal-an attractive strategy for cancer therapy. *Science* **2017**, *355*, 1163-1167.

38. Zhou, B.; Hu, J.; Xu, F.; Chen, Z.; Bai, L.; Fernandez-Salas, E.; Lin, M.; Liu, L.; Yang, C.-Y.; Zhao, Y.; McEachern, D.; Przybranowski, S.; Wen, B.; Sun, D.; Wang, S., Discovery of a small-molecule degrader of bromodomain and extra-terminal (BET) proteins with picomolar cellular potencies and capable of achieving tumor regression. *J. Med. Chem.* **2018,** *61*, 462-481.

39. Zhang, C.; Han, X.-R.; Yang, X.; Jiang, B.; Liu, J.; Xiong, Y.; Jin, J., Proteolysis targeting chimeras (PROTACs) of anaplastic lymphoma kinase (ALK). *Eur. J. Med. Chem.* **2018**, *151*, 304-314.

40. Hu, J.; Hu, B.; Wang, M.; Xu, F.; Miao, B.; Yang, C.-Y.; Wang, M.; Liu, Z.; Hayes, D. F.; Chinnaswamy, K.; Delproposto, J.; Stuckey, J.; Wang, S., Discovery of ERD-308 as a highly potent proteolysis targeting chimera (PROTAC) degrader of estrogen receptor (ER). *J. Med. Chem.* **2019,** *62*, 1420-1442.

41. Lu, J.; Qian, Y.; Altieri, M.; Dong, H.; Wang, J.; Raina, K.; Hines, J.; Winkler, James, D.; Crew, Andrew, P.; Coleman, K.; Crews, Craig, M., Hijacking the E3 ubiquitin ligase cereblon to efficiently target BRD4. *Chem. Biol.* **2015***, 22*, 755-763.

42. Ito, T.; Ando, H.; Suzuki, T.; Ogura, T.; Hotta, K.; Imamura, Y.; Yamaguchi, Y.; Handa, H., Identification of a primary target of thalidomide teratogenicity. *Science* **2010***, 327*, 1345-1350.

43. Lopez-Girona, A.; Mendy, D.; Ito, T.; Miller, K.; Gandhi, A. K.; Kang, J.; Karasawa, S.; Carmel, G.; Jackson, P.; Abbasian, M.; Mahmoudi, A.; Cathers, B.; Rychak, E.; Gaidarova, S.; Chen, R.; Schafer, P. H.; Handa, H.; Daniel, T. O.; Evans, J. F.; Chopra, R., Cereblon is a direct protein target for immunomodulatory and antiproliferative activities of lenalidomide and pomalidomide. *Leukemia* **2012***, 26*, 2326-2335.

44. Goldstein, J. L.; DeBose-Boyd, R. A.; Brown, M. S., Protein sensors for membrane sterols. *Cell* **2006***, 124*, 35-46.

45. DeBose-Boyd, R. A.; Ye, J., SREBPs in lipid metabolism, insulin signaling, and beyond. *Trends Biochem. Sci.* **2018**, *43*, 358-368.

46. Shim, J. S.; Li, R.-J.; Lv, J.; Head, S. A.; Yang, E. J.; Liu, J. O., Inhibition of angiogenesis by selective estrogen receptor modulators through blockade of cholesterol trafficking rather than estrogen receptor antagonism. *Cancer Lett.* **2015***, 362*, 106-115.

47. Brown, M. S.; Goldstein, J. L., The SREBP pathway: regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. *Cell* **1997,** *89* (3), 331-340.

48. Jiang, S.-Y.; Li, H.; Tang, J.-J.; Wang, J.; Luo, J.; Liu, B.; Wang, J.-K.; Shi, X.-J.; Cui, H.-W.; Tang, J.; Yang, F.; Qi, W.; Qiu, W.-W.; Song, B.-L., Discovery of a potent HMG-CoA reductase degrader that eliminates statin-induced reductase accumulation and lowers cholesterol. *Nat. Commun.* **2018,** *9*, 5138.

49. Timms, R. T.; Menzies, S. A.; Tchasovnikarova, I. A.; Christensen, L. C.; Williamson, J. C.; Antrobus, R.; Dougan, G.; Ellgaard, L.; Lehner, P. J., Genetic dissection of mammalian ERAD through

comparative haploid and CRISPR forward genetic screens. *Nat. Commun.* **2016***, 7*, 11786.

50. Schumacher, M. M.; Elsabrouty, R.; Seemann, J.; Jo, Y.; DeBose-Boyd, R. A., The prenyltransferase UBIAD1 is the target of geranylgeraniol in degradation of HMG CoA reductase. *eLife* **2015**, *4*, e05560.

51. Sharpe, L. J.; Cook, E. C. L.; Zelcer, N.; Brown, A. J., The UPS and downs of cholesterol homeostasis. *Trends Biochem. Sci.* **2014***, 39*, 527-535.

52. Ye, Y., The role of the ubiquitin–proteasome system in ER quality control. *Essays Biochem.* **2005***, 41*, 99-112.

53. Buckley, D.L.; Crews, C.M., Small-molecule control of intracellular protein levels through modulation of the ubiquitin proteasome system. *Angew. Chem. Int. Ed.* **2014**, 53, 2312-2330.

54. Tinworth, C.P.; Lithgow, H., Churcher, I., Small molecule-mediated protein knockdown as a new approach to drug discovery. *MedChemComm* **2016**, 7, 2206-2216.

55. Kubowicz, P.; Zelaszczyk, D.; Pekala, E., RNAi in clinical studies. *Curr. Med. Chem.* **2013**, 20, 1801-1816.

56. Burnett, John C.; Rossi, John J., RNA-based therapeutics: current progress and future prospects. *Chem. Biol.* **2012**, 19, 60-71.

57. Fellmann, C.; Gowen, B.G.; Lin, P.-C.; Doudna, J.A.; Corn, J.E., Cornerstones of CRISPR-Cas in drug discovery and therapy. *Nat. Rev. Drug Discov.* **2017**, 16, 89-100.

58. Sun, X.; Gao, H.; Yang, Y.; He, M.; Wu, Y.; Song, Y.; Tong, Y.; Rao, Y., PROTACs: great

opportunities for academia and industry. *Signal Transduct. Target. Ther.* **2019,** *4*, 64.

59. Durrington, P., Dyslipidaemia. *Lancet* **2003**, 362, 717-731.

60. The, L., Lessons from lipitor and the broken blockbuster drug model. *Lancet* **2011**, 378, 1976.

61. Serban, M.-C.; Colantonio, L. D.; Manthripragada, A. D.; Monda, K. L.; Bittner, V. A.; Banach, M.; Chen, L.; Huang, L.; Dent, R.; Kent, S. T.; Muntner, P.; Rosenson, R. S., Statin intolerance and risk of coronary heart events and all-cause mortality following myocardial infarction. *J. Am. Coll. Cardiol.* **2017**, 69, 1386-1395.

62. Giugliano, R.P.; Sabatine, M.S., Are PCSK9 inhibitors the next breakthrough in the cardiovascular field? *J. Am. Coll. Cardiol.* **2015**, 65, 2638-2651.

63. Frank-Kamenetsky, M.; Grefhorst, A.; Anderson, N. N.; Racie, T. S.; Bramlage, B.; Akinc, A.; Butler, D.; Charisse, K.; Dorkin, R.; Fan, Y.; Gamba-Vitalo, C.; Hadwiger, P.; Jayaraman, M.; John, M.; Jayaprakash, K. N.; Maier, M.; Nechev, L.; Rajeev, K. G.; Read, T.; Röhl, I.; Soutschek, J.; Tan, P.; Wong, J.; Wang, G.; Zimmermann, T.; de Fougerolles, A.; Vornlocher, H.-P.; Langer, R.; Anderson, D. G.; Manoharan, M.; Koteliansky, V.; Horton, J. D.; Fitzgerald, K., Therapeutic RNAi targeting PCSK9 acutely lowers plasma cholesterol in rodents and LDL cholesterol in nonhuman primates. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, 105, 11915-11920.

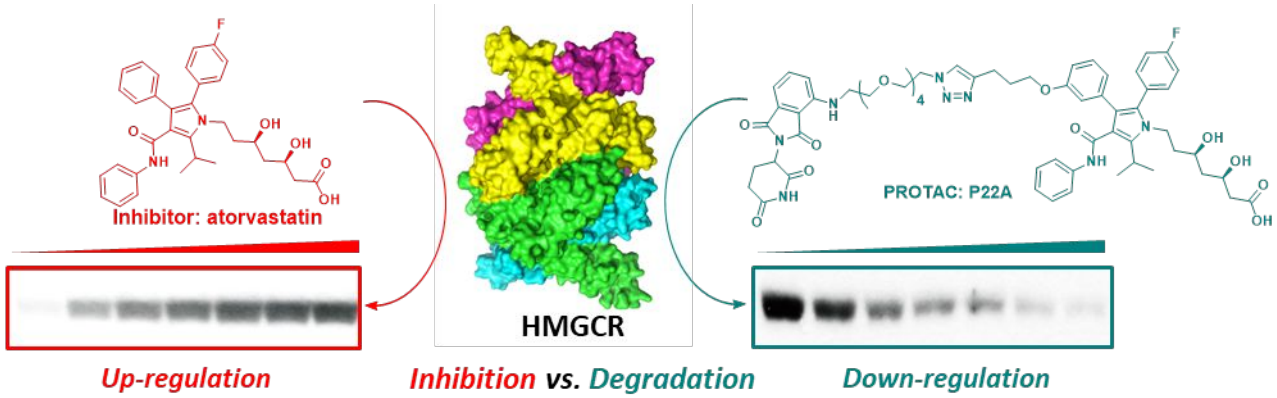
64. Song, B.-L.; DeBose-Boyd, R.A., Ubiquitination of 3-hydroxy-3-methylglutaryl-CoA reductase in permeabilized cells mediated by cytosolic e1 and a putative membrane-bound ubiquitin ligase. *J. Biol. Chem.* **2004**, 279, 28798-28806.

65. Baran, Y.; Gür, B.; Kaya, P.; Ural, A. U.; Avcu, F.; Gündüz, U., Upregulation of multi drug resistance genes in doxorubicin resistant human acute myelogeneous leukemia cells and reversal of the resistance. *Hematology* **2007**, *12*, 511-517.

66. Sisodiya, S. M.; Thom, M., Widespread upregulation of drug-resistance proteins in fatal human status epilepticus. *Epilepsia* **2003**, *44*, 261-264.

67. Nath, S.; Daneshvar, K.; Roy, L. D.; Grover, P.; Kidiyoor, A.; Mosley, L.; Sahraei, M.; Mukherjee, P., MUC1 induces drug resistance in pancreatic cancer cells via upregulation of multidrug resistance genes. *Oncogenesis* **2013**, *2*, e51.

TOC:


Inhibitor: atorvastatin
HMGCR
PROTAC: P22A
Up-regulation
Inhibition vs. Degradation
Down-regulation