Development of Novel Small-Molecule Degraders of FK506-Binding Protein 51

Mao, Tianqi

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Development of Novel Small-Molecule Degraders of FK506-Binding Protein 51

Entwicklung neuer Substanzen zum proteasomalen Abbau des FK506-bindenden Proteins 51

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Abstract

The FK506-binding protein 51 (FKBP51) plays an important role in steroid hormone receptors (SHRs) regulation and is related to many pathologic states and diseases such as stress-related disorder, cancer, metabolic syndrome, and chronic pain. FKBP51 acts on several cellular pathways likely through protein-protein interactions via its multifunctional domains. However, the functional basis of FKBP51 in signalling remains obscure due to the poor characterization of its biochemical properties. Importantly, the current FKBP51 inhibitors are not able to inactivate FKBP51 completely based on the occupancy-driven pharmacology.

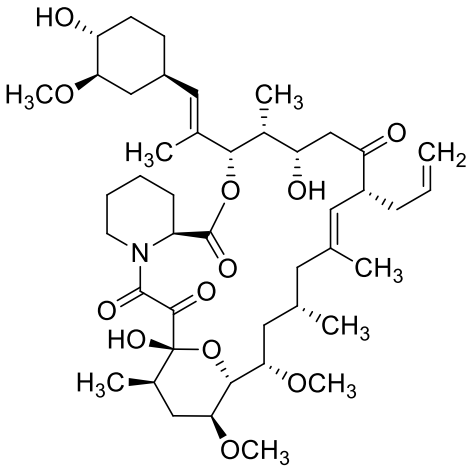
In this thesis, I synthesized a series of small molecules which aim to degrade FK506 binding proteins (FKBPs) via the recently developed proteolysis targeting chimera (PROTAC) strategy. A PROTAC is a heterobifunctional molecule consisting of a ligand for the target protein and a ligand for an E3 ligase, which induces the proteasomal degradation of the target protein. Unlike classical small molecule drugs, PROTACs intend to eliminate the aberrantly functioning protein rather than to inhibit it. By utilizing a selective FKBP51 ligand and non-selective FKBPs ligands developed by our group, two series of PROTACs were designed and synthesized. All of the selective SAFit-PROTACs showed affinities in the nanomolar range for FKBP51, while retaining high selectivity over the close homologue FKBP52. The degradation of cellular FKBP51 was observed upon treatment with the PROTAC **MTQ202**. In turn, I explored rigidified linkers based on **MTQ202** to afford more potent PROTACs. The bicyclic ligand-based PROTACs exhibited outstanding affinities for all of the tested FKBPs in the low nanomolar range to subnanomolar range. The most potent compound, **MTQ509**, showed the highest binding affinity for FKBP51 so far reported (K_i = 0.92 nM).

Zusammenfassung

Das FK506-bindende Protein 51 (FKBP51) spielt eine wichtige Rolle bei der Regulation von Steroidhormonrezeptoren (SHRs) und wird mit einer Vielzahl von Krankheiten assoziiert wie zum Beispiel stressbedingten Störungen, Krebs, metabolischem Syndrom und chronischem Schmerz. FKBP51 ist in verschiedenen zellulären Signalwegen involviert, wobei die multifunktionalen Domänen vermutlich die Signaltransduktion durch Protein-Protein-Interaktionen vermitteln. Bis heute ist die zelluläre Funktion von FKBP51 aufgrund der fehlenden biochemischen Charakterisierung unklar. Die bisher entwickelten FKBP51-Inhibitoren blockieren die Bindungstasche und können FKBP51 nicht vollständig inaktivieren.
Im Rahmen meiner Doktorarbeit habe ich chemische Substanzen synthetisiert, die darauf abzielen, FK506-bindende Proteine (FKBPs) über die kürzlich entwickelte Proteolyse-Targeting-Chimären-Strategie (PROTAC) abzubauen. Ein PROTAC ist ein heterobifunktionelles Molekül, das aus einem Liganden für das Zielprotein und einem Liganden, der an eine E3-Ligase bindet, besteht und den proteasomalen Abbau des Zielproteins induziert. Im Gegensatz zu klassischen Wirkstoffen zielen PROTACs eher darauf ab, das Zielprotein zu eliminieren, anstatt es zu inhibieren. Unter Verwendung eines selektiven FKBP51-Liganden und nicht-selektiver FKBP-Liganden, die von unserer Gruppe entwickelt wurden, wurden zwei Serien von PROTACs entworfen und synthetisiert. Alle selektiven SAFit-PROTACs zeigten Affinitäten im nanomolaren Bereich für FKBP51 und Selektivität gegenüber dem homologen Protein FKBP52. Bei der zellulären Anwendung des PROTAC s **MTQ202** wurde der Abbau von endogenem FKBP51 beobachtet. Zur Erhöhung der Wirksamkeit habe ich ausgehend von **MTQ202** verschiedene Linker untersucht. Die auf bicyclischen Liganden basierenden PROTACs zeigten Affinitäten für alle getesteten FKBPs im niedrigen nanomolaren bis subnanomolaren Bereich. Die potenteste Verbindung, **MTQ509**, zeigte die bisher höchste Bindungsaffinität für FKBP51 (K_i = 0,92 nM).

1. Introduction

1.1 The biology of FKBP51



FK506

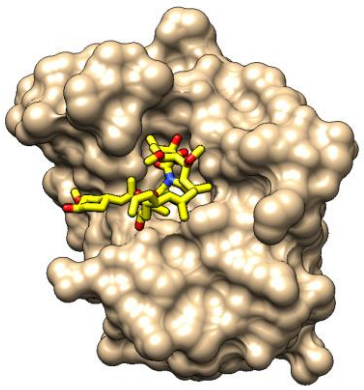


Figure 1. The structure of FK506 and the structure of FKBP12-FK506 complex (PDB ID: 1FKJ)

FK506 binding proteins (FKBPs) are a ubiquitous family of immunophilins that are present in all eukaryotes[1, 2]. This class of proteins features a peptidyl-prolyl cis/trans isomerase (PPIase) domain, to which immunosuppressants like FK506 and rapamycin bind[3, 4]. The PPIase domain also has the ability to catalyze the cis-trans isomerization of Xaa-Pro bonds, regulating folding, activation and degradation of many proteins[5]. Upon binding to immunosuppressive ligands, the PPIase enzymatic activity is abolished.

Except for the canonical members FKBP12 and FKBP12.6, all other FKBPs contain additional functional domains besides the PPIase domain, which are often involved in protein-protein interactions.

1.2 Structure of FKBP51

The FK506 binding protein 51 (FKBP51), a 51 kDa member of the FKBP family, is encoded by the fkbp5 gene and was first identified as a co-chaperone of the heat shock protein 90 (HSP90) in a complex with steroid hormone receptors (SHRs)[6, 7]. FKBP51 is well known for its ability to modulate the activities of steroid hormone receptors, including glucocorticoid receptor (GR) and androgen receptor (AR), which leads to further gene expressions and protein level regulations.

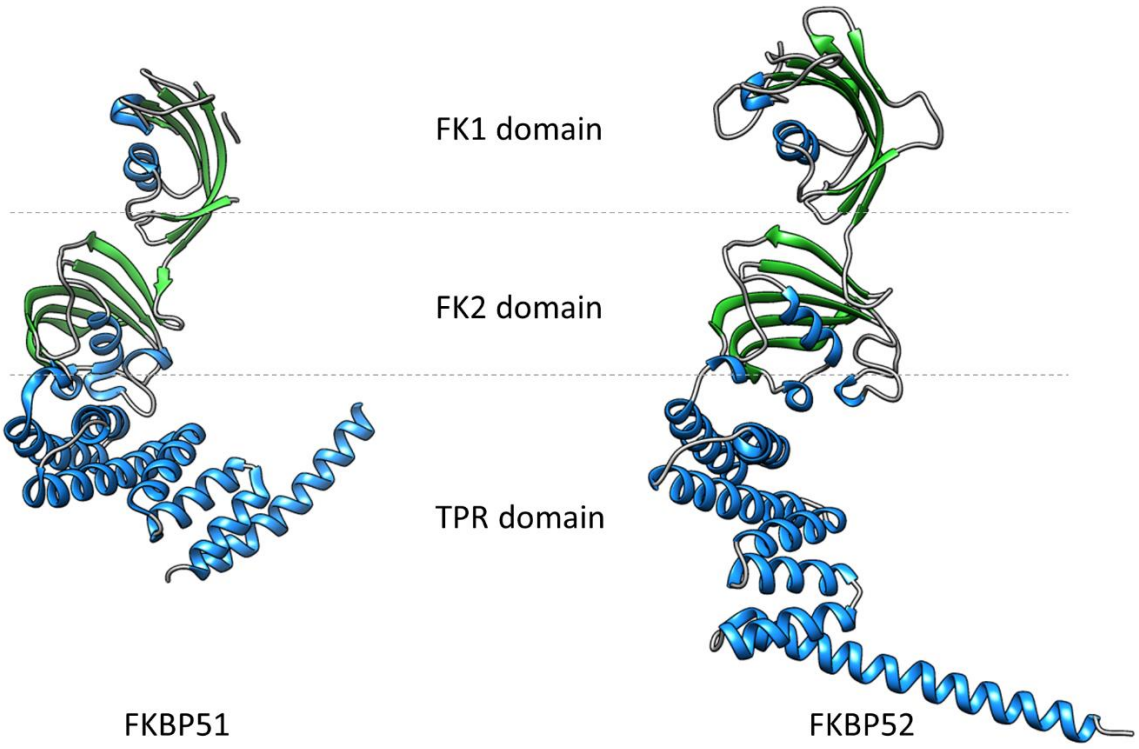


Figure 2. X-ray crystallographic structures of FKBP51 (PDB ID: 1KT0) and FKBP52 (PDB ID: 1Q1C and 1P5Q).

FKBP51 is composed of two FKBP-like domains (FK1 domain and FK2 domain) and a tetratricopeptide repeat domain (TPR domain)[8, 9]. The N-terminal FK1 domain possesses a PPIase catalytic pocket that displays PPIase activity and binds to the immunosuppressant drugs such as FK506 and rapamycin[8, 10]. Besides its catalytic function, some studies of the prototypic homologue FKBP12 show that the PPIase active site is involved in some protein-protein interactions, which implies that FKBP51 may adopt a similar mechanism for the regulation of steroid hormone receptors[8, 11]. The FK2 domain, following the FK1 domain, is structurally similar to FK1 domain but exhibits neither binding ability of immunosuppressant nor catalytic function due to a lack of key residues[12]. The C-terminal TPR domain is the binding site for HSP90 and often participates in mediating protein-protein interactions[13, 14].

FKBP52, the most similar homologue to FKBP51, shares 60% identity and 75% similarity in amino acid sequences with FKBP51, as well as a similar structural architecture, PPIase catalytic activity, FK506-binding ability and Hsp90 binding ability[11, 15]. However, FKBP52 shows antagonistic effect to FKBP51 in modulating SHRs activity[16].

1.3 The function of FKBP51

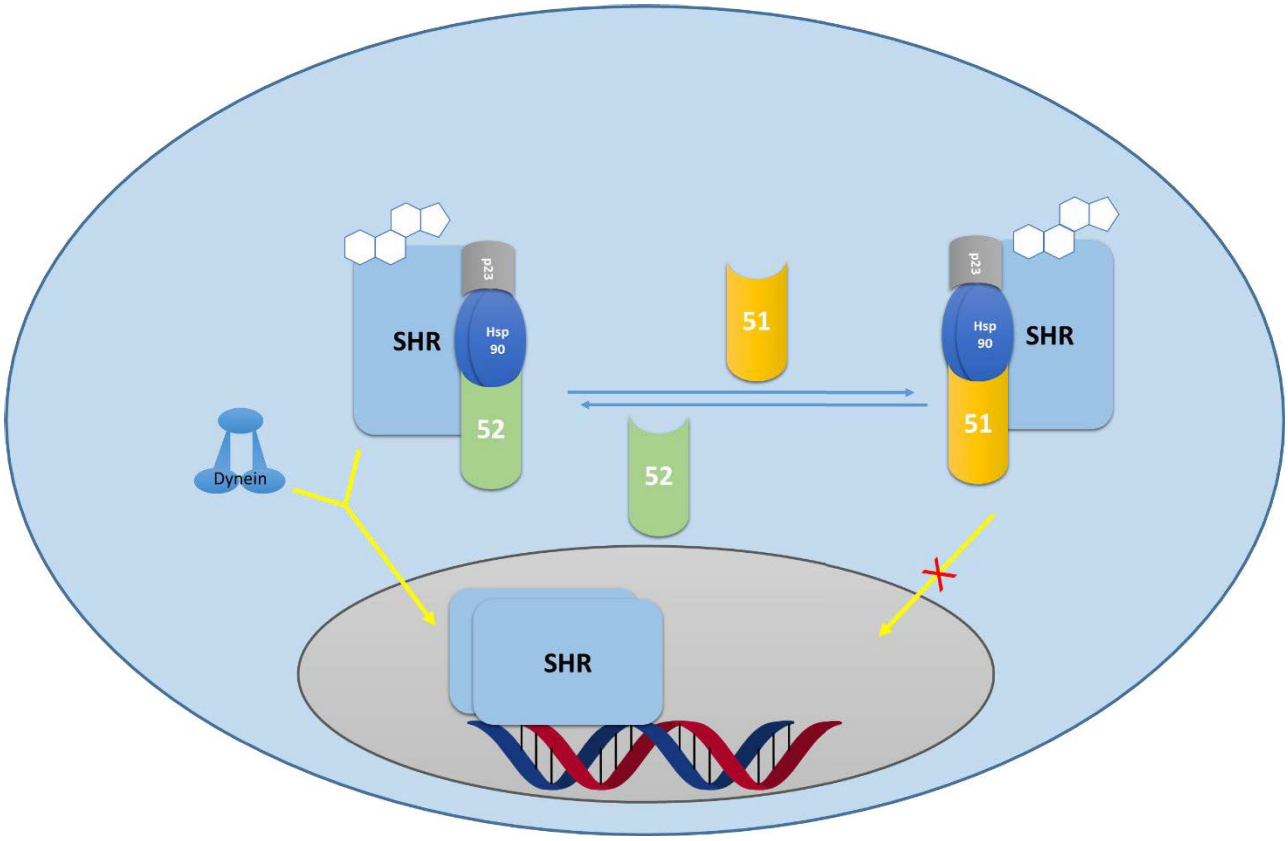


Figure 3. Model for the mechanism of action of FKBP51 and 52 on steroid hormone complex, ligand binding and nuclear translocation.

FKBP51 has been observed to interact with many steroid hormone receptors (SHRs), such as the glucocorticoid receptor (GR), the estrogen receptor (ER), the androgen receptor (AR) and the mineralocorticoid receptor (MR)[17-20]. In most cases, FKBP51 is a negative modulator of SHRs except for AR[21, 22]. Meanwhile, FKBP52 is a positive regulator of SHRs[23-25]. FKBP51 and FKBP52 regulate SHR activity through interaction with the abundant molecular chaperone, 90 kDa heat shock protein (Hsp90), which is involved in numerous protein-protein interactions that modulate important signaling pathways.

According to a proposed mechanism, either FKBP51 or 52 bind to Hsp90 and form a heterocomplex with the co-chaperone p23 and SHR in the cytosol[23, 26]. Upon the steroid binding, FKBP51 and FKBP52 are exchangeable in the heterocomplex due to a ligand-induced conformational change of the receptor[13, 19]. Next, FKBP52 interacts with the dynein-dynactin motor complex by its PPIase pocket, whereas FKBP51 is not able to recruit dynein[23, 27-29]. As a result, the dynein-dynactin motor facilitates SHR translocation from the cytosol into the nucleus, followed by interaction between SHR and response elements leading to enhanced or reduced expression of various genes[30]. Inversely, high cytoplasmic levels of FKBP51 decrease

dynein-dependent SHR nuclear translocation, leading to SHR resistance. This indicates that the expression ratio of FKBP51/FKBP52 is a regulatory factor for steroid receptor regulations[19, 23, 31].

1.4 Risk for diseases

1.4.1 Stress related disorders

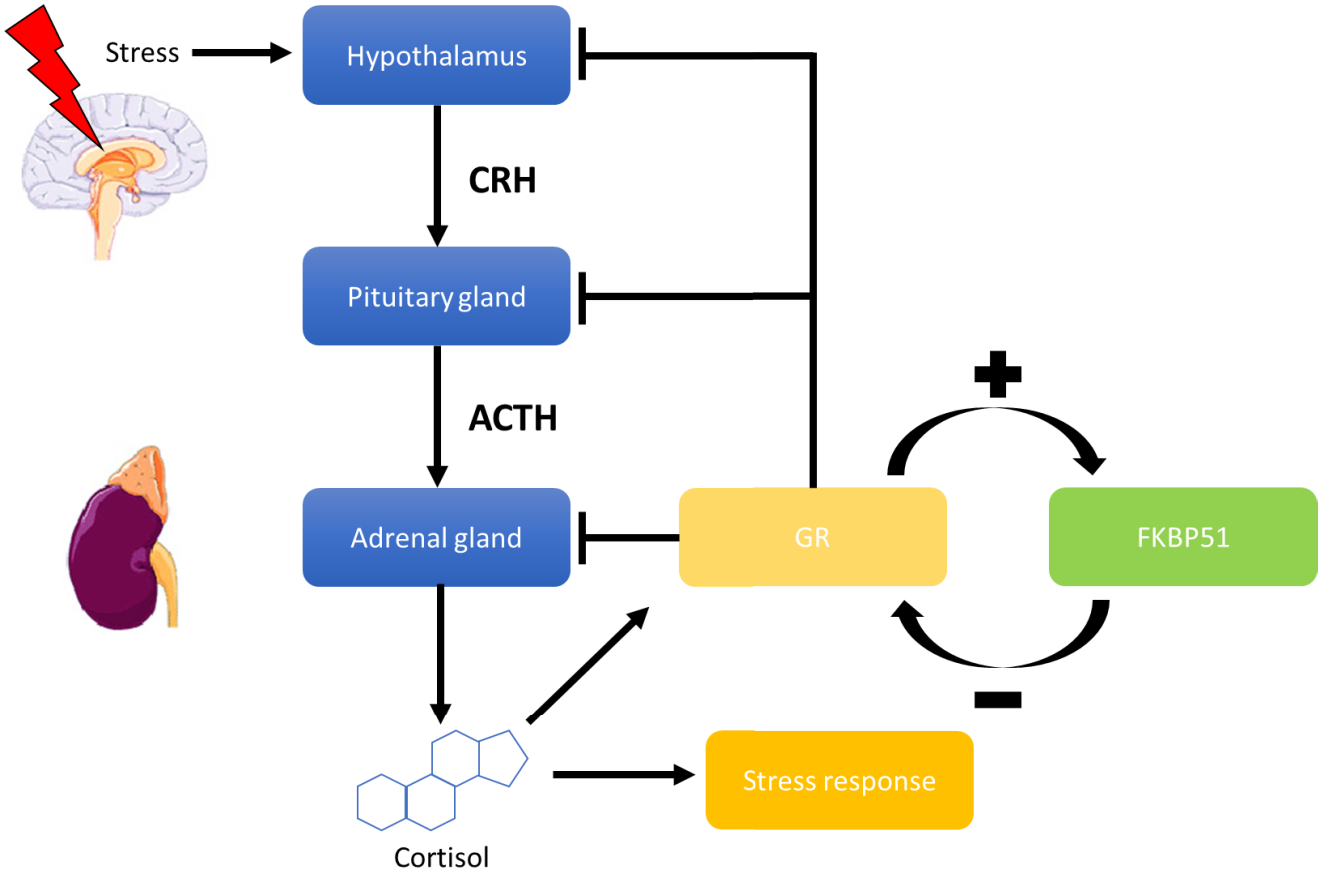


Figure 4. The Hypothalamic–Pituitary–Cortisol System

The hypothalamic–pituitary–adrenal (HPA) axis is one of our main neuroendocrine pathways that regulates stress responses[32, 33]. Upon stress stimulation, neurons in the paraventricular nucleus of the hypothalamus release corticotrophin-releasing hormone (CRH) into the blood vessels connected to the pituitary gland, where adrenocorticotropic hormone (ACTH) is secreted by the stimulus of CRH[34]. In turn, the ACTH is released into the peripheral circulation, stimulating the synthesis and production of glucocorticoid by the adrenal glands. The resulting cortisol, the main glucocorticoid hormone in humans, is secreted into blood and diffuses into the cytoplasm of target cells, where it binds to hormone receptors, such as the mineralocorticoid receptor (MR) and GR.

To prevent a prolonged stress response, the HPA axis is regulated by negative-feedback loops mediated by cortisol to maintain hormone levels[35, 36]. In a stressful situation, the increased cortisol levels in blood and tissues saturate its high affinity receptors MRs, leading to the binding of excess cortisol to its low affinity receptors GRs. The resulting GR activation inhibits the secretion of ACTH/CRH and restores cortisol to basal levels, thus terminating the stress responses[37].

The GR sensitivity is regulated by FKBP51 via an ultra-short negative feedback loop[38]. Enhanced expression of FKBP5, the gene encoding FKBP51, by GR activation reduces the affinity of GR for cortisol and decreases the translocation of GR from the cytosol into the nucleus. The resulting GR resistance diminishes HPA negative feedback, leading to a prolonged stress response after stressor exposure[32, 39].

A well-functioning HPA axis is fundamental to trigger fight-or-flight response and survival. Thus, abnormal changes in FKBP51 levels due to genetic, hormonal or environmental factors can lead to the dysfunction of the Hsp90-FKBP51 complex, which in turn impairs the balance between activation and negative feedback of HPA stress response, and finally can contribute to various psychiatric disorders, such as major depression, post-traumatic stress disorder (PTSD) and anxiety[31, 40-44].

1.4.2 Reproductive Development and Success

The studies of FKBP52 knockout mouse lines showed that FKBP52 plays important roles in the mammalian reproductive system. Male FKBP52 knockout (52KO) mice are still viable but exhibit phenotypes consistent with partial androgen insensitivity syndrome. Although their primary sex organs seem normal, the secondary sex organs are affected, such as smaller prostate glands and seminal vesicles, lower sperm motility and hypospadias[45, 46]. Females are morphologically normal but infertile. This can be attributed to failures in decidualization and embryonic implantation caused by progesterone insensitivity, indicating that FKBP52 plays a crucial role in the female reproduction system[47]. Compare to FKBP52 knockout mice, FKBP51 knockout mice show no obvious morphological and reproductive abnormality.

1.5 Druggability of FKBP51

1.5.1 Non-selective FKBP51 ligands

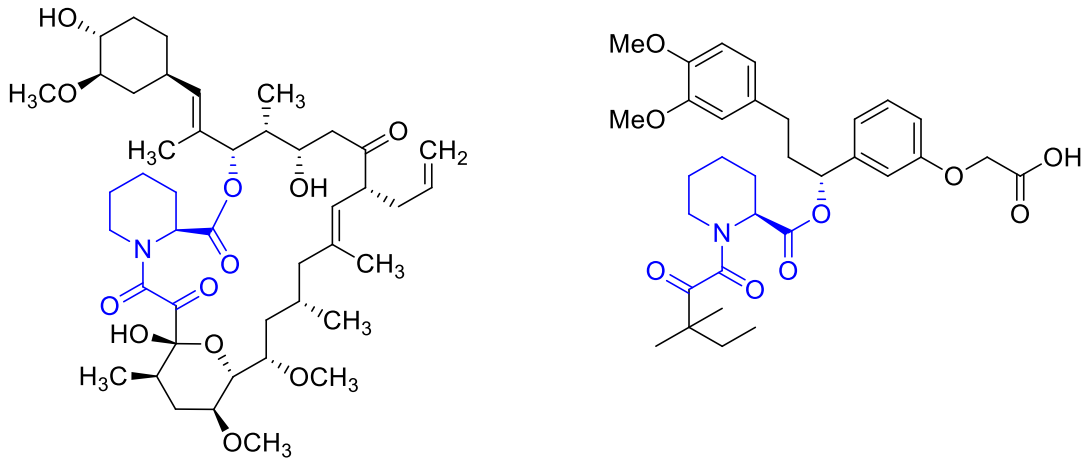


Figure 5. The structures of FK506 and FKBP51/52 ligand SLF

The first synthetic ligand which showed affinity in the micromolar range to FKBP51 and FKBP52 was SLF, an analogue of FK506, which was originally developed as an FKBP12 ligand[9, 48, 49]. Compared to FK506 and rapamycin, SLF has no effector domain, which is responsible for the immunosuppressive activity, but it retains the pipecolic motif derived from the diketoamide pipecolinic core in FK506. Based on the co-crystal structure of SLF and FKBP51, Gopalakrishnan et al. investigated the first structure-activity relationship (SAR) study of SLF derivatives as FKBP51 and FKBP52 ligands[50]. This study revealed that the pipecolate core is essential for the binding affinity. Besides, replacing the carboxylic acid group on the top group with morpholine gives a 15 to 20-fold more potent for FKBP51/52 ligand compare to SLF.

1.5.2 Selective FKBP51 ligands

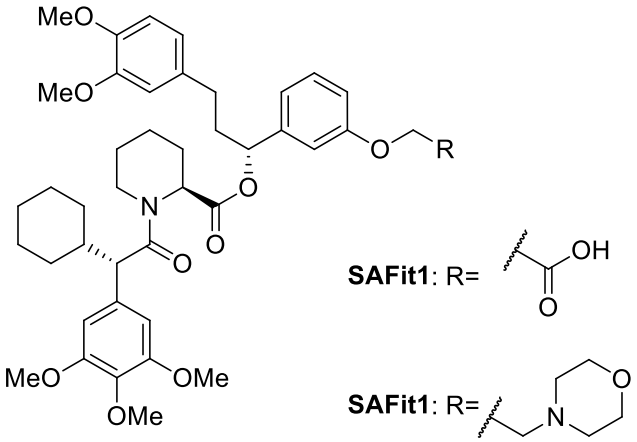


Figure 6. The structures of selective ligands SAFit1 and SAFit2

In 2015, Gaali et al. developed the first selective ligand for FKBP51[51]. In order to distinguish between FKBP51 and FKBP52 pharmacologically, Gaali et al. adopted a chemical genetics approach, which originated from a study to develop artificial selectivity for FKBP12-fusion protein[52]. Based on this method, they expanded the FK506-binding pocket by generating FKBP51F67V and FKBP52F67V mutants. During the development of the complementary ligand for these mutants, they noticed that two of the analogues, iFit1 and iFit2, showed weak affinity to WT FKBP51 but did not bind to WT FKBP52. The FKBP51-iFit1 co-crystal structure revealed that a conformational change compare to the apo form or the FKBP51-FK506 complex was stabilized by iFit1. Upon the influence of the Cα allyl group on iFit1, the Phe67 side chain moves towards Lys58 and Lys60, which is subsequently stabilized by Phe129. Conversely, the side chain of Phe67 in FKBP52 adopted a different conformation because the corresponding residues, Thr58, Trp60 and Val129, are too bulky to undergo similar side chain rearrangements. Intrigued by this finding, a series of Cα-modified derivatives with bulky substituents was synthesized to improve the affinity and selectivity for FKBP51. Eventually, SAFit1 and SAfit2, the two most potent ligands, were developed, which have high affinity (K_i = 4 nM and 6 nM, respectively) and superb specificity (>10,000-fold selectivity for FKBP51 over FKBP52). The SAFit analogues provide a powerful tool for investigations of FKBP51, which is critical for understanding its biological functions.

1.5.3 Bicyclic ligands

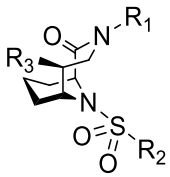


Figure 7. The structure of [4.3.1] bicyclic ligand

Based on the co-crystal structure of FKBP-FK506 and computational design, Babine et al. reported a series of FKBP12 ligands with the rigid bicyclic structure[53], leading to the emergence of FKBP ligands with bridged bicyclic scaffolds. Inspired by these studies, our group developed a series of [4.3.1] bicyclic ligands which mimic the binding conformation of FK506 with the rigidified scaffold instead of the pipecolyl-monocyclic scaffold[54, 55]. The potent bicyclic ligands showed low micromolar to nanomolar affinities to many human FKBPs, including FKBP12, FKBP12.6, FKBP51 and FKBP52.

1.6 Chemical knockdown of protein by small molecule PROTACS

1.6.1 The ubiquitin proteasome system

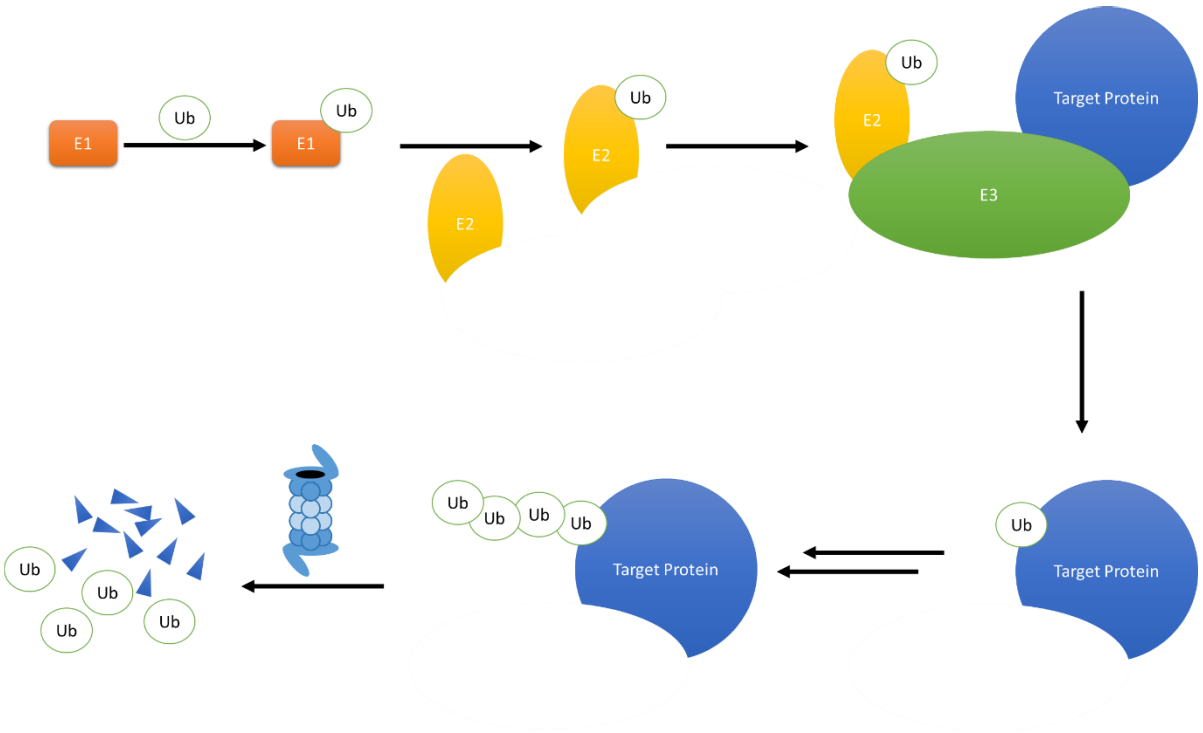


Figure 8. Protein degradation mediated by ubiquitin-proteasome system

The ubiquitin-proteasome system (UPS) plays a crucial role in balancing the intracellular protein levels by degrading proteins. The UPS is a multistep procedure involving ubiquitin, a 9-kDa protein highly conserved from yeast to humans, three kinds of enzyme and the 26S proteasome[56, 57].

Ubiquitination of target proteins is carried out via an enzymatic cascade conducted by the ubiquitin activating enzyme (E1), ubiquitin-conjugating enzyme (E2) and ubiquitin ligase (E3). At the beginning, ubiquitin is attached to E1 via a thioester bond formed by an ATP-dependent reaction. Then the activated ubiquitin is transferred to E2 with the formation of a new high-energy thioester bond. Finally, E3 ubiquitin ligase binds to both ubiquitin-E2 complex and substrate, bringing them in close proximity to catalyze the conjugation of the ubiquitin and the substrate. This ubiquitination process occurs repeatedly in a cyclic manner, resulting in the formation of a polyubiquitinated adduct, which can be recognized and degraded by the 26S proteasome[58, 59].

Currently, there is only one major E1 enzyme, around 50 E2 enzymes and over 600 E3 ligases encoded in the human genome. Unsurprisingly, the variety of E3 ligases confers substrate specificity during ubiquitination and degradation processes.

1.6.2 Proteolysis targeting chimera (PROTAC)

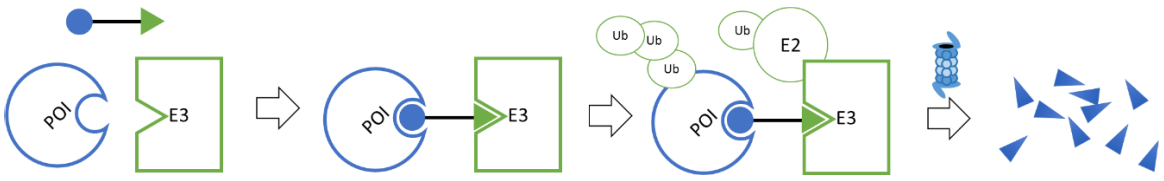
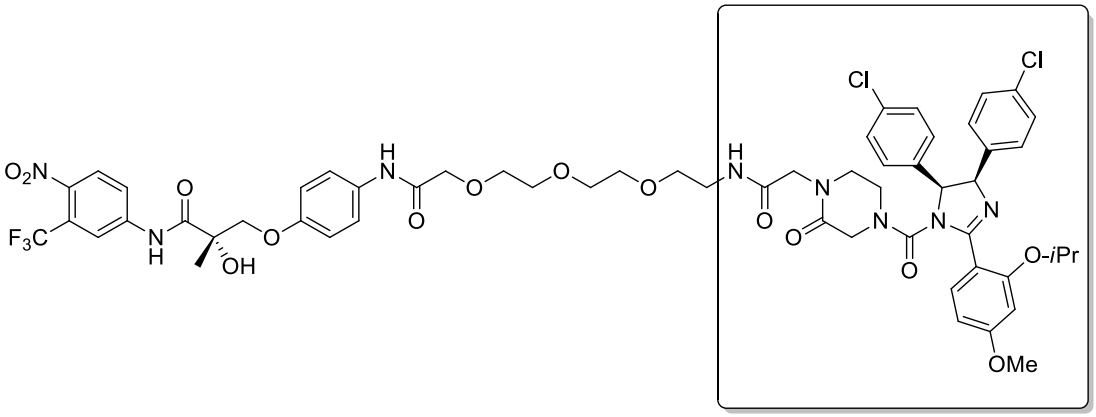


Figure 9. Degradation induced by PROTACs

In the recent decade, proteolysis-targeting chimeras (PROTACs) have emerged as a useful chemical knockdown strategy and have a promising application prospect in clinical therapies for diseases[60-62]. PROTACs are a new class of heterobifunctional molecules consisting of a recognition motif for binding the protein of interest (POI), a recognition motif for recruiting an E3 ligase and a linker to tether the first two together. By binding to the substrate and the E3 ligase complex simultaneously, PROTACs are able to bring them into proximity to induce polyubiquitination of the substrate, leading to the recognition by the 26S proteasome and the proteasomal degradation[63-65].

1.6.3 Recruiting E3 ligases

1.6.3.1 Recruiting E3 ligases MDM2



P1 — **Nutlin**

Targets **mouse double minute 2 homolog** (MDM2)

Figure 10. SARM-nutlin PROTAC

MDM2 is an E3 ligase, which has been found to be overexpressed in many tumors, inducing proteasomal degradation of the tumor suppressor protein p53 and thus drives tumor formation[66]. The first all-small molecule PROTAC **P1** was reported in 2008 by Schneekloth et

al., which recruits MDM2 as the E3 ligase via its potent antagonist nutlin 3 attached to the AR ligand SARM to induce the degradation of ARs[67, 68]. This SARM-nutlin conjugate bound AR with K_i of 4 nM and caused its degradation it in HeLa cells at 10 μM. This cell permeable PROTAC validated the proof-of-concept of small molecule PROTACs.

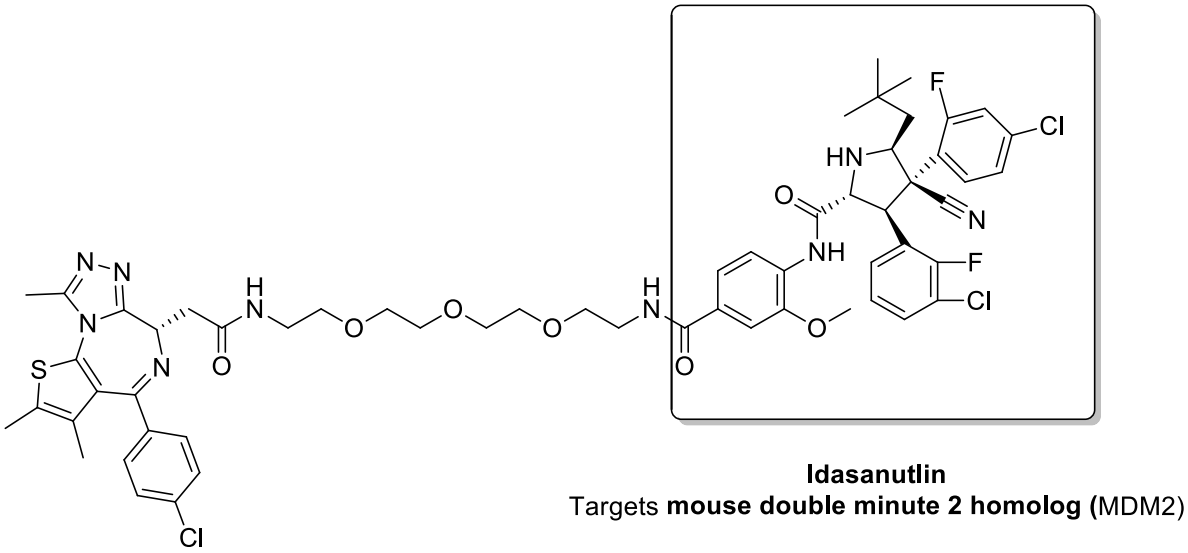


Figure 11. JQ1- idasanutlin PROTAC A1874

Recently, Crews et al. developed a nutlin-based PROTAC **A1874** which degraded the oncoprotein bromodomain-containing protein 4 (BRD4) at nanomolar concentration[69]. **A1874** has been shown to not only be able to induce the degradation of target protein, but also stabilized p53 by the idasanutlin motif, conferring a MDM2-recruiting PROTAC the ability to eliminate the oncogene and activate the tumor suppressor simultaneously. This indicates the potential of nutlin-based PROTACs against certain cancers in anticancer therapie due to its bifunctional mechanism.

1.6.3.2 Recruiting E3 ligases clAP1

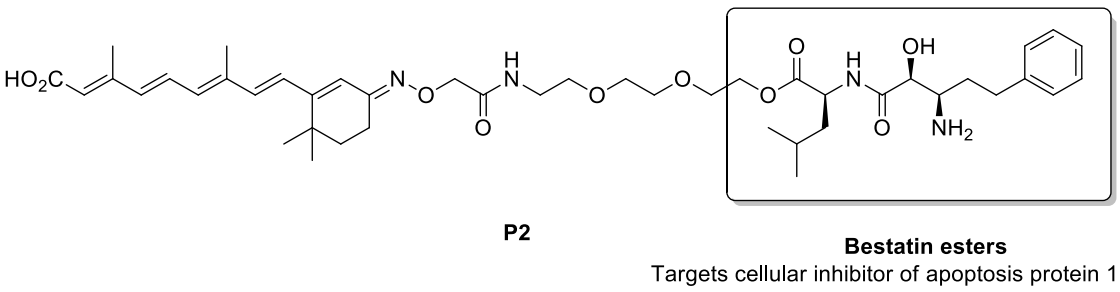


Figure 12. ATRA-MeBS PROTAC

In 2010, Itoh et al. reported PROTACs that linked all-trans retinoic acid (ATRA), an endogenous ligand of CRABP1 and CRABP2, to methyl bestatin (MeBS) by PEG linkers of different lengths[70]. CRABP1 and -2 have been related to Alzheimer's disease and some cancers. The MeBS binds to the BIR3 domain of the E3 ligase Cellular Inhibitor of Apoptosis Protein 1 (cIAP1) and thus facilitates ubiquitination and degradation. The MeBS-based PROTACs were confirmed to induce down-regulation of CRABPs with low micromolar to submicromolar potency in vitro. However, it also led the undesired self-degradation of cIAP1, resulting in the unsustainable down-regulation of the target protein. In later work, this unwanted self-degradation was overcome by a replacement of the ester bond with an amide bond in the linker to afford the PROTAC, which specifically degrade CRABP2[71].

1.6.3.3 Recruiting E3 ligases CRBN

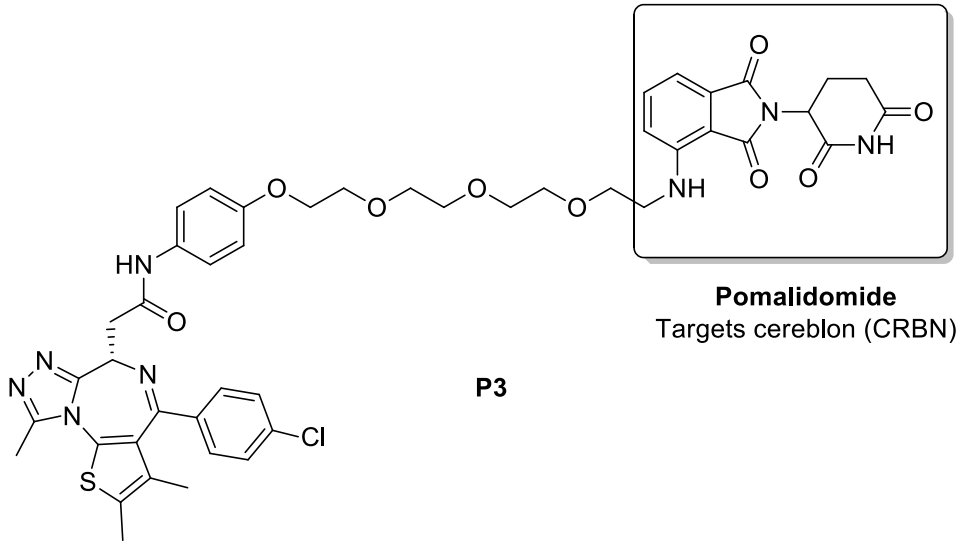


Figure 13. JQ1- pomalidomide PROTAC

Although the small-molecule PROTACs based on MDM2 and cIAP1 E3 ligases are capable of inducing the proteasomal degradation of numerous proteins, these PROTACs were found to be active only in the double-digit-micromolar range. Thus, in terms of potency and target selectivity, recruitment of a novel E3 ligase that causes stronger degradation is needed.

In the past decade, some studies found that thalidomide and its derivatives lenalidomide and pomalidomide are able to bind E3 ubiquitin ligase cereblon (CRBN), a component of a cullin–RING ubiquitin ligase (CRL) complex[72, 73]. Based on these findings, a new PROTACs series coupling the ligand of the oncoprotein BRD4 with pomalidomide to hijack E3 ligase cereblon was developed[74]. The PROTAC ARV-825, which consists of the BRD4-targeting drug OTX015 coupled to pomalidomide via a short alkyl linker, caused almost complete BRD4 protein degradation at 10 nM within 6 h.

1.6.3.4 Recruiting E3 ligases von Hippel–Lindau Protein (VHL)

Von Hippel-Lindau (VHL) is a tumor suppressor protein that binds Cullin RING E3 ubiquitin ligase and subsequently polyubiquitinates hypoxia inducible factor-1α (HIF-1α)[75]. Recruiting E3 ligase Von Hippel-Lindau (VHL) by its peptidic ligands derived from HIF-1α has been known for decades[76]. To address the adverse pharmaceutical properties of the peptide derivatives, the first small molecule ligand for VHL with moderate affinity as well as improved permeability was developed by Buckley et al. in 2012[77]. The hydroxyproline derivative was rationally designed and can bind VHL with IC_{50} value of 4.1 μM.

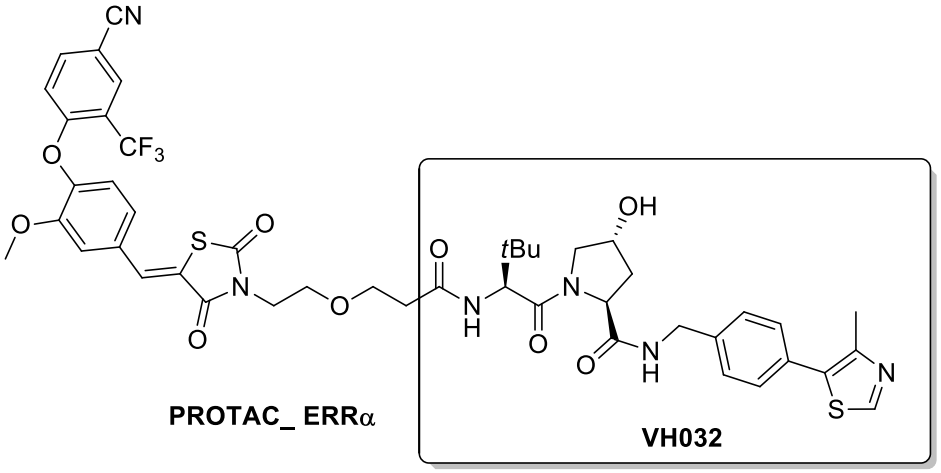


Figure 14. PROTAC_ ERRα

Based on this scaffold, the potent VHL ligand VH032 with nanomolar binding affinity was developed by the structure-guided optimization[78]. Later, VH032 was incorporated into PROTACs targeting estrogen-related receptor alpha (ERRα) and receptor-interacting serine/threonine-protein kinase 2 (RIPK2)[79]. The PROTAC_ERRα reduced the expression of ERRα with a 100nM DC_{50} value and a maximum level of degradation of 86% in MCF-7 cells. The PROTAC_RIPK2 induced a potent degradation of RIPK2 with a 1.4 nM DC_{50} value and a D_{max} of 95% at concentration of 10 nM or higher. The proteasomal degradation was verified by the pretreatment of epoxomicin, leading to inhibited protein knockdown.

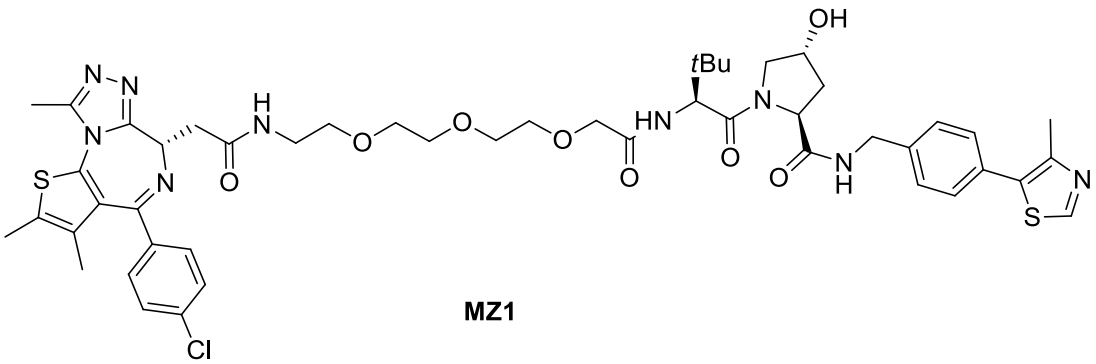


Figure 15. BRD4 targeting PROTAC MZ1

In 2015, Zengerle et al. reported the BRD4 targeting PROTAC MZ1, which tethered the VHL recruiting motif VH032 to JQ1, an inhibitor of bromodomain and extra-terminal (BET) protein[80]. In HeLa cells, MZ1 was able to degrade more than 90% of BET family proteins, including BRD2, BRD3 and BRD4, at 1 μM concentration. Surprisingly, while JQ1 lacks selectivity within the BET protein family, MZ1 showed selectivity for BRD4 over BRD2 and BRD3 in the PROTAC induced degradation at low concentrations. Given the fact that no preferential binding to the bromodomains of BRD4 with MZ1 was observed, they speculated the selective depletion was caused by the preference for polyubiquitination of BRD4 compared to its homologous BRD2/3. This difference could either arise from the steric constraints or the protein-protein interactions between VHL and BRD proteins. Two years later, Gadd et al. solved the co-crystal structure of BRD4 bromodomain-MZ1-VHL ternary complex to elucidate the functional basis of the selective degradation[81]. This research revealed the PROTAC MZ1 folds onto itself and in turn induced extensive protein-protein and protein-ligand interactions within the complex, promoting the cooperative ternary complex formation driven by the surface complementarity between VHL and the BRD4 bromodomain. The development of MZ1 and its analogues provided the proof-of-concept for converting non-selective ligands into selective degraders, which is highly desired for the drug discovery.

1.7 Advantages of degradation by PROTACs over inhibition via small molecules

Although many pharmaceuticals applied so far are small molecules which possess high cell membrane permeability and thereby can target intracellular proteins, some inherent shortcomings of small molecule drugs due to the inhibitory mechanism limited their application and development. The occupancy-based inhibitors act on the target protein by blocking the active binding site to deactivate its functions and thus interfere with the downstream regulation pathways. However, many proteins are difficult to block with cell permeable small molecules via protein-protein interactions, leading to undruggability of over 85% of human proteins based

on inhibitory mechanisms. Besides, reduced bioavailability and adverse side effects caused by off-target effect, drug resistance due to protein mutation and incomplete inhibition remain unresolved issues in drug development.

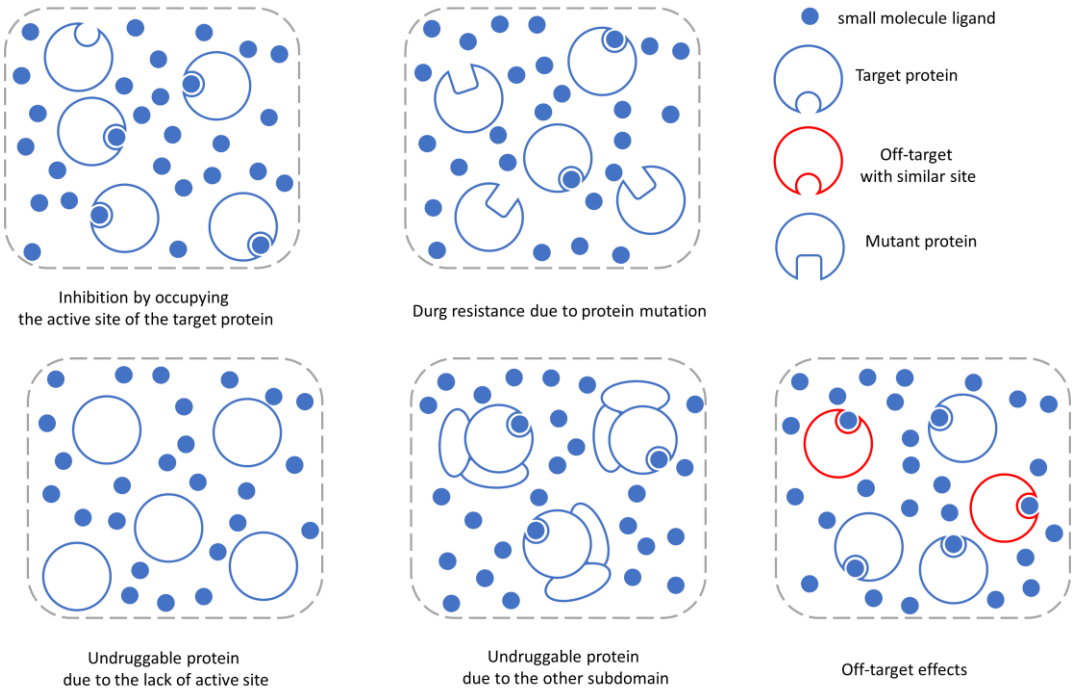


Figure 16. Overview of the shortcomings of small molecule drugs

Compare to the classic small molecular drugs, PROTACs catalyze the intercellular intracellular protein degradation driven by UPS, which may require a lower drug concentration in vivo. Mechanically, PROTACs eliminate the aberrantly functioning protein rather than to inhibit its active site, preventing the remaining subdomains exert their functions via protein-protein interactions.

2 Aim of the Project

FKBP51 has emerged as a promising new therapeutic target for psychiatric disorders and several other diseases[82]. Although many efforts have been made to enhance the potency, small-molecule inhibitors of FKBP51 remain limited in scope with respect to their use. This is due to the occupancy-driven pharmacology.

In this research, we envisioned to develop a new class of small-molecule degraders based on the emerging PROTAC strategy. In order to obtain potent PROTAC molecules, combinations of different target protein ligands, E3 ligase ligands and linkers had to be explored. Therefore, two series of PROTACs which employed our FKBPs ligands with high affinities were designed.

First of all, efficient synthetic procedures for the precursors of ligands and linkers had to be established respectively. Second, the protein binding motifs and the linker had to be assembled to form bifunctional PROTAC molecules while keeping their binding affinities. Third, the abilities to degrade the target protein of the synthesized PROTACs should be well characterized by western blotting. This project aimed to develop promising small-molecule degraders for further optimization of PROTAC-drugs and investigations of FKBPs.

3 Results and Discussion

3.1 The synthesis of the FKBP51 ligand SAFit2

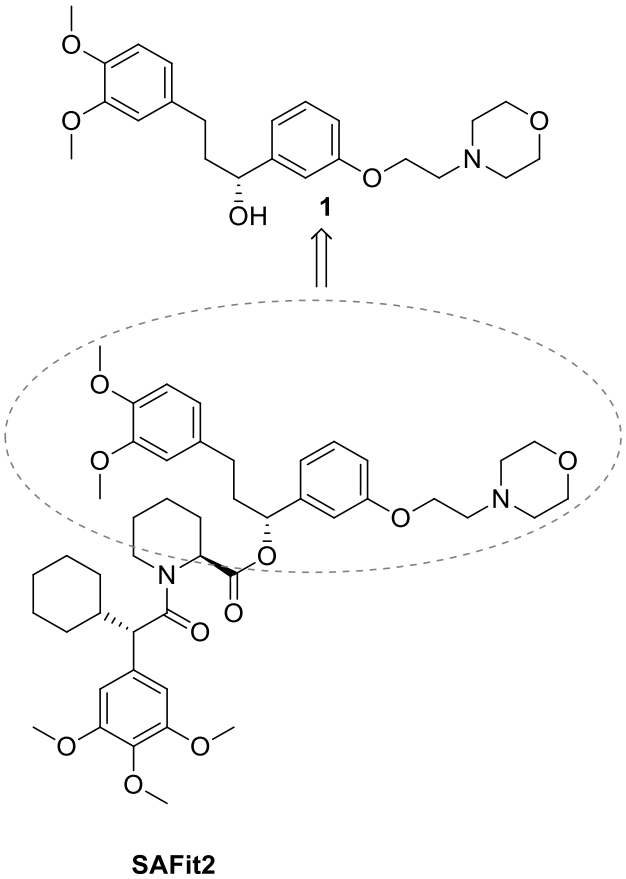
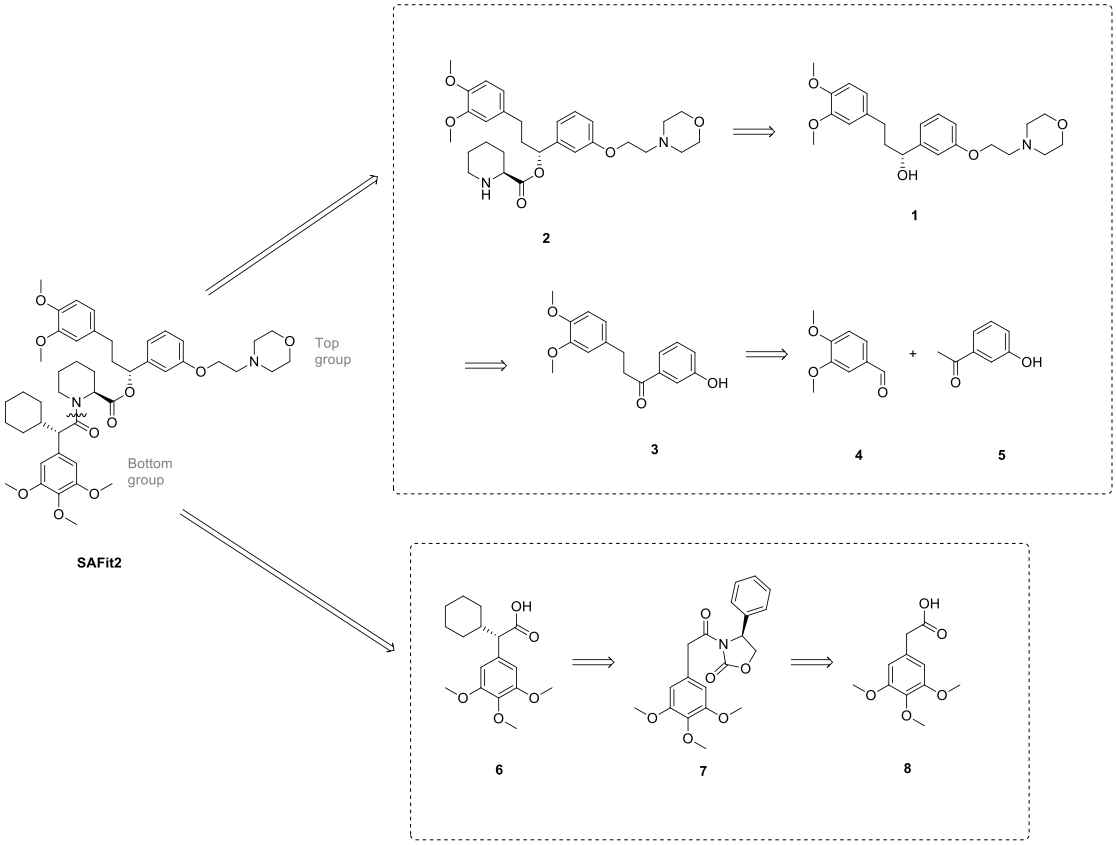


Figure 17. The structure of SAFit2 and its key intermediate 1.

The selective FKBP51 antagonists SAFit1 and SAFit2 developed by our group provided the first pharmacological useful tool to investigate the functions of FKBP51[51]. Compare to SAFit1, SAFit2 has a better application in pharmacological studies in vivo due to the increased brain permeability, while keeping the high affinity and selectivity for FKBP51. However, in comparison to the high request for SAFit2 by our cooperators, the producibility was limited due to the availability of key intermediate **1**. To fulfill the demand for SAFit2, a new synthesis procedure for **1** should be established.

3.1.1 Retrosynthetic analysis and strategy of SAFit2

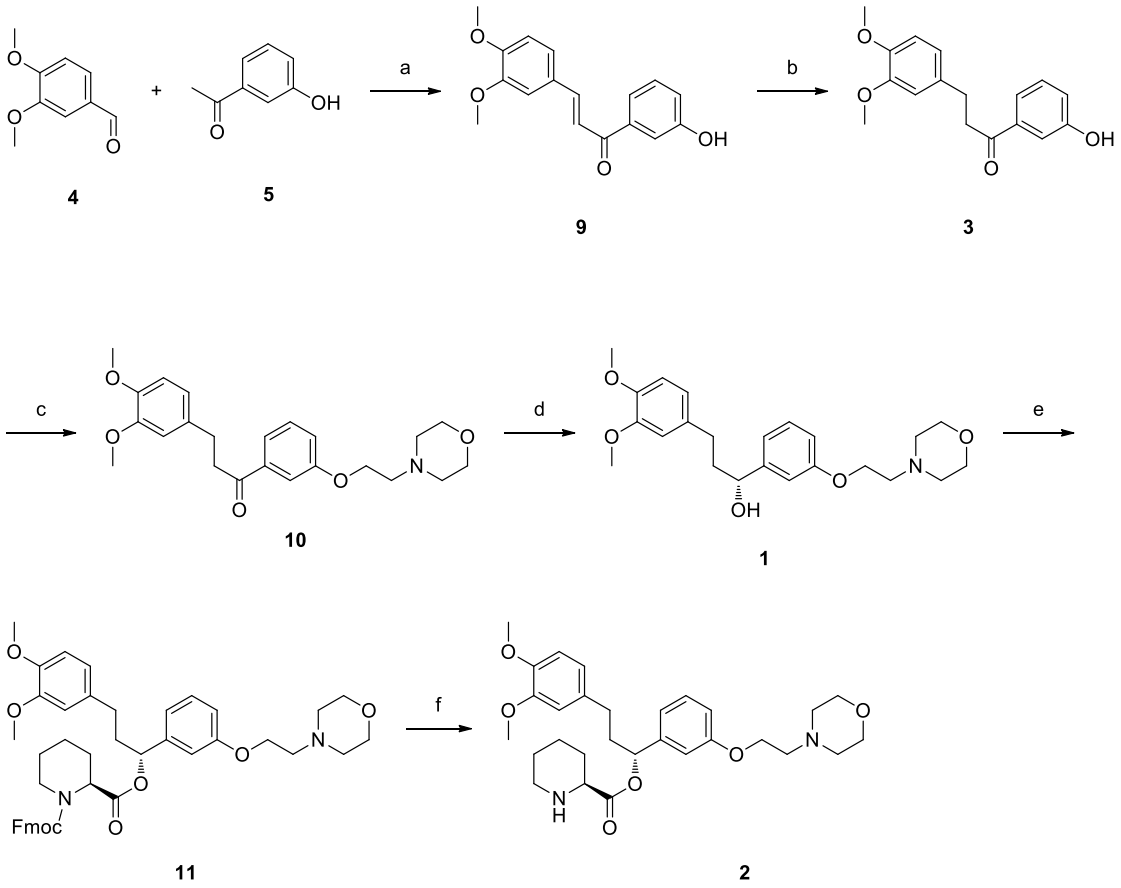


Scheme 1. Retrosynthesis of SAFit2

The retrosynthesis of the SAFit2 is outlined in **Scheme 1**.

Our synthetic strategy towards SAFit2 was derived from our previous work[51, 83]. The SAFit2 molecule can be divided into two parts: the morpholine "top group" **1** and the corresponding trimethoxyphenyl "bottom group" **6**. It was clear that the target molecule SAFit2 could be accessible from coupling pipecolate between top group **1** and bottom group **6**. The top group **1** was envisioned to be derived from **3** through sequential O-alkylation of 2-morpholinoethyl halide and asymmetric reduction of carbonyl. Compound **3** was expected to be obtained by a Claisen-Schmidt condensation between aldehyde **4** and ketone **5** followed by a selective double bond reduction[84]. The bottom group **6** was expected to be carried out by the asymmetric alkylation of **8** with Evans auxiliary to obtain the desired configuration[85].

3.1.2 Synthesis of the top group



Scheme 2: Synthesis of the SAFit top group **1** and the precursor **2**. (a) KOH, H_2O/EtOH, 0°C → RT, overnight. (b) H_2, Lindlar catalyst, 35 bar, MeOH, 7d, 63% for two steps. (c) 2-Morpholinoethyl chloride hydrochloride, K_2CO_3, acetonitrile, RT, overnight, 96% (d) H_2, $RuCl_2$[(S)-xylbinap][(S)-daipen], isopropanol, 35-40 bar, RT, 3d, 86%, ee ≥ 99%. (e) (S)-N-Fmoc-piperidine-2-carboxylic acid, EDC, DMAP, DCM, overnight, RT, 97% (f) 4-Methylpiperidine, DCM, RT, overnight, 73%

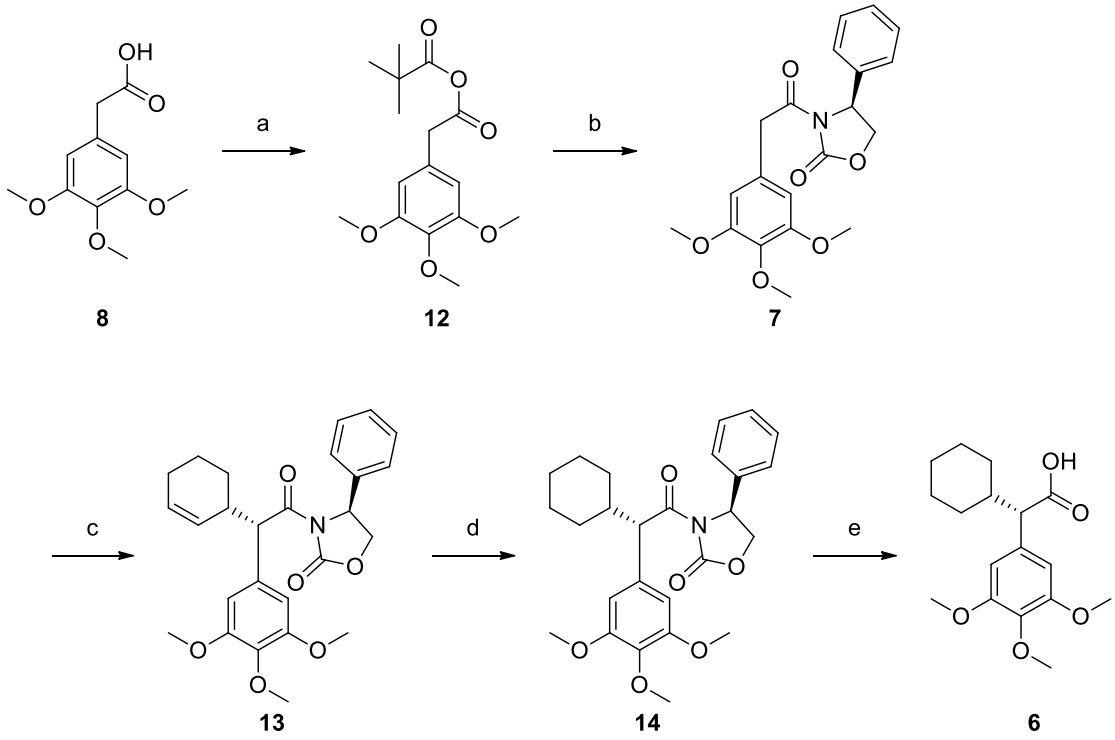
The precursor **2** was prepared via a six-step synthetic route as presented in **Scheme 2**.

The synthesis commenced with the Claisen-Schmidt condensation of commercially available veratraldehyde (3,4-dimethoxybenzaldehyde) and 3-hydroxyacetophenone in presence of potassium hydroxide. The reaction yielded the crude product **9** on large scale (~150 g) which could be used in the next step without further purification.

The chemoselective reduction of the C=C double bond of α, β-unsaturated ketone **9** was carried out in a high pressure autoclave catalyzed by Lindlar catalyst in MeOH. The product could be purified in large scale by recrystallization from MeOH affording **3** in up to 63% yield. The free aromatic alcohol of **3** was subsequently alkylated with 2-morpholinoethyl chloride hydrochloride, a very cheap and commercially available chemical, under mild condition with

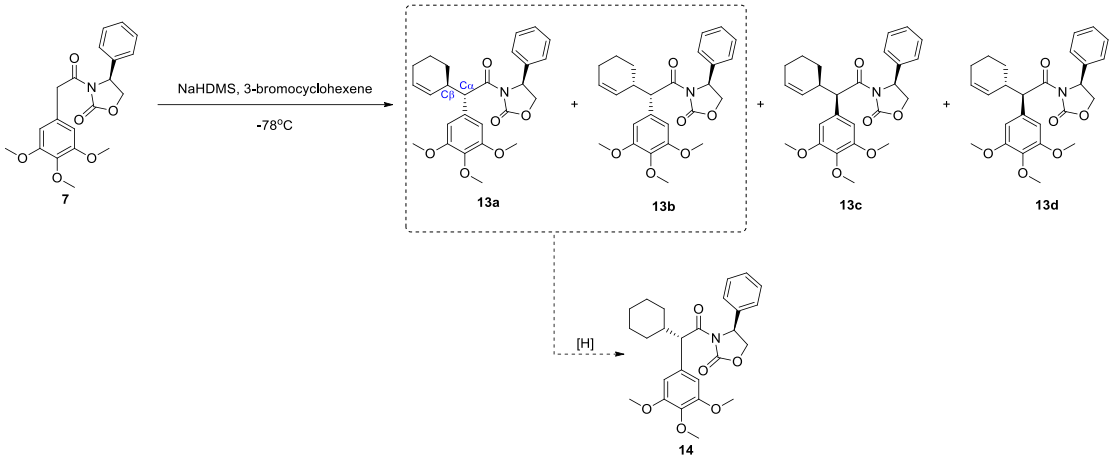
potassium carbonate in acetonitrile at room temperature to afford **10** with high yield of 96% by recrystallization. The chiral alcohol **1** was obtained by the asymmetric hydrogenation using $RuCl_2$[(S)-xylbinap][(S)-daipen] at 35-40 bar hydrogen[86]. Compared to the catalyst previously used in our group, the Si-face selective asymmetric reduction yielded the top group **1** with a higher ee value of >99%. Esterification of the chiral alcohol **1** with commercially available N-Fmoc-L-pipecolinic acid followed by deprotection gave the desired precursor **2** smoothly.

3.1.3 Synthesis of the bottom group



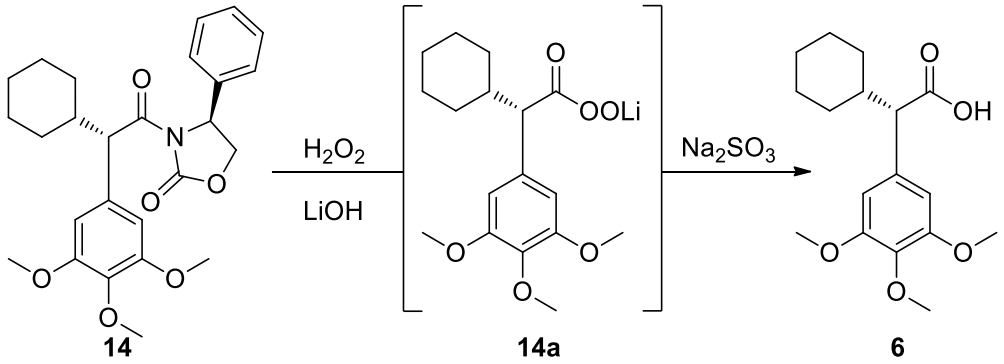
Scheme 3: Synthesis of the SAFit bottom group **6**. (a) Pivaloyl chloride, DIPEA, DCM, 0°C → RT, 2h. (b) (S)-(+)-4-Phenyl-2-oxazolidinone, n-BuLi, THF, -78°C → RT, overnight, 64% for two steps. (c) Bromocyclohexene, NaHMDS, THF, -78°C → 4°C, overnight, 36%. (d) H_2, Pd/C, MeOH, RT, overnight, 99%. (e) LiOH, H_2O_2, THF/H_2O, 0°C → RT, overnight, 80%.

The bottom group **6** was prepared via a five-step synthetic route as presented in **Scheme 3**. To introduce the cyclohexyl group at Cα with the desired configuration, Evan's chiral auxiliary was reacted with the activated trimethoxyphenyl acetic acid **12** to form the imide **7**. The Cα carbon of **7** was then deprotonated by NaHMDS and subsequently alkylated by bromocyclohexene to give **13** as a mixture of four diastereomers.



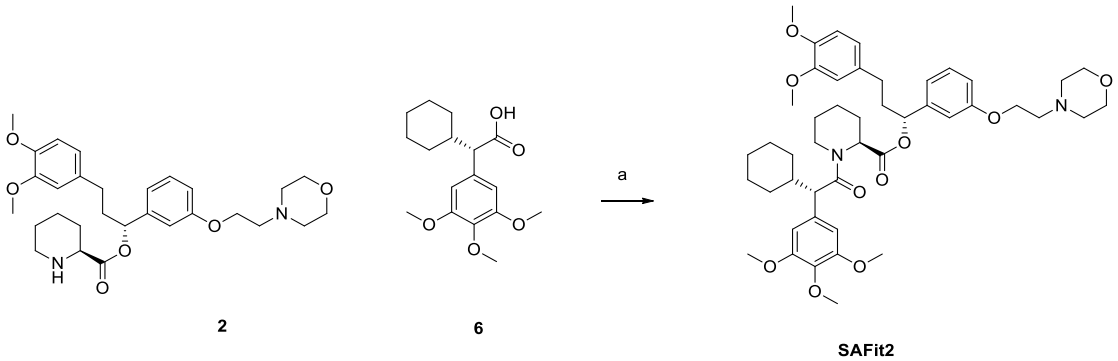
Scheme 4: The separation of diastereomers.

Fortunately, the mixed fraction of the (S)-cyclohexenyl products **13a** and **13b** could be separated completely from the mixture with a moderate yield of 36%. The mixture of the two diastereomers **13a/13b** was inconsequential due to the disappearance of the chiral center at Cβ in the following double bond reduction. The hydrogenation of cyclohexenyl group in H_2 with Pd/C gave **14** with a diastereomeric excess of >99%. Finally, the oxazolidinone chiral auxiliary was cleaved by H_2O_2 under basic condition to regenerate the carboxylic group.



Scheme 5: The cleavage of oxazolidinone chiral auxiliary.

3.1.4 Synthesis of SAFit2



Scheme 6: Synthesis of SAFit2 (a) HATU, DIPEA, DCM/DMF = 2/1, RT, 16h, 80%.

Eventually, the coupling between **2** and bottom group **6** with HATU gave the desired SAFit2 in 80% yield. By our procedure we established for the synthesis of key intermediate **1**, a larger scale of SAFit2 can be obtained rapidly and economically, which enabled pharmacological studies of FKBP51[87, 88]. With the SAFit2 we synthesized, the research carried out by Balsevich et al. has shown that chronic treatment with SAFit2 has the same effects of FKBP51 deletion, including weight regulation and glucose tolerance. In a shorter term of treatment, glucose tolerance was remarkably improved. In this research, a substrate of AKT2 which mediates glucose uptake was identified mechanistically as a new association between FKBP51 and AS160. Balsevich et al. proposed that FKBP51 is involved in the mediation between stress and T2D (type 2 diabetes), indicating a potential target for therapeutic approaches.

3.2 Design of SAFit based PROTACs

In order to degrade FKBP51 specifically, we use the SAFit motif as the protein of interest (POI) ligand of our PROTACs. Based on the co-crystal structure of the FKBP51-FK1 domain complexed with the SAFit analogue iFit4 (PDB ID: 4TW7[51]), the methoxy groups and the morpholyl group on the aromatic rings of the top group are solvent exposed and are not involved in direct interactions with the binding pocket, making them suitable sites for tethering to E3 ubiquitin without impairing affinity to FKBP51. Replacement of alkyl groups with a propargyl group allows an easy conjugation of SAFit ligand with a polyethylene glycol (PEG) linker via click reaction.

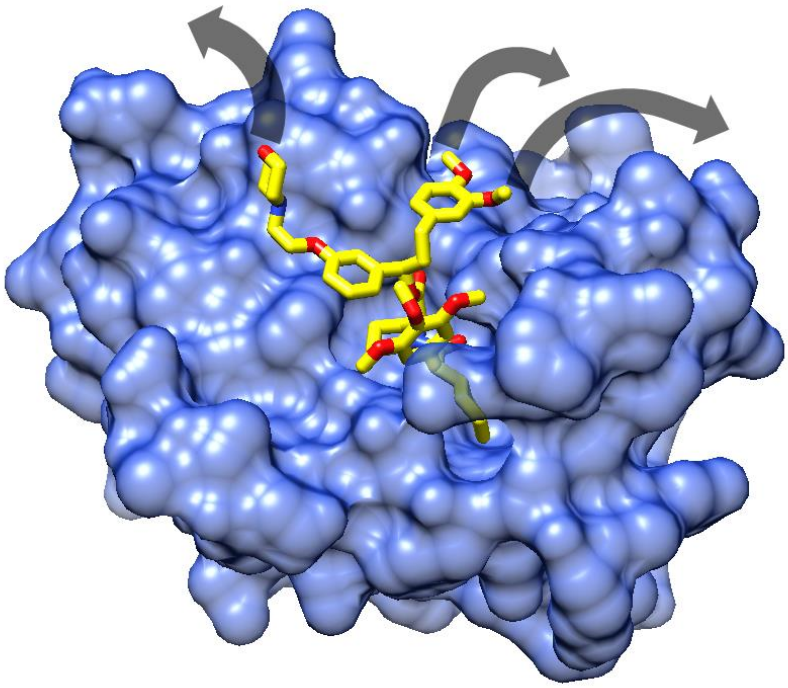


Figure 18. Exemplary co-crystal structure to guide the linker attachment for PROTACs. The exit vectors for linking are indicated by the arrows. (PDB ID: 4TW7)

Among numerous E3 ligases, VHL and CRBN are the two most popular E3 ligases being utilized by present PROTACs to mediate ubiquitination and subsequent protein degradation via the proteasome[89, 90]. As a result, various potent ligands of VHL and CRBN have been studied and developed[78, 91 92, 93]. VH032, a potent and specific VHL ligand, and pomalidomide, a commercially available thalidomide analogue, which can recruit CRBN, where chosen to be conjugated to our SAFit motif via a linker respectively.

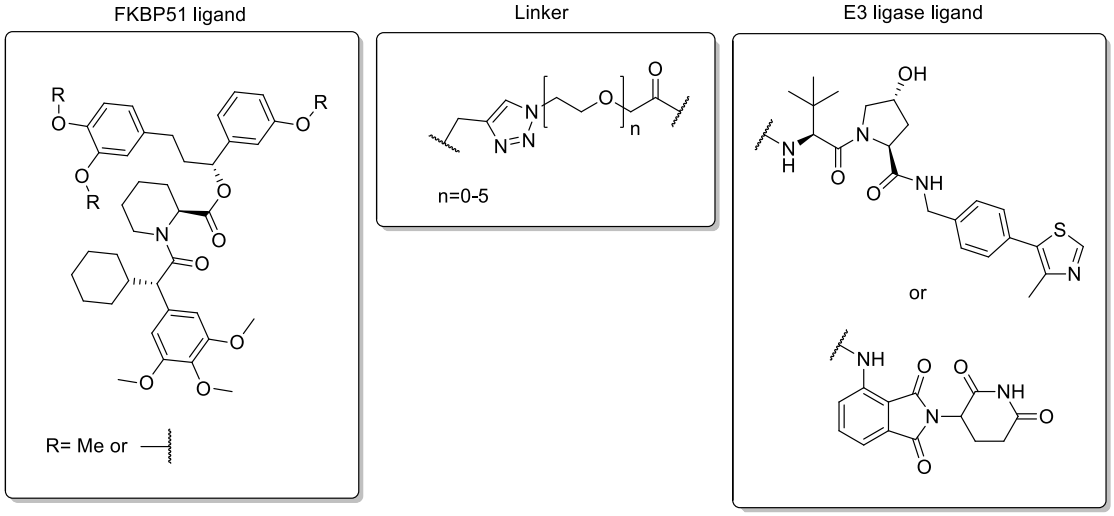


Figure 19. Structures of SAFit-based PROTACs.

The linker of PROTACs connects the E3 ligase recognition motif and the small molecule ligand for the target protein. The length and configuration of the linker can impact the efficiency of a PROTAC to degrade a target protein[94]. An appropriate linker is usually crucial for inducing an optimal protein-protein interaction between a protein of interest and an E3-E2-ubiquitin complex to promote ubiquitination of target protein resulting in efficient proteasomal degradation.

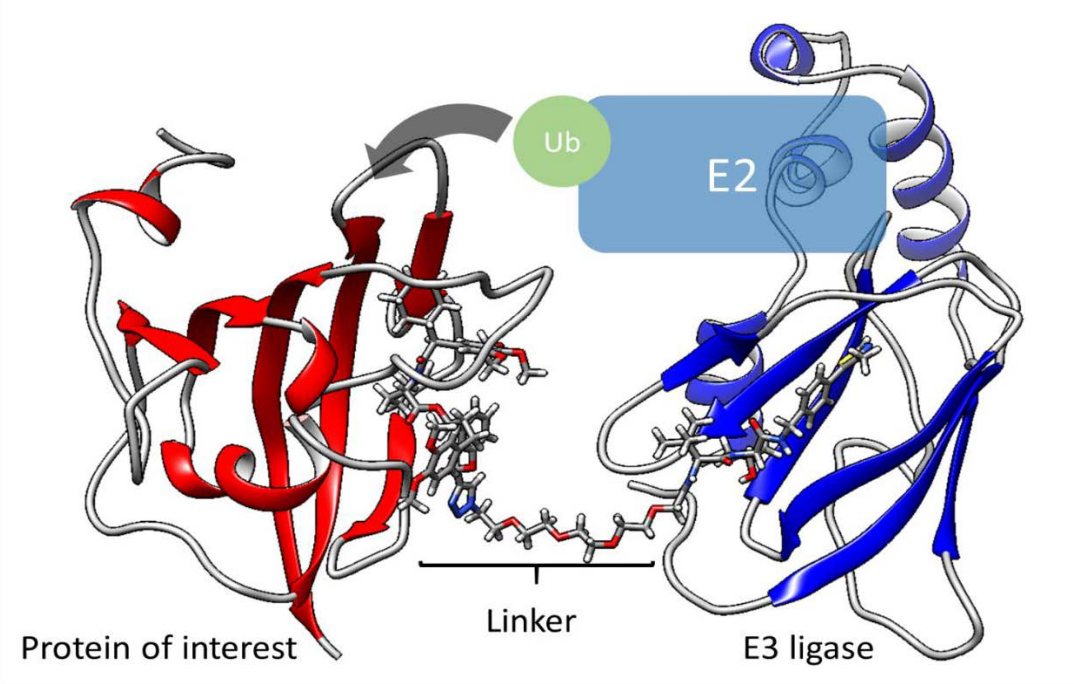
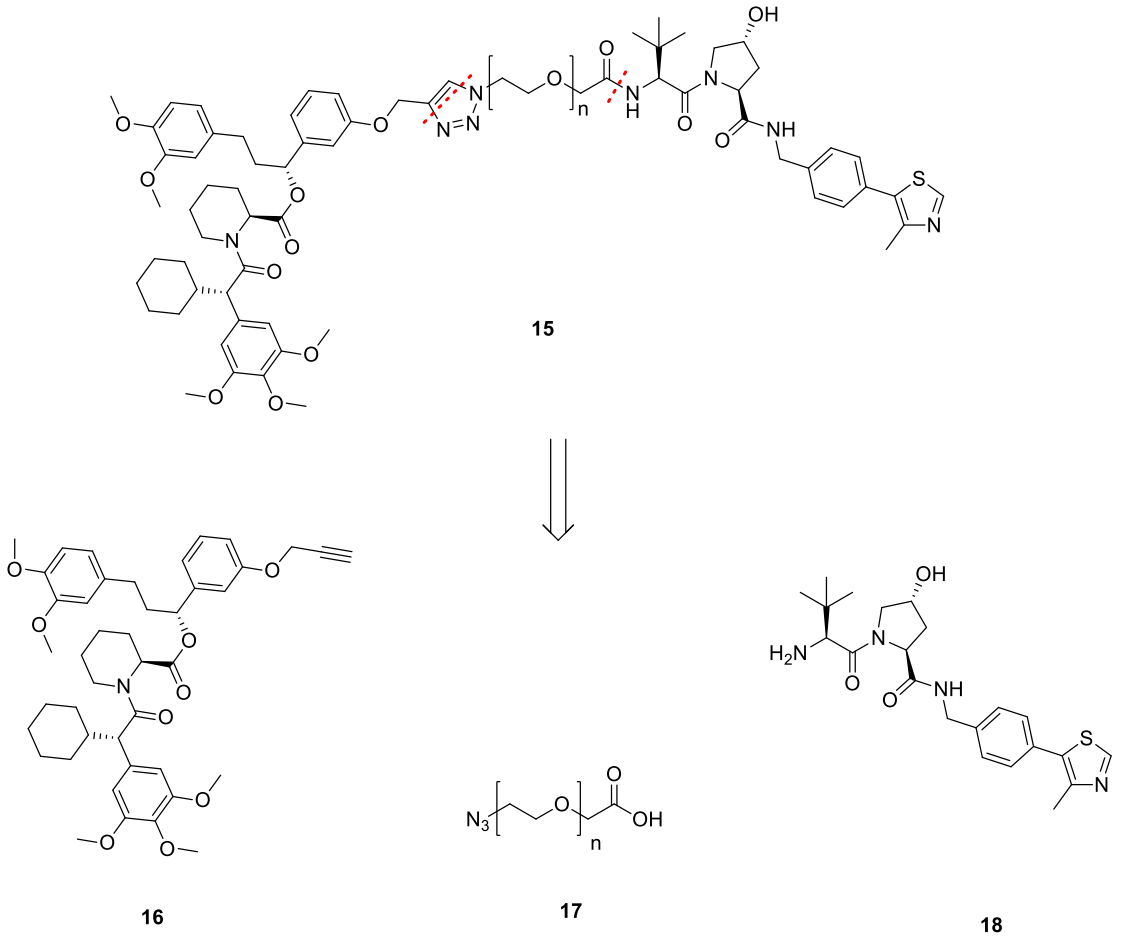


Figure 20. The proposed influence of a PROTAC linker in protein-protein interaction (PDB ID: 4TW7, 4W9H).

So far, due to the difficulty to predict the optimal length of the linker of a PROTAC, most of current PROTACs were developed by selecting linker with lengths from 12 to over 20 carbon randomly[77, 95-97]. Thus, to explore the influence of the distance between protein ligand and E3 recruiting motif, we chose polyethylene glycol (PEG) containing different numbers of repeat units to tether the other two moieties.

3.2.1 Synthesis of SAFit based PROTACs

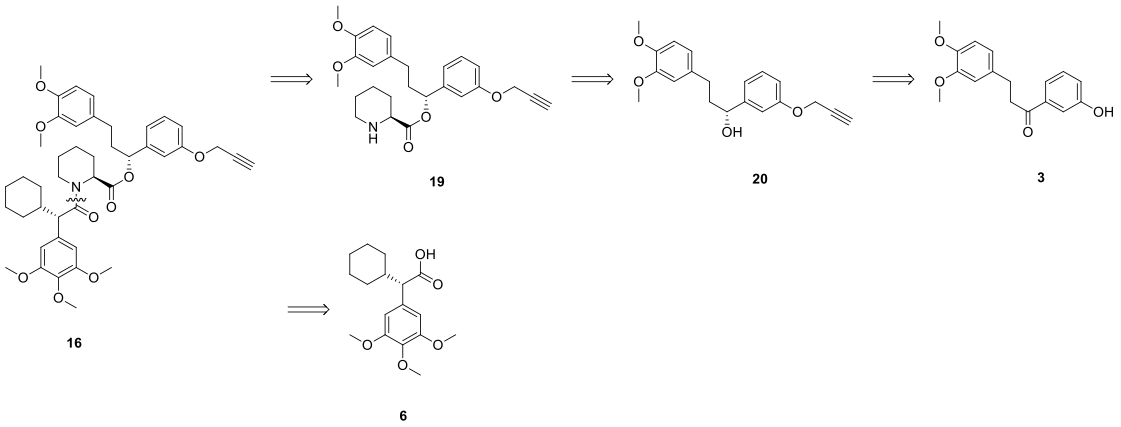


Scheme 7: Disconnection of SAFit-based PROTAC structure (only the VHL analogue is shown).

The SAFit-based PROTAC could be deconstructed to three parts: SAFit analogue (FKBP ligand), azido acid (PEG linker) and VH032 or pomalidomide (E3 ligase ligand).

3.2.2 FKBP ligand

3.2.2.1 Retrosynthetic analysis and strategy



Scheme 8: Retrosynthesis of the SAFit analogue **16**.

The retrosynthesis of the SAFit analogue **16** is outlined in **Scheme 8**.

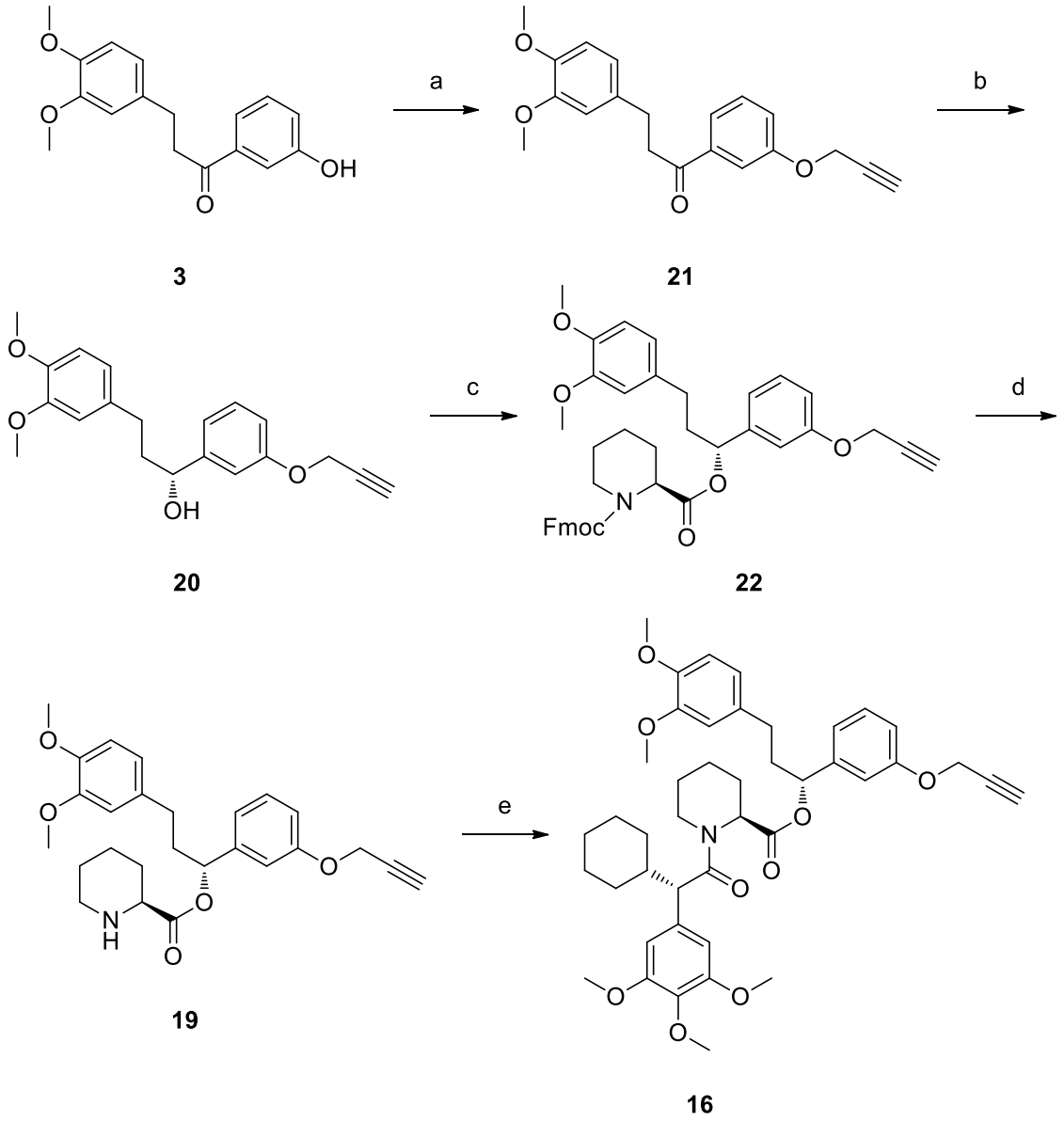
Our synthetic strategy towards the SAFit analogue **16** was derived from our previous work[51]. It was clear that the target molecule **16** could be accessible from coupling the top group **19** and bottom group **6**. The synthesis of **6** was reported by our group. Compound **19** could be obtained by esterification of **20** with protected L-pipecolic acid. The unsaturated chiral alcohol **20** was envisioned to be derived from **3** through sequential asymmetric reduction of carbonyl and O-alkylation of phenolic hydroxyl group.

3.2.2.2 Synthesis of the SAFit analogue 16

The free aromatic alcohol of **3** was subsequently alkylated with propargyl bromide under mild condition with potassium carbonate in acetone at room temperature to afford **21**.

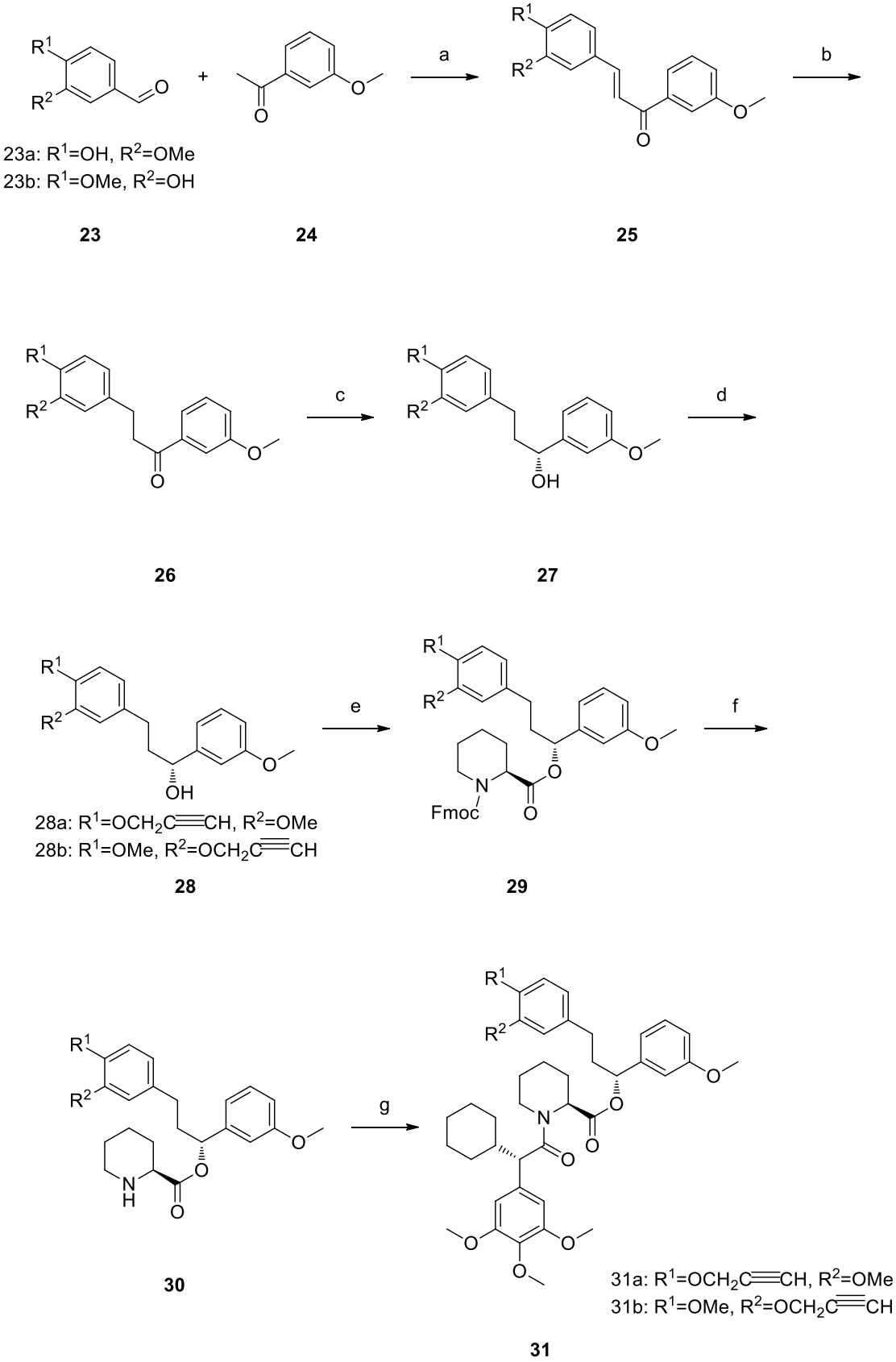
Due to the poor selectivity of carbonyl group and alkynyl group in catalytic hydrogenation, **21** was then subjected to (R)-stereoselective Corey-Bakshi-Shibata reduction of the carbonyl group with borane at -40°C[98, 99]. **20** was obtained with enantiomeric excess of 94%. Higher reaction temperature (-15°C) decrease the ee% of product, while no conversion was observed at a lower temperature (-78°C).

Esterification of the chiral alcohol with commercially available N-Fmoc-L-pipecolinic acid followed by deprotection gave the desired O-propargyl top group **19**. The desired product could be obtained by coupling **19** and bottom group **6** with HATU.



Scheme 9: Synthesis of the SAFit analogue **16**. (a) Propargyl bromide, K_2CO_3, acetone, RT, overnight, 90%; (b) (S)-(−)-2-Methyl-CBS-oxazaborolidine, BH_3-THF, THF, -40°C, 5h, quant., ee = 94%; (c) (S)-N-Fmoc-piperidine-2-carboxylic acid, EDC, DMAP, DCM, overnight, RT, 86%; (d) 4-Methylpiperidine, DCM, RT, overnight, 73%; (e) **6**, HATU, DIPEA, DCM/DMF, RT, overnight, 67%.

3.2.2.3 Synthesis of the SAFit analogues 31a and 31b.



Scheme 10: Synthesis of the SAFit analogues **31a** and **31b**. (a)KOH, H_2O/EtOH, 10°C → 50°C, overnight, 39-63%; (b) Zn, NH_4OAc, H_2O/EtOH, RT, 64%; (c) H_2, $RuCl_2$[(S)-dm-

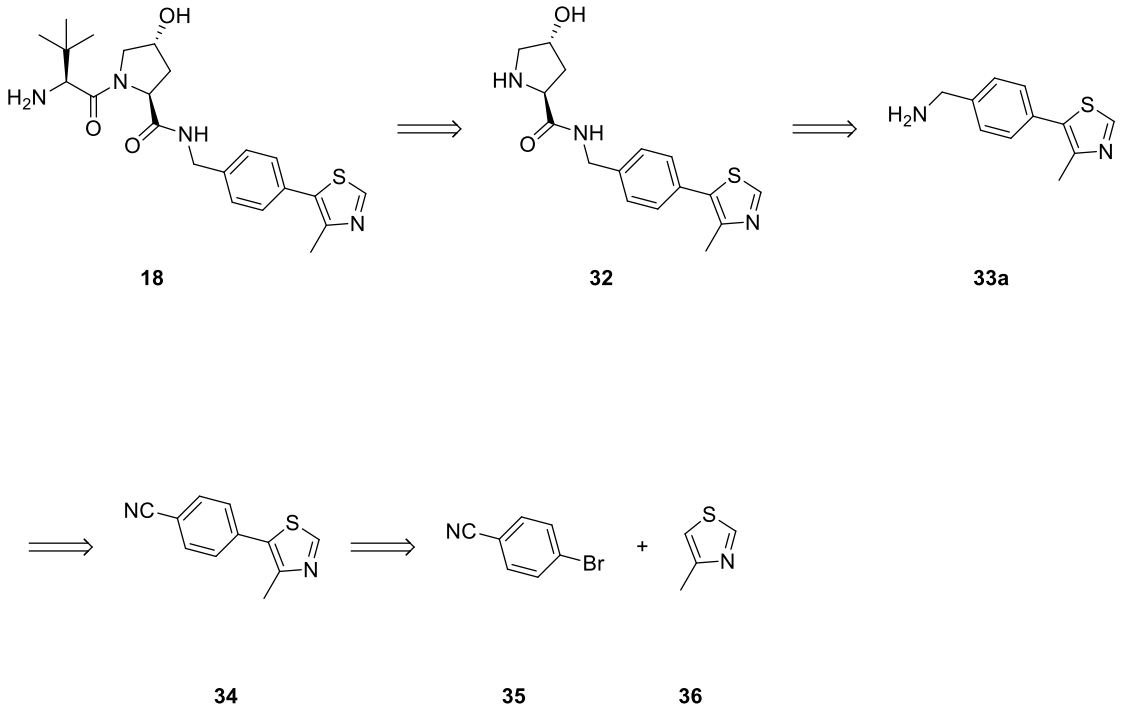
segphos®][(S)-daipen], K_2CO_3, *i*-PrOH, 10 bar, RT, 3d, 94-99%; (d) propargyl bromide, K_2CO_3, acetone, RT, overnight, 89-93%; (e) (S)-N-Fmoc-piperidine-2-carboxylic acid, EDC, DMAP, DCM, overnight, RT, 95-96%; (f) 4-methylpiperidine, DCM, RT, overnight, 89-96%; (g) **6**, HATU, DIPEA, DCM/DMF, RT, overnight, 65-67%.

The students Christian Walz and Michael Walz contributed to these experiments.
Similar to the synthesis described above, the first step was the coupling of commercially available vanillin **23a**/isovanillin **23b** and 3-methoxyacetophenone via Claisen-Schmidt condensation in KOH/EtOH.
However, due to the deprotonation of vanillin in alkaline condition, the reaction rate decreased and could not be completed. The mesomeric effect increases the electron density of the carbon of the aldehyde, which makes the nucleophilic attack of **23** more difficult. Therefore, a higher temperature (50°C) and increased KOH concentration were applied to complete the reaction.
Instead of high pressure hydrogenation, the selective reduction of the conjugated double bond was carried out in a Zn/NH_4OAc system to give **26,** which was then subjected to asymmetric hydrogenation catalyzed by the low-cost catalyst $RuCl_2$[(S)-dm-segphos®][(S)-daipen] under a relatively low pressure (10 bar)[100].
Similar to the procedure as described above, the phenolic hydroxyl group was alkylated with propargyl bromide followed by esterification and deprotection to afford the key intermediate **30**. Coupling reaction between 3**0** and bottom group with HATU gave the desired product **31**.

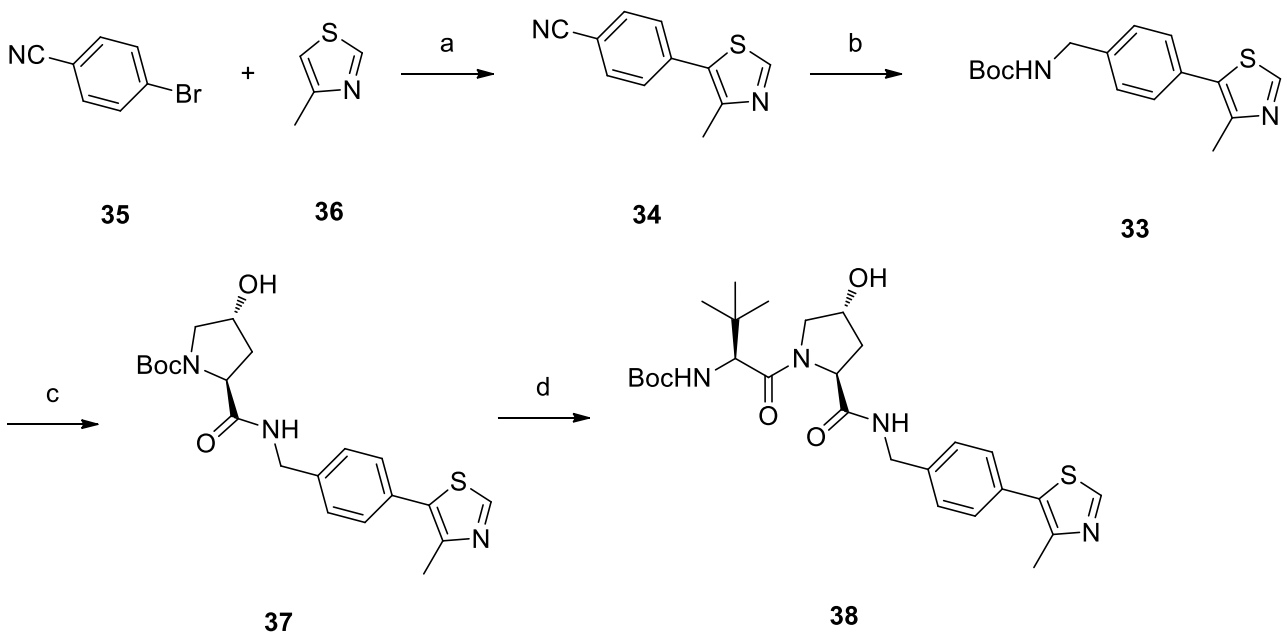
3.2.3 E3 ligase ligand

3.2.3.1 Retrosynthetic analysis and strategy

The retrosynthesis of the VH032 is outlined in **Scheme 11**.
The synthesis of the dipeptide-based VHL ligand **18** was carried out by peptide synthesis with Boc-protected L-hydroxyproline and L-tert-leucine starting from **34**. **34** was obtained by Heck cross-coupling between **35** and **36** follow by nitrile reduction. Under most conditions, palladium-catalyzed cross-coupling of thiazoles with aryl halides results in alkylation at the C2-position of the thiazole. The regioselective cross coupling in the presence of KOAc was anticipated to provide the C5-arylated thiazole product[101].



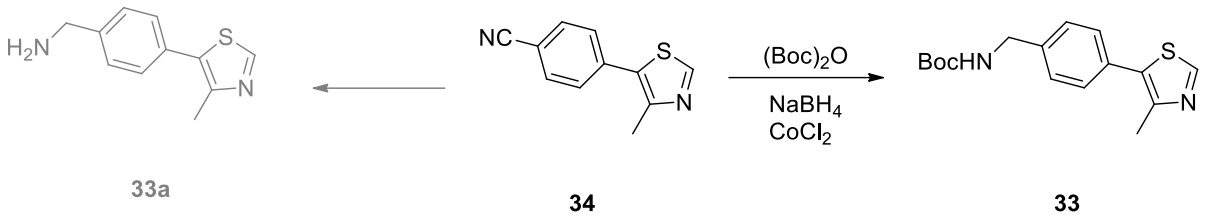
Scheme 11: Retrosynthesis of the VH032 precursor **18**[78].



Scheme 12: Synthesis of the VH032 precursor **38**. (a) $Pd(OAc)_2$, KOAc, dimethylacetamide, 120°C, 19h, 82%; (b) Boc_2O, $CoCl_2$, $NaBH_4$, MeOH, 0°C → RT, 58%; (c) TFA/DCM = 1/1; then Boc-Hyp-OH, EDC, HOBt, DIPEA, DMF, 0°C → RT, 68%; (d) TFA/DCM = 1/1; then Boc-Tle-OH, EDC, HOBt, DIPEA, DMF, 0°C → RT, 70%.

3.2.3.2 Synthesis

The first step was a Heck reaction of commercially available 4-methylthiazole and 4-bromobenzonitrile in the presence of palladium acetate affording **34** in 81% yield.



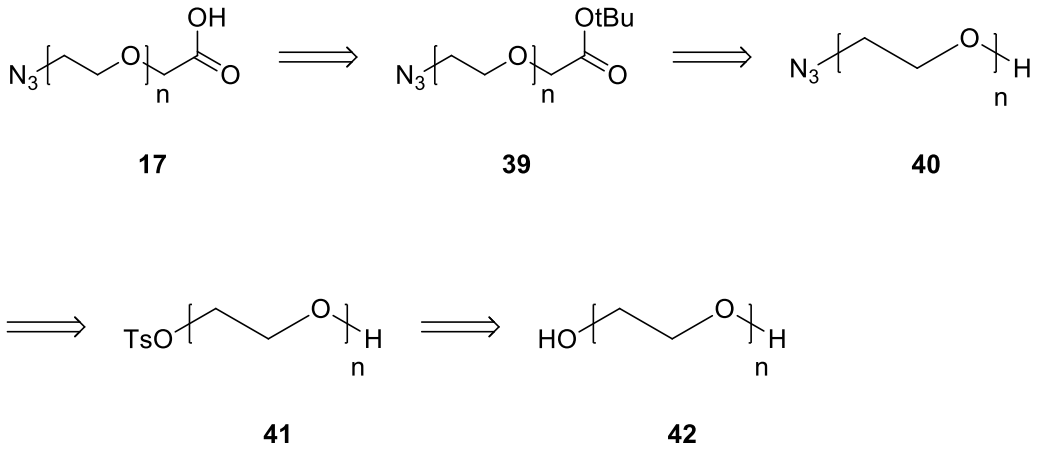
33a **34** **33**

Attempts to reduce the nitrile with $NaBH_4$ in the presence of $CoCl_2$ at 0°C gave amine **33a**, albeit in a low yield (less than 30%) of due to the difficult separation of the product caused by the free primary amine group. In order to improve the yield and stability of the reduction product, in situ N-Boc protection was applied to give **33**[102].

After in situ deprotection, **33** was further coupled successively with N-protected L-hydroxyproline and N-protected L-tert-leucine to give the desired VHL ligand **38** protected with Boc.

3.2.4 Linker

3.2.4.1 Retrosynthetic analysis and strategy



17 **39** **40**

41 **42**

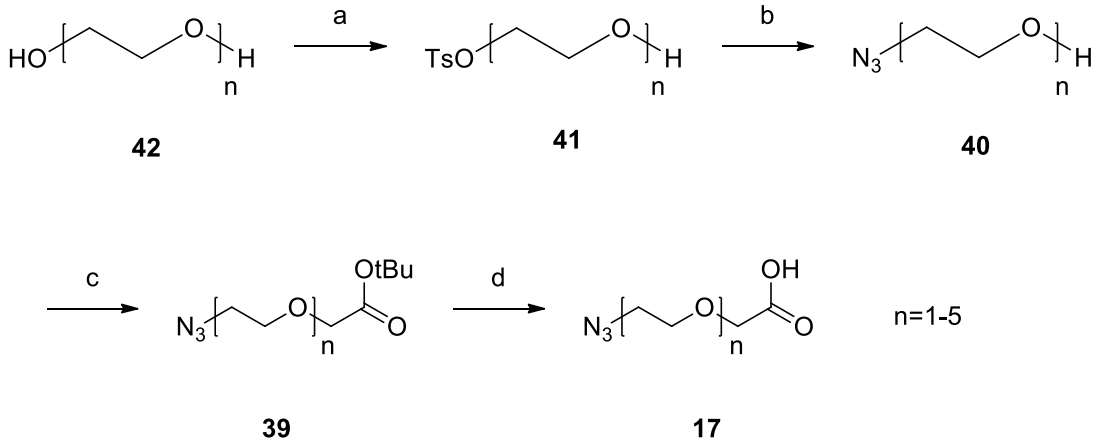
Scheme 13: Retrosynthesis of the linkers[103].

The retrosynthesis of the azido acids is outlined in **Scheme 13**. Compound **17** could be carried out from **40** via substitution with tert-butyl bromoacetate followed by deprotection. **40** could be obtained by monotosylation of diol followed by substitution of tosyl by sodium azide.

3.2.4.2 Synthesis

The azido acids were prepared by a 4-step synthetic route as shown in **Scheme 14**. The synthesis of compound **41** was carried out by mono tosylation of commercially available diols mediated

by silver(I) oxide[104]. The azido group was simply introduced by nucleophilic substitution with sodium azide in DMF at room temperature. **40** was alkylated with tert-butyl bromoacetate and further deprotected to give azido acids **17** in different lengths. The low yield of the deprotection indicated that a lower reaction temperature is required.



42 **41** **40**

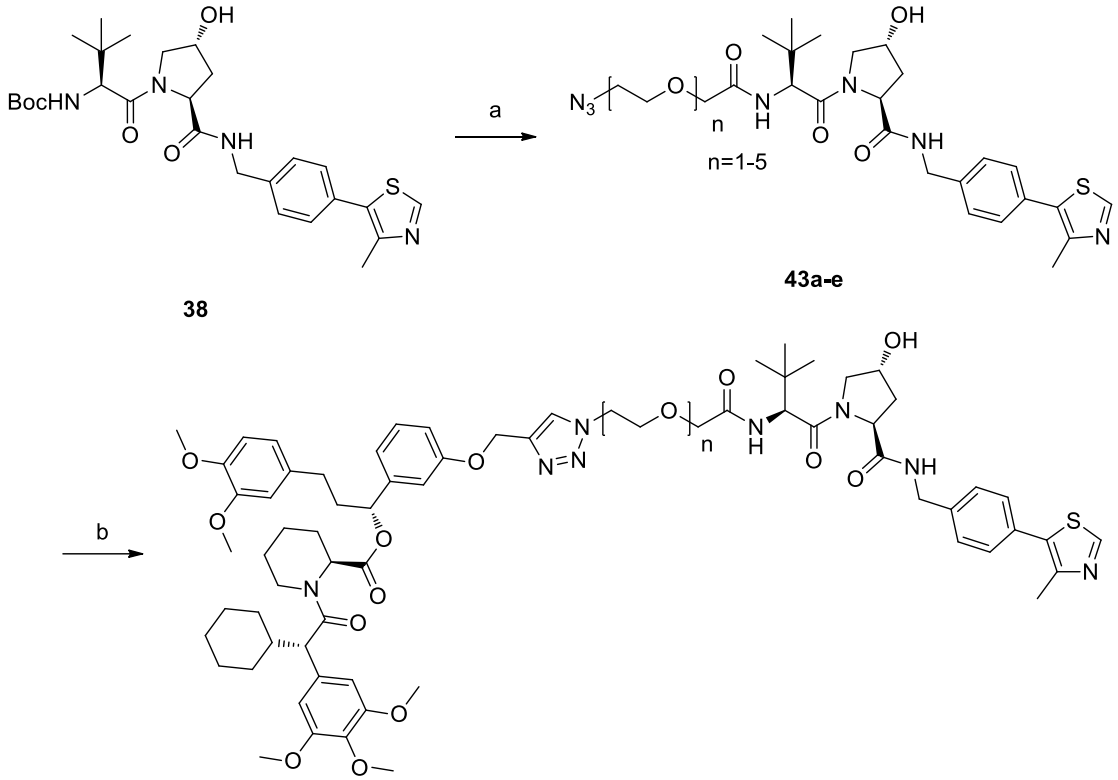
39 **17**

Scheme 14: Synthesis of the linkers **17**. (a) 1 eq. TsCl, Ag_2O, KI, DCM, 0°C, 30min, 70-78%; (b) NaN_3, DMF, RT, overnight, 86-95%; (c) tert-butyl bromoacetate, tert-BuOK, tert-BuOH, RT, overnight, 63-90%; (d) TFA/DCM, RT, 30min, 20-51%.

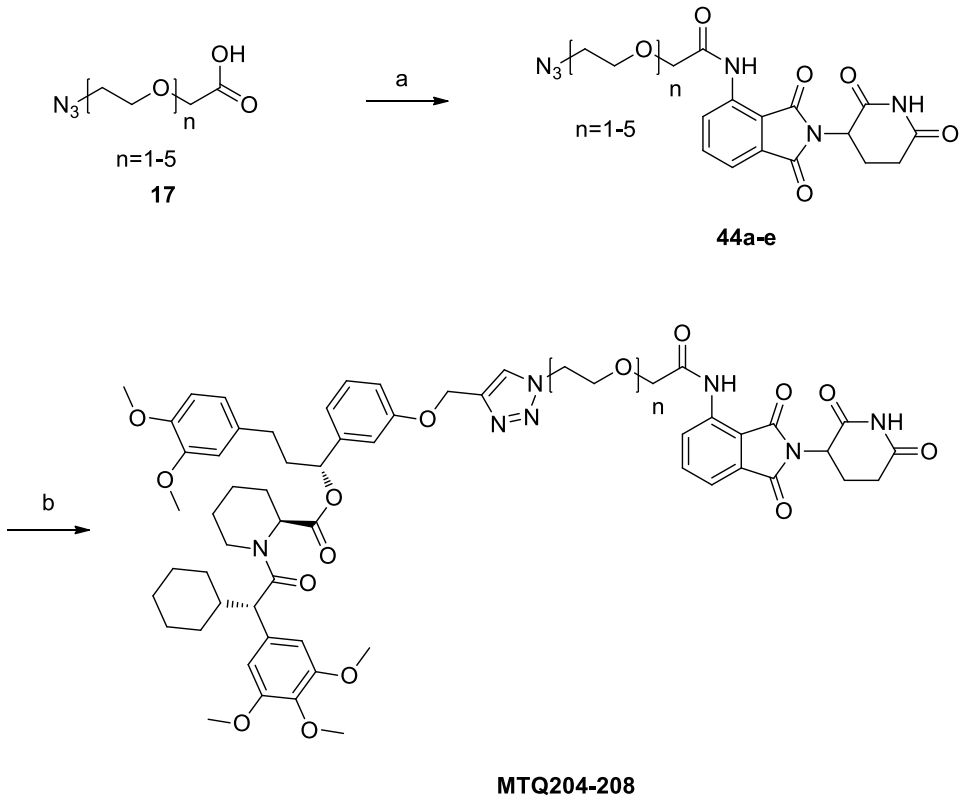
3.2.5 Assembly of PROTAC molecules

Considering the variety of the FKBP51 ligands sought to be tested as PROTACs, the azido acid and E3 ligase ligand should be assembled first. The Boc group was removed via in situ deprotection by TFA to afford **VH032**, which was then coupled to azido acid **17** with HATU. The resulting azido-PEG-VH032 **43** was conjugated with the SAFit analogues **16** or **31a/b** by a copper-catalyzed click reaction[105] to give the SAFit-based PROTACs **MTQ199-203**.

Compare to the aliphatic amine group of VH032, the aromatic amine group of pomalidomide exhibits lower reactivity in acylation reactions. Therefore, for the CRBN-based PROTACs, the azido-PEG-acid was first activated by oxalyl chloride to form an acid chloride. After activation, substoichiometric amount of pomalidomide was added to afford the desired azido-PEG-pomalidomide **44**, which in turn was conjugated to the SAFit analogues. The resultant CRBN-based PROTACs **MTQ204-208** were obtained with good yield.



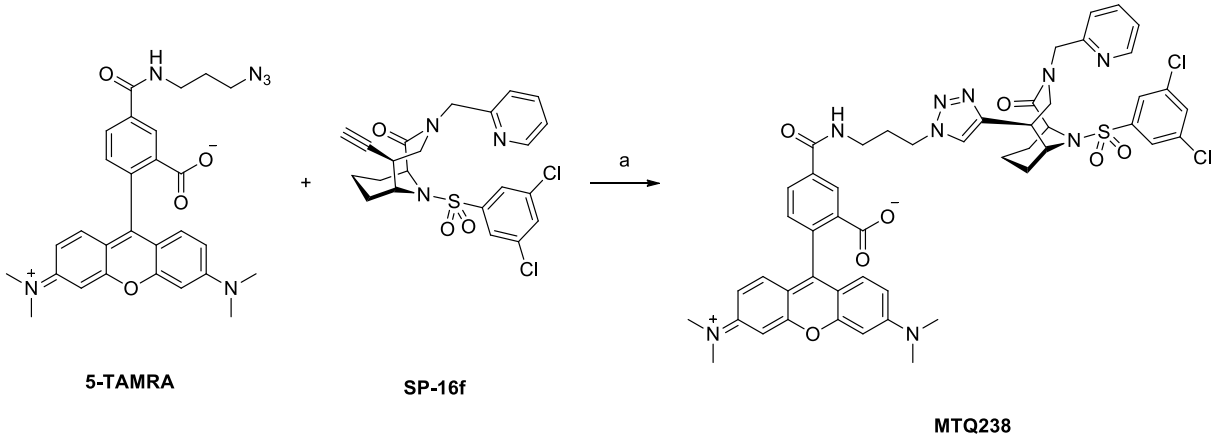
Scheme 15: Synthesis of the VHL-based PROTACs. (a) TFA/DCM = 1/1, RT, 30min; then **17**, HATU, DIPEA, DCM, RT, overnight, 68-91%; (b) **16** or **31a/b**, $CuSO_4$, (+)-Sodium L-ascorbate, t-BuOH/H_2O/DMSO, RT, overnight,74-89%. (Only shown for **16**)



Scheme 16: Synthesis of the CRBN-based PROTACs. (a) $(COCl)_2$, DMF, DCM, 0°C to RT, 3h; then pomalidomide, DMF, 0°C to RT, overnight, 34-99%; (b) **16** or **31a/b**, $CuSO_4$, (+)-Sodium L-ascorbate, t-BuOH/H_2O/DMSO, RT, overnight, 68-78%. (Only shown for **16**)

3.2.6 Biochemical characterization

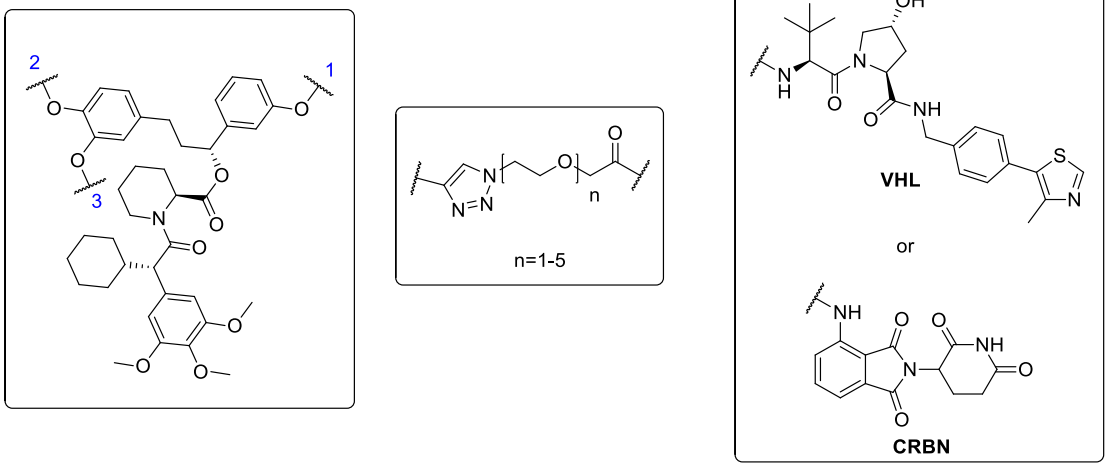
3.2.6.1 Synthesis of the fluorescent tracer



Scheme 17: Synthesis of the fluorescent tracer **MTQ238**. (a) TBTA, $CuSO_4$, (+)-Sodium L-ascorbate, t-BuOH/H_2O = 43:1, 37°C, 6h, 23%.

The fluorescent tracer **MTQ238** was obtained via the click reaction of bicyclic ligand **SP-16f** and 5-TAMRA fluorophore[55].

3.2.6.2 Evaluation of binding affinity



FKBP51 ligand Linker E3 ligase ligand

Nr.	SAFit Attachment point	E3-ligase Ligand	PEG linker length	K_i (nM)		
				FKBP51FK1	FKBP52FK1	FKBP12
SAFit1[51]	1	/	/	4 ± 0.3	>50000	90 ± 11
SAFit2[51]	1	/	/	6 ± 2	>50000	87 ± 6
16	1	/	(propargyl)	32 ± 7	>3200	563 ± 220
31a	2	/	(propargyl)	70 ± 18	>3200	1867 ± 362
31b	3	/	(propargyl)	149 ± 39	>3200	1701 ± 256
MTQ199	1	VHL	1	25 ± 6	>3200	408 ± 168
MTQ200	1	VHL	2	24 ± 7	>3200	677 ± 87
MTQ201	1	VHL	3	23 ± 7	>3200	436 ± 131
MTQ202	1	VHL	4	24 ± 8	>3200	532 ± 164
MTQ203	1	VHL	5	12 ± 3	>3200	814 ± 313
MTQ204	1	CRBN	1	19 ± 5	>3200	306 ± 161
MTQ205	1	CRBN	2	16 ± 5	>3200	256 ± 24
MTQ206	1	CRBN	3	22 ± 6	>3200	206 ± 45
MTQ207	1	CRBN	4	18 ± 4	>3200	256 ± 66
MTQ208	1	CRBN	5	5 ± 1	>3200	226 ± 65
MTQ229	1	VHL	0	36 ± 9	>3200	610 ± 181
MTQ235	1	CRBN	0	32 ± 12	>3200	563 ± 220
MTQ328	2	VHL	1	47 ± 19	>3200	409 ± 69
MTQ329	2	VHL	2	60 ± 16	>3200	584 ± 79
MTQ330	2	VHL	3	86 ± 26	>3200	410 ± 79
MTQ331	2	VHL	4	59 ± 22	>3200	368 ± 110
MTQ332	2	VHL	5	34 ± 10	>3200	455 ± 126
MTQ333	2	CRBN	1	22 ± 6	>3200	111 ± 48
MTQ334	2	CRBN	2	21 ± 10	>3200	177 ± 26

Table 1. Binding affinities of SAFit-based PROTACs for FKBP51, FKBP52 and FKBP12. The FP-assays were performed by Stephanie Merz.

Nr.	SAFit Attachment point	E3-ligase Ligand	PEG linker length	K_i (nM)		
				FKBP51FK1	FKBP52FK1	FKBP12
MTQ335	2	CRBN	3	18 ± 4	>3200	111 ± 12
MTQ336	2	CRBN	4	24 ± 6	>3200	81 ± 21
MTQ337	2	CRBN	5	25 ± 6	>3200	162 ± 47
MTQ338	3	VHL	1	100 ± 29	>3200	1345 ± 220
MTQ339	3	VHL	2	59 ± 20	>3200	640 ± 319
MTQ340	3	VHL	3	54 ± 12	>3200	601 ± 61
MTQ341	3	VHL	4	50 ± 11	>3200	642 ± 287
MTQ342	3	VHL	5	36 ± 9	>3200	506 ± 203
MTQ343	3	CRBN	1	26 ± 9	>3200	1535 ± 382
MTQ344	3	CRBN	2	26 ± 8	>3200	542 ± 113
MTQ345	3	CRBN	3	34 ± 12	>3200	430 ± 107
MTQ346	3	CRBN	4	40 ± 11	>3200	287 ± 49
MTQ347	3	CRBN	5	30 ± 7	>3200	368 ± 57

Table 1. (continued)

The binding affinity of synthesized PROTACs including their precursors are assessed by a competitive binding fluorescence polarization assay (FP assay)[9]. A bicyclic FKBP ligand linked to a 5-TAMRA fluorophore was used as the tracer **MTQ238**. The binding affinity was measured by the competition ability of ligand with the tracer for FKBPs[9, 55].

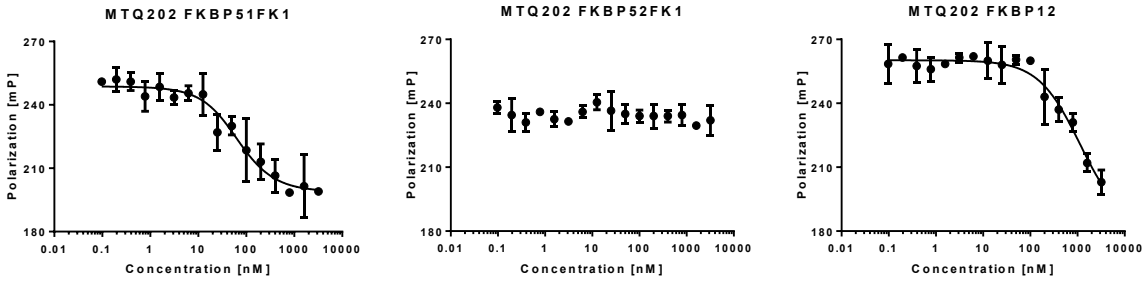


Figure 21: Biochemical characterization of **MTQ202**.

As shown in **table 1**, most of the SAFit analogues and their corresponding PROTACs derivatives retained the binding ability but partially lose their affinity for both FKBP51 and FKBP12 compare to the prototype compounds SAFit1 and SAFit2. All of them maintain the high selectivity for FKBP51 over FKBP52. The three propargyl SAFit analogues, **16**, **31a** and **31b**, exhibited 3 to 13-fold decrease in binding affinity compared to SAFit2 (K_i = 11 nM in the same assay). Interestingly, after coupling to the linker and E3 ligase motif, the resulting triazole product shows a higher affinity for FKBP51 and FKBP12 compare to its precursor. Conjugation on the attachment point 1 gives better affinity than the other two attachment points. Compared

to VHL-based PROTACs, CRBN-based PROTACs exhibit higher affinity for both FKBP51 and FKBP12. Generally, a longer linker leads to a better binding affinity, indicating a modest steric hindrance around the attachment point.

Among all of the PROTACs, **MTQ208** shows the most potent binding affinity with K_i value of 5 nM which is comparable to SAFit1.

3.3 Bicyclic ligand based PROTACs

3.3.1 Design of bicyclic ligand-based PROTACs

Knocking down FKBP51 or FKBP52 by chemical approach specifically remains a topic of interest for therapeutic concerns. So far, SAFits are the only known potent inhibitors for FKBP51 with high selectivity. Unfortunately, the specific inhibition of FKBP52 by a classic small molecule ligand is currently impossible due to lack of a selective ligand for FKBP52 over FKBP51. As described before, additional selectivity is achievable by the cooperative interactions within target protein-PROTAC-E3 ligase ternary complex. Thus, integrating a non-selective FKBPs ligand into a PROTAC may induce the selectivity for a certain FKBP. Therefore, we designed a series of PROTACs with [4.3.1] bicyclic FKBPs ligand as the POI recognition motif, expecting to turn a non-selective ligand to a selective protein degrader for FKBP51 or FKBP52.

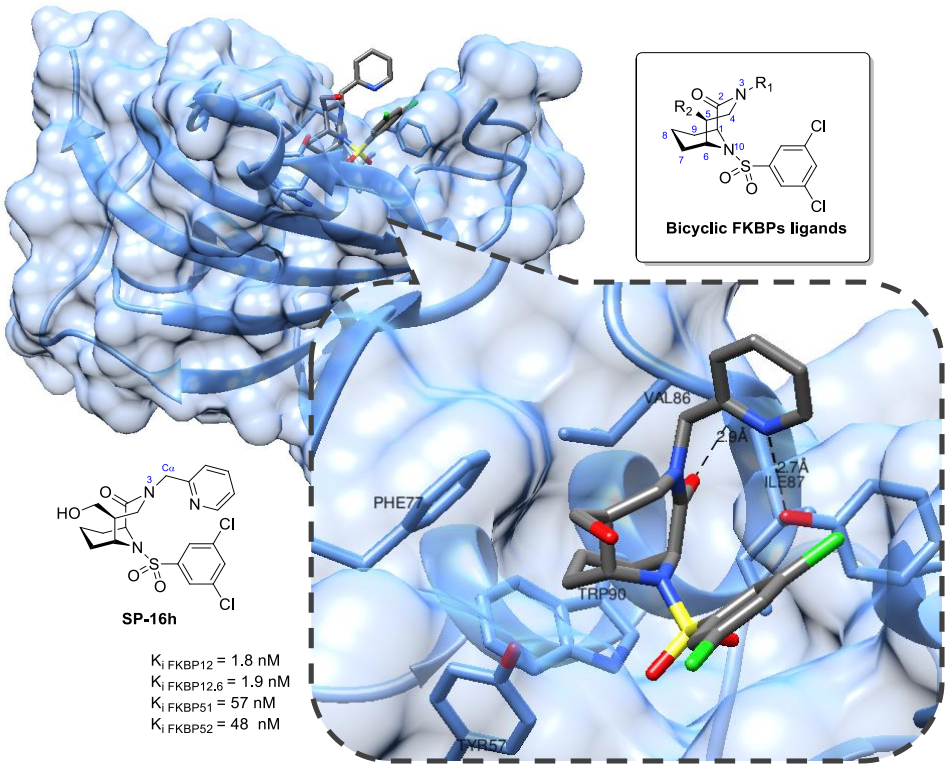


Figure 22: The [4.3.1] bicyclic ligands and the co-crystal structure of **SP-16h** in complex with FKBP51 (PDB ID: 5OBK)

The [4.3.1] bicyclic 3,5-dichlorophenylsulfonamides have shown high potency in the nanomolar to subnanomolar concentration range against 11 different human and microbial FKBPs, including FKBP12, FKBP12.6, FKBP51 and FKBP52[55]. Upon analyzing the binding mode of bicyclic ligand **SP-16h** with FKBP51, we found the pyridine substituent on N^3 formed a key hydrogen bond with Try113, indicating the Cα of the substituent is an ideal attachment point for the conjugating to PROTAC molecule due to the similarity between the pyridine group and the triazole group obtained via click reaction. To tether the bicyclic scaffold to an E3 ligase ligand, a propargyl group is introduced as the substituent on N^3 to conjugate to the linker via click reaction. As shown in **Figure 23**, the triazole derivative **SP-11v**, a bicyclic ligand with high similarity to our products resulted from a click reaction and showed moderate to high affinities for several FKBPs ($K_{i\ FKBP12}$ = 5 nM, $K_{i\ FKBP12.6}$ = 36 nM, $K_{i\ FKBP51}$ = 1.1 μM, $K_{i\ FKBP52}$ = 0.5 μM). In addition, the replacement of the hydrogen at Cα with a (S)- or (R)-methyl group can sterically restrict the free rotation of the substituent, leading to a favorable binding conformation of the ligand in the active site.

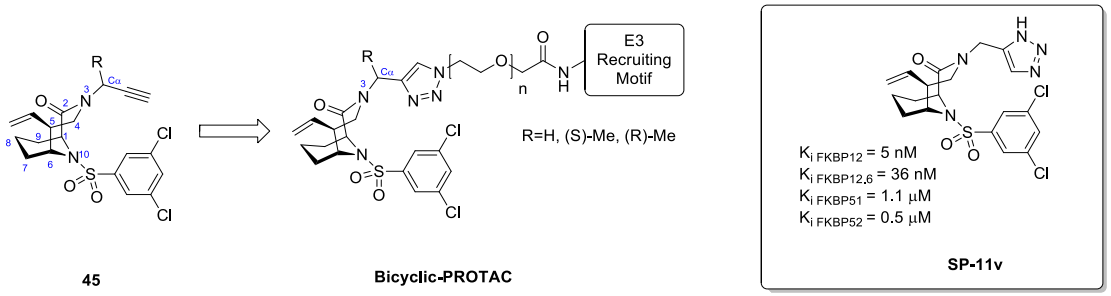
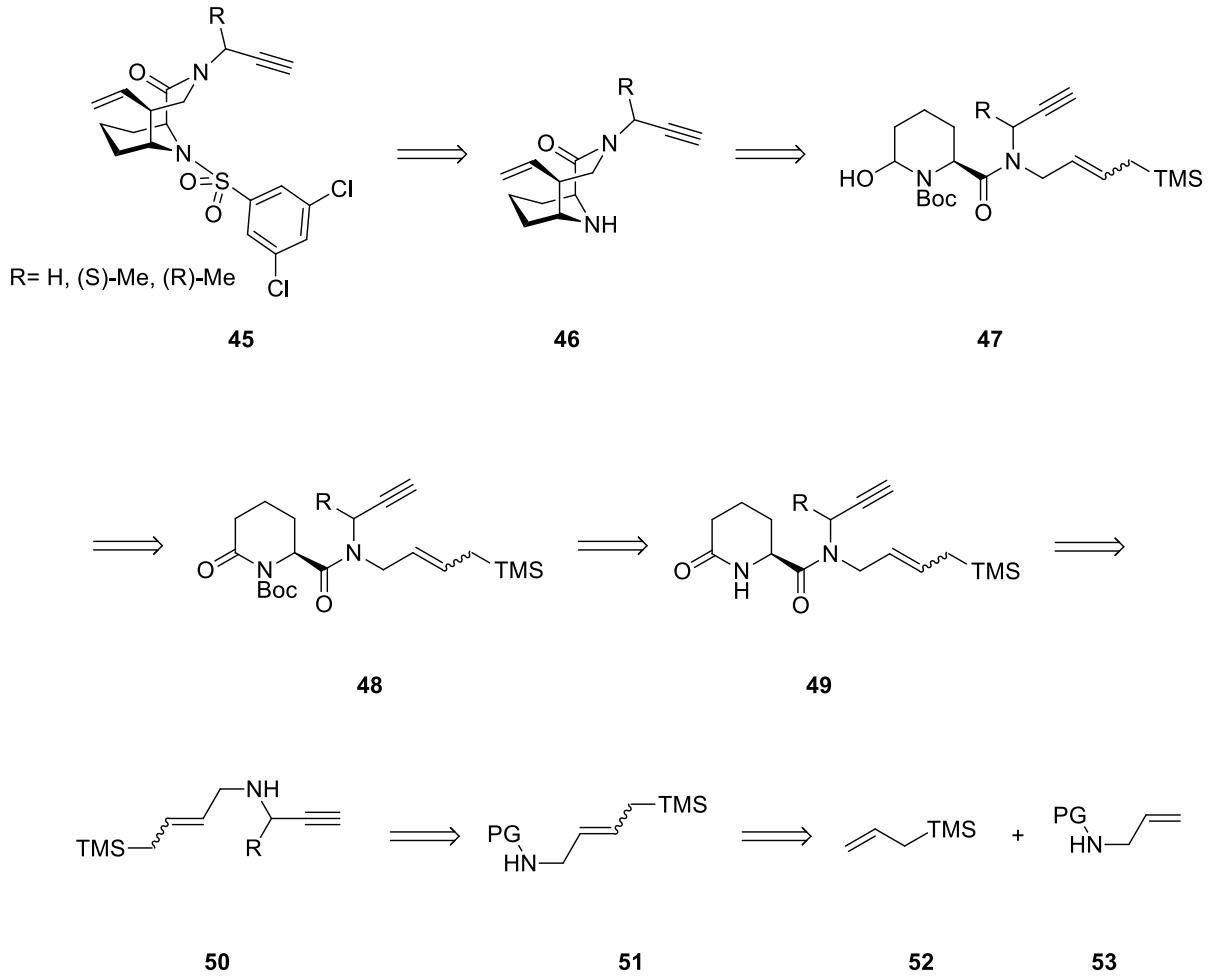


Figure 23: The PROTACs based on the bicyclic ligands. The similar ligand **SP-11v** showed high affinities for FKBP12/12.6 and moderate affinities for FKBP51/52.

3.3.2 Synthesis of bicyclic compounds

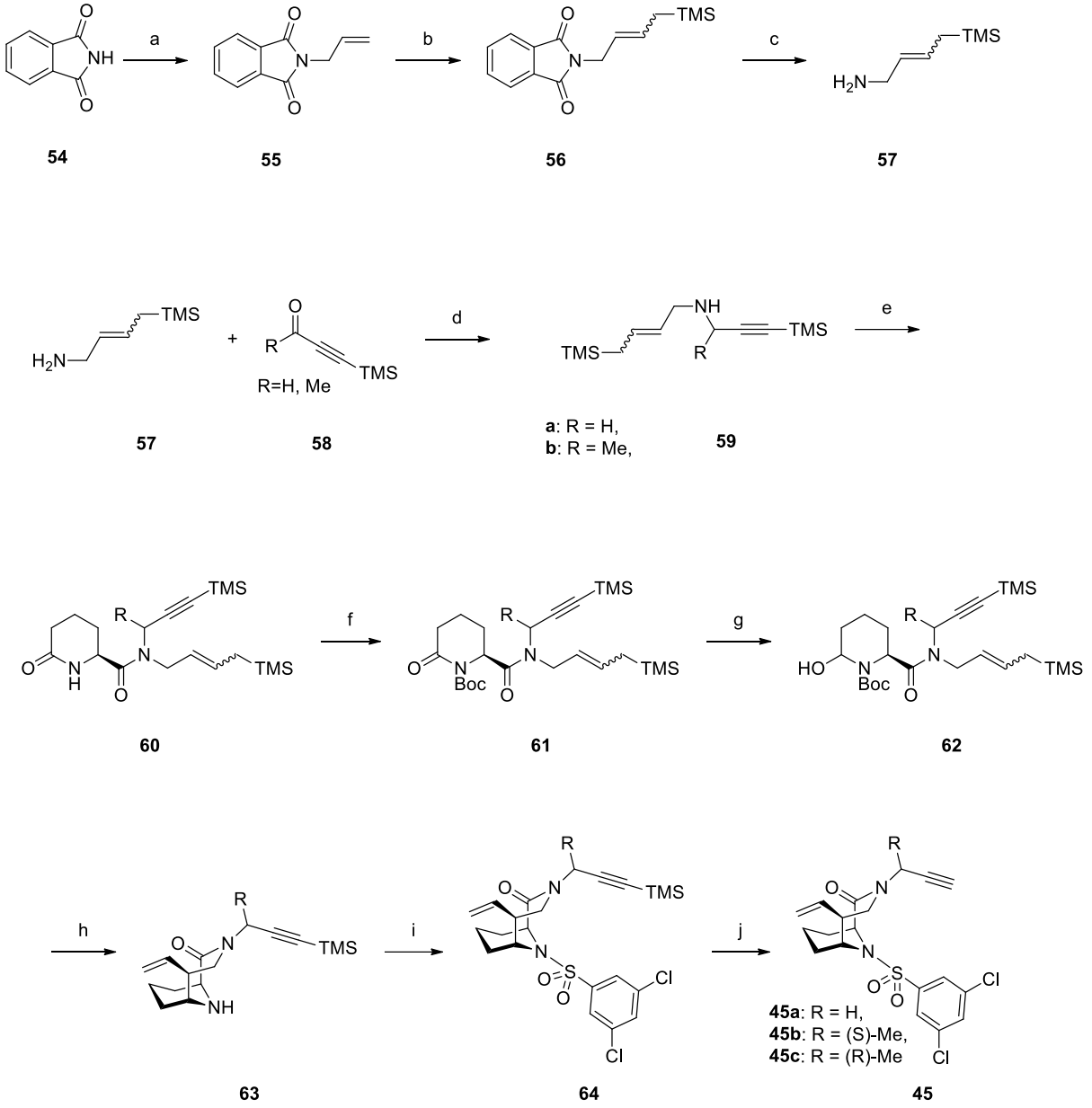
3.3.2.1 Retrosynthetic analysis and strategy



Scheme 18: Retrosynthesis of the bicyclic ligand precursor **45**.

The retrosynthesis of the derivatives **45** is outlined in Scheme **18**. The target molecule **45** could be synthesized by N-sulfonylation of **46** with 3,5-dichlorobenzenesulfonyl chloride. The formation of the bicyclic moiety of compound **46** was expected to be accessible from hydroxyl carbamate **47** and that the ring-closing reaction to be achieved throught the N-acyliminium cyclization reported by our group[54]. Amide bond formation between **50** and commercially available (S)-6-oxo-2-piperidinecarboxylic acid followed by Boc protection of the amide would generate **48**, which could undergo a selective reduction to give **47**. Compound **51** could be obtained by metathesis of allyl-TMS with protected allylamine.

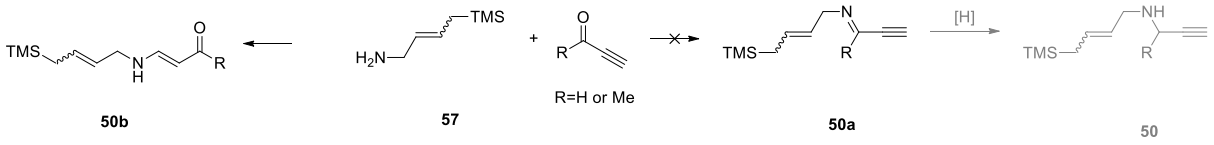
3.3.2.2 Synthesis



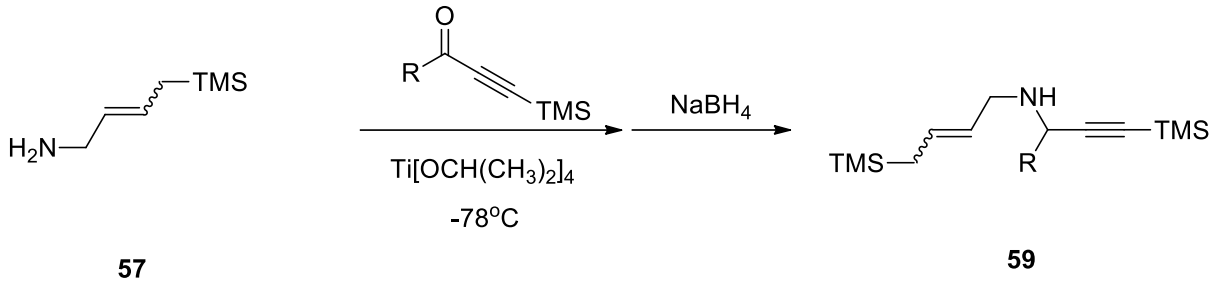
Scheme 19: Synthesis of the key intermediate **45a-c**. (a) K_2CO_3, allyl bromide, DMF, RT, 3 h, 99%; (b) allyl-TMS, Grubbs I, DCM, 60 °C, 4 h, 66%; (c) NH_2NH_2, MeOH, 70 °C, 24 h; (d) Titanium(IV) isopropoxide, EtOH, -78°C, 5h; then $NaBH_4$, -78°C → RT, 1h, 64-68%; (e) (S)-6-Oxopiperidine-2-carboxylic acid, HATU, DIPEA, RT, DMF, 2 h; (f) Boc_2O, DIPEA, DMAP, DCM, RT, overnight, 60% for two steps; (g) DIBAL, THF, −78 °C, 15 min; (h) HF-pyridine, DCM, −78 °C, 1 h, 50-64% for two steps; (i) 3,5-Dichlorobenzenesulfonyl chloride, DIPEA, MeCN, RT, overnight; (j) K_2CO_3, MeOH, 1.5h, 50-58% for two steps.

On the basis of our previous research work, we developed the synthesis for N-propargyl bicyclic compounds[54, 55]. The synthesis commenced with allylation of phtalimide. The resulting allyl-

phtalimide, **55**, was transformed by metathesis with allylTMS and Grubbs first-generation catalyst and subsequently deprotected to afford the amine **57**.



Scheme 20: Undesired 1,4-Michael addition product.



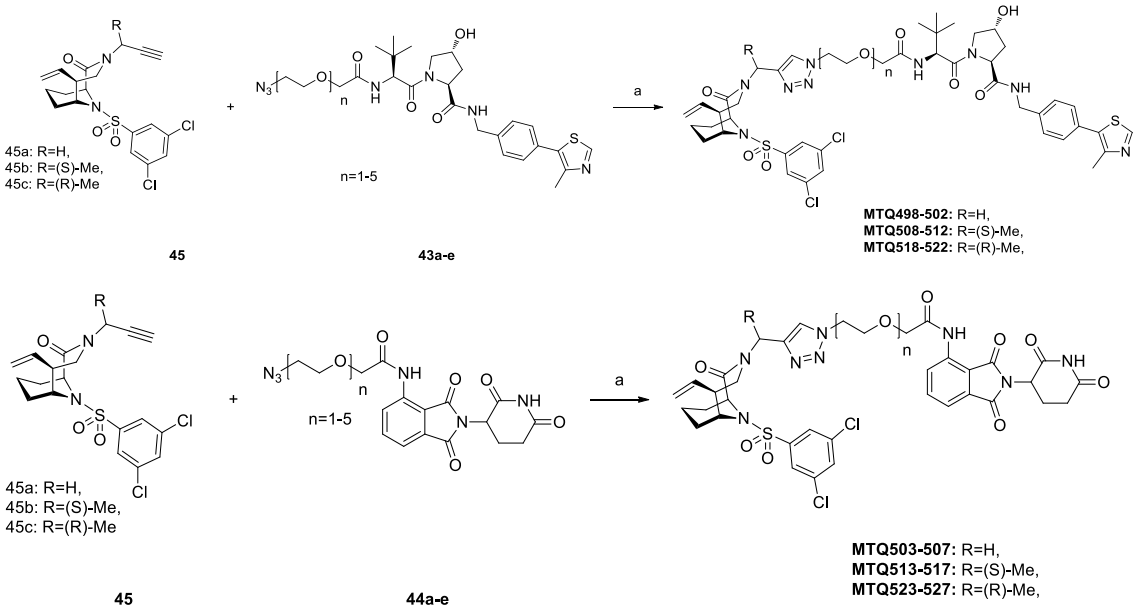
Scheme 21: Synthesis of **59** via promoted reductive amination

Unfortunately, exposure of amine **57** to acetylenic ketone under room temperature resulted in rapid formation of the 1,4-Michael addition product **50b**.

Thus, we use titanium(IV) isopropoxide and TMS-protected alkynyl ketone or aldehyde to promote the reductive amination of ketone to avoid the undesirable byproduct[106].

The resulting building block **59** was reacted with commercially available (S)-6-oxo-2-piperidinecarboxylic acid and subsequently Boc-protected to furnish **61**, the precursor for the key chemoselective reduction and asymmetric N-acyliminium cyclization. The S/R methyl diastereomers could be separated by chromatography after Boc protection. The chemoselective reduction of **61** gave instable intermediate hydroxyl carbamate **62**, which was immediately subjected to cyclization by the treatment with hydrofluoric acid in pyridine resulted in bicyclic building block **63**. Surprisingly, the trimethylsilylacetylenic group survived the HF- pyridine treatment. Reaction of **63** with dichlorphenyl sulfonyl chloride followed by deprotection furnished the desired N-propargyl bicyclic compound **45**.

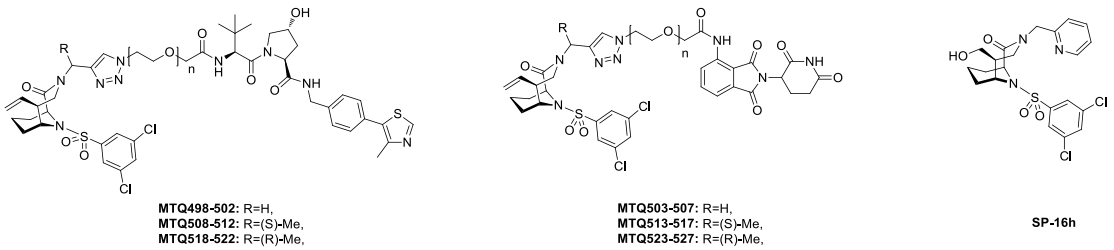
Like for the synthesis of SAFit-based PROTACs, the bicyclic PROTACs were obtained by the Cu-catalyzed click reaction between the propargyl FKBPs ligand and the azido-PEG-E3 ligase ligand.



Scheme 22: Synthesis of the bicyclic PROTACs. (a) $CuSO_4$, (+)-Sodium L-ascorbate, t-BuOH/H_2O/DMSO, RT, overnight, 47-97%.

3.3.3 Biochemical characterization

3.3.3.1 Evaluation of binding affinity



Nr.	R	E3-ligase	linker length	K_i (nM)			
				FKBP51FK1	FKBP52FK1	FKBP12	FKBP12.6
SP-16h[55]	/	/	/	57 ± 5	48 ± 3	1.8 ±0.1	1.9 ± 0.3
45a	H	/	/	544 ± 95	383 ± 83	36 ± 5	467 ± 41
45b	(S)-Me	/	/	2157 ± 738	1364 ± 422	150 ± 34	904 ± 73
45c	(R)-Me	/	/	3312 ± 1436	2923 ± 826	217 ± 31	1657 ± 115
MTQ498	H	VHL	1	80 ± 16	115 ± 29	4.7 ± 0.4	67 ± 5
MTQ499	H	VHL	2	8.8 ± 2.5	11 ± 3	0.1 ± 0.03	0.75 ± 0.2
MTQ500	H	VHL	3	20 ± 5	26 ± 7	0.6 ± 0.1	10 ± 0.8
MTQ501	H	VHL	4	31 ± 5	55 ± 11	1.4 ± 0.2	19 ± 1

Table 2. Binding affinities of bicyclic PROTACs for FKBP51, FKBP52 and FKBP12. The FP-assays were performed by Stephanie Merz and Christian Meyners.

Nr.	R	E3-ligase	linker length	K_i (nM)			
				FKBP51FK1	FKBP52FK1	FKBP12	FKBP12.6
MTQ502	H	VHL	5	18 ± 3	29 ± 8	1.6 ± 0.3	16 ± 1
MTQ503	H	CRBN	1	8 ± 1	1.9 ± 0.5	0.2 ± 0.03	0.46 ± 0.1
MTQ504	H	CRBN	2	25 ± 6	43 ± 11	2.1 ± 0.2	17 ± 1.3
MTQ505	H	CRBN	3	17 ± 4	16 ± 5	2.4 ± 0.2	21 ± 2
MTQ506	H	CRBN	4	28 ± 5	30 ± 8	1.5 ± 0.2	19 ± 2
MTQ507	H	CRBN	5	12 ± 2	60 ± 12	0.7 ± 0.1	10 ± 1.2
MTQ508	(S)-Me	VHL	1	5.3 ± 1.5	6.3 ± 2	0.6 ± 0.1	2.5 ± 0.6
MTQ509	(S)-Me	VHL	2	0.9 ± 0.2	0.23 ± 0.5	0.01 ± 0.005	0.05 ± 0
MTQ510	(S)-Me	VHL	3	1.2 ± 0.3	2.9 ± 1.2	0.2 ± 0.02	1.9 ± 0.3
MTQ511	(S)-Me	VHL	4	1.1 ± 0.4	2.2 ± 0.9	0.1 ± 0.03	1.5 ± 0.3
MTQ512	(S)-Me	VHL	5	1.9 ± 0.5	1.7 ± 0.6	0.2 ± 0.02	1.7 ± 0.3
MTQ513	(S)-Me	CRBN	1	2.3 ± 0.4	0.7 ± 0.3	0.2 ± 0.02	0.41 ± 0.2
MTQ514	(S)-Me	CRBN	2	4.4 ± 1.2	5.7 ± 1.7	0.4 ± 0.1	2.4 ± 0.4
MTQ515	(S)-Me	CRBN	3	3.2 ± 0.9	5.0 ± 1.2	0.5 ± 0.1	4.8 ± 0.5
MTQ516	(S)-Me	CRBN	4	4.2 ± 0.8	8.4 ± 2.3	0.5 ± 0.1	3.7 ± 0.5
MTQ517	(S)-Me	CRBN	5	2.1 ± 0.4	4.9 ± 1.1	0.4 ± 0.05	1.5 ± 0.3
MTQ518	(R)-Me	VHL	1	676 ± 275	1619 ± 639	95 ± 8	678 ± 75
MTQ519	(R)-Me	VHL	2	745 ± 108	1726 ± 274	131 ± 13	454 ± 39
MTQ520	(R)-Me	VHL	3	355 ± 58	1513 ± 314	95 ± 9	385 ± 34
MTQ521	(R)-Me	VHL	4	317 ± 34	772 ± 174	67 ± 10	279 ± 25
MTQ522	(R)-Me	VHL	5	357 ± 85	609 ± 133	54 ± 5	294 ± 29
MTQ523	(R)-Me	CRBN	1	506 ± 313	1333 ± 214	84 ± 9	533 ± 57
MTQ524	(R)-Me	CRBN	2	387 ± 74	539 ± 93	62 ± 5	515 ± 40
MTQ525	(R)-Me	CRBN	3	590 ± 387	1685 ± 284	108 ± 14	675 ± 76
MTQ526	(R)-Me	CRBN	4	357 ± 48	817 ± 307	91 ± 10	360 ± 31
MTQ527	(R)-Me	CRBN	5	537 ± 68	783 ± 240	158 ± 13	622 ± 51

Table 2. (continued)

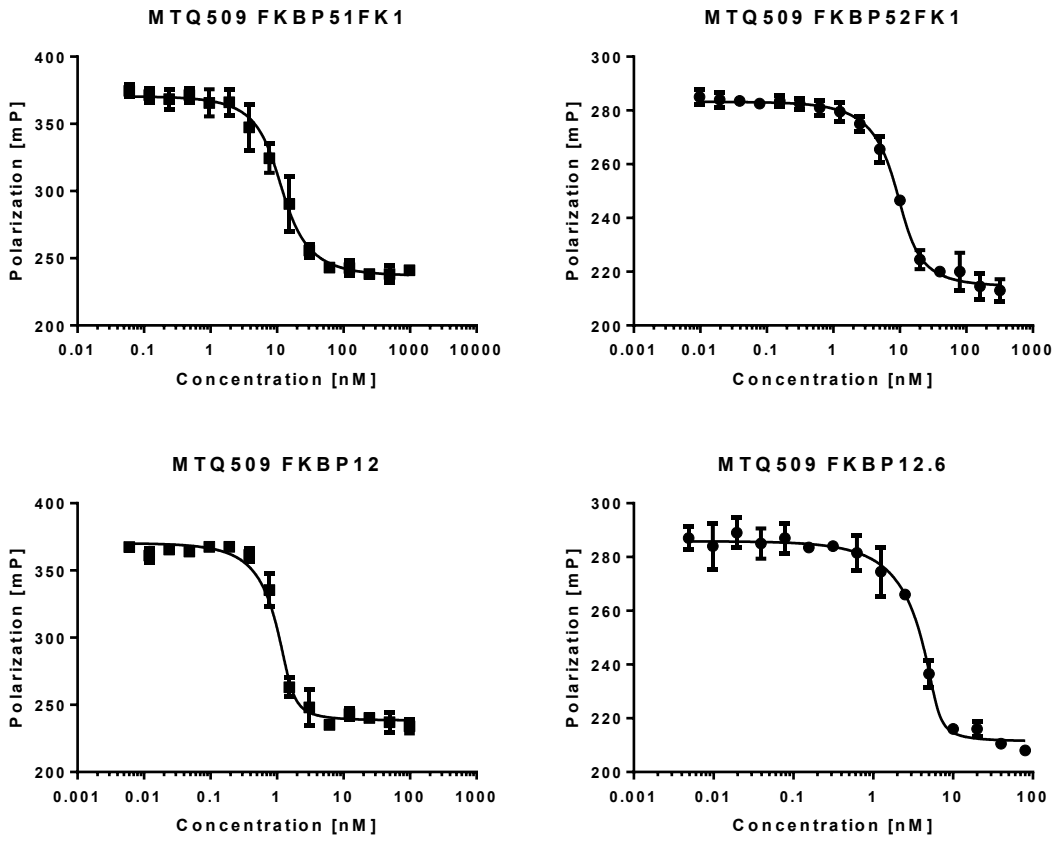


Figure 24: Biochemical characterization of **MTQ509**.

Compared to its pyridine analogue **SP-16h**[55] ($K_{i\ FKBP51}$ = 57 ± 5 nM, $K_{i\ FKBP52}$ = 48 ± 3 nM, $K_{i\ FKBP12}$ = 1.8 ± 0.1 nM), the N-propargyl bicyclic compounds **45a**, **45b** and **45c** showed quite weak binding affinity in the low micromolar to high nanomolar range for FKBPs. Surprisingly, upon conjugating with azido analogues by click reaction, most of the resulting PROTACs resume the potent affinities for all of FKBPs tested, indicating a heterocycle is essential for high affinity binding. With the (S)-methyl group as R substituent, the PROTACs **MTQ508-517** showed outstanding affinities for all of the tested FKBPs in the low nanomolar range to subnanomolar range. For the PROTAC series without methyl substituent, compounds **MTQ498-507** displayed high binding affinities, which are comparable to the pyridine bicyclic analogue. Where R = (R)-methyl group, the PROTACs **MTQ518-527** showed submicromolar affinities for FKBP51/52 and moderate affinities for FKBP12, indicating an R-configuration substituent is unfavorable. For the VHL-based PROTACs, the molecule with the shortest linker always shows the worst binding affinity compare to the other analogues. When the linker has two PEG units, the PROTAC exhibits the best affinity in the series. Then, with the extension of the linker length, the affinities are deceased gradually. For the CRBN-based PROTACs, the shortest linker affords the most potent compound.

3.4 Evaluation of degradation of FKBP51

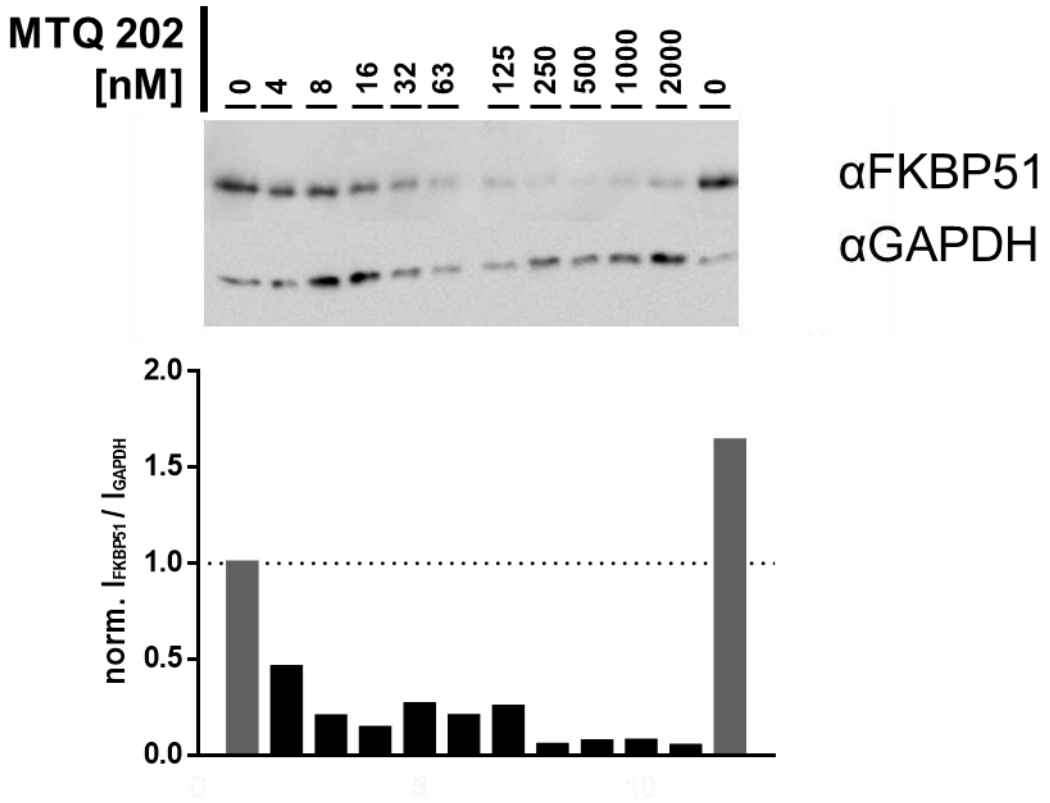


Figure 25. Western blotting showed the degradation of FKBP51 by the treatment of MTQ202 in HEK293T cells.

To profile the degradation activity of our PROTACs, the levels of FKBP51 in several cell lines were monitored by Western blot analysis using GAPDH as the control. Andreas Hähle, Yannick Kristiansen and Thomas Geiger contributed to these experiments.

After 24h incubation time, degradation of FKBP51 in HEK293T cell line was observed with **MTQ202**, the VHL-based PROTAC with 4-PEG-linker and attachment point 1, in the concentration range of 63 to 2000 nM, while no obvious degradation of FKBP51 was observed with any other PROTACs tested.

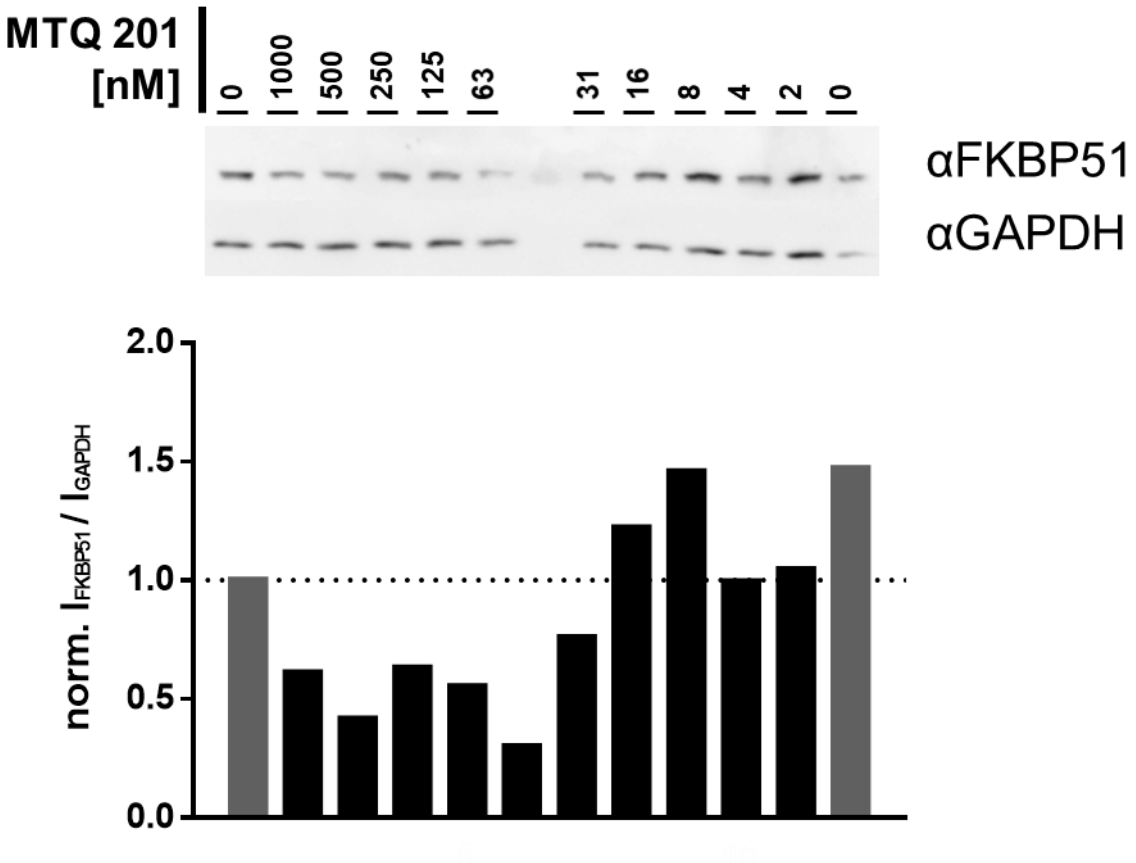


Figure 26. Western blotting has not shown clear evidence of the degradation of FKBP51 by the treatment of MTQ201 in HEK293T cells.

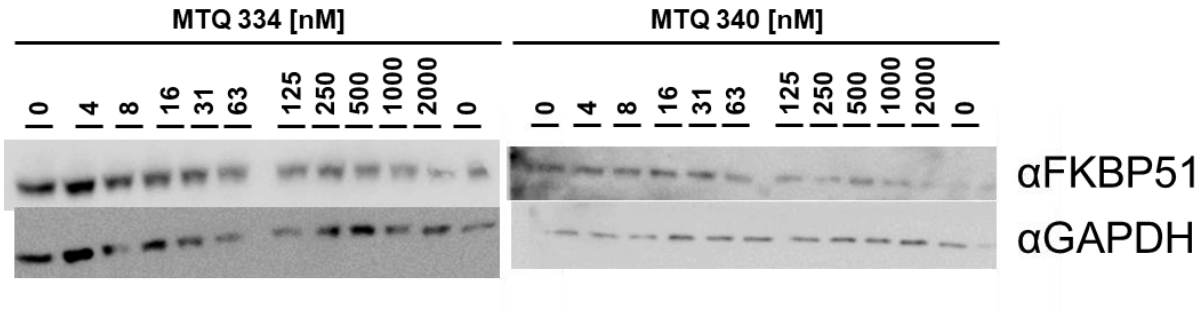


Figure 27. Degradation of FKBP51 has not been observed by the treatment of other SAFit-based PROTACs with various attachment positions and linker lengths in HEK293T cells.

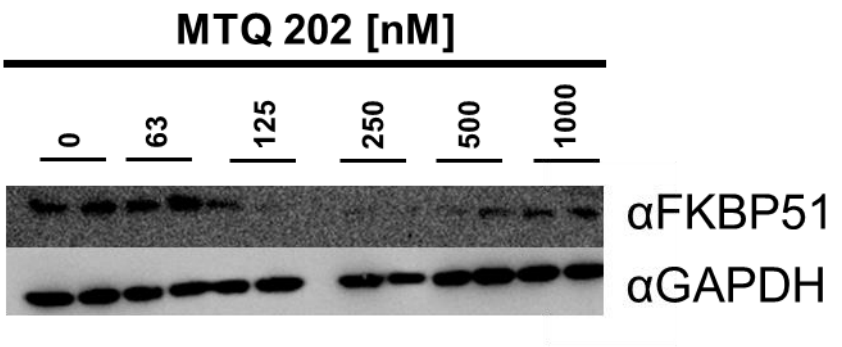


Figure 28. MTQ202 was able to induce the degradation of FKBP51 in Hela cells.

Degradation of FKBP51 with **MTQ202** was also observed in Hela cells. As shown above, the level of FKBP51 starts to decrease with 125 nM **MTQ202**. The re-appearance of protein levels upon treatment of high concentrations of **MTQ202** was likely due to the “hook effect", where the high concentration of the PROTAC consumes E3 ligase and POI to form PROTAC-E3 and PROTAC-POI binary complexes, thereby disrupting the desired E3-PROTAC-POI ternary complexes[79].

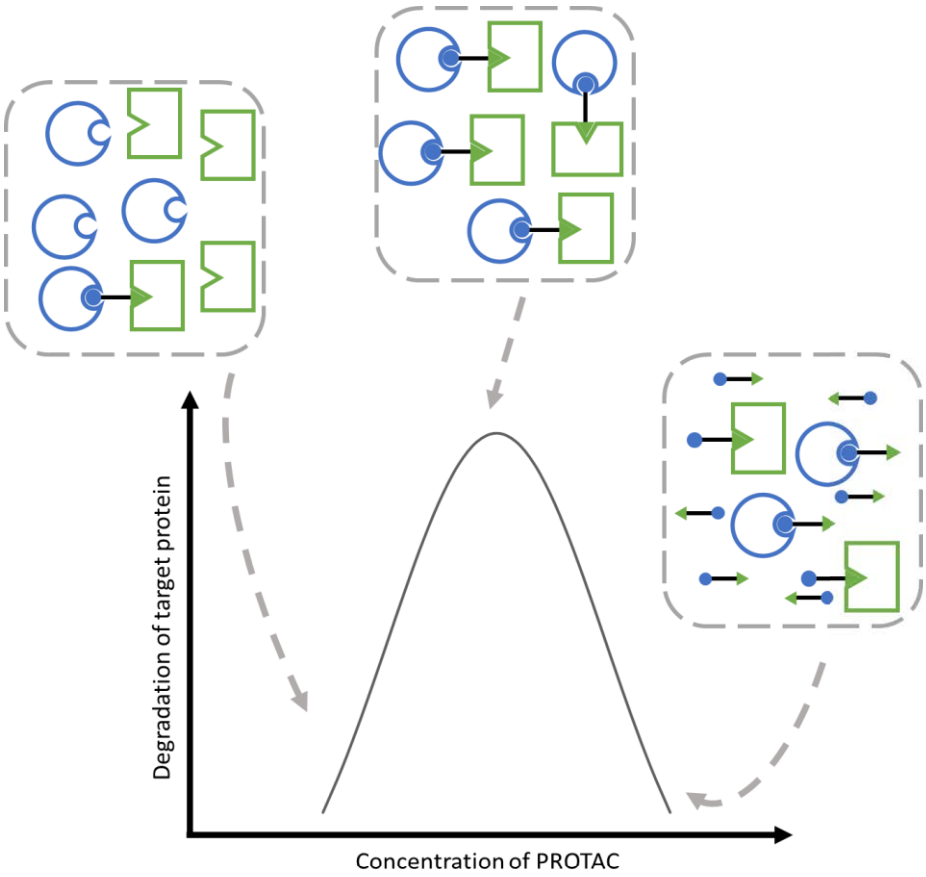


Figure 29. The "hook effect" of PROTACs.

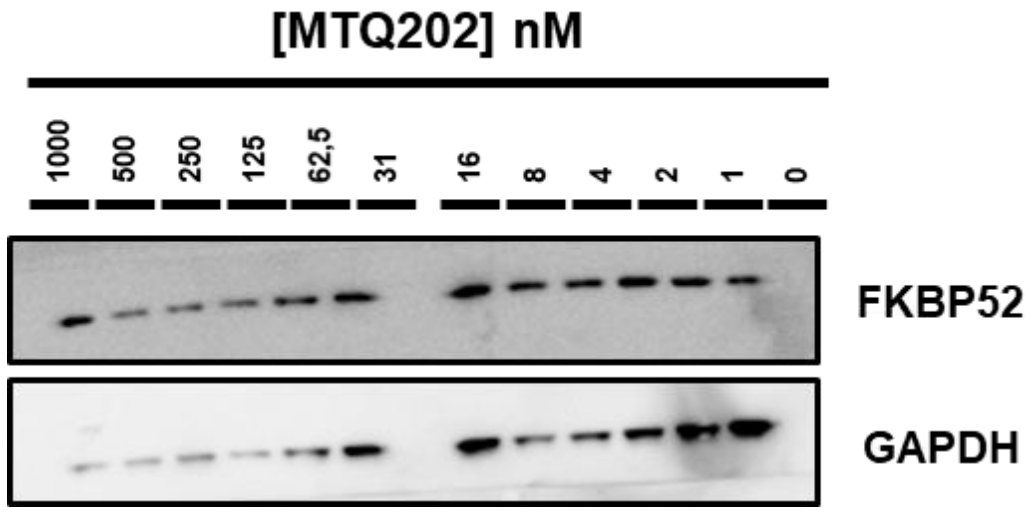


Figure 30. No obvious degradation of FKBP52 was shown in western blotting by the treatment of MTQ202 in HEK293 cells.

As expected, **MTQ202** was not able to reduce the concentraction of FKBP52 due to the lack of affinity for FKBP52.

Among all the PROTACs I synthesized, the degradation of FKBPs was only observed with the SAFit-based PROTAC **MTQ202**. Either one PEG unit shorter (**MTQ201**) or longer (**MTQ203**) in the PROTAC with same POI ligand and E3 ligase ligand did not show a slight effect on the degradation. This result indicated the impact of the linker length for the ability of a PROTAC. A linker, which is too short might lead to a preference of forming dimer complex (PROTAC-protein or PROTAC-E3), which in turn prevents the formation of protein-PROTAC-E3 ligase ternary complex. Inversely, a longer which is too long would confer excess steric freedom, leading to the failure of stabilizing protein-protein interaction.

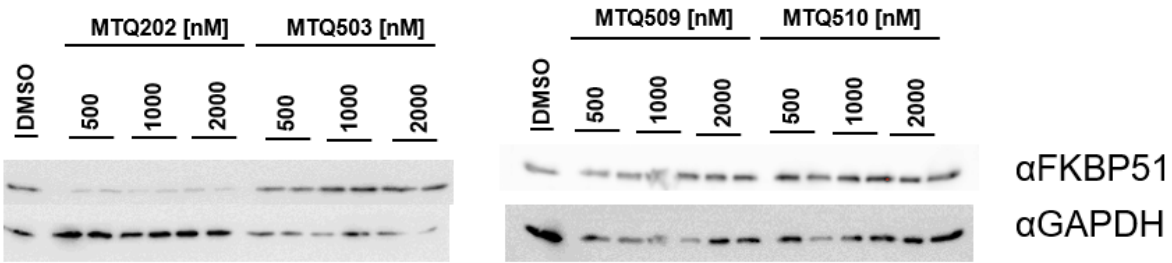


Figure 31. Bicyclic-based PROTACs with high affinities for FKBP51 have not exhibited abilities to degrade FKBP51 in HEK293T cells.

Although parts of the bicyclic ligand-based PROTACs possess potent affinity for FKBPs, none of them showed activity to promote the protein degradation in western blotting so far, indicating binding affinity is not the only influence factor of PROTACs.

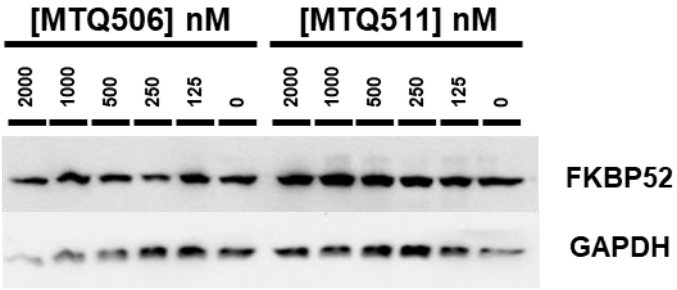


Figure 32. FKBP52 was not degraded in HEK293 cells by the treatment of bicyclic-based PROTACs with affinities for FKBP52.

3.5 Improvement of PROTACs by conformational restriction

3.5.1 Design

Among the PROTACs I made, SAFit-based PROTAC **MTQ202** shows a moderate ability to induce degradation of our target protein FKBP51. Although it provides a proof of principle for a chemical knockout strategy, our PROTACs had to become more potent to provide an efficient tool for degrading FKBP51.

In an effort to enhance the degrading capability of **MTQ202**, we designed a series of conformationally restricted linker by methylation. By the addition of a methyl group to a carbon on the linker of **MTQ202**, the conformations the linker can adopt will be restricted due to the rotational preferences. The resulting spatial constraints can induce a preferred conformation of protein-protein interaction between E3-E2-ubiquitin complex and FKBP51, leading to a more efficient degradation of the target protein[107].

3.5.2 Retrosynthetic analysis and strategy

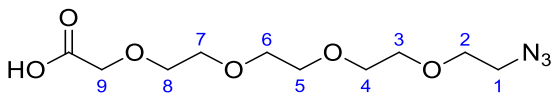


Figure 33. Potential positions for modification.

As shown above, 9 carbon positions on the PEG chain of the MTQ202-linker are possible to be modified by a methyl group substituent, which can be allocated into two groups: the methyl

group locates on the odd number of carbon (X = 1, 3, 5, 7, 9) or the even number of carbon (X = 2, 4, 6, 8). For each position, two configurations ((S)- or (R)-) of methyl group are possible.

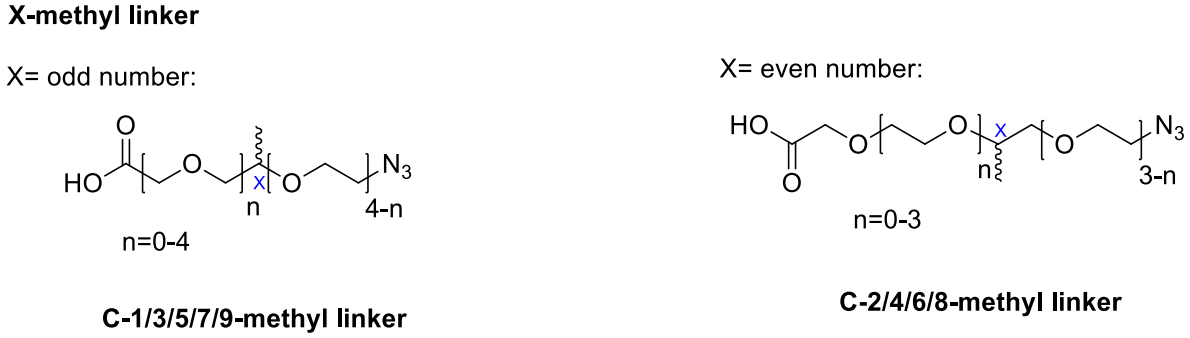
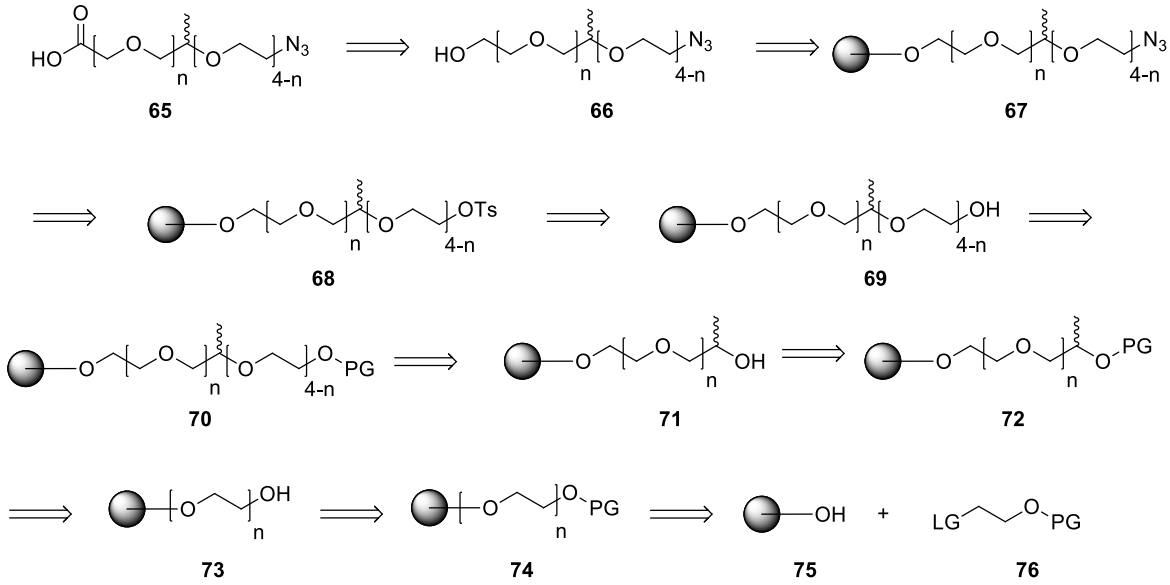


Figure 34. Potential positions for methylation.

3.5.2.1 Odd numbered methyl linker

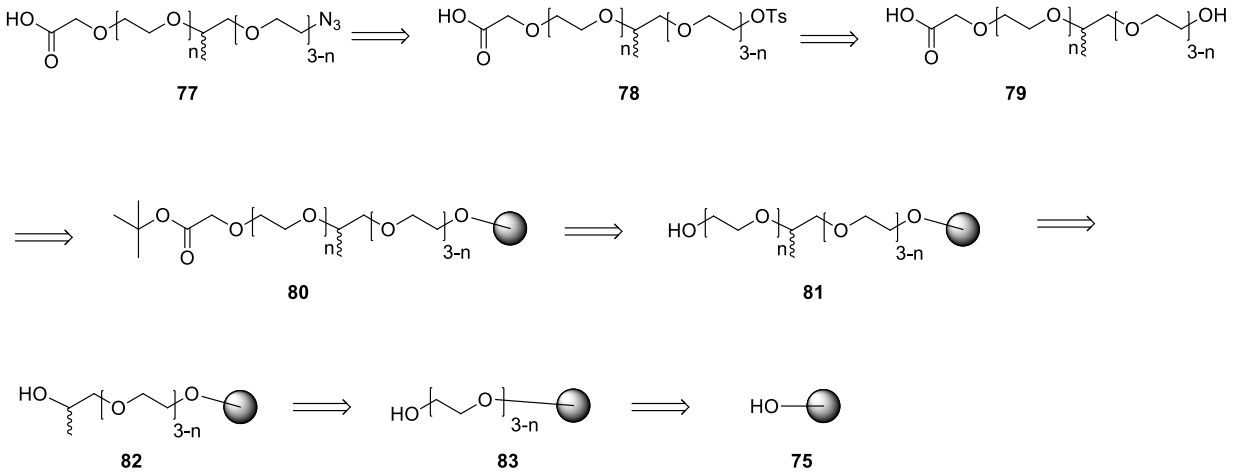


Scheme 23: Retrosynthesis of the odd-numbered methyl linkers **65**.

The retrosynthesis of the odd-numbered methyl linkers **65** is outlined in **Scheme 23**. The target azido acid **65** can be obtained by the oxidation of azido alcohol **66**. According to our synthesis route of the linkers, the azido group can be introduced by the nucleophile substitution of a tosyl group, which can be obtained by the tosylation of a hydroxyl group. This indicates the key intermediate is a diol, which require regioselectivity in the reactions. To synthesize PEG linkers with methyl groups at different positions systematically, we considered to employ a solid phase synthetic approach to extend the linker from one end to the other by adding building blocks. Thus, the hydroxyl group of **66,** which will be oxidized to carboxylic acid is inactivated by

attaching to the solid support. The extension of the linker chain can be achieved by repeated substitution reactions of an electrophile containing a protected terminal hydroxyl group, followed by deprotection to expose the new hydroxyl group at the growing end.

3.5.2.2 Even numbered methyl linker



Scheme 24: retrosynthesis of the even-numbered methyl linker **77**.

Similar to the synthesis route above, the even-numbered methyl linker can be obtained by the solid-phase synthesis but starts at another end. The hydroxyl group which will be tosylated should be attached to the resin to allow the introduction of chiral methyl group and the carboxylic acid group.

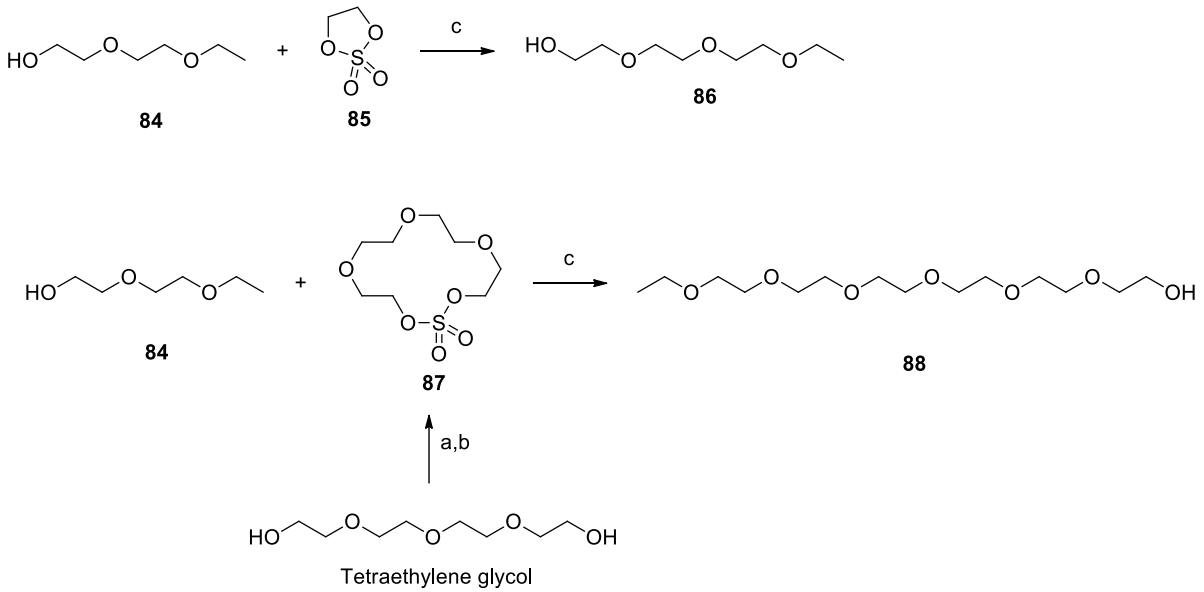
3.5.3 Optimization of synthesis conditions in liquid phase

Although PEGs are a widely utilized polymer in drug development and nanotechnology due to its biocompatibility, an efficient PEG-chain elongation remains a challenge due to the low yield of the available ether synthesis methods.

Regarding the difficulties of monitoring the reaction progress and overall yield, the synthesis route and conditions were firstly optimized in liquid phase. Diethylene glycol monoethyl ether (**84**), a 2-unit PEG chain with one end capped by ethyl, was employed as the substitute for the intermediate bound to resin.

First, we investigated the feasibility of using the cyclic sulfate as the suitable building block to elongate the PEG chain. Cyclic sulfates have been widely utilized in the monofunctionalization of diols, where the sulfate group acts as both the leaving group during the nucleophilic reaction and the protection group of hydroxyl group after the ring-opening reaction. Furthermore, cyclic sulfates can be prepared conveniently by the cyclization of low-cost polyethylene glycol with

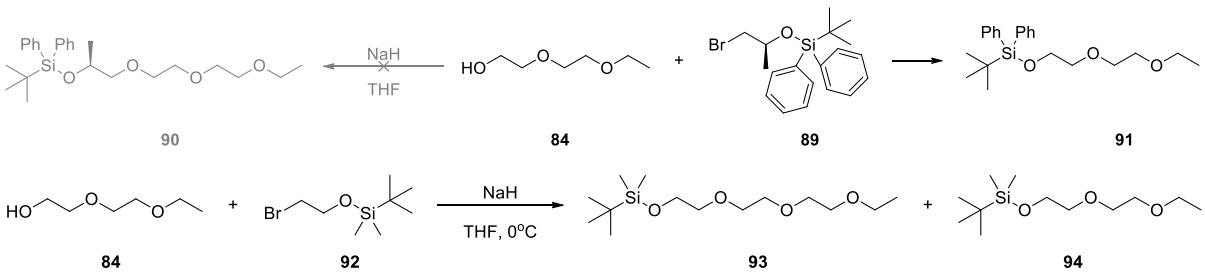
thionyl chloride in the presence of TEA and DMAP followed by the oxidation to corresponding sulfate[108].



Scheme 25: The ring-opening reaction of the cyclic sulfate **85** or **87** with diethylene glycol monoethyl ether **84**. (a) $SOCl_2$, DIPEA, DMAP, DCM, 0°C, 2h, 68%; (b) $NaIO_4$, $RuCl_3$, DCM/MeCN/H_2O = 2/2/3, 0°C, 1h, 92%; (c) NaH, THF, 0°C, 6h; then 20% H_2SO_4/ dioxane = 1/2, 60°C, overnight. 85% for **86**, 92% for **88**.

Here, we employed the smallest and commercially available cyclic sulfates, 1,3,2-dioxathiolane 2,2-dioxide **85**, and the 14-membered cyclic sulfates **87** (with 4 PEG units) as the electrophiles to explore the utility. As shown above, after the substitution with diethylene glycol monoethyl ether **84** and the subsequent deprotection in acidic condition, the extended PEG chains were obtained with the yield of 85% and 92%, respectively. This shows the cyclic sulfate is an ideal building block to extend PEG chain into different lengths flexibly with high yield.

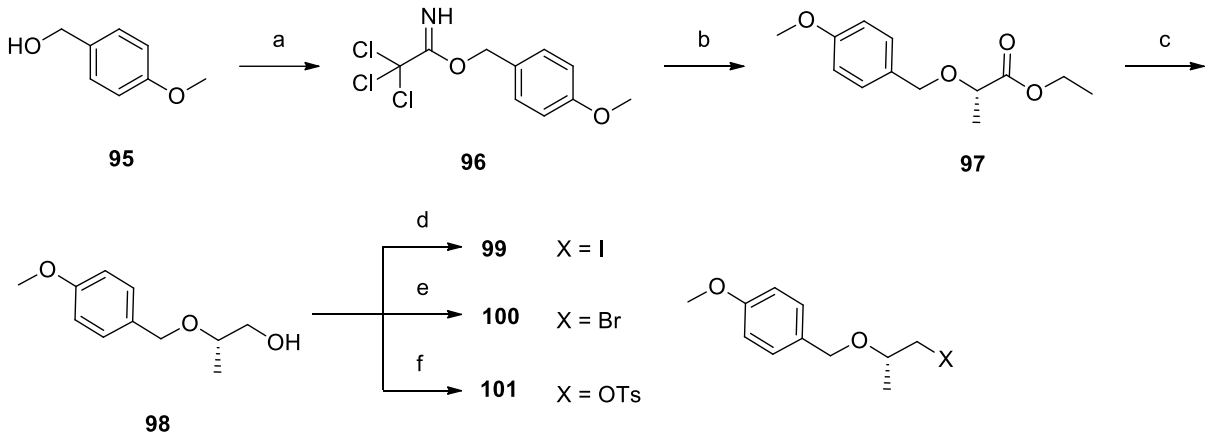
In order to introduce the asymmetric methyl group into our linker, we prepared several protected alcohols with different leaving groups.



Scheme 26: Side reactions of ether synthesis with TBDPS- or TBDMS-protected alcohols.

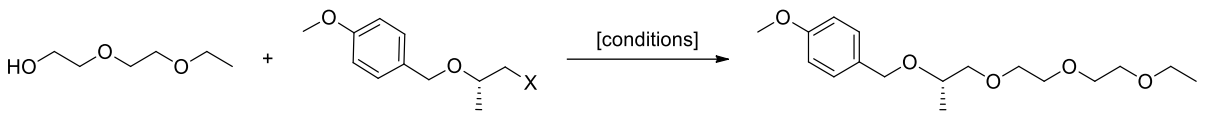
First, we tried to conjugate the diethylene glycol monoethyl ether with the TBDPS-protected alcohol **89** under the basic condition. Unfortunately, the undesired side product **91** was detected by LC-MS. A replacement of TBDPS group with TBDMS group did not prevent the side reaction.

After replacing the silyl protecting groups with the 4-methoxybenzyl group, the undesired side product was no longer detected by LC-MS, indicating a benzyl derivative is more suitable in this substitution reaction. To optimize the reaction condition, a series of 4-methoxybenzyl protected alcohols were synthesized and reacted with **84** at different conditions.



Scheme 27: The synthesis of 4-methoxybenzyl protected alcohols **99-101**. (a) NaH, Cl_3CCN, Et_2O 0°C → RT, 1.5h; (b) (-)-Ethyl L-lactate, D-(+)-10-camphorsulfonic acid, DCM, RT, overnight, 86% for 2 steps; (c) $LiAlH_4$, EtO_2, 0°C → RT, 87%; (d) I_2, PPh_3, imidazole, DCM, 0°C→RT, 1d, 73%; (e) CBr_4, PPh_3, DCM, 0°C → RT, 1d, 70%; (f) *p*-TsCl, TEA, DMAP, DCM, 0°C → RT, 1d, 71%.

As shown below, the best result was obtained in the presence of t-BuOK while a tosyl group was employed as the leaving group.

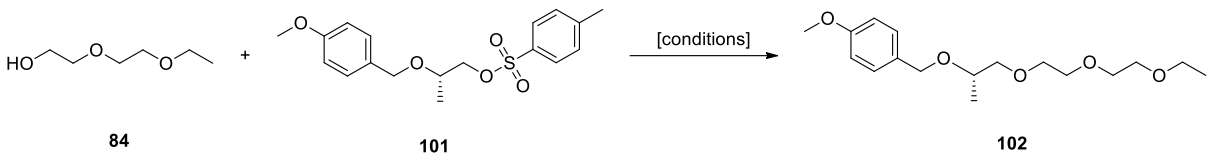


84 | **99 X = I**, **100 X = Br**, **101 X = OTs** | **102**

Entry	-X (3eq.)	base (2eq.)	% product (determined by LC, @220nm)
1	I	NaH	3
2	I	tBuOK	1
3	I	KOH	4
4	I	TEA	no reaction
5	I	DIPEA	no reaction
6	I	DBU	no reaction
7	Br	NaH	7
8	Br	tBuOK	11
9	Br	KOH	12
10	Br	TEA	no reaction
11	Br	DIPEA	no reaction
12	Br	DBU	no reaction
13	OTs	NaH	11
14	OTs	tBuOK	19
15	OTs	KOH	16
16	OTs	TEA	no reaction
17	OTs	DIPEA	no reaction
18	OTs	DBU	no reaction

Scheme 28: The screening of leaving groups and bases. General conditions: 1 eq. 2-(2-ethoxyethoxy)ethanol + 3 eq. R-X + 2 eq. base, THF, 60°C, 16h.

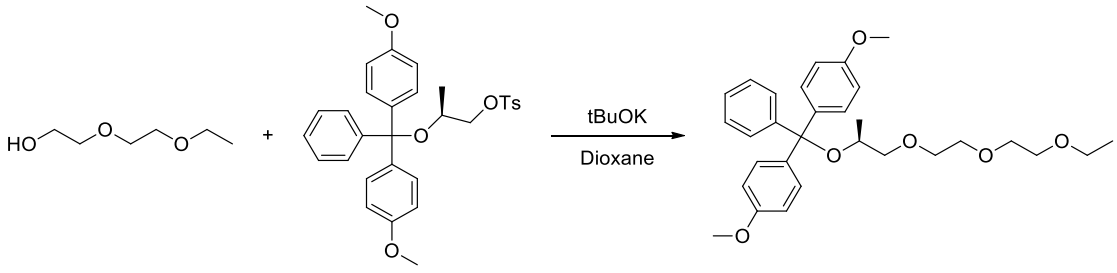
Using t-BuOK as the base, the reaction was performed in several different solvent systems. Among all of the solvents tested, dioxane gave the highest yield determined by LC.



84 101 102

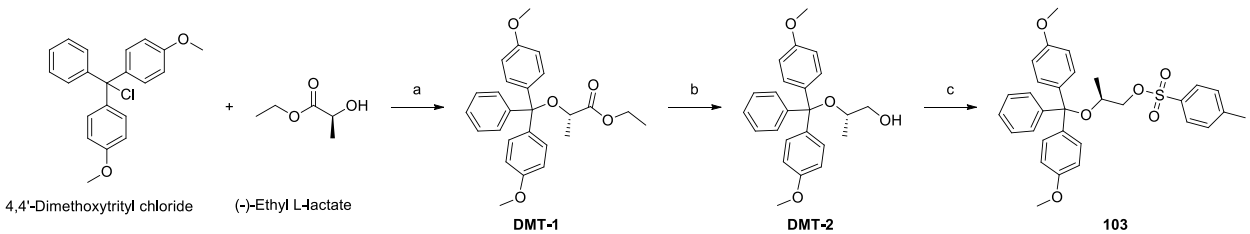
Entry	solvent	% product (determined by LC, @220nm
1	Dioxane	17
2	DMF	14
3	DMSO	13
4	NMP	13
5	Toluene	10

Scheme 29: The screening of solvents. General conditions: 1 eq. 2-(2-ethoxyethoxy)ethanol + 3 eq. R-OTs + 2 eq. tBuOK, 60°C, 16h



84 103 104

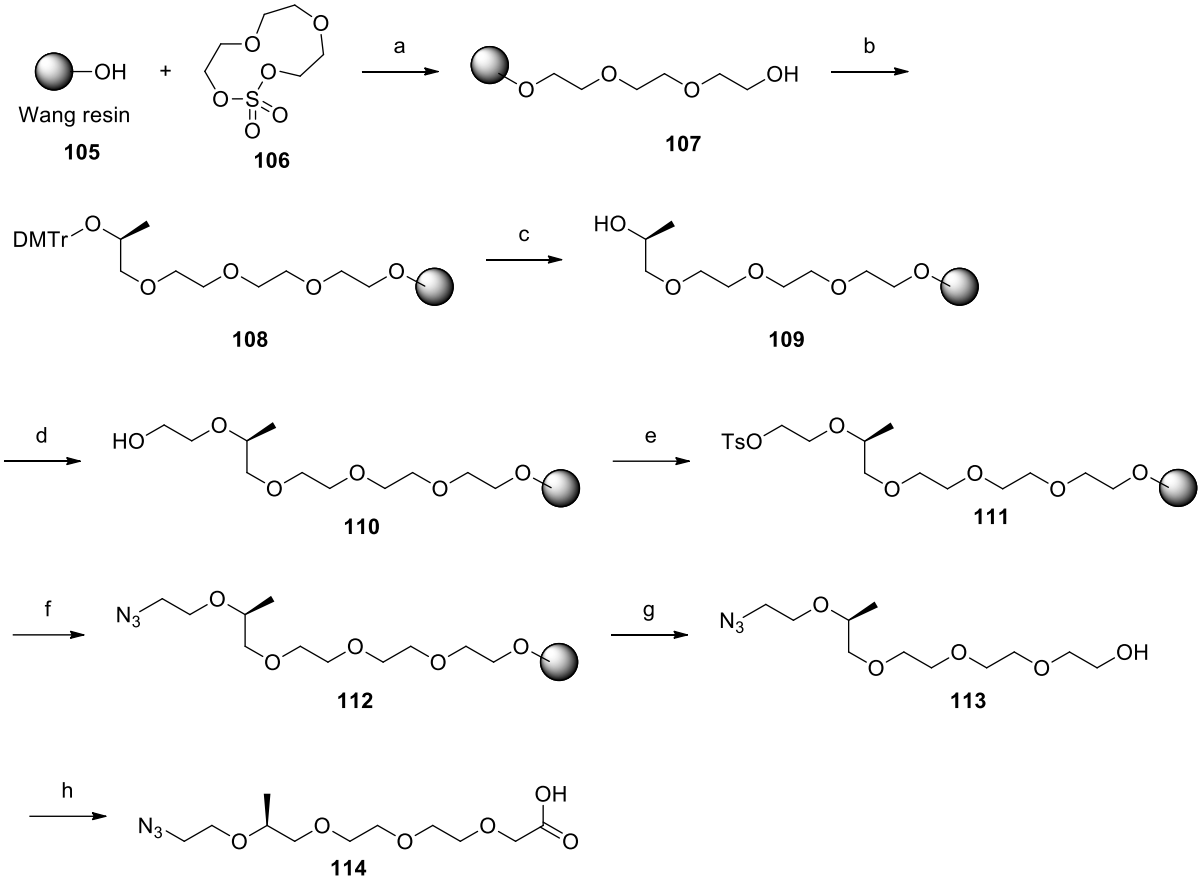
In order to prevent the cleavage of the product from resin during the deprotection, we employed a DMTr group, which has less stability to acids, to protect the hydroxyl group in the nucleophile substitution. The reaction under the optimized condition gave the moderate yield (49%) of desired product.



4,4'-Dimethoxytrityl chloride (-)-Ethyl L-lactate DMT-1 DMT-2 103

Scheme 30: The synthesis of DMTr protected alcohol **103**. (a) Pyridine, RT, 16h, 95%; (b) $LiAlH_4$, THF, 0°C → RT, 1.5h, 98%; (c) *p*-TsCl, TEA, DMAP, DCM, 0°C → RT, overnight, 67%.

3.5.4 Synthesis of the linkers by solid support



Scheme 31: The synthesis of azido acid **114**. (a) tBuOK, THF, 0°C-RT, overnight; then TsOH/H_2O/Dioxane, 60°C, 4h, THF; (b) **103**, tBuOK, dioxane, 60°C, 2 times; (c) 1% TFA in DCM, RT, 4 times; (d) 1,3,2-dioxathiolane 2,2-dioxide, t-BuOK, THF, 0°C → RT, overnight; then TsOH/H_2O/Dioxane, 60°C, 4h; (e) TsCl, TEA, DMAP 0°C → RT; (f) NaN_3, DMF, 60°C, 4h; (g) 10% TFA in DCM, 5 times; (h) Jones reagent, acetone, 0°C, 4h. 7% for 9 steps.

The Wang resin was chosen to be used in the solid support synthesis because it has moderate acid-tolerance and affords a free hydroxyl group after cleavage, which will be oxidized to the carboxyl group of our azido-acid linker.

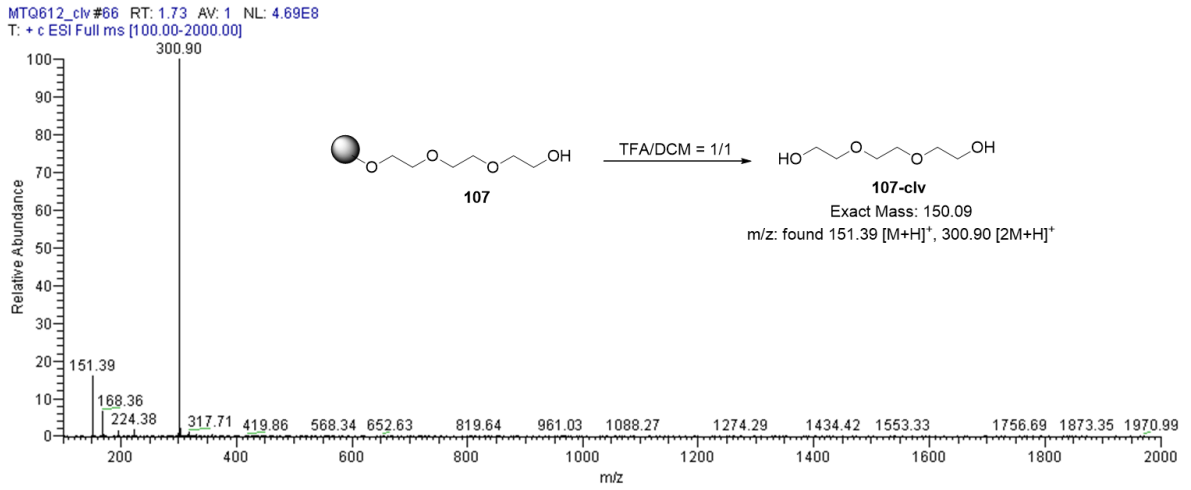


Figure 35. Mass spectrometry data of the product cleaved from 107 with 50% TFA in DCM.

The synthesis started with the nucleophilic ring-opening reactions of the macrocyclic sulfate **106** with deprotonated Wang resin. After the hydrolysis, a PEG chain attached on solid support with 3 units was obtained. The verification of the reaction product was performed by mass spectrometry to check the presence of desired product. After removing solvent of reaction, a small part of the washed resin was taken to a vial with 1 ml 50% TFA solution in DCM. The vial was shaked for a few minutes to cleave the product from the resin. The measurement result of the concentrated supernatant by mass spectrometry showed that the nucleophilic reaction and the ring-opening reaction were successfully achieved.

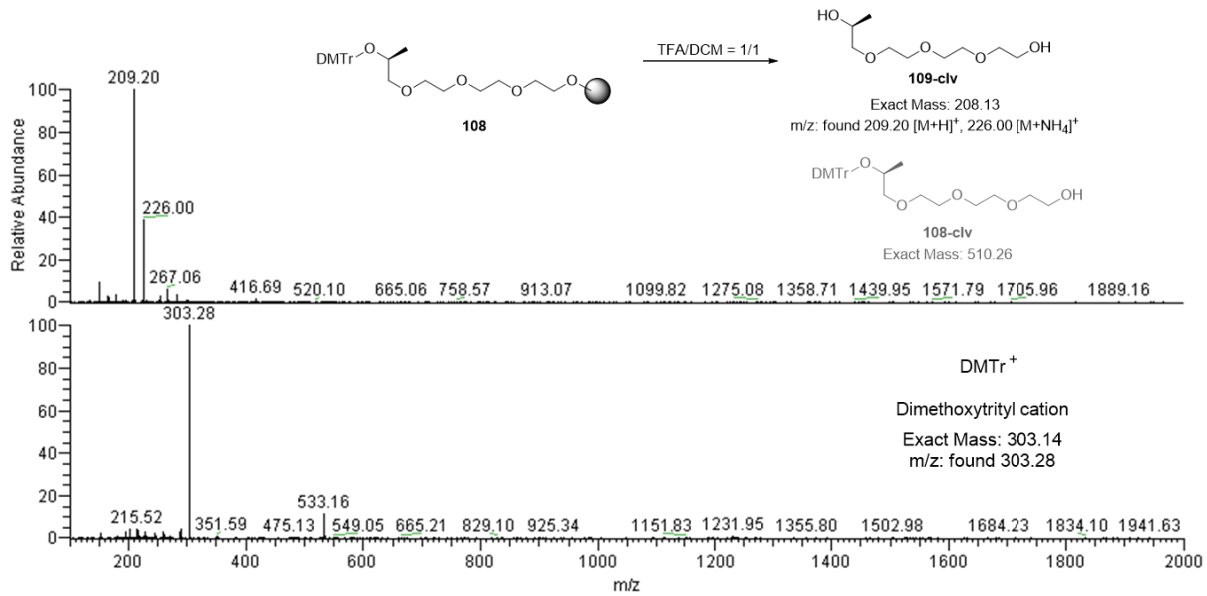


Figure 36. Mass spectrometry data of the product cleaved from 108 with 50% TFA in DCM.

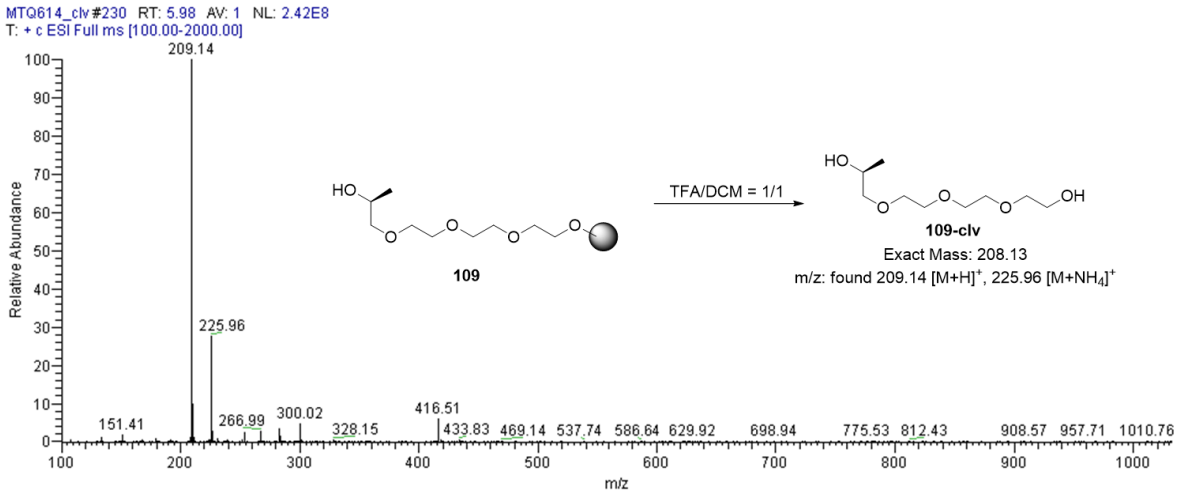


Figure 37. Mass spectrometry data of the product cleaved from 109 with 50% TFA in DCM.

The chiral methyl group was introduced by the nucleophilic substitution of **103** with the deprotonated **107**. Instead of the protected product **108-clv**, the deprotected alcohol **109-clv** was detected, while an intensive signal of dimethoxytrityl cation was shown. This indicated the instability of DMTr group under acidic condition.

The deprotection of DMTr group of **100** was conducted in diluted acidic solution (1% TFA in DCM) which did not cause obvious cleavage of Wang resin. Dimethoxytrityl cation was absent if **108** was deprotected completely.

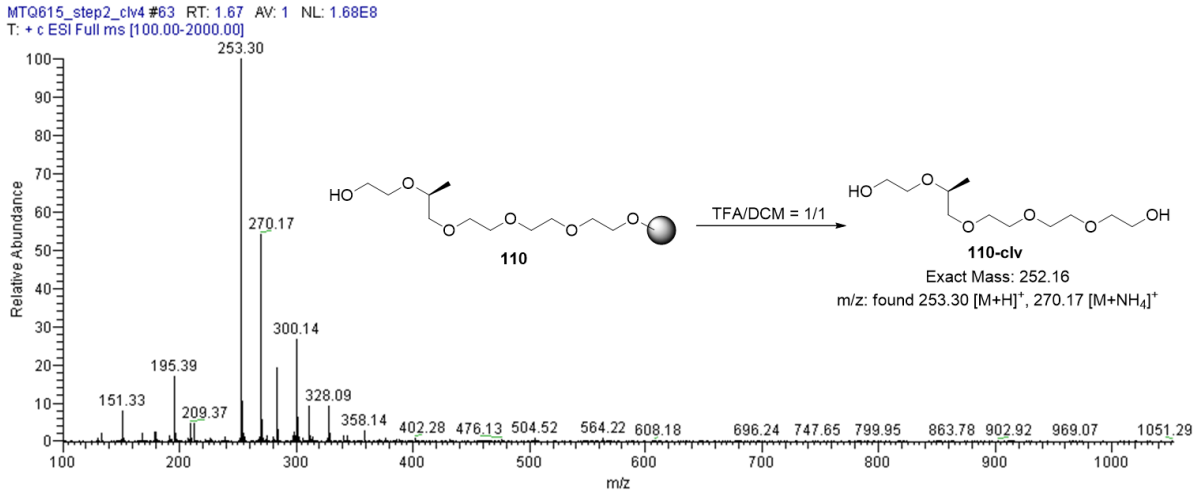


Figure 38. Mass spectrometry data of the product cleaved from 110 with 50% TFA in DCM.

To finish the extension of PEG chain, the resulting compound **109** reacted with a smaller cyclic sulfate followed by the deprotection, tosylation and substitution by sodium azide.

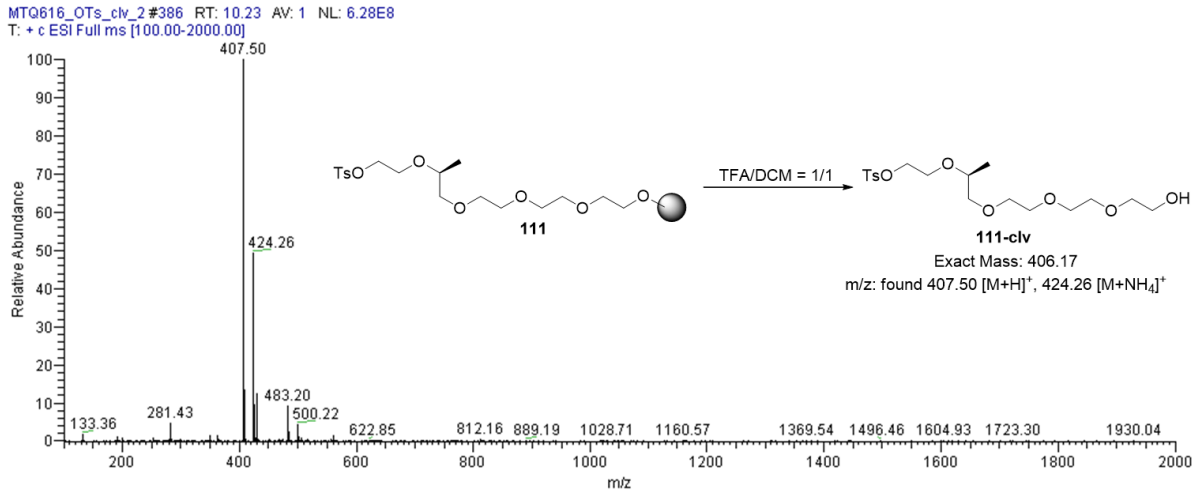


Figure 39. Mass spectrometry data of the product cleaved from 111 with 50% TFA in DCM.

The cleavage of Wang resin was completed in 10% TFA in DCM to afford azido alcohol **113** which was oxidized to give the desired methyl-linker **114**.

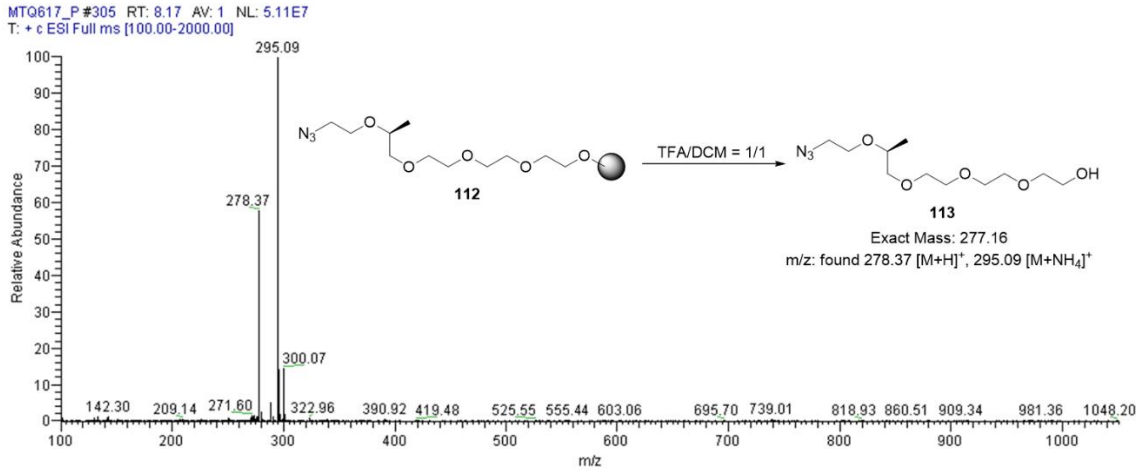


Figure 40. Mass spectrometry data of the product cleaved from 112 with 50% TFA in DCM.

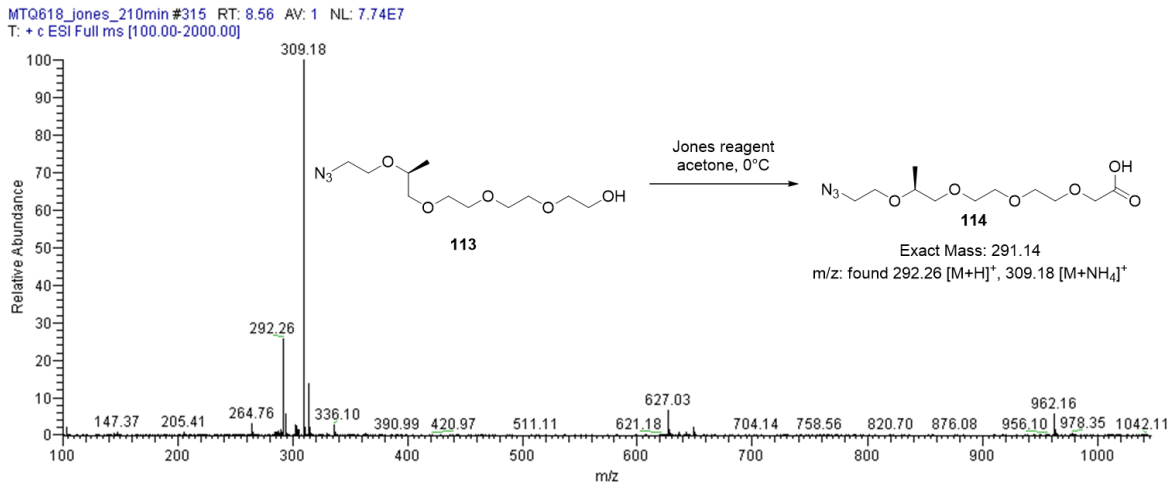
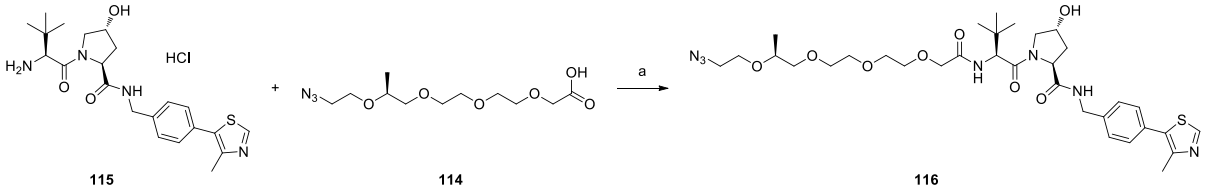


Figure 41. Mass spectrometry data of the oxidized product of 113.



Scheme 32: The conjugation of azido acid **114** with VH032. (a) HATU, DIPEA, DCM, RT, overnight, 41%.

The conjugation reaction between **114** and VH032 gave the key intermediate **116**, which will be coupled to SAFit analogue via click reaction in further studies to afford a new class of PROTACs.

4 Summary and Outlook

In order to induce specific degradation of target protein, a new class of PROTACs was designed based on the selective FKBP51 ligands SAFit1 and SAFit2. The SAFit analogue was proposed to conjugate with two E3 ligase ligands, VH032 and pomalidomide, via PEG linkers at different attachment points respectively. By the synthetic route we established, a series of SAFit-based PROTACs molecules, **MTQ199-208** and **MTQ328-347**, were obtained. Competition binding fluorescence polarization assays showed that all of the compounds retain high selectivity for FKBP51 over the close homologue FKBP52 while exhibiting nanomolar range affinities. Upon the treatment with the PROTAC **MTQ202** on several cell lines, the degradation of FKBP51 was observed in western blot assays. The other synthesized PROTACs with different linkers, E3 ligase ligands or attachment points showed no abilities to influence the level of FKBP51. We postulated the structure of a PROTAC molecular is crucial to induce the formation of protein-PROTAC-E3 ligase ternary complex, which promote the degradation of target protein.

Illuminated by PROTAC MZ1, a non-selective FKBPs ligand featured with [4.3.1] bicyclic structure was introduced into the PROTACs in order to induce a selective degradation of our target protein FKBP51. Base on the co-crystal structure of **SP-16h** in complex with FKBP51, a series of bicyclic ligand-based PROTACs was designed and synthesized. Although no evidence of protein knock-down was observed by western blot assays, some of these compounds exhibited outstanding affinities for all of the tested FKBPs in the low nanomolar range to subnanomolar range. Compared to a (R)-methyl group or a hydrogen, a (S)-methyl group on the Cα enhances the affinities of a ligand for FKBPs. The most potent compound, **MTQ509**, showed the highest binding affinity for FKBP51 so far reported (K_i = 0.92 nM). The binding mode of **MTQ509** to FKBPs need to be clarified by further crystallography studies.

Base on SAFit-like PROTACs, we postulated that a FKBP51-PROTAC-E3 ligase ternary complex can be induced by a linker with conformational restriction. In order to obtain potent PROTACs for FKBP51, we designed a series of methyl PEG linker and the synthetic route was confirmed. A library of new linkers will be established in further studies to investigate the impact of the structure of PROTACs aim to degrade FKBP51 specifically.

5 List of Abbreviations

ACTH	Adrenocorticotropic Hormone
AR	Androgen Receptor
BET	Bromodomain And Extra-Terminal
BRD	Bromodomain-Containing Protein
CH	Cyclohexane
cIAP1	Cellular Inhibitor Of Apoptosis Protein 1
CRBN	Cereblon
CRH	Corticotrophin-Releasing Hormone
DCM	Dichlormethane
DIPEA	N,N-Diisopropylethylamine
DMAP	4-(Dimethylamino)Pyridine
DMF	Dimethylformamide
DMSO	Dimethyl Sulfoxide
DMTr	4,4′-Dimethoxytrityl
EA	Ethyl Acetate
EDC	N-(3-Dimethylaminopropyl)-N′-Ethylcarbodiimide Hydrochloride
ERRα	Estrogen-Related Receptor Alpha
FKBP	FK506 Binding Protein
FP assay	Fluorescence Polarization assay
GR	Glucocorticoid Receptor
HATU	1-[Bis(Dimethylamino)Methylene]-1H-1,2,3-Triazolo[4,5-B]Pyridinium 3-Oxid Hexafluorophosphate
HOBt	1-Hydroxybenzotriazol
HPA	Hypothalamic–Pituitary–Adrenal
HR-MS	High-Resolution Mass Spectrometry
Hsp90	Heat Shock Protein 90
iFit	Induced Fit
LC-MS	Liquid Chromatography–Mass Spectrometry
MeOH	Methanol
MR	Mineralocorticoid Receptor
NMR	*Nuclear Magnetic Resonance*
PDB	Protein Data Bank
PEG	Polyethylene Glycol
POI	Protein Of Interest

PPIase	Peptidyl-Prolyl Cis/Trans Isomerase
PROTAC	Proteolysis Targeting Chimera
PTSD	Post-Traumatic Stress Disorder
SAFit	Selective Antagonist Of FKBP51 By Induced Fit
SAR	Structure-Activity Relationship
SHR	Steroid Hormone Receptor
TEA	Triethylamine
TFA	Trifluoroacetic Acid
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
Ub	Ubiquitin
UPS	Ubiquitin-Proteasome System
VHL	Von Hippel-Lindau

6 Experimental Section

General Methods

All of the reactions were performed in round bottom glass flasks under argon atmosphere (ALPHAGAZTM 1 Argon, 99.999 %) if no additional information was indicated. When water- or air sensitive reagents were used, the reaction was carried out with extra dry solvent in the reaction vessel which was heated with a heat gun, evacuated in vaccum, sealed with a silicone or rubber septum and filled with argon.

NMR spectroscopy

NMR spectroscopy were performed by the Department of Chemistry and Pharmacy of LMU or the NMR Department of TU Darmstadt. ^{1}H, ^{13}C, DEPT, 2D COSY, NOESY, HSQC, and HMBC NMR spectra were recorded either on a Brucker AC 300, a Bruker XL400, a Bruker Avance II 300 MHz, a Bruker Avance III, a Bruker DRX 500 or a Bruker AMX 600 NMR spectrometer at room temperature. Chemical shifts are reported in ppm (δ) and residual protio solvent peaks were used as internal references (^{1}H NMR: chloroform-*d*: δ = 7.26 ppm, DMSO-d_6: 2.50 ppm; ^{13}C: chloroform-*d*: δ = 77 ppm, DMSO-d_6: 39.52 ppm). Multiplicities are reported as singlet (s), doublet (d), triplet (t), quartet (q) or multiplet (m). Coupling constants (J) are reported in Hertz (Hz).

LC-MS

Liquid chromatography–mass spectrometry (LC-MS) was measured by the following instruments:

Pump: Beckman Coulter System Gold 126 solvent module

Autosampler: Beckman Coulter System Gold 508 autosampler

Detector: Beckman Coulter System Gold 166 detector or System Gold Diode Array Detector Module 168; wavelength: 220 nm or 280 nm

Column: YMC-Pack Pro C8 3 μm 120Å, 100x4.6 mm from YMC.

Eluents: Eluent A: 0.1 % formic acid in water; Eluent B: 0.1 % formic acid in acetonitrile

Standard method: 0 % B to 100 % B in 19 min.

MS: Thermo Finnigan LCQ Deca XP Plus, mode: Electrospray ionization (ESI).

Analytic HPLC

Pump: Beckman Coulter System Gold 126 solvent module or Dionex P580 pump

Autosampler: Beckman Coulter System Gold 508 autosampler or Dionex ASI-100 automated sample injector

Detector: Beckman Coulter System Gold Diode Array Detector Module 168 or Dionex UVD 340U Photodiode Array Detector; wavelength: 220 nm, 280 nm

Column: Phenomenex Kinetex 5 μm C18 100 Å, 250 x 4.6 mm or Phenomenex Jupiter 4μ Proteo 90Å, 250 x 4.6 mm

Eluents: Eluent A: 0.1 % TFA in water; Eluent B: 0.1 % TFA in acetonitrile

Method: Gradients are given in percentage B

Chiral HPLC

Pump: Beckman Coulter System Gold 126 solvent module

Autosampler: Beckman Coulter System Gold 508 autosampler

Detector: Beckman Coulter System Gold Diode Array Detector Module 168; wavelength: 220 nm, 280 nm

Column: DAICEL Chiralcel OD-H

Eluents: Eluent A: *n*-Hexane; Eluent B: *i*-Propanol

Semi-Preparative HPLC

Pump: Beckman Coulter System Gold Programmable Solvent Module 126 NMP

Detector: Beckman Coulter Programmable Detector Module 166; wavelength: 254 nm

Collector: Beckman Coulter SC 100 fraction collector

Column: Phenomenex Jupiter 10μ Proteo 90 Å, 250 x 21.2 mm 10 micron

Eluents: Eluent A: 0.1 % TFA in water; Eluent B: 0.1 % TFA in acetonitrile

Method: Gradients are given in percentage B

HR-MS

High resolution mass spectrometry (HR-MS) was performed by the Mass Spectrometry Department of TU Darmstadt. Mass spectra were recorded on a Bruker Daltonics Impact II, quadrupol-time-of-flight spectrometer.

Flash chromatography

Flash chromatography was performed either with an Interchim Puriflash 430 system or manually. The manual column chromatography was performed with silica gel 60 (Carl Roth, 0.04-0.063 mm, 230-400 mesh).

Thin layer chromatography

Thin layer chromatography (TLC) was performed on aluminium sheets coated with silica gel (Merck Millipore, Silica gel 60 F254). UV-active compounds were detected by UV lamps (λ = 254 nm or 365 nm). Non-UV-active compounds were detected by TLC staining solutions.

Reagents and solvents

All of the reagents and solvents bought from commercial sources were used without further purification unless noted additionally.

Solvent	CAS-number	Grade	Company
Acetone	67-64-1	ROTISOLV® HPLC	Carl Roth
Acetonitril	75-05-8	ROTISOLV® HPLC Gradient	Carl Roth
Cyclohexane	110-82-7	ROTISOLV® HPLC or > 99,5 %, zur Synthese	Carl Roth
Dichloromethane	75-09-2	ROTISOLV > 99,9 %	Carl Roth
Diethylether	60-29-7	ROTIPURAN® ≥99,5 %, p.a.	Carl Roth
Dimethylformamide	68-12-2	> 99,8 % zur Peptidsynthese	Carl Roth
Ethyl acetate	141-78-6	ROTISOLV® HPLC or Extra Pure, SLR	Carl Roth or Fisher Scientific
i-Propanol	67-63-0	ROTISOLV® HPLC	Carl Roth
Methanol	67-56-1	ROTISOLV® HPLC	Carl Roth
n-Hexane	110-54-3	ROTISOLV® HPLC	Carl Roth
Tetrahydrofuran	109-99-9	ROTIDRY® ≥99,9 % (≤50 ppm H_2O)	Carl Roth

Fluorescence Polarization Assay

The competition binding fluorescence polarization assay was performed as described[9] by Stephanie Merz and Christian Meyners.

Chemical Synthesis

General method A for synthesis of monotosylate alcohol

To a stirred solution of diol **15-17** (1 eq.) in DCM were added Ag_2O (1.5 eq.), TsCl (1 eq.), and KI (0.2 eq.). The reaction mixture was stirred at 0°C for 30 min and then filtered through a small pad of silica gel and washed with EtOAc. Evaporation of the solvent, followed by column chromatography, gave the desired monotosylate product.

General method B for synthesis of azido alcohol

To a stirred solution of monotosylate alcohol **41** (1 eq.) in DMF sodium azide (2 eq.) was added. The mixture was stirred for 16 h at room temperature. Dichloromethane was added and the mixture was washed with brine. The organic phase was dried over magnesium

sulfate, filtered, and evaporated under reduced pressure. The crude was purified by flash chromatography to give a yellow oil.

General Method C for synthesis of azides

To a stirred solution of alcohol **40** (1 eq.) in tert-butanol, potassium tert-butoxide (1.5 eq.) was added. The mixture was stirred at 30°C for 15 min and tert-butyl bromoacetate (2 eq.) was added. The mixture was stirred at 30°C for 16 h, and then evaporated under reduced pressure. The crude was dissolved in dichloromethane and washed with brine. The organic phase was dried over magnesium sulfate, filtered, and evaporated under reduced pressure. The crude product was purified by flash chromatography.

General Method D for synthesis of azido acids

Azides **39** (1 eq.) was dissolved in dichloromethane/TFA (1/1, v/v) and the mixture was stirred at room temperature for 30 min. The mixture was evaporated under reduced pressure, and then the crude was dissolved in water. The pH was adjusted to 10 by addition of an aqueous sodium hydroxide solution (1 M). The solution was washed with ethyl acetate and the pH was adjusted to 2 by addition an aqueous hydrochloric acid solution (3 M). The aqueous layer was extracted with ethyl acetate. The combined extracts were dried over magnesium sulfate, filtered, and evaporated under reduced pressure. The crude product was purified by flash chromatography.

General Method E

37 (90 mg, 0.170 mmol, 1 eq.) was dissolved in a solution of TFA/DCM (v/v = 1:1, 3mL) and stirred at RT under Ar. Then the solvent was removed in reduced pressure and the given brown oil was added to a solution of azido acid (0.170 mmol, 1 eq.) in DCM (4 ml). HATU (97 mg, 0.255 mmol, 1.5 eq.) was added and the pH adjusted to >9 by addition of DIPEA (0.148 ml, 0.850 mmol, 5 eq.). After stirring overnight at RT the reaction mixture was extracted with water. The organic phase was dried over magnesium sulfate and evaporated to dryness. The crude product was purified by flash column chromatography.

General Method F

Oxalyl chloride (2M in DCM, 3.9 eq.) was added dropwise into a solution of azido acid (3 eq.) and DMF (1 drop) in DCM (2 ml) at 0°C. After stirring at 0°C → RT for 3h, the solvent and oxalyl chloride were removed under reduced pressure. The resulting product was dissolved in DMF (1 ml) and added into a solution of pomalidomide (1 eq.) in DMF at 0°C. The reactants were stirred at 0°C → RT for 5h. Then the mixture was diluted by DCM (100mL) and washed with brine (25 mL x 3). The organic phase was dried over magnesium sulfate, filtered and concentrated under reduced pressure. Purification by flash column chromatography gave a light yellow solid.

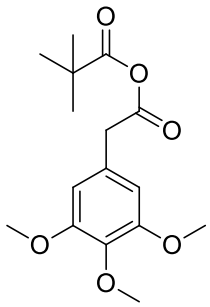
General Method G

To a solution of **16/31a/31b/45** (1 eq.) and **43/44** (1 eq.) in t-BuOH-H2O (1:1,1 mL) at RT were added copper(II) sulfate pentahydrate (1M in H_2O, 5.5 μl, 5.5 μmol) and (+)-Sodium L-ascorbate (1M in H_2O, 5.5 μl, 5.5 μmol). The reaction mixture was stirred at RT overnight. Then the mixture was diluted by DCM (50mL) and washed with brine (25 mL x 2). The organic phase was dried over magnesium sulfate, filtered and concentrated under reduced pressure.

General Method H

To a solution of carboxylic acid **6** (1 eq.) in DCM/DMF (2/1), HATU (1.1 eq.), DIPEA (3 eq.) and **19** (1 eq.) were added. After stirring for 16 h, the mixture was poured into brine and extracted 3 times with Et_2O. The combined organic layer was washed 3 times with brine, dried over magnesium sulfate, filtered and condensed. The crude product was purified by flash chromatography.

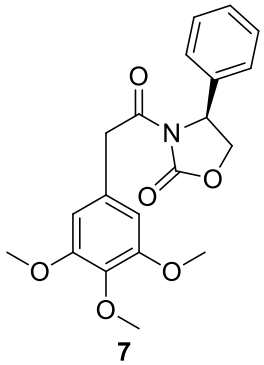
2-(3,4,5-Trimethoxyphenyl)acetic pivalic anhydride (12)



12

DIPEA (0.425 ml, 2.43 mmol, 1.1 eq.) and pivaloyl chloride (0.3 ml, 2.43 mmol, 1.1 eq.) were added to a solution of 2-(3,4,5-trimethoxyphenyl)acetic acid (0.5 g, 2.21 mmol, 1 eq.) in DCM (4 mL) at 0°C. The solution was stirred at 0°C for 30 min and RT for 1h. Then it was quenched by the addition of saturated NH_4Cl solution and the product was extracted with DCM (50 ml). The combined organics were dried over magnesium sulfate, filtered and then the solvent was removed to obtain the pivalic acid mixed anhydride.

(S)-4-Phenyl-3-(2-(3,4,5-trimethoxyphenyl)acetyl)oxazolidin-2-one (7)



7

BuLi (2.5M in THF, 0.97 ml, 2.42 mmol, 1.1 eq.) was added into a solution of (S)-4-phenyloxazolidin-2-one (0.36 g, 2.20 mmol, 1 eq.) in THF (8 mL) at -78°C and stirred at that temperature for 1h. The solution of pivalic acid mixed anhydride in DCM (1 mL) was added and stirred for 16h at -78°C to RT. Then it was quenched by the addition of saturated NH_4Cl solution and the product was extracted with EtOAc. The combined organics were dried over magnesium sulfate, filtered and the solvent was removed under reduced pressure. Purification by flash column chromatography (c-Hexane:EtOAc, gradient EtOAc 0 to 30%) gave a yellow oil (535mg, 1.43 mmol, 65%)

TLC [cyclohexane/EtOAc 6/4]: R_f = 0.28.

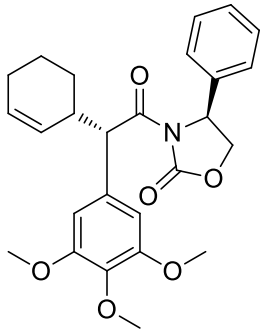
HPLC [30-100% Solvent B, 20 min]: R_t = 12.92 min, purity (220 nm) = 98%.

^{1}H NMR: (400 MHz, Chloroform-*d*) = 7.32 (m, 3 H), 7.18 (m, 2 H), 6.45 (s, 2 H), 5.43 (dd, J = 4.0, 8.8 Hz, 1 H), 4.69 (t, J = 8.9 Hz, 1 H), 4.27 (d, J = 17.7 Hz, 1 H), 4.25 (dd, J = 4.0, 8.8 Hz, 1 H), 4.15 (d, J = 14.7 Hz, 1 H), 3.82 (s, 3 H), 3.77 (s, 6 H).

^{13}C NMR: (101 MHz, Chloroform-*d*) = 170.58, 153.68, 153.25, 138.81, 137.23, 129.22, 128.86, 128.84, 126.12, 106.76, 69.99, 60.95, 57.88, 56.15, 41.81.

MS (ESI+): m/z: found 371.97 $[M+H]^+$, calculated 372.14$[C_{20}H_{21}NO_6+H]^+$

(S)-3-((S)-2-((R)-Cyclohex-2-en-1-yl)-2-(3,4,5-trimethoxyphenyl)acetyl)-4-phenyloxazolidin-2-one (13)



13

NaHMDS (1.0M in THF, 2.11 ml, 5.25 mmol, 1.5 eq.) was added dropwise to a solution of **7** (0.52 g, 1.41 mmol, 1 eq.) in THF (20 mL) at -78°C. The mixture was stirred for 1 h, then 3-bromocyclohex-1-ene (1.13 g, 7.04 mmol, 5 eq.) was added dropwise. The reaction mixture was stirred at -78°C for 1 h and then at 0°C for 16 h. Then it was quenched by the addition of saturated NH_4Cl solution and the product was extracted with EtOAc. The combined organics were dried over magnesium sulfate, filtered and the solvent was removed. Purification by flash column chromatography (c-Hexane:EtOAc, gradient EtOAc 0 to 10%) gave a light yellow oil (191mg, 0.43 mmol, 31%)

TLC [cyclohexane/EtOAc 6/4]: R_f = 0.31.

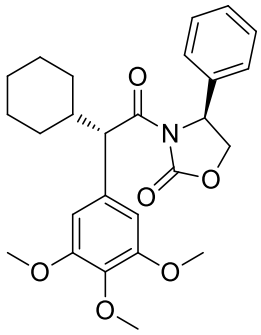
HPLC [60-80% Solvent B, 20 min]: R_t = 14.15 min, purity (220 nm) = 98%.

^{1}H NMR: (800 MHz, Chloroform-*d*) = 7.41 (m, 2 H), 7.35 (m, 3 H), 6.67 (s, 2 H), 5.62 (d, 1 H, J = 10.0 Hz), 5.39 (dd, J = 9.0, 3.8 Hz, 1 H), 5.05 (d, J = 10.0 Hz, 1 H), 4.84 (d, J = 11.3 Hz, 1 H), 4.60 (t, J = 9.0 Hz, 1 H), 4.23 (dd, J = 9.0, 3.8 Hz, 1 H), 3.85 (s, 6 H), 3.83 (s, 3 H), 2.80 (m, 1 H), 1.95 (m, 2 H), 1.66 (m, 1 H), 1.43 (m, 1 H), 1.33 (m, 1 H), 1.12 (m, 1 H).

^{13}C NMR: (201 MHz, Chloroform-*d*) = 173.63, 153.47, 153.22, 139.40, 137.37, 132.77, 129.50, 129.31, 129.25, 128.92, 128.54, 126.19, 126.07, 106.16, 69.54, 60.97, 58.25, 56.30, 56.26, 53.71, 39.67, 26.22, 25.41, 20.54.

MS (ESI+): m/z: found 452.01 $[M+H]^+$, calculated 452.21 $[C_{26}H_{29}NO_6+H]^+$

(S)-3-((S)-2-Cyclohexyl-2-(3,4,5-trimethoxyphenyl)acetyl)-4-phenyloxazolidin-2-one (14)



14

13 (0.19 g, 0.43 mmol, 1 eq.) was dissolved in MeOH (10 mL) and the solution was degassed with Ar.
Then palladium on carbon (45 mg, 0.042 mmol, 0.1 eq.) was added. In the following step H_2 was bubbled through the reaction mixture for 5 min. The reaction was then stirred under H_2 atmosphere for 16 h. Then the dark suspension was filtered through celite and the solvent was removed under reduced pressure. Purification by flash column chromatography (c-Hexane:EtOAc, gradient EtOAc 0 to 13%) gave a yellow oil (190mg, 0.42 mmol, 99%).

TLC [cyclohexane/EtOAc 8/2]: $R_f = 0.23$.

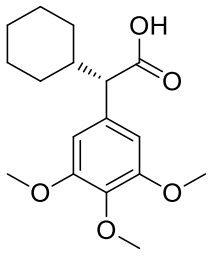
HPLC [65-80% Solvent B, 20 min]: $R_t = 14.72$ min, purity (220 nm) $\geq 99\%$.

^{1}H NMR: (800 MHz, DMSO-d_6) δ 7.41 (t, J = 7.6 Hz, 2H), 7.35 – 7.32 (m, 3H), 6.59 (s, 2H), 5.46 (dd, J = 8.7, 3.6 Hz, 1H), 4.77 (d, J = 10.6 Hz, 1H), 4.68 (t, J = 8.8 Hz, 1H), 4.15 (dd, J = 8.9, 3.6 Hz, 1H), 3.74 (s, 6H), 3.64 (s, 3H), 1.94 – 1.88 (m, 1H), 1.55 – 1.51 (m, 3H), 1.28 – 1.25 (m, 1H), 1.19 – 1.15 (m, 1H), 1.11 – 1.02 (m, 3H), 0.94 – 0.89 (m, 1H), 0.81 – 0.75 (m, 1H).

^{13}C NMR: (201 MHz, DMSO-d_6) δ 173.30, 153.98, 153.09, 140.37, 137.16, 133.30, 129.19, 128.63, 126.46, 106.57, 70.08, 60.41, 57.89, 56.28, 54.02, 41.82, 31.25, 30.26, 26.26, 25.65.

MS (ESI+): m/z: found 454.03 $[M+H]^+$, calculated 454.22 $[C_{26}H_{31}NO_6+H]^+$

(S)-2-Cyclohexyl-2-(3,4,5-trimethoxyphenyl)acetic acid (6)



6

Lithium hydroxide (41.6 mg, 1.74 mmol) and hydrogen peroxide solution (30% w/w in water, 199 μl, 1.93 mmol) were added to a solution of **14** in THF/H_2O (10 mL, 8:5) at 0°C. After stirring for 16h, the reaction was quenched by the addition of saturated $NaSO_3$ solution and extracted 3 times with DCM. The aqueous phase was acidified with conc. HCl to PH = 2 and

extracted with DCM. The combined organic layer was dried over magnesium sulfate, filtered and concentrated under reduced pressure to afford a white foam (95mg, 0.31 mmol, 80%).

TLC [cyclohexane/EtOAc+1%AcOH 6/4]: R_f = 0.27.

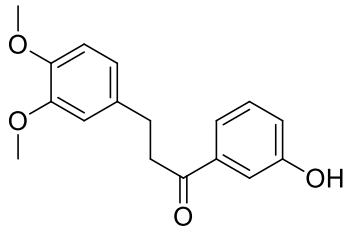
HPLC [0-100% Solvent B, 20 min]: R_t = 17.57 min, purity (220 nm) = 90%.

^{1}H NMR: (599 MHz, Chloroform-*d*) δ 6.54 (s, 2H), 3.84 (s, 6H), 3.82 (s, 3H), 3.13 (d, J = 10.7 Hz, 1H), 1.96 – 1.87 (m, 2H), 1.77 – 1.73 (m, 1H), 1.67 – 1.61 (m, 2H), 1.39 – 1.29 (m, 2H), 1.17 – 1.13 (m, 2H), 1.07 (ddd, J = 12.7, 3.8, 1.7 Hz, 1H), 0.75 (ddd, J = 23.9, 11.7, 3.2 Hz, 1H).

^{13}C NMR: (151 MHz, Chloroform-*d*) δ 179.64, 153.28, 137.44, 132.97, 105.72, 60.98, 59.10, 56.28, 41.03, 32.08, 30.42, 26.40, 26.07.

MS (ESI+): m/z: found 309.00 $[M+H]^+$, calculated 309.17 $[C_{17}H_{24}O_5+H]^+$

3-(3,4-dimethoxyphenyl)-1-(3-hydroxyphenyl)propan-1-one (3)



3

3,4-Dimethoxybenzaldehyde (100 g, 602 mmol, 1.0 eq.) and 1-(3-hydroxyphenyl)ethanone (82.0 g, 602 mmol, 1.0 eq.) were dissolved in EtOH (800 mL) and cooled to 3°C. A solution of KOH (135 g, 2.41 mol, 4.0 eq.) in cold H_2O (640 mL) was added dropwise over 1 h to the reaction mixture, which was then stirred over night while warming to RT. Orange solids precipitated and the resulting suspension was poured onto ice (1.5 L). After acidification with conc. HCl to pH = 2, the precipitated clots were resuspended *via* sonification and pounding. Filtration of the resulting suspension yielded orange solids, which were dissolved in EtOAc and dried over $MgSO_4$. After removal of the solvent *in vacuo*, (E)-3-(3,4-dimethoxyphenyl)-1-(3-hydroxyphenyl)prop-2-en-1-one **8** (153 g) was obtained as crude product with slight impurities and used without further purification in the next reaction step. For the subsequent reduction reaction, an autoclave (Modell II, Roth) was flushed with argon and then charged with crude product **8** (25.6 g, 90 mmol, 1.0 eq.; pureness assumed), Lindlar catalyst (400 mg, 3.76 mmol, 42 mol%) and MeOH (150 mL). The autoclave was again flushed with argon, then closed and filled with hydrogen gas (35 bar). After stirring for 7 d at RT, the reaction mixture was filtered through celite and the solvent was removed *in vacuo*. The obtained yellow solid was purified via recrystallization from MeOH (70 mL) yielding white solids, which were washed with cold MeOH (2×10 mL) and cold hexane (2×15 mL). **3** (16.3 g, 56.9 mmol, 63%) was obtained as white powder.

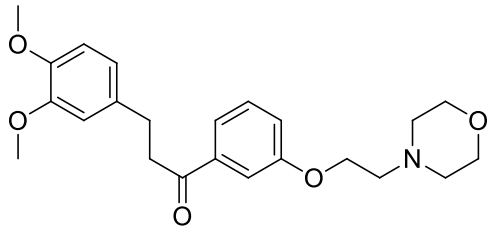
TLC [cyclohexane/EtOAc 2/1]: R_f = 0.20.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.55 – 7.46 (m, 2H), 7.31 (t, *J* = 7.9 Hz, 1H), 7.07 (dd, *J* = 8.1, 2.6 Hz, 1H), 6.82 – 6.72 (m, 3H), 6.24 (s, 1H), 3.85 (d, *J* = 2.4 Hz, 6H), 3.26 (t, *J* = 7.6 Hz, 2H), 3.00 (t, *J* = 7.6 Hz, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 200.10, 156.48, 149.00, 147.52, 138.37, 133.88, 130.04, 120.70, 120.34, 114.70, 112.04, 111.56, 56.08, 55.98, 40.93, 29.98.

HPLC [0%–100% eluent B, 20 min]: R_t = 15.8 min, 92% purity

3-(3,4-dimethoxyphenyl)-1-(3-(2-morpholinoethoxy)phenyl)propan-1-one (10)



10

K_2CO_3 (31.1 g, 225 mmol, 4 eq.) was added to a suspension of **3** (16.1 g, 56.3 mmol, 1.0 eq.) in acetonitrile (280 mL) at RT. 2-Morpholinoethyl chloride hydrochloride (11.5 g, 61.9 mmol, 1.1 eq.) was added to the reaction mixture. After stirring at RT overnight, the mixture was filtered and the filtrate was evaporated under reduced pressure. The residue was purified by recrystallization from *i*-propanol affording morpholine **10** (21.57 g, 21.6 mmol, 96%) as a white powder.

TLC [EtOAc + 2% MeOH]: R_f = 0.30.

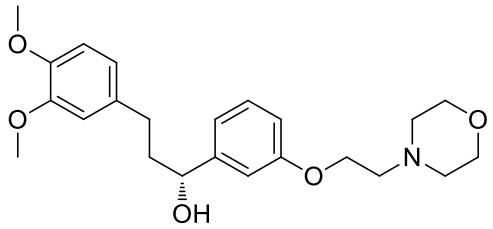
HPLC [0%–100% eluent B, 20 min]: Rt = 9.9 min, ≥ 99% purity

^{1}H NMR (300 MHz, Chloroform-*d*) δ 7.56 – 7.46 (m, 2H), 7.35 (t, *J* = 7.9 Hz, 1H), 7.11 (dd, *J* = 8.1, 2.5 Hz, 1H), 6.78 (dd, *J* = 5.1, 2.0 Hz, 3H), 4.15 (t, *J* = 5.7 Hz, 2H), 3.86 (d, *J* = 3.9 Hz, 6H), 3.76 – 3.69 (m, 4H), 3.26 (t, *J* = 7.6 Hz, 2H), 3.01 (t, *J* = 7.6 Hz, 2H), 2.81 (t, *J* = 5.7 Hz, 2H), 2.62 – 2.50 (m, 4H).

^{13}C NMR (75 MHz, Chloroform-*d*) δ 199.23, 159.14, 149.09, 147.59, 138.44, 134.02, 129.74, 120.96, 120.33, 120.11, 113.30, 112.05, 111.55, 67.06, 66.16, 57.72, 56.10, 56.01, 54.24, 40.94, 29.99.

MS (ESI+): m/z: found 400.28 $[M+H]^+$, calculated $[C_{23}H_{29}NO_5+H]^+$

(R)-3-(3,4-dimethoxyphenyl)-1-(3-(2-morpholinoethoxy)phenyl)propan-1-ol (1)



1

An autoclave (Modell II, Roth) was flushed with argon and then charged with **10** (2 g, 5 mmol, 1.0 eq.), $RuCl_2$[(S)-xylbinap][(S)-daipen] (61 mg, 0.05 mmol, 1 mol%), K_2CO_3 (692 mg, 5 mmol, 1 eq.) and i-propanol (25 mL). The autoclave was again flushed with argon, then closed and filled with hydrogen gas (35-40 bar). After stirring for 3 d at RT, the reaction mixture was filtered through celite and the solvent was removed *in vacuo*. The obtained yellow solid was purified by chromatography (EtOAc + 2% MeOH + 1% TEA) yielding **1** (1.7 g, 4.3 mmol, 86%)

TLC [EtOAc + 2% MeOH + 0.1% TEA]: $R_f = 0.14$.

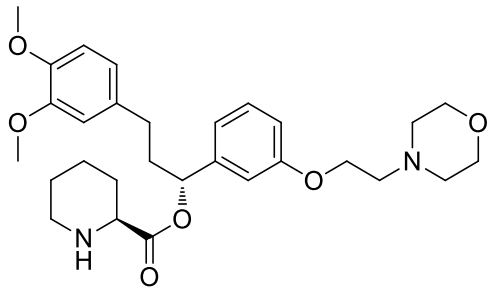
HPLC [0%–100% eluent B, 20 min]: R_t = 9.1 min, ≥ 99% purity

^{1}H NMR (300 MHz, Chloroform-*d*) δ 7.25 (t, J = 8.2 Hz, 1H), 6.97 – 6.88 (m, 2H), 6.85 – 6.77 (m, 2H), 6.76 – 6.69 (m, 2H), 4.65 (dd, J = 7.5, 5.5 Hz, 1H), 4.10 (t, J = 5.6 Hz, 2H), 3.89 – 3.81 (m, 6H), 3.77 – 3.67 (m, 4H), 2.80 (t, J = 5.6 Hz, 2H), 2.75 – 2.53 (m, 6H), 2.18 – 1.90 (m, 2H).

^{13}C NMR (75 MHz, Chloroform-*d*) δ 158.92, 148.93, 147.29, 146.68, 134.49, 129.57, 120.29, 118.60, 113.63, 112.21, 111.93, 111.42, 73.70, 66.81, 65.66, 57.68, 56.02, 55.92, 54.08, 40.74, 31.74.

ee ≥ 99%

(S)-(R)-3-(3,4-dimethoxyphenyl)-1-(3-(2-morpholinoethoxy)phenyl)propyl piperidine-2-carboxylate (2)



2

1 (519 mg, 1.3 mmol, 1.0 eq.) and (S)-N-Fmoc-piperidine-2-carboxylic acid (500 mg, 1.4 mmol, 1.1 eq.) were dissolved in DCM (6.5 mL) followed by the addition of EDC (273 mg, 1.4 mmol, 1.1 eq.) and DMAP (32 mg, 0.26 mmol, 0.2 eq.) at RT. After stirring for 16h, the mixture was concentrated under reduced pressure and purified by flash column chromatography (cyclohexane:EtOAc, gradient EtOAc 0 to 15%) to give **11** as a yellow oil (926 mg, 1.26 mmol,

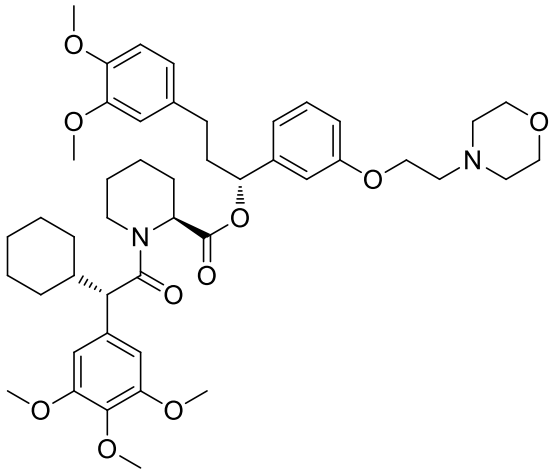
97%).
11 (880 mg, 1.2mmol, 1 eq.) was dissolved in 6 mL 4-methylpiperidine/DCM (v/v= 1:9). The solution was stirred at RT overnight. Then the solution was diluted with 30 ml DCM, and washed with saturated NH_4Cl solution (20 mL x 3). The organic layer was dried over magnesium sulfate, filtered and concentrated under reduced pressure. Purification by flash column chromatography (DCM → DCM + 5% MeOH) gave **2** (198 mg, 0.45 mmol, 73%) as a yellow oil.

TLC [DCM + 5% MeOH]: $R_f = 0.58$.

^{1}H NMR (599 MHz, Chloroform-*d*) δ 7.25 – 7.22 (m, 1H), 6.92 – 6.90 (m, 1H), 6.88 – 6.86 (m, 1H), 6.82 (ddd, *J* = 8.3, 2.6, 0.9 Hz, 1H), 6.77 (d, *J* = 8.1 Hz, 1H), 6.68 – 6.64 (m, 2H), 5.75 (dd, *J* = 8.0, 5.6 Hz, 1H), 4.10 (t, *J* = 5.8 Hz, 2H), 3.85 (d, *J* = 4.7 Hz, 6H), 3.74 – 3.72 (m, 4H), 3.40 (dd, *J* = 9.9, 3.3 Hz, 1H), 3.09 (dt, *J* = 12.1, 3.6 Hz, 1H), 2.80 (t, *J* = 5.7 Hz, 2H), 2.69 – 2.59 (m, 2H), 2.59 – 2.56 (m, 4H), 2.55 – 2.50 (m, 1H), 2.26 – 2.21 (m, 1H), 2.07 – 2.02 (m, 2H), 1.79 (dd, *J* = 9.0, 4.7 Hz, 1H), 1.67 – 1.56 (m, 2H), 1.52 – 1.43 (m, 2H).

^{13}C NMR (151 MHz, Chloroform-*d*) δ 172.62, 158.95, 149.01, 147.47, 141.90, 133.71, 129.72, 120.27, 119.18, 113.99, 113.23, 111.81, 111.42, 75.86, 67.04, 65.87, 58.71, 57.81, 56.07, 56.01, 54.25, 45.65, 38.08, 31.52, 29.22, 25.69, 24.12.

(S)-(R)-3-(3,4-dimethoxyphenyl)-1-(3-(2-morpholinoethoxy)phenyl)propyl 1-((S)-2-cyclohexyl-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2-carboxylate (SAFit2)



SAFit2 was synthesized from **2** (120 mg, 0. 23 mmol) according to the General Method H. Purification by flash column chromatography (cyclohexane → EtOAc + 2% MeOH + 0.1% TEA) gave a white solid (150 mg, 0.18 mmol, 80%).

TLC [EtOAc + 2% MeOH + 1% TEA]: R_f=0.41.

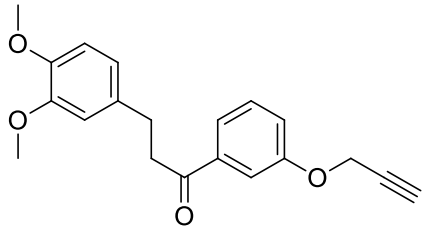
HPLC [30-100% Solvent B, 20 min]: R_t = 14.77 min, purity (220 nm) = 98%.

^{1}H NMR (800 MHz, Chloroform-*d*) (3:1 mixture of rotamers) δ 7.29 (t, *J* = 7.9 Hz, 0.25H), 7.10 (t, *J* = 7.9 Hz, 0.75H), 6.96 (d, *J* = 7.7 Hz, 0.25H), 6.90 (s, 0.25H), 6.87 (dd, *J* = 8.1, 2.1 Hz, 0.25H), 6.79 (d, *J* = 8.2 Hz, 0.25H), 6.77 – 6.74 (m, 1.5H), 6.70 – 6.67 (m, 1H), 6.66 – 6.61 (m, 1.75H), 6.48 (s, 1.5H), 6.43 – 6.37 (m, 1.25H), 5.83 – 5.77 (m, 0.25H), 5.55 (dd, *J* = 8.1, 5.6 Hz, 0.75H), 5.48 – 5.44 (m, 0.75H), 4.72 (d, *J* = 5.3 Hz, 0.25H), 4.58 – 4.51 (m,

0.25H), 4.18 – 4.04 (m, 2H), 3.94 (d, *J* = 13.6 Hz, 0.75H), 3.87 – 3.81 (m, 9H), 3.77 (s, 6H), 3.71 (s, 4H), 3.37 (d, *J* = 9.8 Hz, 0.75H), 2.98 (d, *J* = 9.7 Hz, 0.25H), 2.84 (s, 1.5H), 2.78 (td, J = 13.4, 2.7 Hz, 1H), 2.75 – 2.51 (m, 4.5H),2.47 (ddd, *J* = 14.3, 9.4, 5.3 Hz, 1H), 2.41 – 2.35 (m, 1H), 2.31 – 2.25 (m, 1H), 2.10 – 2.06 (m, 1H), 1.96 (dtd, *J* = 13.9, 8.8, 5.4 Hz, 1H), 1.90 – 1.85 (m, 1H), 1.85 – 1.80 (m, 1H), 1.70 – 1.55 (m, 6H), 1.45 – 1.42 (m, 1H), 1.35 – 1.23 (m, 3H), 1.18 – 1.11 (m, 2H), 1.02 – 0.86 (m, 1H), 0.79 – 0.54 (m, 1H).

^{13}C NMR (201 MHz, Chloroform-*d*) (major) δ 172.45, 170.67, 153.15, 148.94, 147.39, 142.01, 136.90, 133.74, 133.56, 129.71, 120.37, 113.86, 113.11, 111.88, 111.37, 105.86, 77.35, 75.74, 60.91, 57.70, 56.45, 56.17, 56.04, 55.96, 55.17, 54.10, 52.13, 43.74, 41.48, 38.20, 32.94, 31.18, 30.78, 27.04, 26.91, 26.70, 26.35, 26.31, 25.67, 21.10.

3-(3,4-Dimethoxyphenyl)-1-(3-(prop-2-yn-1-yloxy)phenyl)propan-1-one (21)



21

To a solution of **3** (3 g, 10.48 mmol, 1 eq.) and K_2CO_3 (4.35 g, 20.96 mmol, 3 eq.) in acetone (50 mL) was added 3-bromoprop-1-yne (80% wt in toluene, 1.356 mL, 13.62 mmol, 1.3 eq.) and stirred at RT for 16h. Then the mixture was filtered and the precipitate was washed with DCM. The solvent was removed under reduced pressure and the crude product was recrystallized from methanol to obtain white crystal (3.07g, 9.4 mmol, 90%).

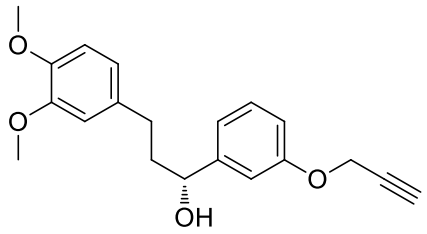
TLC [cyclohexane/EtOAc 6/4]: R_f = 0.63.

HPLC [0-100% Solvent B, 20 min]: R_t = 18.60 min, purity (220 nm) ≥ 99%.

^{1}H NMR (300 MHz, Chloroform-*d*) δ 7.60 – 7.53 (m, 2H), 7.41 – 7.32 (m, 1H), 7.20 – 7.12 (m, 1H), 6.81 – 6.73 (m, 3H), 4.72 (d, J = 2.3 Hz, 2H), 3.86 (s, 3H), 3.84 (s, 3H), 3.26 (t, J = 7.4 Hz, 2H), 3.00 (t, J = 7.6 Hz, 2H), 2.53 (t, J = 2.3 Hz, 1H).

^{13}C NMR (75 MHz, Chloroform-*d*) δ 198.91, 157.76, 148.94, 147.45, 138.31, 133.83, 129.68, 121.45, 120.19, 120.16, 113.68, 111.90, 111.42, 78.09, 75.94, 55.95, 55.85, 40.76, 29.84.

(R)-3-(3,4-Dimethoxyphenyl)-1-(3-(prop-2-yn-1-yloxy)phenyl)propan-1-ol (20)



20

To a stirred solution of **21** (250 mg, 0.77 mmol, 1 eq.) and (S)-(−)-2-methyl-CBS-oxazaborolidine solution (1M in toluene, 464 μl, 0.46 mmol, 0.6 eq.) in dry THF (15 mL) borane tetrahydrofuran complex solution (1M in THF, 1.16 mL, 1.16 mmol, 1.5 eq.) was added at -40°C and the mixture was stirred at the same temperature under Ar atmosphere for 5h. Then 2 mL MeOH was added, and the mixture was warmed to room temperature. Saturated NH_4Cl solution was added and the mixture was extracted with EtOAc. The crude product was concentrated and purified by flash column chromatography (cyclohexane → 1% MeOH in DCM). **20** was afforded as a colorless oil (253mg, 0.77 mmol, 100%).

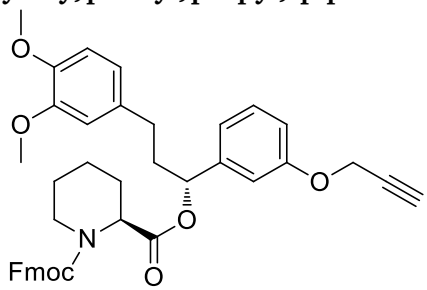
TLC [cyclohexane/EtOAc 6/4]: R_f = 0.49.

HPLC [0-100% Solvent B, 20 min]: R_t = 17.10 min, purity (220 nm) = 97%.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.29 (t, J = 7.9 Hz, 1H), 7.01 – 6.96 (m, 2H), 6.90 (ddd, J = 8.2, 2.5, 0.9 Hz, 1H), 6.81 – 6.77 (m, 1H), 6.75 – 6.70 (m, 2H), 4.70 (d, J = 2.4 Hz, 2H), 4.68 (dd, J = 7.8, 5.4 Hz, 1H), 3.86 (s, 3H), 3.86 (s, 3H), 2.75 – 2.57 (m, 2H), 2.51 (t, J = 2.4 Hz, 1H), 2.15 – 1.96 (m, 2H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ 157.90, 149.01, 147.37, 146.57, 134.46, 129.71, 120.34, 119.28, 114.04, 112.72, 111.93, 111.43, 78.68, 75.68, 73.87, 56.08, 55.98, 55.95, 40.70, 31.74.

(S)-1-((9H-Fluoren-9-yl)methyl) 2-((R)-3-(3,4-dimethoxyphenyl)-1-(3-(prop-2-yn-1-yloxy)phenyl)propyl) piperidine-1,2-dicarboxylate (22)



22

20 (240 mg, 0.735 mmol, 1.0 eq.) and (S)-N-Fmoc-piperidine-2-carboxylic acid (284 mg, 0.809 mmol, 1.1 eq.) were dissolved in DCM (3.5 mL) followed by the addition of EDC (155 mg, 0.809 mmol, 1.1 eq.) and DMAP (18 mg, 0.147 mmol, 0.2 eq.) at RT. After stirring for 16h, the mixture was concentrated under reduced pressure and purified by flash column chromatography (cyclohexane:EtOAc, gradient EtOAc 0 to 15%) to give a yellow oil (419mg, 0.63 mmol, 86%)

TLC [cyclohexane/EtOAc 6/4]: R_f = 0.51.

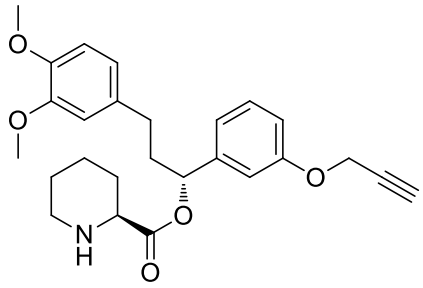
HPLC [50-100% Solvent B, 20 min]: R_t = 20.91 min, purity (220 nm) ≥ 99%.

^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.80 – 7.69 (m, 2H), 7.60 (t, J = 8.4 Hz, 1H), 7.51 – 7.17 (m, 6H), 6.98 – 6.84 (m, 3H), 6.74 (d, J = 8.1 Hz, 1H), 6.67 – 6.57 (m, 2H), 5.82 – 5.76 (m, 1H), 5.08 – 4.84 (m, 1H), 4.70 – 4.57 (m, 2H), 4.49 – 4.07 (m, 4H), 3.86 – 3.78 (m, 6H), 3.20 – 2.95 (m, 1H), 2.62 – 2.42 (m, 3H), 2.37 – 2.29 (m, 1H), 2.26 – 2.15 (m, 1H), 2.08 – 2.04 (m, 1H), 1.77 – 1.66 (m, 3H), 1.53 – 1.40 (m, 1H), 1.34 – 1.27 (m, 1H).

^{13}C NMR (126 MHz, Chloroform-*d*) δ 171.01, 157.82, 156.47, 156.03, 149.01, 147.46, 144.22, 144.13, 143.98, 143.93, 141.85, 141.60, 141.39, 133.62, 133.50, 129.73, 127.76, 127.14, 125.16, 125.03, 120.22, 120.04, 119.89, 119.77, 114.43, 113.59, 113.37, 111.88, 111.81, 111.50, 78.57, 76.53, 76.26, 75.78, 75.71, 67.87, 60.45, 56.03, 55.92, 55.02, 54.65, 47.35, 42.12, 42.00, 38.16, 31.23, 27.11, 26.92, 24.89, 24.66, 21.11, 20.91, 20.80, 14.30.

MS (ESI+): m/z: found 682.09 $[M+Na]^+$, calculated 682.28 $[M+Na]^+$

(S)-(R)-3-(3,4-Dimethoxyphenyl)-1-(3-(prop-2-yn-1-yloxy)phenyl)propyl piperidine-2-carboxylate (19)



19

22 was dissolved in 3mL 4-methylpiperidine/DCM (v/v= 1:9). The solution was stirred at RT overnight.
Then the solution was diluted with 100 ml DCM, and washed with saturated NH_4Cl solution. The organic layer was dried over magnesium sulfate, filtered and concentrated under reduced pressure. Purification by flash column chromatography (cyclohexane → cyclohexane: EtOAc =2:8 + 1% TEA) gave a yellow oil (198mg, 0.45 mmol, 73%).

TLC [EtOAc + 1% TEA]: R_f = 0.23.

HPLC [0-100% Solvent B, 20 min]: R_t = 14.37 min, purity (220 nm) = 96%.

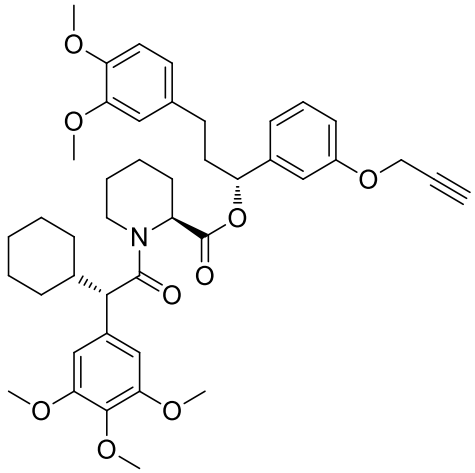
^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.19 (t, J = 8.0 Hz, 1H), 6.90 – 6.85 (m, 2H), 6.85 – 6.80 (m, 1H), 6.70 (d, J = 8.1 Hz, 1H), 6.62 – 6.56 (m, 2H), 5.70 (dd, J = 7.8, 5.7 Hz, 1H), 4.61 (d, J = 2.4 Hz, 2H), 3.78 (s, 3H), 3.77 (s, 3H), 3.29 (dd, J = 9.9, 3.2 Hz, 1H), 2.99 (dt, J = 11.0, 3.4 Hz, 1H), 2.61 – 2.45 (m, 3H), 2.44 (t, J = 2.4 Hz, 1H), 2.22 – 2.12 (m, 1H), 2.04 – 1.93 (m, 2H), 1.77 – 1.69 (m, 1H), 1.58 – 1.46 (m, 2H), 1.45 – 1.32 (m, 2H).

^{13}C NMR (126 MHz, Chloroform-*d*) δ 172.92, 157.79, 149.04, 147.49, 142.06, 133.72, 129.67, 120.27, 119.86, 114.19, 113.47, 111.86, 111.50, 78.60, 75.71, 75.51, 58.87, 56.04, 55.98,

55.95, 45.78, 38.05, 31.41, 29.39, 25.96, 24.26.

MS (ESI+): m/z: found 438.02 $[M+H]^+$, calculated 438.23 $[M+H]^+$

(S)-(R)-3-(3,4-Dimethoxyphenyl)-1-(3-(prop-2-yn-1-yloxy)phenyl)propyl 1-((S)-2-cyclohexyl-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2-carboxylate (16)



16

16 was synthesized from **19** (180 mg, 0. 41 mmol) according to the General Method H. Purification by flash column chromatography (cyclohexane:EtOAc, gradient EtOAc 0 to 20%) gave a white solid (200mg, 0.275 mmol, 67%).

TLC [cyclohexane/EtOAc 6/4]: $R_f = 0.45$.

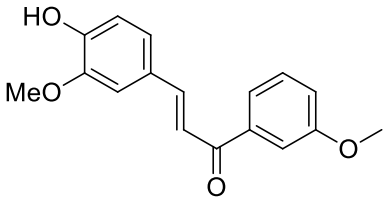
HPLC [0%–100% eluent B, 20 min]: Rt = 18.5 min, ≥ 99% purity

^{1}H NMR (500 MHz, DMSO-d_6, 353K) δ 7.27 (m, 1H), 7.06 – 6.58 (m, 8H), 5.58 (dd, J = 7.9, 5.6 Hz, 1H), 5.30 (dd, J = 6.0, 2.7 Hz, 1H), 4.84 – 4.72 (m, 2H), 4.16 (d, J = 13.8 Hz, 1H), 3.84 – 3.59 (m, 16H), 3.46 – 3.35 (m, 1H), 2.89 – 2.75 (m, 1H), 2.62 (m, 1H), 2.50 – 2.33 (m, 2H), 2.29 – 2.12 (m, 1H), 2.12 – 1.76 (m, 3H), 1.73 – 1.37 (m, 6H), 1.34 – 0.66 (m, 8H).

^{13}C NMR (126 MHz, DMSO-d_6, 353K) δ 172.04, 170.21, 157.33, 152.67, 149.15, 147.54, 141.90, 137.02, 133.63, 133.59, 129.30, 120.29, 118.87, 114.22, 113.19, 113.07, 112.98, 106.84, 79.18, 77.71, 74.92, 59.85, 56.35-55.50, 52.97, 51.84, 42.99, 40.96, 37.32, 31.69, 30.35, 29.82, 26.33-25.02, 20.43.

HRMS: m/z: found 728.37910 $[M+H]^+$, calculated 728.37931 $[M+H]^+$

(E)-3-(4-hydroxy-3-methoxyphenyl)-1-(3-methoxyphenyl)prop-2-en-1-one (25a)



25a

4-Hydroxy-3-methoxybenzaldehyde (vanillin) (22.8 g, 0.15 mmol, 1 eq.) was dissolved in ethanol (150 mL) and 1-(3-methoxyphenyl)ethan-1-one (22.5 g, 20.6 mL, 0.15 mmol, 1 eq.) was added. The mixture was cooled to 0 °C. Potassium hydroxide (33.7 g, 0.6 mmol, 4 eq.) was dissolved in water (100 mL) and the solution was cooled to ~10°C. The KOH solution was added slowly to the reaction mixture. To dissolve the formed precipitation, ethanol (260 mL) was added. The solution was warmed to room temperature and stirred overnight. After 25 h, potassium hydroxide (8.4 g, 0.15 mmol, 1 eq.) was added and the solution was heated to 40 °C. After 28.5 h potassium hydroxide (8.4 g, 0.15 mmol, 1 eq.) was added, the solution was heated to 50°C and stirred overnight. Most of the ethanol was removed under vacuum and the solution was stirred at 40°C for another day. Water was added to dissolve the precipitation and conc. HCl (~80 mL) was added to acidify (pH<2) the reaction mixture. The mixture was extracted three times with 250 mL DCM. The combined organic phases were washed with brine, dried over magnesium sulfate, filtered, and the solvent was removed under vacuum. The crude product was filtered through a silica gel pad with pure DCM, then with cyclohexane:EtOAc = 3/2. The combined product fractions were dissolved in NaOH (1 M) and washed with DCM. The formed precipitation was filtered and washed with DCM. The precipitation was dissolved in water, acidified with conc. HCl and extracted with DCM. The combined organic phases were dried over magnesium sulfate, filtered, and the solvent was removed under vacuum to give 16.5 g (57.9 mmol, 39%) **25a** as a brown-yellow oil which became solid after 2 days.

TLC [cyclohexane/EtOAc 6/4]: R_f = 0.41.

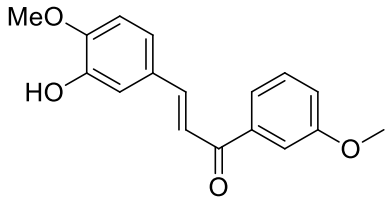
HPLC [20-80% Solvent B, 20 min]: R_t = 13.66 min, purity (220 nm) = 99%.

^{1}H-NMR (300 MHz, Chloroform-*d*): δ = 7.75 (d, J = 15.6 Hz, 1H), 7.58 (d, J = 7.6 Hz, 1H), 7.55 – 7.51 (m, 1H), 7.40 (m, 1H), 7.35 (d, J= 15.6 Hz, 1H), 7.19 (dd, J = 8.2, 1.9 Hz, 1H), 7.14 – 7.08 (m, 2H), 6.95 (d, J = 8.2 Hz, 1H), 6.20 (s, 1H), 3.92 (s, 3H), 3.86 (s, 3H) ppm.

^{13}C-NMR (75 MHz, Chloroform-*d*): δ = 190.45, 159.92, 148.52, 146.98, 145.40, 139.96, 129.58, 127.51, 123.53, 121.04, 119.81, 119.01, 115.03, 113.07, 110.18, 56.09, 55.55 ppm.

Mass (ESI+): m/z: calculated 285.11 $[C17H16O4+H]^+$, found 285.17 $[M+H]^+$.

(E)-3-(3-hydroxy-4-methoxyphenyl)-1-(3-methoxyphenyl)prop-2-en-1-one (25b)



25b

Isovanillin (22.8 g, 150 mmol) and 3′-Methoxyacetophenone (22.5 g, 150 mmol) were dissolved in 150 mL EtOH and cooled to 0°C in an ice bath. KOH (33.7 g, 600 mmol) was dissolved in 100 mL H_2O and added to the aforementioned ketone/aldehyde solution. Another 200 mL EtOH were added. The reaction mixture was allowed to warm to RT and stirred for 1 d. The solution was poured into ice and the solution was acidified with conc. HCl to pH < 2. The solution was extracted with ethyl acetate, dried over $MgSO_4$, filtered and concentrated in vacuum to dryness. The resulting solid was dissolved in DCM and filtered by a silica gel pad. The resulting residue was recrystallized in methanol to afford **25b** (26.6 g, 93.6 mmol, 63%).

TLC [cyclohexane/EtOAc 6/4]: Rf = 0.41.

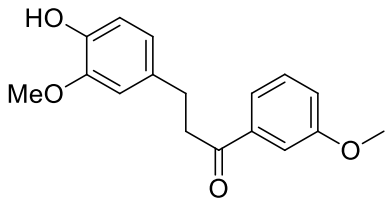
HPLC [0%–100% eluent B, 20 min]: Rt = 13.6 min, 91% purity

Mass: (ESI^+), m/z: calculated 285.11 $[C_{17}H_{16}O_4 + H]^+$, found 285.18 $[M + H]^+$

^{1}H-NMR (300 MHz, Chloroform-*d*): δ 7.74 (d, *J* = 15.6 Hz, 1H), 7.59 (d, *J* = 7.9 Hz, 1H), 7.53 (s, 1H), 7.39 (dd, *J* = 15.8, 8.8 Hz, 2H), 7.28 (d, *J* = 2.1 Hz, 1H), 7.13 (t, *J* = 2.5 Hz, 2H), 6.88 (d, *J* = 8.3 Hz, 1H), 5.68 (s, 1H), 3.94 (s, 3H), 3.88 (s, 3H) ppm.

^{13}C-NMR (75 MHz, Chloroform-*d*): δ 144.78, 129.51, 122.72, 120.97, 120.36, 119.18, 113.02, 112.76, 110.58, 56.03, 55.48 ppm.

3-(4-hydroxy-3-methoxyphenyl)-1-(3-methoxyphenyl)propan-1-one (26a)



26a

25a (2.3 g, 8.23 mmol, 1 eq.) was dissolved in ethanol (820 mL). Ammoniumacetate (NH_4OAc) (31.7 g, 412 mmol, 50 eq.) was dissolved in water (66 mL) and was added to the reaction mixture. Zinc powder (1.6 g, 24.69 mmol, 3 eq.) was added in two portions within 2 min. The mixture was stirred at room temperature for 12 – 15 min. After filtration, the solvent was removed under vacuum. The solution was diluted with water (40 mL) and was extracted

with DCM. The combined organic phases were dried over magnesium sulfate, filtered and the solvent was removed under vacuum. The crude product was purified by column chromatography (cyclohexane:EtOAc, gradient EtOAc 20 to 40%) to give 6.1 g (21.13 mmol, 64%) **26a**.

TLC [cyclohexane/EtOAc 6/4]: Rf = 0.58.

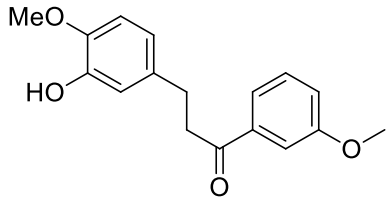
HPLC [20-80% Solvent B, 20 min]: Rt = 12.54 min, purity (220 nm) = 99%.

^{1}H-NMR (300 MHz, Chloroform-*d*): δ = 7.53 (d, J = 7.7 Hz, 1H), 7.50 – 7.46 (m, 1H), 7.35 (t, J = 7.9 Hz, 1H), 7.10 (dd, J = 8.5, 2.9 Hz, 1H), 6.85 (d, J = 7.8 Hz, 1H), 6.74 (d, J = 8.9 Hz, 2H), 5.34 (s, 1H), 3.86 (s, 3H), 3.84 (s, 3H), 3.26 (t, J = 7.6 Hz, 2H), 3.00 (t, J = 7.6 Hz, 2H) ppm.

^{13}C-NMR (75 MHz, Chloroform-*d*): δ = 199.41, 159.95, 146.57, 144.08, 138.40, 133.26, 129.68, 120.98, 120.78, 119.61, 114.50, 112.46, 111.30, 55.99, 55.54, 41.01, 30.09 ppm.

Mass (ESI+): m/z: calculated 287.13 $[C17H18O4+H]^+$, found 286.96 $[M+H]^+$.

3-(3-hydroxy-4-methoxyphenyl)-1-(3-methoxyphenyl)propan-1-one (26b)



26b

A 0.05 M solution of ketone **25b** (7.0 g, 24.61 mmol) in 95% ethanol was added to 55 mL water containing ammonium acetate (94.9 g, 1230.6 mmol) at room temperature. The solution was stirred vigorously while zinc powder (3.2 g, 73.85 mmol) was added in five equal portions at intervals of 15 min. Stirring was continued for further 15 min. The suspended material was removed by filtration and washed with ethanol and the filtrate was evaporated under reduced pressure nearly to dryness. The residue was poured into ice-cooled water and it was extracted with DCM (5 x 100 mL), dried over $MgSO_4$, filtered and concentrated in vacuum to dryness. The crude product was purified by chromatography (cyclohexane:EtOAc, gradient EtOAc 10 to 30%) to afford ketone **4** (4.5 g, 13.8 mmol, 64%).

TLC [cyclohexane/EtOAc 8/2]: R_f = 0.23.

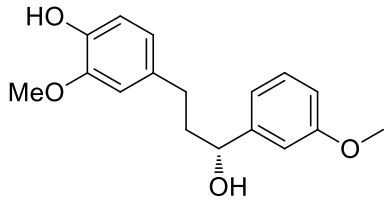
HPLC [0%–100% eluent B, 20 min]: R_t = 13.4 min, 97% purity

^{1}H-NMR (300 MHz, Chloroform-*d*): δ 7.53 (d, *J* = 7.6 Hz, 1H), 7.49 (s, 1H), 7.35 (t, *J* = 7.9 Hz, 1H), 7.10 (d, *J* = 8.2 Hz, 1H), 6.83 (s, 1H), 6.75 (q, *J* = 8.2 Hz, 2H), 5.64 (s, 1H), 3.86 (s, 3H), 3.85 (s, 3H), 3.25 (t, *J* = 7.7 Hz, 2H), 2.97 (t, *J* = 7.6 Hz, 2H) ppm.

^{13}C-NMR (75 MHz, Chloroform-*d*): δ 199.15, 159.84, 145.60, 144.99, 138.28, 134.56, 129.56, 120.68, 119.77, 119.57, 114.59, 112.25, 110.78, 56.02, 55.43, 40.67, 29.62 ppm.

Mass: (ESI^+), m/z: calculated 287.13 $[C_{17}H_{18}O_4 + H]^+$, found 287.08 $[M + H]^+$

(R)-4-(3-hydroxy-3-(3-methoxyphenyl)propyl)-2-methoxyphenol (27a)



27a

Ketone **26a** (5.4 g, 18.9 mmol) was suspended in 90 mL isopropanol and the solution was degassed with argon in an autoclave. K_2CO_3 (2.6 g, 18.9 mmol) and the catalyst $RuCl_2$[(*S*)-(DM-SEGPHOS)][(*S*)-DAIPEN] (182 mg, 0.15 mmol) were added. The autoclave was closed and flushed with H_2 for three times. A pressure of 10 bar H_2 was applied and the mixture was stirred for 2 d. The solution was filtered and the filtrate was concentrated in vacuum. The crude product was purified by chromatography (cyclohexane:EtOAc, gradient EtOAc 10 to 40%) to give 5.1 g (17.7 mmol, 94%) alcohol **27a**.

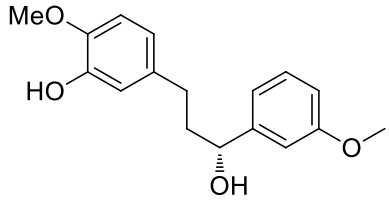
TLC [cyclohexane/EtOAc 6/4]: R_f=0.42.

^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.20 – 7.15 (m, 1H), 6.86 – 6.81 (m, 2H), 6.76 – 6.71 (m, 2H), 6.61 – 6.57 (m, 2H), 5.46 (s, 1H), 4.57 (dd, *J* = 7.8, 5.2 Hz, 1H), 3.76 (s, 3H), 3.72 (s, 3H), 2.65 – 2.44 (m, 2H), 2.06 – 1.82 (m, 3H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 159.90, 146.53, 146.47, 143.82, 133.78, 129.63, 121.07, 118.36, 114.39, 113.14, 111.58, 111.17, 73.92, 55.99, 55.35, 40.78, 31.84.

HPLC [0%–100% eluent B, 20 min]: R_t = 11.4 min, 95% purity

(R)-5-(3-hydroxy-3-(3-methoxyphenyl)propyl)-2-methoxyphenol (27b)



27b

Ketone **26b** (3.32 g, 11.62 mmol) was suspended in 100 mL isopropanol and the solution was degassed with argon in an autoclave. K_2CO_3 (1.61 g, 11.62 mmol) and the catalyst $RuCl_2$[(*S*)-(DM-SEGPHOS)][(*S*)-DAIPEN] (0.11 g, 0.09 mmol) were added. The autoclave was closed and flushed with H_2 for three times. A pressure of 10 bar H_2 was applied and the mixture was stirred for 2 d. The solution was filtered and the filtrate was concentrated in vacuum. The crude product was purified by chromatography (cyclohexane:EtOAc, gradient EtOAc 20 to 40%) to give 3.30 g (11.44 mmol, 98.8%) alcohol **27b**.

TLC [cyclohexane/EtOAc 6/4]: R_f = 0.43.

HPLC [0%–100% eluent B, 20 min]: Rt = 10.8 min, 99% purity

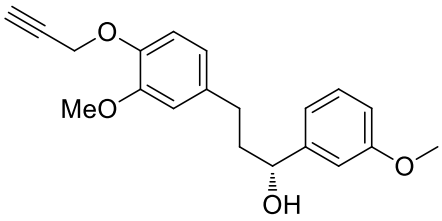
ee = 99%

HR-MS: (ESI$^+$), m/z: calculated 311.13 [$C_{17}H_{20}O_4$ + Na]$^+$, found 311.13 [M + Na]$^+$

^{1}H-NMR (300 MHz, Chloroform-*d*): 7.27 (t, *J* = 8.1 Hz, 1H), 6.93 (dd, *J* = 4.4, 1.8 Hz, 2H), 6.83 (ddd, *J* = 8.2, 2.5, 1.0 Hz, 1H), 6.80 – 6.75 (m, 2H), 6.68 (dd, *J* = 8.2, 2.0 Hz, 1H), 4.66 (dd, *J* = 7.7, 5.4 Hz, 1H), 3.87 (s, 3H), 3.82 (s, 3H), 2.63 (td, *J* = 9.0, 6.6 Hz, 2H), 2.16 – 1.95 (m, 2H) ppm.

^{13}C-NMR (75 MHz, Chloroform-*d*): 159.77, 146.38, 145.51, 144.81, 135.11, 129.49, 119.73, 118.26, 114.75, 113.08, 111.37, 110.73, 73.71, 56.02, 55.23, 40.48, 31.38 ppm.

(R)-3-(3-methoxy-4-(prop-2-yn-1-yloxy)phenyl)-1-(3-methoxyphenyl)propan-1-ol (28a)



28a

TLC [cyclohexane/EtOAc 6/4]: R_f = 0.47.

Mass (ESI+): m/z: calculated 309.15 [$C_{20}H_{22}O_4$+H–H_2O]$^+$, found 309.17 [M+H–H_2O]$^+$.

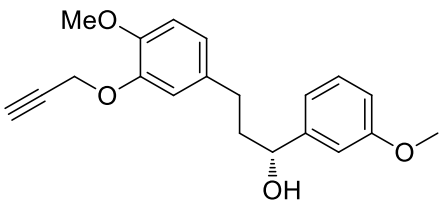
HPLC [0%–100% eluent B, 20 min]: R_t = 13.9 min, 98% purity

ee = 93%.

^{1}H-NMR (300 MHz, Chloroform-*d*): δ = 7.56 (t, J = 8.1 Hz, 1H), 7.29 – 7.17 (m, 3H), 7.16 – 7.08 (m, 1H), 7.06 – 7.00 (m, 2H), 5.02 (d, J = 2.4 Hz, 2H), 4.96 (dd, J = 7.7, 5.4 Hz, 1H), 4.14 (s, 3H), 4.10 (s, 3H), 3.08 – 2.86 (m, 2H), 2.79 (t, J = 2.4 Hz, 1H), 2.47 – 2.20 (m, 2H) ppm.

^{13}C-NMR (75 MHz, Chloroform-*d*): δ = 159.90, 149.75, 146.44, 145.06, 136.15, 129.63, 120.25, 118.33, 114.90, 113.12, 112.41, 111.57, 78.97, 75.68, 73.89, 57.08, 55.96, 55.33, 40.58, 31.81 ppm.

(R)-3-(4-methoxy-3-(prop-2-yn-1-yloxy)phenyl)-1-(3-methoxyphenyl)propan-1-ol (28b)



28b

To a solution of **27b** (9.98 g, 34.6 mmol) and K_2CO_3 (47.84 g, 346.1 mmol) in 150 mL acetone,

3-Bromprop-1-yne (3.71 mL, 4.94 g, 41.5 mmol) was added and stirred at RT for 4 d. The solution was filtered and the filter cake wash washed with acetone. The combined solution was concentrated in vacuum and purified by chromatography (cyclohexane:EtOAc, gradient EtOAc 30 to 40%) to give 10.5 g (32.0 mmol, 93%) alcohol **28b**.

TLC [cyclohexane/EtOAc 8/2]: $R_f = 0.13$.

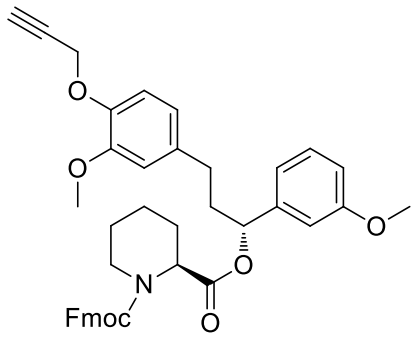
HPLC [0%–100% eluent B, 20 min]: Rt = 13.4 min, ≥ 99% purity

Mass: (ESI^+), m/z: calculated 309.15 $[C_{20}H_{22}O_4+H–H_2O]^+$, found 309.13 $[M + H – H_2O]^+$

^{1}H-NMR (300 MHz, Chloroform-*d*): 7.25 (t, *J* = 8.1 Hz, 1H), 6.91 (dd, *J* = 4.6, 2.4 Hz, 2H), 6.88 (s, 1H), 6.85 – 6.77 (m, 3H), 4.73 (d, *J* = 2.4 Hz, 2H), 4.64 (dd, *J* = 7.8, 5.3 Hz, 1H), 3.84 (s, 3H), 3.80 (s, 3H), 2.66 (td, *J* = 8.8, 6.6 Hz, 2H), 2.50 (t, *J* = 2.4 Hz, 1H), 2.13 – 1.94 (m, 3H) ppm.

^{13}C-NMR (75 MHz, Chloroform-*d*): 159.78, 147.95, 146.60, 146.41, 134.27, 129.50, 121.83, 118.23, 115.13, 112.97, 111.92, 111.48, 78.79, 75.71, 73.61, 56.81, 55.98, 55.23, 40.47, 31.47 ppm.

(S)-1-((9H-fluoren-9-yl)methyl) 2-((R)-3-(3-methoxy-4-(prop-2-yn-1-yloxy)phenyl)-1-(3-methoxyphenyl)propyl) piperidine-1,2-dicarboxylate (29a)



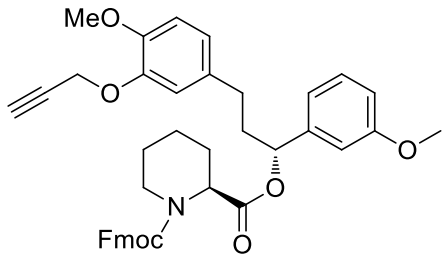
EDC-HCl (1.20 g, 6.25 mmol), DMAP (0.18 g, 1.56 mmol) and (S)-N-Fmoc-piperidine-2-carboxylic acid (1.83 g, 5.21 mmol) were dissolved in 52 mL DCM at 0°C. Alcohol **28a** (1.70 g, 5.21 mmol) was added and the mixture was stirred at 0°C for 15 min and at RT overnight. The solution was concentrated in vacuum and purified by chromatography (cyclohexane:EtOAc, gradient EtOAc 20 to 30%) to afford ester **29a** (3.3 g, 5.00 mmol, 96%) as a yellow oil.

TLC [cyclohexane/EtOAc 6/4]: $R_f = 0.67$.

^{1}H NMR (rotamers represent) (500 MHz, Chloroform-*d*) δ 7.84 – 7.71 (m, 2H), 7.63 (t, *J* = 7.3 Hz, 1H), 7.54 – 7.45 (m, 1H), 7.45 – 7.30 (m, 3H), 7.29 – 7.20 (m, 2H), 6.97 – 6.88 (m, 3H), 6.87 – 6.79 (m, 1H), 6.71 – 6.59 (m, 2H), 5.87 – 5.74 (m, 1H), 5.11 – 4.87 (m, 1H), 4.74 (d, *J* = 2.2 Hz, 2H), 4.54 – 4.10 (m, 4H), 3.85 – 3.71 (m, 6H), 3.22 – 2.95 (m, 1H), 2.66 – 2.46 (m, 3H), 2.39 – 2.19 (m, 2H), 2.07 – 2.02 (m, 1H), 1.80 – 1.68 (m, 3H), 1.55 – 1.43 (m, 1H), 1.30 – 1.27 (m, 1H).

^{13}C NMR (126 MHz, $CDCl_3$) (major) δ 171.05, 159.81, 156.48, 149.75, 145.17, 144.21, 143.99, 141.73, 141.39, 135.28, 129.72, 127.77, 127.14, 125.16, 120.07, 118.81, 114.87, 113.48, 112.39, 112.33, 78.92, 76.42, 75.69, 67.88, 57.04, 55.92, 55.30, 54.65, 47.34, 42.14, 38.11, 31.34, 26.91, 24.90, 20.91.

(S)-1-((9H-fluoren-9-yl)methyl) 2-((R)-3-(4-methoxy-3-(prop-2-yn-1-yloxy)phenyl)-1-(3-methoxyphenyl)propyl) piperidine-1,2-dicarboxylate (29b)



29b

EDC-HCl (0.71 g, 3.68 mmol), DMAP (0.11 g, 0.92 mmol) and (S)-N-Fmoc-piperidine-2-carboxylic acid (1.08 g, 3.06 mmol) were dissolved in 30 mL DCM at 0°C. Alcohol **28b** (1.00 g, 3.06 mmol) was added and the mixture was stirred at 0°C for 15 min and at RT overnight. The solution was concentrated in vacuum and purified by chromatography (cyclohexane:EtOAc, gradient EtOAc 0 to 20%) to afford ester **29b** (1.92 g, 2.91 mmol, 95%) as a yellow oil.

TLC [cyclohexane/EtOAc 8/2]: R_f = 0.23.

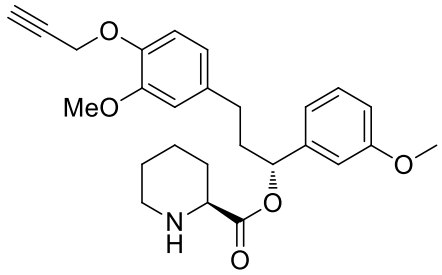
HPLC [50%–100% eluent B, 20 min]: Rt = 15.6 min, ≥ 99% purity

Mass (ESI^+), m/z: calculated 682.28 $[C_{41}H_{41}NO_7 + Na]^+$, found 682.29 $[M+ Na]^+$

^{1}H-NMR (300 MHz, Chloroform-*d*): 7.75 (dd, *J* = 13.6, 7.5 Hz, 2H), 7.60 (t, *J* = 6.1 Hz, 1H), 7.53 - 7.16 (m, 6H), 6.91 (d, *J* = 7.7 Hz, 1H), 6.87 (d, *J* = 2.2 Hz, 1H), 6.85 – 6.62 (m, 4H), 5.76 (s, 1H), 5.08 – 4.85 (m, 1H), 4.73 – 4.67 (m, 2H), 4.51 – 4.04 (m, 4H), 3.83 (s, 3H), 3.75 (d, *J* = 18.1 Hz, 3H), 3.08 (dt, *J* = 43.4, 12.5 Hz, 1H), 2.61 – 2.40 (m, 3H), 2.38 – 2.14 (m, 2H), 2.05 (t, *J* = 7.1 Hz, 1H), 1.70 (dt, *J* = 9.7, 5.2 Hz, 3H), 1.55 – 1.15 (m, 6H) ppm.

^{13}C-NMR (75 MHz, Chloroform-*d*): 170.92, 159.71, 148.09, 146.64, 144.12, 143.91, 141.67, 141.29, 133.40, 129.62, 127.66, 127.05, 125.08, 121.73, 119.95, 118.86, 118.71, 115.02, 113.35, 112.41, 112.31, 111.93, 78.71, 75.73, 67.79, 56.81, 55.96, 55.20, 54.92, 54.55, 47.25, 42.02, 41.92, 38.01, 31.00, 27.04, 26.93, 26.82, 24.82, 24.58, 20.82, 20.72 ppm.

(S)-(R)-3-(4-(ethynyloxy)-3-methoxyphenyl)-1-(3-methoxyphenyl)propyl piperidine-2-carboxylate (30a)



30a

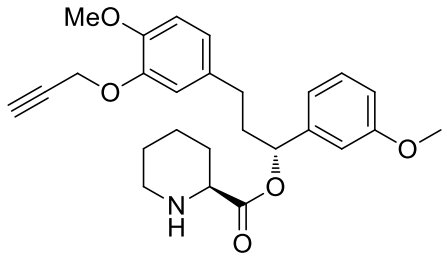
Ester **29a** (3.25 g, 4.93 mmol) was dissolved in 25 mL 4-methylpiperidine/DCM (v/v=1:9) and the mixture was stirred at RT for 2 h. Then the solution was diluted with DCM, washed with sat. NH_4Cl, dried over $MgSO_4$ and concentrated in vacuum. The crude product was purified by chromatography (cyclohexane:EtOAc with 1% TEA, gradient EtOAc 0 to 80%) to afford **30a** (2.09 g, 4.78 mmol, 96%).

TLC [EtOAc + 1% TEA]: $R_f = 0.28$.

^{1}H NMR (300 MHz, Chloroform-*d*) δ 7.24 (t, J = 7.9 Hz, 1H), 6.96 – 6.87 (m, 2H), 6.87 – 6.77 (m, 2H), 6.72 – 6.62 (m, 2H), 5.77 (dd, J = 7.9, 5.7 Hz, 1H), 4.71 (d, J = 2.4 Hz, 2H), 3.83 (s, 3H), 3.78 (s, 3H), 3.36 (dd, J = 9.7, 3.2 Hz, 1H), 3.05 (dt, J = 11.8, 3.4 Hz, 1H), 2.71 – 2.50 (m, 3H), 2.48 (t, J = 2.4 Hz, 1H), 2.32 – 2.17 (m, 1H), 2.12 – 2.02 (m, 2H), 1.85 – 1.71 (m, 1H), 1.66 – 1.37 (m, 4H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 172.89, 159.73, 149.73, 145.15, 141.84, 135.32, 129.62, 120.12, 118.85, 114.84, 113.24, 112.43, 112.25, 78.86, 75.67, 75.59, 58.79, 57.00, 55.93, 55.28, 45.70, 37.92, 31.47, 29.34, 25.90, 24.20.

(S)-(R)-3-(4-methoxy-3-(prop-2-yn-1-yloxy)phenyl)-1-(3-methoxyphenyl)propyl piperidine-2-carboxylate (30b)



30b

Ester **29b** (1.82 g, 2.76 mmol) was dissolved in 13.5 mL 4-methylpiperidine/DCM (v/v=1:9) and the mixture was stirred at RT for 2 h. Then the solution was diluted with DCM, washed with sat. NH_4Cl, dried over $MgSO_4$ and concentrated in vacuum. The crude product was purified by chromatography (cyclohexane:EtOAc with 1% TEA, gradient EtOAc 40 to 60%) to afford

30b (1.07 g, 2.45 mmol, 89%).

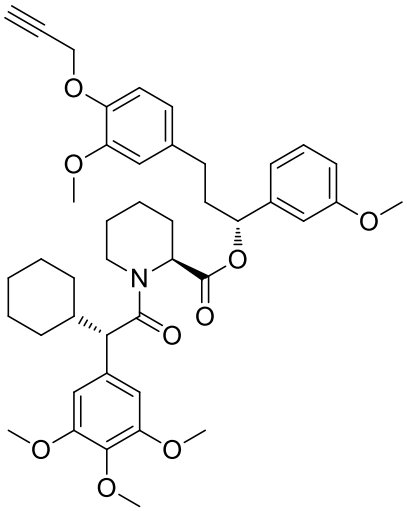
TLC [cyclohexane/EtOAc 6/4]: R_f = 0.13.

HPLC [0%–100% eluent B, 20 min]: R_t = 12.0 min, 98% purity

^{1}H-NMR (300 MHz, Chloroform-*d*): 7.28 – 7.19 (m, 1H), 6.90 (d, *J* = 7.7 Hz, 1H), 6.87 – 6.78 (m, 4H), 6.74 (dd, *J* = 8.2, 1.9 Hz, 1H), 5.75 (dd, *J* = 7.9, 5.7 Hz, 1H), 4.74 (d, *J* = 2.4 Hz, 2H), 3.83 (s, 3H), 3.79 (s, 3H), 3.37 (dd, *J* = 9.7, 3.2 Hz, 1H), 3.06 (dt, *J* = 12.0, 3.4 Hz, 1H), 2.69 – 2.53 (m, 3H), 2.51 (t, *J* = 2.4 Hz, 1H), 2.32 – 2.20 (m, 2H), 2.12 – 1.99 (m, 3H), 1.83 – 1.75 (m, 1H), 1.63 – 1.40 (m, 4H) ppm.

^{13}C-NMR (75 MHz, Chloroform-*d*): 172.73, 159.68, 148.13, 146.66, 141.82, 133.51, 129.54, 121.78, 118.79, 115.11, 113.16, 112.40, 111.95, 78.72, 75.76, 75.48, 58.68, 56.88, 55.97, 55.23, 45.59, 37.86, 31.18, 29.21, 25.78, 24.10 ppm.

(S)-(R)-3-(3-methoxy-4-(prop-2-yn-1-yloxy)phenyl)-1-(3-methoxyphenyl)propyl 1-((S)-2-cyclohexyl-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2-carboxylate (31a)



31a

31a was synthesized from **30a** (853 mg, 1.95 mmol) according to the General Method H. Purification by flash column chromatography (cyclohexane:EtOAc, gradient EtOAc 0 to 30%) gave a white solid (894 mg, 1.23 mmol, 63%).

TLC [cyclohexane/EtOAc 6/4]: R_f = 0.45.

HPLC [50-100% Solvent B, 20 min]: R_t = 14.26 min, purity (254 nm) ≥ 99%.

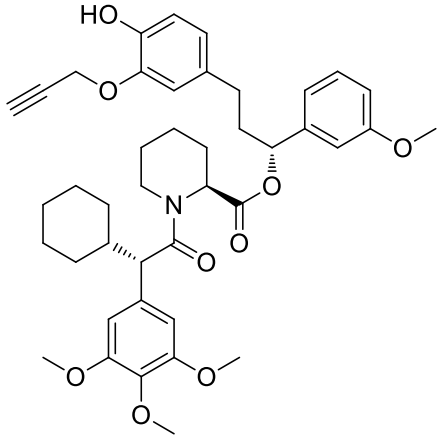
^{1}H NMR (500 MHz, Chloroform-*d*) (3:1 mixture of rotamers) δ 7.23 (t, *J* = 7.9 Hz, 0.25H), 7.04 (t, *J* = 7.9 Hz, 0.75H), 6.91 – 6.76 (m, 2H), 6.70 – 6.62 (m, 1H), 6.59 – 6.54 (m, 2H), 6.45 – 6.30 (m, 3H), 5.75 (t, *J* = 6.9 Hz, 0.25H), 5.51 (dd, *J* = 7.9, 5.8 Hz, 0.75H), 5.41 (d, *J* = 4.7 Hz, 0.75H), 4.67 – 4.63 (m, 2H), 4.49 (d, *J* = 13.5 Hz, 0.25H), 3.89 (d, *J* = 13.7 Hz, 1H), 3.78 – 3.74 (m, 6H), 3.70 – 3.62 (m, 9H), 3.31 (d, *J* = 9.9 Hz, 0.75H), 2.90 (d, *J* = 9.6 Hz, 0.25H), 2.71 (td, *J* = 13.3, 2.7 Hz, 1H), 2.54 – 2.30 (m, 3H), 2.22 (d, *J* = 14.0 Hz, 1H),

2.06 – 1.99 (m, 1H), 1.92 – 1.72 (m, 3H), 1.62 – 1.48 (m, 6H), 1.28 – 1.17 (m, 3H), 1.14 – 0.89 (m, 3H), 0.86 – 0.45 (m, 2H).

^{13}C NMR (126 MHz, $CDCl_3$) (major) δ 172.37, 170.65, 159.56, 153.18, 149.75, 145.14, 141.82, 136.99, 135.27, 133.75, 129.62, 120.29, 118.52, 114.91, 113.18, 112.50, 112.44, 105.87, 78.94, 75.78, 75.68, 60.85, 57.07, 56.15, 55.97, 55.26, 52.12, 43.77, 41.53, 38.05, 32.90, 31.21, 30.77, 26.85, 26.71, 26.33, 26.29, 25.70, 21.09.

HRMS: m/z: found 728.37926 $[M+H]^+$, calculated 728.37931 $[M+H]^+$

(S)-(R)-3-(4-methoxy-3-(prop-2-yn-1-yloxy)phenyl)-1-(3-methoxyphenyl)propyl 1-((S)-2-cyclohexyl-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2-carboxylate (31b)



31b

31b was synthesized from **30b** (500 mg, 1.15 mmol) according to the General Method H. Purification by flash column chromatography (cyclohexane:EtOAc, gradient EtOAc 0 to 40%) gave a white solid (546 mg, 0.75 mmol, 65%).

TLC [cyclohexane/EtOAc 6/4]: $R_f = 0.60$.

HPLC [50%–100% eluent B, 20 min]: R_t = 12.4 min, 98% purity

Mass (ESI^+): m/z: calculated 728.38 $[C_{43}H_{53}NO_9 + H]^+$, found 728.23 $[M+ H]^+$

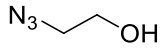
^{1}H NMR (500 MHz, Chloroform-*d*) (3:1 mixture of rotamers) δ 7.29 (t, *J* = 7.9 Hz, 0.25H), 7.10 (t, *J* = 7.9 Hz, 0.75H), 6.95 – 6.71 (m, 4H), 6.69 (dd, *J* = 8.2, 1.9 Hz, 0.75H), 6.63 – 6.60 (m, 0.75H), 6.49 (s, 1.5H), 6.42 (d, *J* = 5.6 Hz, 1H), 5.83 – 5.76 (m, 0.25H), 5.55 (dd, *J* = 7.9, 5.9 Hz, 0.75H), 5.47 (d, *J* = 5.0 Hz, 0.75H), 4.74 – 4.72 (m, 2H), 4.70 (d, *J* = 2.3 Hz, 0.25H), 4.55 (d, *J* = 13.7 Hz, 0.25H), 3.95 (d, *J* = 14.0 Hz, 0.75H), 3.86 – 3.77 (m, 7H), 3.75 (d, *J* = 7.5 Hz, 4H), 3.71 (s, 4H), 3.37 (d, *J* = 9.9 Hz, 0.75H), 2.96 (d, *J* = 9.7 Hz, 0.25H), 2.78 (td, *J* = 13.4, 2.7 Hz, 1H), 2.63 – 2.34 (m, 3H), 2.28 (d, *J* = 9.8 Hz, 1H), 2.13 – 2.07 (m, 1H), 2.00 – 1.76 (m, 3H), 1.72 – 1.51 (m, 6H), 1.43 – 1.26 (m, 3H), 1.22 – 1.03 (m, 3H), 1.00 – 0.64 (m, 2H).

^{13}C NMR (126 MHz, $CDCl_3$) (major) δ 172.36, 170.60, 159.56, 153.16, 148.19, 146.74, 141.88, 137.02, 133.72, 133.47, 129.58, 122.00, 118.51, 115.26, 113.13, 112.51, 112.04,

105.92, 78.87, 75.89, 75.68, 60.84, 56.95, 56.15, 56.08, 55.24, 55.10, 52.10, 43.75, 41.49, 37.98, 32.89, 30.94, 30.77, 26.83, 26.70, 26.31, 26.27, 25.69, 21.07.

HRMS: m/z: found 728.37899 $[M+H]^+$, calculated 728.37931 $[M+H]^+$

2-Azidoethanol (40a)



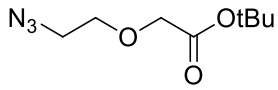
40a

2-Chloroethanol (4.16 mL, 62.10 mmol) was added to a solution of NaN_3 (4.97 g, 76.38 mmol) and NaOH (0.25 g, 6.21 mmol) in water (20 mL). The mixture was stirred at room temperature for 3 days, and sodium sulfate (5.8 g) was added. After 10 min, the mixture was extracted with DCM (3 x 70 mL). The combined extracts were dried in magnesium sulfate and concentrated. The residue was distilled to give light yellow oil (2.97g, 34.14 mmol, 55%)

^{1}H NMR (300 MHz, Chloroform-*d*) δ 3.73 (q, J = 5.4 Hz, 2H), 3.44 – 3.34 (m, 2H), 2.97 (t, J = 5.6 Hz, 1H).

^{13}C NMR (75 MHz, Chloroform-*d*) δ 61.34, 53.47.

Tert-butyl 2-(2-azidoethoxy)acetate (39a)



39a

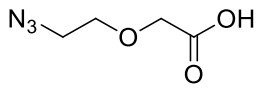
39a was synthesized from **40a** (2.87 g, 32.96 mmol) according to the General Method C. Purification by flash column chromatography (0% to 6% EtOAc in cyclohexane) gave a light yellow oil (4.18 g, 20.76 mmol, 63%).

TLC [CH:EA 8:2]: R_f = 0.47.

^{1}H NMR (300 MHz, Chloroform-*d*) δ 3.70 – 3.65 (m, 2H), 3.65 – 3.60 (m, 14H), 3.58 – 3.53 (m, 2H), 3.38 – 3.31 (m, 2H), 2.89 (s, 1H).

^{13}C NMR (75 MHz, Chloroform-*d*) δ 72.61, 70.70, 70.62, 70.57, 70.33, 70.03, 61.71, 50.70.

2-(2-Azidoethoxy)acetic acid (17a)



42a

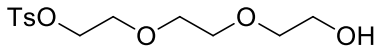
17a was synthesized from **39a** (2 g, 9.94 mmol) according to the General Method D. Purification by flash column chromatography (cyclohexane to 2% MeOH in DCM) gave a light yellow oil (0.292 g, 1.99 mmol, 20%).

TLC [(DCM:MeOH 95:5) +1% HCOOH]: R_f = 0.58.

^{1}H NMR (300 MHz, Chloroform-*d*) δ 10.14 (s, 1H), 4.19 (s, 2H), 3.79 – 3.68 (m, 2H), 3.49 – 3.40 (m, 2H).

^{13}C NMR (75 MHz, Chloroform-*d*) δ 175.39, 70.48, 68.07, 50.75.

2-(2-(2-Hydroxyethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (41c)



41c

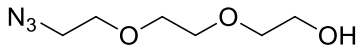
41c was synthesized from triethylene glycol (3 g, 20 mmol) according to the General Method A. Purification by flash column chromatography (0% to 100% EtOAc in cyclohexane) gave a light yellow oil (4.7 g, 15.60 mmol, 78%).

TLC [CH:EA 2:8]: R_f = 0.32.

^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.77 (d, J = 8.3 Hz, 2H), 7.32 (d, J = 8.0 Hz, 2H), 4.16 – 4.13 (m, 2H), 3.70 – 3.66 (m, 4H), 3.58 (s, 4H), 3.56 – 3.53 (m, 2H), 2.42 (s, 3H).

^{13}C NMR (126 MHz, Chloroform-*d*) δ 144.96, 133.05, 129.92, 128.02, 72.59, 70.83, 70.33, 69.26, 68.76, 61.78, 21.69.

2-(2-(2-Azidoethoxy)ethoxy)ethanol (40c)



40c

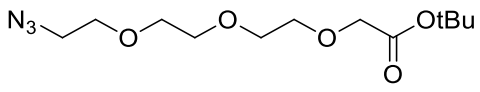
40c was synthesized from **41c** (4.7 g, 15.44 mmol) according to the General Method B. Purification by flash column chromatography (0% to 80% EtOAc in cyclohexane) gave a light yellow oil (2.54 g, 14.51 mmol, 94%).

TLC [CH:EA 2:8]: R_f = 0.29.

^{1}H NMR (300 MHz, Chloroform-*d*) δ 3.75 – 3.69 (m, 2H), 3.69 – 3.64 (m, 6H), 3.62 – 3.57 (m, 2H), 3.42 – 3.34 (m, 2H), 2.42 (s, 1H).

^{13}C NMR (75 MHz, Chloroform-*d*) δ 72.62, 70.77, 70.49, 70.14, 61.86, 50.77.

Tert-butyl 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)acetate (39c)



39c

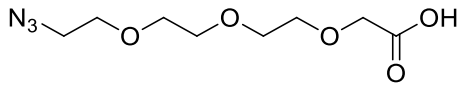
39c was synthesized from **40c** (1.40 g, 8 mmol) according to the General Method C. Purification by flash column chromatography (0% to 30% EtOAc in cyclohexane) gave a light yellow oil (2.10 g, 7.20 mmol, 90%).

TLC [CH:EA 6:4]: R_f = 0.54.

^{1}H NMR (500 MHz, Chloroform-*d*) δ 3.99 (s, 2H), 3.70 – 3.62 (m, 10H), 3.36 (t, J = 5.1 Hz, 2H), 1.45 (s, 9H).

^{13}C NMR (126 MHz, Chloroform-*d*) δ 169.70, 81.56, 70.81, 70.75, 70.73, 70.09, 69.14, 50.78, 28.18.

2-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)acetic acid (17c)



17c

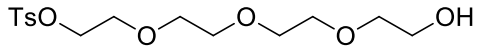
17c was synthesized from **39c** (2.06 g, 7.12 mmol) according to the General Method D. Purification by flash column chromatography (cyclohexane to 2% MeOH and 0.4% HCOOH in DCM) gave a light yellow oil (1.30 g, 2.85 mmol, 40%).

TLC [(DCM:MeOH 95:5) +1% HCOOH]: R_f = 0.33.

^{1}H NMR (300 MHz, Chloroform-*d*) δ 9.95 (s, 1H), 4.15 (s, 2H), 3.74 – 3.71 (m, 2H), 3.70 – 3.62 (m, 8H), 3.39 – 3.33 (m, 2H).

^{13}C NMR (75 MHz, Chloroform-*d*) δ 173.85, 71.26, 70.68, 70.52, 70.44, 70.07, 68.54, 50.68.

2-(2-(2-(2-Hydroxyethoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (41d)

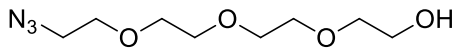


41d

41d was synthesized from tetraethylene glycol (0.93 g, 4.8 mmol) according to the General Method A. Purification by flash column chromatography (0% to 100% EtOAc in cyclohexane) gave a light yellow oil (1.20 g, 3.46 mmol, 72%).

TLC [CH:EA 2:8]: R_f = 0.12.

2-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)ethanol (40d)



40d

40d was synthesized from **41d** (1.00 g, 2.87 mmol) according to the General Method B. Purification by flash column chromatography (0% to 80% EtOAc in cyclohexane) gave a light yellow oil (0.65 g, 2.47 mmol, 86%).

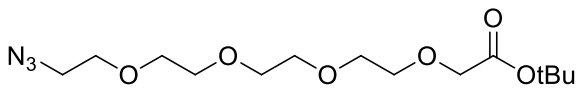
TLC [CH:EA 2:8]: R_f = 0.20.

^{1}H NMR (300 MHz, Chloroform-*d*) δ 3.73 – 3.68 (m, 2H), 3.65 (s, 10H), 3.61 – 3.56 (m, 2H),

3.41 – 3.34 (m, 2H), 2.83 (s, 1H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 72.51, 70.64, 70.60, 70.52, 70.28, 69.97, 61.64, 50.64.

Tert-butyl 14-azido-3,6,9,12-tetraoxatetradecan-1-oate (39d)



39d

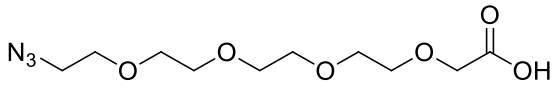
39d was synthesized from **40d** (1.40 g, 8 mmol) according to the General Method C. Purification by flash column chromatography (0% to 30% EtOAc in cyclohexane) gave a light yellow oil (0.79 g, 6.96 mmol, 87%).

TLC [CH:EA 6:4]: R_f = 0.30.

^{1}H NMR (300 MHz, Chloroform-*d*) δ 3.98 (s, 2H), 3.71 – 3.60 (m, 14H), 3.39 – 3.32 (m, 2H), 1.44 (s, 9H).

^{13}C NMR (75 MHz, Chloroform-*d*) δ 169.71, 81.56, 70.79, 70.78, 70.75, 70.71, 70.68, 70.65, 70.08, 69.11, 50.76, 28.17.

14-Azido-3,6,9,12-tetraoxatetradecan-1-oic acid (17d)



17d

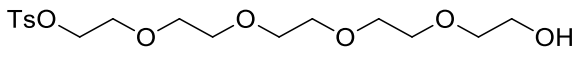
17d was synthesized from **39d** (0.74 g, 2.22 mmol) according to the General Method D. Purification by flash column chromatography (cyclohexane to 2% MeOH and 0.4% HCOOH in DCM) gave a light yellow oil (0.31 g, 1.13 mmol, 51%).

TLC [(DCM:MeOH 95:5) +1% HCOOH]: R_f = 0.60.

^{1}H NMR (300 MHz, Chloroform-*d*) δ 8.85 (s, 1H), 4.13 (s, 2H), 3.74 – 3.60 (m, 14H), 3.39 – 3.32 (m, 2H).

^{13}C NMR (75 MHz, Chloroform-*d*) δ 173.14, 71.22, 70.67, 70.62, 70.54, 70.46, 70.33, 69.99, 68.69, 50.66.

14-Hydroxy-3,6,9,12-tetraoxatetradecyl 4-methylbenzenesulfonate (41e)



41e

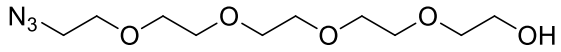
41e was synthesized from pentaethylene glycol (1.50 g, 6.30 mmol) according to the General Method A. Purification by flash column chromatography (0% to 100% EtOAc in cyclohexane) gave a light yellow oil (1.74 g, 4.41 mmol, 70%).

TLC [EA]: R_f = 0.14.

^{1}H NMR (300 MHz, Chloroform-*d*) δ 7.72 (d, J = 8.3 Hz, 2H), 7.27 (d, J = 8.5 Hz, 2H), 4.13 – 4.04 (m, 2H), 3.65 – 3.48 (m, 18H), 2.78 (s, 1H), 2.37 (s, 3H).

^{13}C NMR (75 MHz, Chloroform-*d*) δ 144.82, 133.04, 129.85, 127.97, 72.53, 70.73, 70.59, 70.54, 70.51, 70.33, 69.30, 68.68, 61.70, 21.64.

14-Azido-3,6,9,12-tetraoxatetradecan-1-ol (40e)



40e

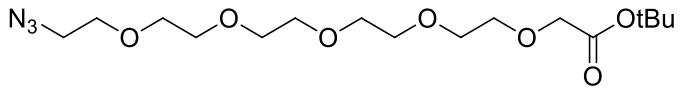
40e was synthesized from **41e** (1.61 g, 4.11 mmol) according to the General Method B. Purification by flash column chromatography (0% to 100% EtOAc in cyclohexane) gave a light yellow oil (1.02 g, 3.90 mmol, 95%).

TLC [DCM:MeOH]: R_f = 0.53.

^{1}H NMR (300 MHz, Chloroform-*d*) δ 3.70 – 3.65 (m, 2H), 3.65 – 3.60 (m, 14H), 3.58 – 3.53 (m, 2H), 3.38 – 3.31 (m, 2H), 2.89 (s, 1H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 72.61, 70.70, 70.62, 70.57, 70.33, 70.03, 61.71, 50.70.

Tert-butyl 17-azido-3,6,9,12,15-pentaoxaheptadecan-1-oate (39e)



39e

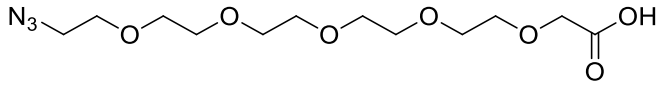
39e was synthesized from **40e** (0.974 g, 3.70 mmol) according to the General Method C. Purification by flash column chromatography (0% to 30% EtOAc in cyclohexane) gave a light yellow oil (1.15 g, 3.03 mmol, 82%).

TLC [CH:EA 4:6]: R_f = 0.28.

^{1}H NMR (300 MHz, Chloroform-*d*) δ 3.98 (s, 2H), 3.71 – 3.57 (m, 18H), 3.40 – 3.30 (m, 2H), 1.44 (s, 9H).

^{13}C NMR (75 MHz, Chloroform-*d*) δ 169.69, 81.54, 70.77, 70.75, 70.72, 70.65, 70.07, 69.10, 50.75, 28.16.

17-Azido-3,6,9,12,15-pentaoxaheptadecan-1-oic acid (17e)



17e

17e was synthesized from **39e** (1.11 g, 2.91 mmol) according to the General Method D. Purification by flash column chromatography (cyclohexane to 2% MeOH and 0.4% HCOOH in

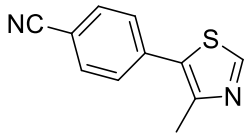
DCM) gave a light yellow oil (0.41 g, 1.28 mmol, 44%).

TLC [(DCM:MeOH 95:5) +1% HCOOH]: R_f= 0.33.

^{1}H NMR (300 MHz, Chloroform-*d*) δ 8.97 (s, 1H), 4.13 (s, 2H), 3.75 – 3.57 (m, 18H), 3.35 (t, J = 4.3 Hz, 2H).

^{13}C NMR (75 MHz, Chloroform-*d*) δ 172.81, 71.24, 70.70, 70.64, 70.61, 70.48, 70.39, 70.02, 68.80, 50.70.

4-(4-Methylthiazol-5-yl)benzonitrile (34)



34

4-Bromobenzonitrile (2 g, 10.99 mmol, 1 eq.), 4-methylthiazole (1.09 g, 10.99 mmol, 1 eq.), potassium acetate (2.16 g, 21.98 mmol, 2 eq.), palladium (II) acetate (0.025 g, 0.110 mmol, 0.01 eq.) were dissolved in dimethylacetamide (20 mL) and stirred under argon. The mixture was heated to 120°C and stirred for 19 hours, then diluted with 500 mL EtOAc, and washed 4 times with 70 mL brine. The first wash was then back extracted with 100 mL EtOAc, and then washed 4 times with 20 mL brine. The combined organic layer was dried over magnesium sulfate, filtered and concentrated under vacuum. The crude product was concentrated and purified by column chromatography (cyclohexane:EtOAc, gradient EtOAc 0 to 40%). **34** was afforded as a yellow solid (1.8 g, 8.99 mmol, 82%).

TLC [cyclohexane/EtOAc 6/4]: R_f = 0.27.

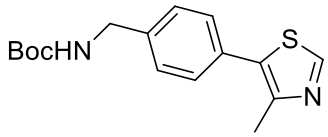
HPLC [0-100% Solvent B, 20 min]: R_t = 15.40 min, purity (220 nm) = 97%.

^{1}H NMR (599 MHz, Chloroform-*d*) δ 8.77 (s, 1H), 7.74 – 7.71 (m, 2H), 7.58 – 7.55 (m, 2H), 2.57 (s, 3H).

^{13}C NMR (151 MHz, Chloroform-*d*) δ 151.71, 150.06, 136.91, 132.64, 130.29, 129.84, 118.57, 111.69, 16.44..

MS (ESI+): 201.01 $[M+H]^+$, calculated 201.05 $[M+H]^+$

Tert-butyl 4-(4-methylthiazol-5-yl)benzylcarbamate (33)



33

To a suspension of **34** (1.3 g, 6.49 mmol, 1 eq.), di-tert-butyl dicarbonate (4.25 g, 19.47 mmol, 3 eq.) and cobalt(II) chloride (0.421 g, 3.25 mmol, 0.5 eq.) in methanol was added sodium

borohydride (1.228 g, 32.5 mmol, 5 eq.) over 30 min at 0°C. The reaction was allowed to proceed for 30 min at RT. The mixture was filtered through celite and the filter cake was washed with 50 mL EtOAc. The crude product was concentrated and purified by flash column chromatography (DCM → DCM+1% MeOH). **33** was afforded as a yellow solid (1.14 g, 3.74 mmol, 58%).

TLC [cyclohexane/EtOAc 6/4]: R_f = 0.49.

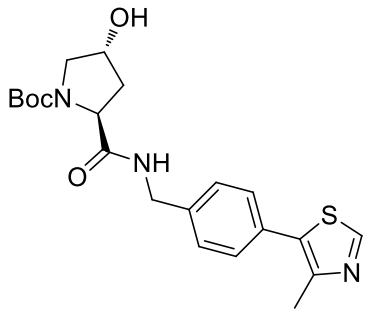
HPLC [0-100% Solvent B, 20 min]: R_t = 16.55 min, purity (220 nm) = 96%.

^{1}H NMR (599 MHz, Chloroform-*d*) δ 8.69 (s, 1H), 7.41 – 7.38 (m, 2H), 7.34 (d, J = 8.1 Hz, 2H), 4.99 (s, 1H), 4.35 (d, J = 5.2 Hz, 2H), 2.52 (s, 3H), 1.46 (s, 9H).

^{13}C NMR (151 MHz, Chloroform-*d*) δ 156.03, 150.42, 148.39, 139.12, 131.84, 130.89, 129.60, 127.85, 79.77, 44.38, 28.53, 16.09.

MS (ESI+): m/z: found 305.04 $[M+H]^+$, calculated 305.13 $[M+H]^+$

(2S,4R)-Tert-butyl 4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidine-1-carboxylate (37)



37

A solution of TFA (15 ml) and DCM (15 mL) was degassed by Ar for 5 min. Then **33** (940 mg, 3.09 mmol, 1 eq.) was added into the solution and stirred at RT for 60 min. Then the solvent was removed in reduced pressure to give the corresponding deprotected intermediate (TFA salt) without further purification.
(2S,4R)-1-(Tert-butoxycarbonyl)-4-hydroxypyrrolidine-2-carboxylic acid (0.929 g, 4.02 mmol, 1.3 eq.), EDC (0.770 g, 4.02 mmol, 1.3 eq.) and HOBt (0.615 g, 4.02 mmol, 1.3 eq.) were dissolved in DMF (15 mL) and cooled to 0 °C. DIPEA (2,70 ml, 15.45 mmol, 5 eq.) was added, and the solution was stirred for 30 min. Then the deprotected intermediate (in 1 mL DMF) was added, and the solution was allowed to warm slowly to room temperature. After 20 hours the mixture was poured into 100 mL brine and extracted 4 times with EtOAc. The combined organic layer was dried over magnesium sulfate, filtered and concentrated under reduced pressure. Purification by flash column chromatography (cyclohexane to 5% MeOH/DCM) gave a light yellow form (880 mg, 2.11 mmol, 68%)

TLC [DCM + 5% MeOH]: R_f = 0.26.

HPLC [0-100% Solvent B, 20 min]: R_t = 12.48 min, purity (220 nm) >99%.

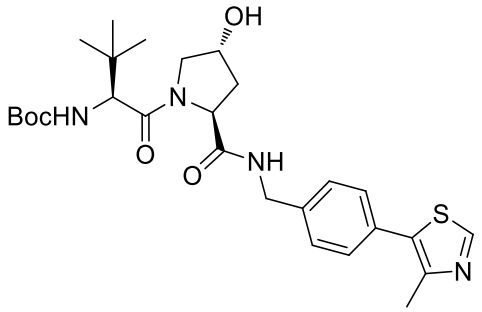
1H NMR (300 MHz, DMSO-d_6) δ 8.97 (s, 1H), 8.46 (q, J = 7.2, 6.7 Hz, 1H), 7.44 – 7.35 (m, 4H), 5.00 (d, J = 3.5 Hz, 1H), 4.41 – 4.14 (m, 4H), 3.52 – 3.36 (m, 1H), 3.33 – 3.25 (m, 1H),

2.44 (s, 3H), 2.14 – 2.02 (m, 1H), 1.87 (ddd, J = 14.2, 7.8, 4.6 Hz, 1H), 1.41 (s, 3H), 1.26 (s, 6H).

13C NMR (75 MHz, DMSO-d_6) δ 172.50, 172.24, 153.92, 153.52, 151.42, 147.76, 139.42, 131.04, 129.93, 128.78, 128.67, 128.08, 127.42, 78.64, 78.46, 68.50, 67.81, 58.92, 55.01, 54.74, 41.80, 41.52, 28.10, 27.87, 15.87, 15.83.

MS (ESI+): m/z: found 417.96 $[M+H]^+$, calculated 418.18 $[M+H]^+$

Tert-butyl ((S)-1-((2S,4R)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)carbamate (38)



38

37 (650 mg, 1.56 mmol, 1 eq.) was dissolved in a solution of TFA/DCM (2 ml, 1:1) and stirred at RT under Ar overnight. Then the solvent was removed in reduced pressure to give the corresponding deprotected intermediate (TFA salt) without further purification.
(S)-2-((Tert-butoxycarbonyl)amino)-3,3-dimethylbutanoic acid (397 mg, 1.72 mmol, 1.1 eq.), EDC (329 mg, 1.72 mmol, 1.1 eq.) and HOBt (263 mg, 1.72 mmol, 1.1 eq.) were dissolved in DMF (1 mL) and cooled to 0 °C. DIPEA (1.36 ml, 7.8 mmol, 5 eq.) was added, and the solution was stirred for 15 min. Then the deprotected intermediate (in 0.5 mL DMF) was added, and the solution was allowed to warm slowly to room temperature. After 20 hours the mixture was poured into brine and extracted 4 times with EtOAc. The combined organic layer was dried over magnesium sulfate, filtered and concentrated under reduced pressure. Purification by flash column chromatography (cyclohexane to 5% MeOH in DCM) gave a light yellow solid (580 mg, 1.09 mmol, 70%)

TLC [DCM + 5% MeOH]: R_f = 0.23.

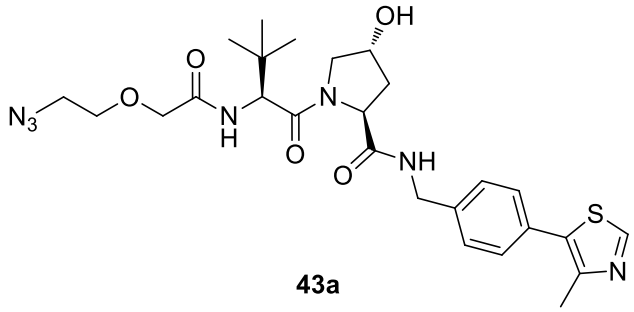
HPLC [0-100% Solvent B, 20 min]: R_t = 14.97 min, purity (220 nm) = 97%.

^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.60 (s, 1H), 7.42 (s, 1H), 7.30 – 7.22 (m, 4H), 5.17 (d, J = 9.0 Hz, 1H), 4.66 (t, J = 7.8 Hz, 1H), 4.46 (dd, J = 15.1, 6.1 Hz, 2H), 4.24 (dd, J = 15.0, 5.2 Hz, 1H), 4.12 (d, J = 9.3 Hz, 1H), 3.92 (d, J = 11.1 Hz, 1H), 3.64 (br, s, 1H), 3.55 (dd, J = 11.0, 3.2 Hz, 1H), 2.43 (s, 3H), 2.42 – 2.37 (m, 1H), 2.07 – 1.99 (m, 1H), 1.33 (s, 9H), 0.85 (s, 9H).

^{13}C NMR (126 MHz, Chloroform-*d*) δ 172.39, 170.85, 156.25, 150.29, 148.41, 138.15, 131.61, 130.86, 129.45, 128.01, 80.26, 70.05, 58.87, 58.54, 56.51, 43.19, 36.05, 35.09, 28.32, 26.31, 15.99.

MS (ESI+): m/z: found 531.02 $[M+H]^+$, calculated 531.26 $[M+H]^+$

(2S,4R)-1-((S)-2-(2-(2-Azidoethoxy)acetamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (43a)



43a

43a was synthesized from **38** and **17a** according to the General Method E. Purification by flash column chromatography (cyclohexane to 5% MeOH in DCM) gave a white foam (77 mg, 0.14 mmol, 81%).

TLC [DCM:MeOH 95:5]: R_f = 0.29.

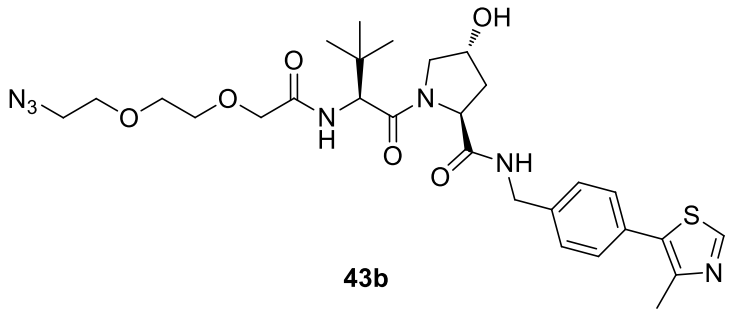
HPLC [0-100% Solvent B, 20 min]: R_t = 10.9 min, purity (220 nm) ≥ 99%.

^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.65 (s, 1H), 7.48 (t, J = 5.9 Hz, 1H), 7.35 – 7.29 (m, 4H), 7.20 (d, J = 8.8 Hz, 1H), 4.69 (t, J = 7.9 Hz, 1H), 4.54 – 4.46 (m, 3H), 4.30 (dd, J = 15.1, 5.4 Hz, 1H), 4.02 – 3.94 (m, 3H), 3.71 – 3.59 (m, 3H), 3.49 – 3.35 (m, 2H), 2.48 (s, 3H), 2.44 – 2.40 (m, 1H), 2.12 – 2.03 (m, 1H), 0.95 (s, 9H).

^{13}C NMR (126 MHz, Chloroform-*d*) δ 171.18, 171.07, 169.69, 150.40, 148.50, 138.24, 131.71, 130.93, 129.53, 128.10, 70.59, 70.33, 70.17, 58.81, 57.29, 56.84, 50.76, 43.25, 36.32, 35.28, 26.44, 16.09.

MS (ESI+): m/z: found 558.08 $[M+H]^+$, calculated 558.25 $[M+H]^+$

(2S,4R)-1-((S)-2-(2-(2-(2-Azidoethoxy)ethoxy)acetamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (43b)



43b

43b was synthesized from **38** and (2-(2-azidoethoxy)ethoxy)acetic acid according to the General Method E. Purification by flash column chromatography (cyclohexane to 5% MeOH in DCM) gave a light yellow foam (99 mg, 0.15 mmol, 91%).

TLC [DCM:MeOH 95:5]: R_f = 0.32.

HPLC [0-100% Solvent B, 20 min]: R_t = 11.0 min, purity (220 nm) ≥ 99%.

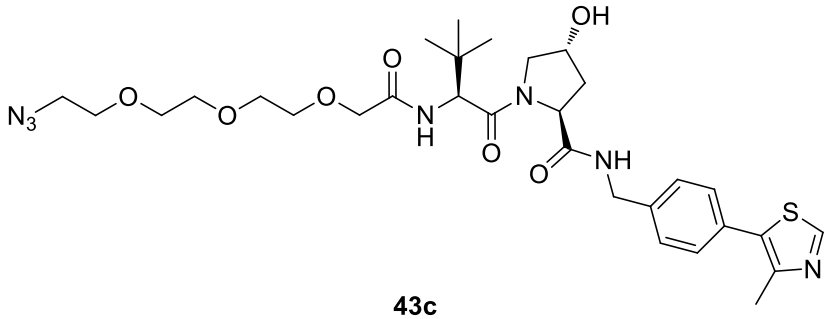
^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.60 (s, 1H), 7.36 (t, J = 5.9 Hz, 1H), 7.30 – 7.25 (m, 4H), 7.20 (d, J = 8.6 Hz, 1H), 4.63 (t, J = 7.9 Hz, 1H), 4.48 – 4.42 (m, 3H), 4.27 (dd, J =

15.0, 5.4 Hz, 1H), 3.97 – 3.87 (m, 3H), 3.69 – 3.54 (m, 9H), 3.36 – 3.27 (m, 2H), 2.43 (s, 3H), 2.42 – 2.37 (m, 1H), 2.08 – 2.00 (m, 1H), 0.89 (s, 9H).

^{13}C NMR (126 MHz, Chloroform-*d*) δ 171.20, 170.90, 170.33, 150.29, 148.43, 138.15, 131.60, 130.88, 129.45, 128.05, 71.08, 70.36, 70.33, 70.10, 70.04, 58.65, 57.15, 56.67, 50.55, 43.18, 36.11, 35.09, 26.37, 16.01.

MS (ESI+): m/z: found 602.12 $[M+H]^+$, calculated 602.28 $[M+H]^+$

(2S,4R)-1-((S)-14-Azido-2-(tert-butyl)-4-oxo-6,9,12-trioxa-3-azatetradecan-1-oyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (43c)



43c

43c was synthesized from **38** and **17c** according to the General Method E. Purification by flash column chromatography (cyclohexane to 5% MeOH in DCM) gave a light yellow foam (85 mg, 0.13 mmol, 77%).

TLC [DCM:MeOH 95:5]: R_f = 0.18.

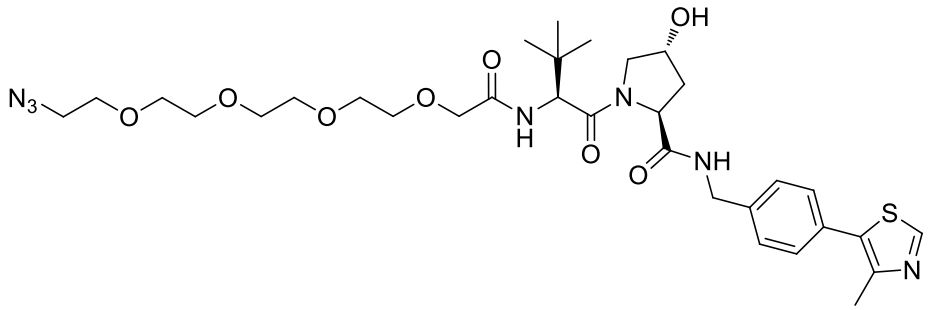
HPLC [0-100% Solvent B, 20 min]: R_t = 11.0 min, purity (220 nm) = 99%.

^{1}H NMR (300 MHz, Chloroform-*d*) δ 8.60 (s, 1H), 7.39 (t, J = 5.9 Hz, 1H), 7.32 – 7.25 (m, 4H), 7.23 (d, J = 8.5 Hz, 1H), 4.63 (t, J = 7.9 Hz, 1H), 4.50 – 4.39 (m, 3H), 4.27 (dd, J = 15.1, 5.4 Hz, 1H), 3.98 – 3.88 (m, 3H), 3.63 – 3.52 (m, 11H), 3.33 – 3.24 (m, 2H), 2.43 (s, 3H), 2.42 – 2.32 (m, 1H), 2.09 – 1.98 (m, 1H), 0.89 (s, 9H).

^{13}C NMR (75 MHz, Chloroform-*d*) δ 171.25, 171.05, 170.43, 150.39, 148.49, 138.27, 131.69, 130.91, 129.52, 128.10, 71.22, 70.74, 70.55, 70.36, 70.13, 70.10, 58.76, 57.16, 56.77, 50.73, 43.23, 36.26, 35.24, 26.46, 16.11.

MS (ESI+): m/z: found 646.09 $[M+H]^+$, calculated 646.30 $[M+H]^+$

(2S,4R)-1-((S)-17-Azido-2-(tert-butyl)-4-oxo-6,9,12,15-tetraoxa-3-azaheptadecan-1-oyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (43d)



43d

43d was synthesized from **38** and **17d** according to the General Method E. Purification by flash column chromatography (cyclohexane to 5% MeOH in DCM) gave a yellow oil (79 mg, 0.12 mmol, 68%).

TLC [DCM:MeOH 95:5]: R_f = 0.35.

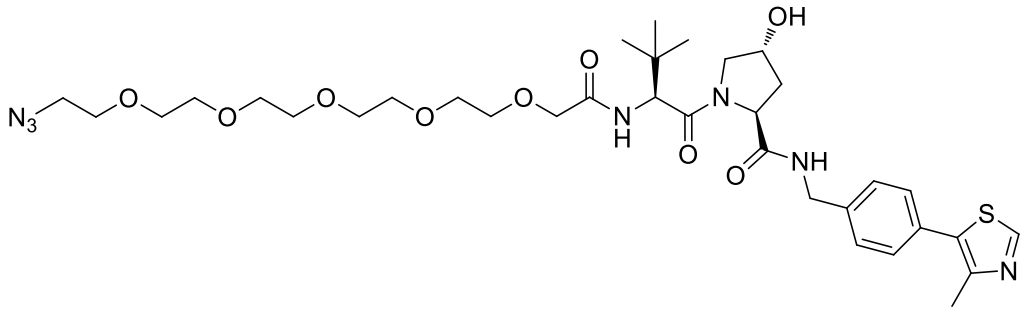
HPLC [0-100% Solvent B, 20 min]: R_t = 11.2 min, purity (220 nm) ≥ 99%.

^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.67 (s, 1H), 7.48 (t, J = 5.9 Hz, 1H), 7.36 – 7.31 (m, 4H), 7.28 (d, J = 8.8 Hz, 1H), 4.70 (t, J = 7.8 Hz, 1H), 4.55 – 4.47 (m, 3H), 4.34 (dd, J = 15.1, 5.5 Hz, 1H), 3.99 (t, J = 9.2 Hz, 3H), 3.68 – 3.61 (m, 15H), 3.38 – 3.34 (m, 2H), 2.50 (s, 3H), 2.43 (ddd, J = 12.8, 7.7, 4.7 Hz, 1H), 2.14 – 2.06 (m, 1H), 0.96 (s, 9H).

^{13}C NMR (126 MHz, Chloroform-*d*) δ 171.33, 171.20, 170.49, 150.48, 148.58, 138.40, 131.80, 131.00, 129.61, 128.19, 71.31, 70.81, 70.76, 70.74, 70.55, 70.48, 70.21, 70.17, 58.89, 57.25, 56.88, 50.84, 43.32, 36.43, 35.39, 26.56, 16.20.

MS (ESI+): m/z: found 690.25 $[M+H]^+$, calculated 690.33 $[M+H]^+$

(2S,4R)-1-((S)-20-Azido-2-(tert-butyl)-4-oxo-6,9,12,15,18-pentaoxa-3-azaicosan-1-oyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (43e)



43e

43e was synthesized from **38** and **17e** according to the General Method E. Purification by flash column chromatography (cyclohexane to 5% MeOH in DCM) gave a yellow oil (93 mg, 0.13 mmol, 74%).

TLC [DCM:MeOH 95:5]: R_f = 0.28.

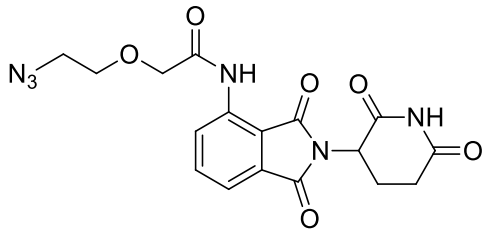
HPLC [0-100% Solvent B, 20 min]: R_t = 11.4 min, purity (220 nm) ≥ 99%.

^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.68 (s, 1H), 7.49 (t, J = 5.9 Hz, 1H), 7.38 – 7.28 (m, 5H), 4.71 (t, J = 7.9 Hz, 1H), 4.56 – 4.48 (m, 3H), 4.35 (dd, J = 15.1, 5.4 Hz, 1H), 4.05 – 3.94 (m, 3H), 3.72 – 3.57 (m, 21H), 3.37 (t, J = 5.1 Hz, 2H), 2.51 (s, 3H), 2.45 (ddd, J = 12.9, 7.9, 4.7 Hz, 1H), 2.15 – 2.07 (m, 1H), 0.97 (s, 9H).

^{13}C NMR (126 MHz, Chloroform-*d*) δ 171.23, 171.09, 170.44, 150.37, 148.48, 138.30, 131.70, 130.90, 129.52, 128.11, 71.22, 70.73, 70.69, 70.66, 70.64, 70.60, 70.47, 70.42, 70.12, 70.06, 58.77, 57.15, 56.80, 50.75, 43.23, 36.29, 35.27, 26.47, 16.10.

MS (ESI+): m/z: found 734.23 $[M+H]^+$, calculated 734.35 $[M+H]^+$

2-(2-Azidoethoxy)-N-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)acetamide (44a)



44a

44a was synthesized from **17a** (70 mg, 0.483 mmol) according to the General Method F. Purification by flash column chromatography (0% to 60% EtOAc in cyclohexane) gave a light yellow solid (57 mg, 0.14 mmol, 90%).

TLC [CH:EA 4:6]: R_f = 0.37.

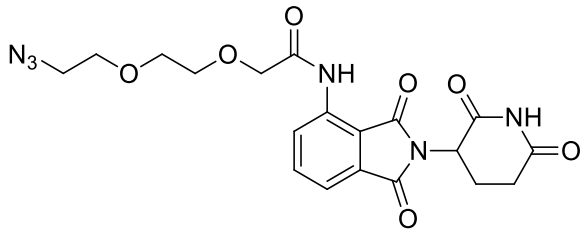
HPLC [0-100% Solvent B, 20 min]: R_t = 11.1 min, purity (220 nm) = 97%.

MS (ESI+): m/z: found 400.96 $[M+H]^+$, calculated 401.12 $[M+H]^+$

^{1}H NMR (500 MHz, DMSO-d_6) δ 11.15 (s, 1H), 10.36 (s, 1H), 8.73 (d, J = 8.5 Hz, 1H), 7.89 (t, J = 7.9 Hz, 1H), 7.65 (d, J = 7.3 Hz, 1H), 5.18 (dd, J = 12.7, 5.4 Hz, 1H), 4.27 (s, 2H), 3.83 (t, J = 4.7 Hz, 2H), 3.61 – 3.58 (m, 2H), 2.98 – 2.84 (m, 1H), 2.68 – 2.55 (m, 2H), 2.17 – 2.05 (m, 1H).

^{13}C NMR (126 MHz, DMSO-d_6) δ 172.73, 169.72, 168.98, 168.23, 166.68, 136.55, 135.92, 131.32, 124.47, 118.42, 116.20, 70.00, 69.78, 49.95, 49.01, 30.94, 21.93.

2-(2-(2-Azidoethoxy)ethoxy)-N-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)acetamide (44b)



44b

44b was synthesized from (2-(2-Azidoethoxy)ethoxy)acetic acid (95 mg, 0.503 mmol) according to the General Method F. Purification by flash column chromatography (0% to 60% EtOAc in cyclohexane) gave a light yellow solid (25 mg, 0.056 mmol, 34%).

TLC [CH:EA 4:6]: R_f = 0.28.

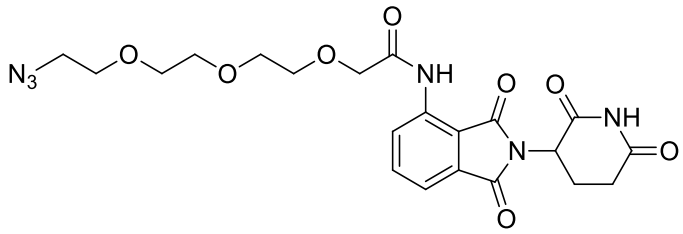
HPLC [0-100% Solvent B, 20 min]: R_t = 11.4 min, purity (220 nm) = 96%.

^{1}H NMR (500 MHz, Chloroform-*d*) δ 10.38 (s, 1H), 8.79 (d, J = 8.4 Hz, 1H), 8.33 (s, 1H), 7.69 – 7.61 (m, 1H), 7.51 (d, J = 7.3 Hz, 1H), 4.90 (dd, J = 12.4, 5.3 Hz, 1H), 4.14 (s, 2H), 3.80 – 3.70 (m, 4H), 3.68 – 3.62 (m, 2H), 3.32 (t, J = 5.1 Hz, 2H), 2.86 – 2.63 (m, 3H), 2.12 – 2.05 (m, 1H).

^{13}C NMR (126 MHz, Chloroform-*d*) δ 171.05, 169.42, 168.58, 168.06, 166.89, 136.90, 136.45, 131.49, 125.44, 118.95, 116.31, 71.75, 71.24, 70.71, 70.30, 50.80, 49.40, 31.50, 22.77.

MS (ESI+): m/z: found 445.04$[M+H]^+$, calculated 445.15 $[M+H]^+$

2-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)-N-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)acetamide (44c)



44c

44c was synthesized from **17c** (95 mg, 0.407 mmol) according to the General Method F. Purification by flash column chromatography (0% to 80% EtOAc in cyclohexane) gave a light yellow solid (56 mg, 0.056 mmol, 35%).

TLC [CH:EA 2:8]: R_f = 0.30.

HPLC [0-100% Solvent B, 20 min]: R_t = 11.5 min, purity (220 nm) = 97%.

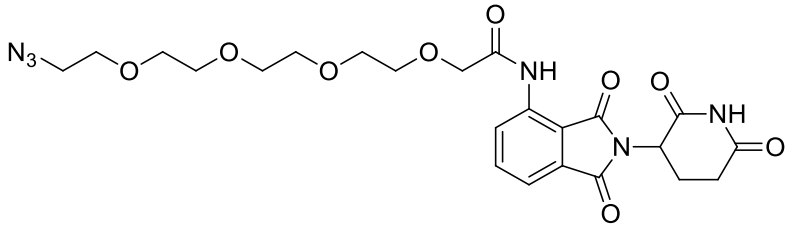
^{1}H NMR (500 MHz, Chloroform-*d*) δ 10.43 (s, 1H), 8.83 (d, J = 8.4 Hz, 1H), 8.70 (s, 1H), 7.75 – 7.64 (m, 1H), 7.55 (d, J = 7.3 Hz, 1H), 4.94 (dd, J = 12.3, 5.4 Hz, 1H), 4.19 (s, 2H), 3.84 –

3.76 (m, J = 2.5 Hz, 4H), 3.72 – 3.60 (m, 6H), 3.37 – 3.31 (m, 2H), 2.91 – 2.69 (m, 3H), 2.13 (ddd, J = 9.4, 5.0, 2.2 Hz, 1H).

^{13}C NMR (126 MHz, Chloroform-*d*) δ 171.24, 169.56, 168.49, 168.18, 166.87, 136.83, 136.36, 131.45, 125.32, 118.84, 116.24, 71.64, 71.11, 70.84, 70.75, 70.72, 70.08, 50.75, 49.34, 31.44, 22.71.

MS (ESI+): m/z: found 489.13$[M+H]^+$, calculated 489.17 $[M+H]^+$

14-Azido-N-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)-3,6,9,12-tetraoxatetradecan-1-amide (44d)



44d

44d was synthesized from **17d** (105 mg, 0.379 mmol) according to the General Method F. Purification by flash column chromatography (0% to 80% EtOAc in cyclohexane) gave a light yellow solid (66 mg, 0.123 mmol, 99%).

TLC [CH:EA 2:8]: R_f = 0.26.

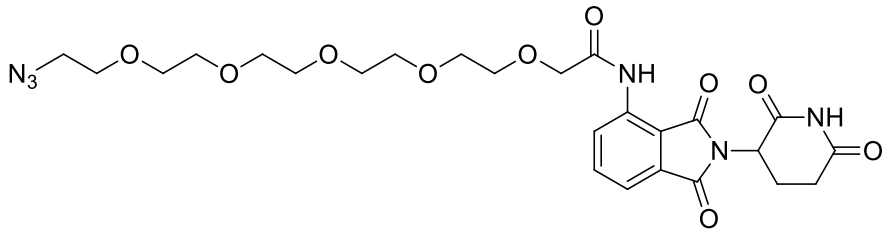
HPLC [0-100% Solvent B, 20 min]: R_t = 11.6 min, purity (220 nm) = 91%.

^{1}H NMR (500 MHz, Chloroform-*d*) δ 10.45 (s, 1H), 8.85 – 8.75 (m, 2H), 7.73 – 7.64 (m, 1H), 7.54 (d, J = 7.2 Hz, 1H), 4.94 (dd, J = 12.2, 5.4 Hz, 1H), 4.18 (d, J = 1.5 Hz, 2H), 3.79 (th, J = 5.2, 2.8 Hz, 4H), 3.67 – 3.61 (m, 10H), 3.36 (t, J = 5.1 Hz, 2H), 2.91 – 2.68 (m, 3H), 2.17 – 2.10 (m, 1H).

^{13}C NMR (126 MHz, Chloroform-*d*) δ 171.25, 169.52, 168.48, 168.17, 166.85, 136.82, 136.34, 131.46, 125.27, 118.82, 116.24, 71.63, 71.06, 70.74, 70.64, 70.61, 70.58, 69.99, 50.72, 49.34, 31.43, 22.74.

MS (ESI+): m/z: found 533.17$[M+H]^+$, calculated 533.20 $[M+H]^+$

17-Azido-N-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)-3,6,9,12,15-pentaoxaheptadecan-1-amide (44e)



44e

44e was synthesized from **17e** (105 mg, 0.425 mmol) according to the General Method F. Purification by flash column chromatography (0% to 80% EtOAc in cyclohexane) gave a light yellow solid (34 mg, 0.059 mmol, 55%).

TLC [EA]: R_f = 0.37.

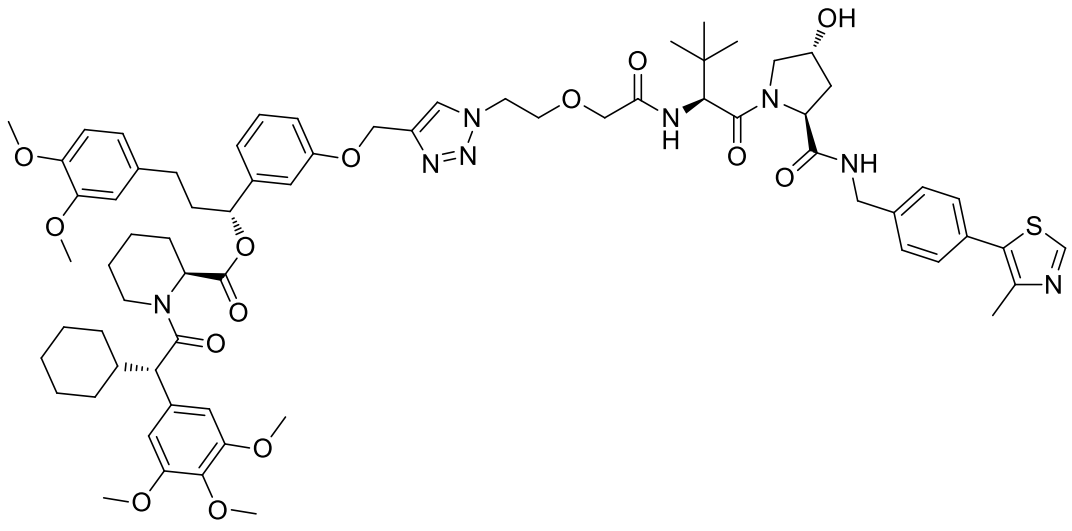
HPLC [0-100% Solvent B, 20 min]: R_t = 11.6 min, purity (220 nm) = 97%.

^{1}H NMR (500 MHz, Chloroform-*d*) δ 10.47 (s, 1H), 8.84 (d, J = 8.5 Hz, 1H), 8.78 (s, 1H), 7.71 (t, J = 7.9 Hz, 1H), 7.56 (d, J = 7.3 Hz, 1H), 4.94 (dd, J = 12.3, 5.4 Hz, 1H), 4.18 (d, J = 2.5 Hz, 2H), 3.80 (s, 4H), 3.70 – 3.62 (m, 14H), 3.36 (t, J = 5.1 Hz, 2H), 2.94 – 2.67 (m, 3H), 2.17 – 2.11 (m, 1H).

^{13}C NMR (126 MHz, Chloroform-*d*) δ 171.03, 169.38, 168.41, 168.00, 166.78, 136.76, 136.25, 131.40, 125.21, 118.74, 116.17, 71.61, 70.98, 70.67, 70.62, 70.53, 70.47, 69.93, 50.69, 49.29, 31.39, 22.69.

MS (ESI+): m/z: found 576.22 $[M+H]^+$, calculated 577.23 $[M+H]^+$

(S)-(R)-3-(3,4-Dimethoxyphenyl)-1-(3-((1-(2-(2-(((S)-1-((2S,4R)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-2-oxoethoxy)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)propyl 1-((S)-2-cyclohexyl-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2-carboxylate (MTQ199)



MTQ199

MTQ199 was synthesized from **43a** (7 mg, 0. 0137 mmol) according to the General Method G. Purification by flash column chromatography (0% to 5% MeOH in DCM) gave a white solid (13 mg, 0.010 mmol, 74%).

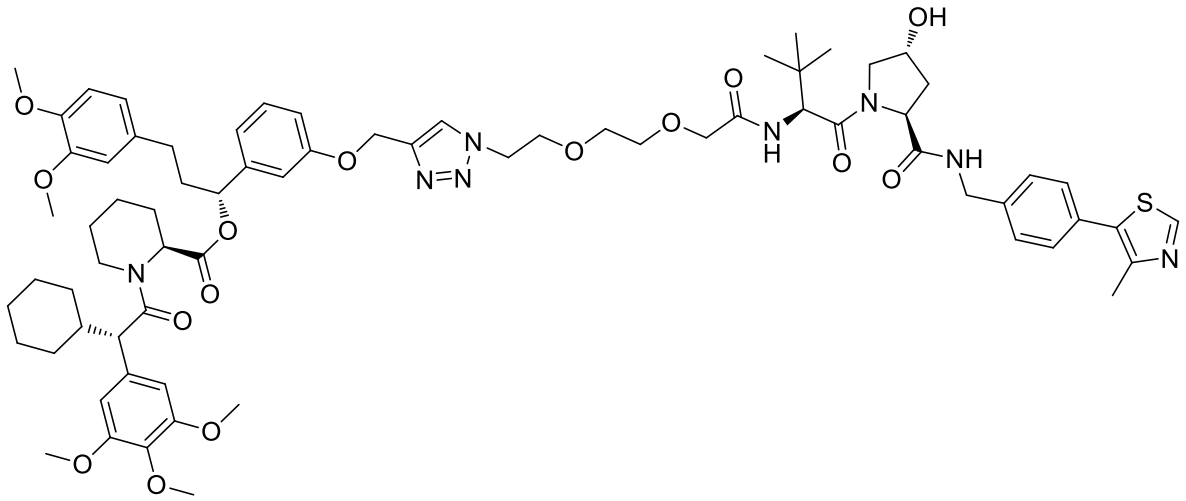
TLC [DCM:MeOH 95:5]: R_f = 0.25.

HPLC [0-100% Solvent B, 20 min]: R_t = 17.4 min, purity (220 nm) = 99%.

^{1}H NMR (300 MHz, Chloroform-*d*) (mixture of rotamers) δ 8.69 (s, 1H), 7.79 (s, 0.75H), 7.74 (s, 0.25H), 7.42 – 7.26 (m, 5H), 7.20 – 7.04 (m, 2H), 7.02 – 6.73 (m, 4H), 6.69 – 6.58 (m, 2H), 6.50 – 6.46 (m, 2H), 5.58 (dd, *J* = 7.8, 5.7 Hz, 1H), 5.44 (d, *J* = 3.5 Hz, 1H), 5.17 (s, 0.5H), 5.14 (s, 1.5H), 4.79 – 4.20 (m, 8H), 4.06 – 3.86 (m, 6H), 3.85 – 3.80 (m, 8H), 3.77 (s, 2H), 3.71 (s, 4H), 3.64 – 3.57 (m, 1H), 3.38 (d, *J* = 9.9 Hz, 0.75H), 2.97 (d, *J* = 9.6 Hz, 0.25H), 2.90 – 2.70 (m, 1H), 2.64 – 2.53 (m, 1H), 2.51 (s, 3H), 2.49 – 2.22 (m, 4H), 2.13 – 1.95 (m, 4H), 1.88 – 1.79 (m, 4H), 1.74 – 1.68 (m, 2H), 1.62 – 1.50 (m, 3H), 1.37 – 1.28 (m, 2H), 1.22 – 1.06 (m, 3H), 0.93 (s, 9H).

HRMS: m/z: found 1285.62133 $[M+H]^+$, calculated 1285.62135 $[M+H]^+$

(S)-(R)-3-(3,4-Dimethoxyphenyl)-1-(3-((1-(2-(2-(2-(((S)-1-((2S,4R)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-2-oxoethoxy)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)propyl 1-((S)-2-cyclohexyl-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2-carboxylate (MTQ200)



MTQ200

MTQ200 was synthesized from **43b** (8 mg, 0. 0137 mmol) according to the General Method G. Purification by flash column chromatography (0% to 5% MeOH in DCM) gave a white solid (13 mg, 0.010 mmol, 74%).

TLC [DCM:MeOH 95:5]: R_f = 0.24.

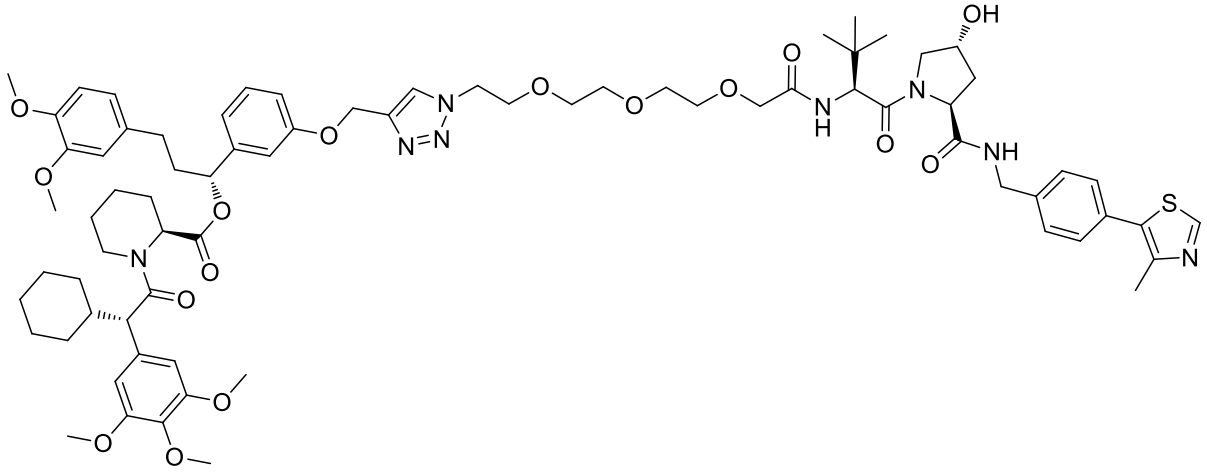
HPLC [0-100% Solvent B, 20 min]: R_t = 17.6 min, purity (220 nm) = 99%.

^{1}H NMR (500 MHz, Chloroform-*d*) (mixture of rotamers) δ 8.66 (s, 1H), 7.97 (s, 0.75H), 7.85 (s, 0.25H), 7.36 – 7.31 (m, 3H), 7.30 – 7.26 (m, 2H), 7.25 – 7.08 (m, 2H), 6.99 – 6.96 (m, 0.5H), 6.86 – 6.73 (m, 2.75H), 6.69 – 6.59 (m, 2H), 6.49 (s, 1.5H), 6.46 – 6.41 (m, 1.25H),

5.54 (dd, J = 8.0, 5.7 Hz, 1H), 5.45 (d, J = 5.0 Hz, 1H), 5.17 (s, 0.5H), 5.14 (s, 1.5H), 4.70 – 4.40 (m, 7H), 4.34 – 4.24 (m, 1H), 4.05 – 3.92 (m, 3H), 3.91 – 3.86 (m, 2H), 3.85 – 3.81 (m, 9H), 3.77 (s, 2H), 3.71 (s, 4H), 3.67 – 3.56 (m, 5H), 3.38 (d, J = 9.8 Hz, 0.75H), 2.99 (d, J = 9.6 Hz, 0.25H), 2.78 (td, J = 13.4, 2.7 Hz, 1H), 2.64 – 2.52 (m, 1H), 2.50 (s, 3H), 2.49 – 2.23 (m, 4H), 2.06 – 1.92 (m, 2H), 1.90 – 1.80 (m, 2H), 1.66 – 1.51 (m, 5H), 1.48 – 1.37 (m, 1H), 1.34 – 1.28 (m, 2H), 1.21 – 1.09 (m, 3H), 0.96 (s, 9H), 0.91 – 0.52 (m, 3H).

HRMS: m/z: found 1329.64806 $[M+H]^+$, calculated 1329.64756 $[M+H]^+$

(S)-(R)-3-(3,4-Dimethoxyphenyl)-1-(3-((1-((S)-13-((2S,4R)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidine-1-carbonyl)-14,14-dimethyl-11-oxo-3,6,9-trioxa-12-azapentadecyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)propyl 1-((S)-2-cyclohexyl-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2-carboxylate (MTQ201)



MTQ201

MTQ201 was synthesized from **43c** (9 mg, 0. 0137 mmol) according to the General Method G. Purification by flash column chromatography (0% to 5% MeOH in DCM) gave a white solid (15 mg, 0.011 mmol, 81%).

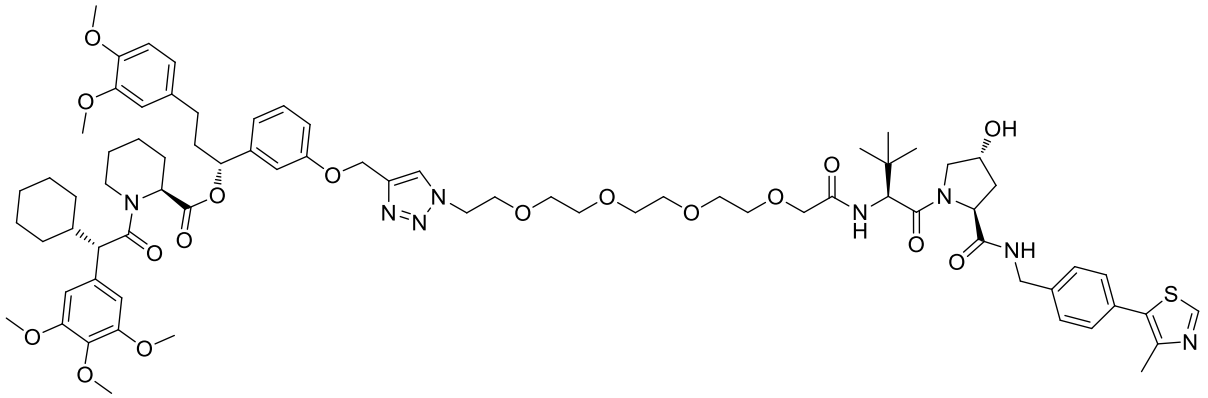
TLC [DCM:MeOH 95:5]: R_f = 0.24.

HPLC [30-100% Solvent B, 20 min]: R_t = 17.4 min, purity (220 nm) = 99%.

^{1}H NMR (300 MHz, Chloroform-*d*) (mixture of rotamers) δ 8.66 (s, 1H), 7.81 (s, 0.75H), 7.79 (s, 0.25H), 7.37 – 7.30 (m, 5H), 7.24 – 6.98 (m, 2H), 6.96 – 6.82 (m, 1H), 6.81 – 6.71 (m, 2H), 6.69 – 6.57 (m, 2H), 6.53 – 6.38 (m, 3H), 5.57 (dd, J = 7.6, 5.8 Hz, 1H), 5.46 (d, J = 4.3 Hz, 1H), 5.16 (s, 0.5H), 5.13 (s, 1.5H), 4.76 – 4.46 (m, 7H), 4.38 – 4.27 (m, 1H), 4.05 (d, J = 11.3 Hz, 1H), 3.98 (d, J = 2.7 Hz, 2H), 3.86 – 3.81 (m, 10H), 3.76 (s, 2H), 3.70 (s, 4H), 3.67 – 3.57 (m, 10H), 3.38 (d, J = 9.7 Hz, 0.75H), 3.00 (d, J = 9.8 Hz, 0.25H), 2.87 – 2.70 (m, 1H), 2.63 – 2.52 (m, 1H), 2.51 (s, 3H), 2.50 – 2.22 (m, 4H), 2.14 – 2.05 (m, 2H), 1.95 – 1.82 (m, 2H), 1.63 – 1.50 (m, 4H), 1.43 – 1.31 (m, 2H), 1.21 – 1.06 (m, 4H), 0.95 (s, 9H), 0.88 – 0.52 (m, 4H).

HRMS: m/z: found 1373.67337 $[M+H]^+$, calculated 1373.67378 $[M+H]^+$

(S)-(R)-3-(3,4-Dimethoxyphenyl)-1-(3-((1-((S)-16-((2S,4R)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidine-1-carbonyl)-17,17-dimethyl-14-oxo-3,6,9,12-tetraoxa-15-azaoctadecyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)propyl 1-((S)-2-cyclohexyl-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2-carboxylate (MTQ202)



MTQ202

MTQ202 was synthesized from **43d** (10 mg, 0. 0137 mmol) according to the General Method G. Purification by flash column chromatography (0% to 5% MeOH in DCM) gave a white solid (17 mg, 0.012 mmol, 89%).

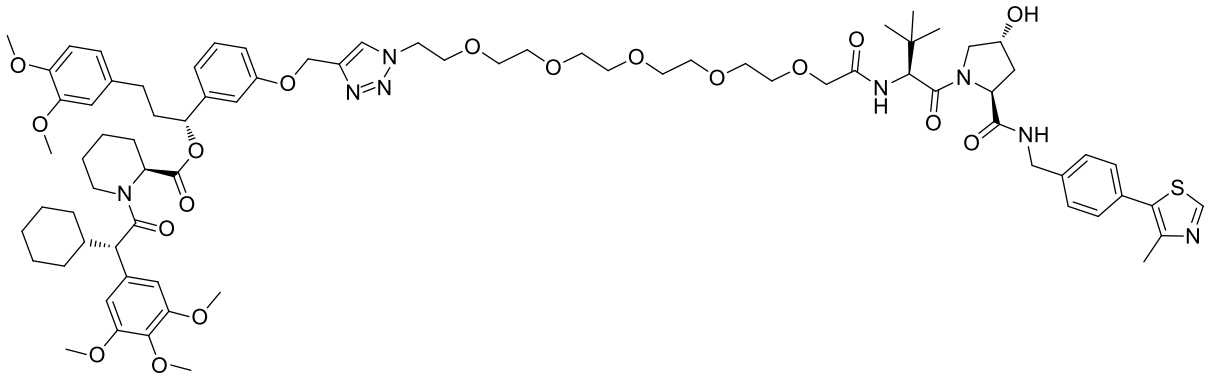
TLC [DCM:MeOH 95:5]: R_f = 0.29.

HPLC [0-100% Solvent B, 20 min]: R_t = 17.6 min, purity (220 nm) = 99%.

^{1}H NMR (500 MHz, Chloroform-*d*) (mixture of rotamers) δ 8.66 (s, 1H), 7.81 (s, 1H), 7.39 – 7.28 (m, 5.5H), 7.23 (d, *J* = 8.7 Hz, 1H), 7.13 – 6.95 (m, 1.5H), 6.87 – 6.78 (m, 1H), 6.77 – 6.74 (m, 1.5H), 6.70 – 6.59 (m, 2H), 6.49 (s, 1.5H), 6.46 – 6.41 (m, 1H), 5.58 (dd, *J* = 7.9, 5.7 Hz, 1H), 5.46 (d, *J* = 4.7 Hz, 1H), 5.17 (s, 0.5H), 5.13 (s, 1.5H), 4.73 – 4.69 (m, 1H), 4.57 – 4.48 (m, 5H), 4.37 – 4.30 (m, 1H), 4.07 (d, *J* = 11.4 Hz, 1H), 4.03 – 3.90 (m, 3H), 3.87 – 3.81 (m, 10H), 3.76 (s, 2H), 3.70 (s, 4H), 3.66 – 3.55 (m, 14H), 3.38 (d, *J* = 9.8 Hz, 1H), 2.78 (td, *J* = 13.4, 2.7 Hz, 1H), 2.64 – 2.51 (m, 2H), 2.50 (s, 3H), 2.49 – 2.33 (m, 2H), 2.31 – 2.23 (m, 1H), 2.14 – 2.06 (m, 2H), 1.99 – 1.81 (m, 3H), 1.66 – 1.54 (m, 5H), 1.48 – 1.28 (m, 3H), 1.22 – 1.05 (m, 3H), 0.95 (s, 9H), 0.88 – 0.53 (m, 2H).

HRMS: m/z: found 1417.69922 $[M+H]^+$, calculated 1417.69999 $[M+H]^+$

(S)-(R)-3-(3,4-Dimethoxyphenyl)-1-(3-((1-((S)-19-((2S,4R)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidine-1-carbonyl)-20,20-dimethyl-17-oxo-3,6,9,12,15-pentaoxa-18-azahenicosyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)propyl 1-((S)-2-cyclohexyl-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2-carboxylate (MTQ203)



MTQ203

MTQ203 was synthesized from **43e** (10 mg, 0. 0137 mmol) according to the General Method G. Purification by flash column chromatography (0% to 5% MeOH in DCM) gave a white solid (16 mg, 0.011 mmol, 81%).

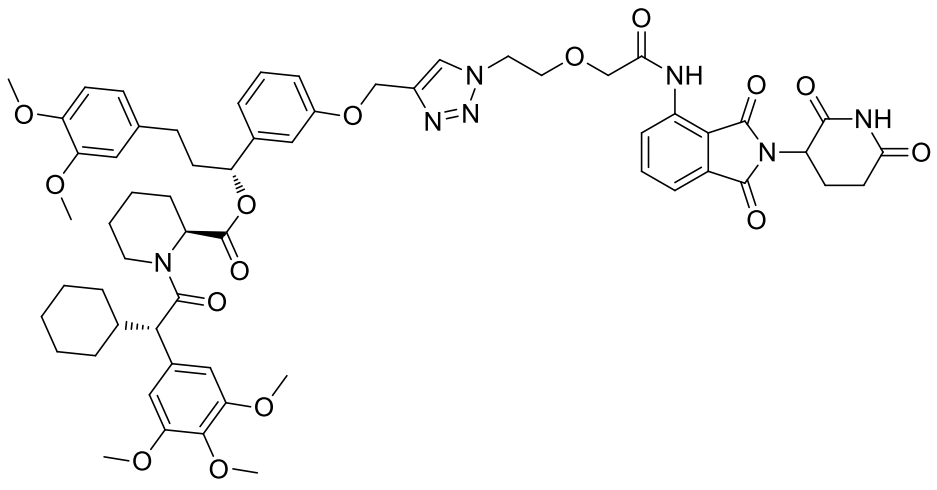
TLC [DCM:MeOH 95:5]: R_f = 0.23.

HPLC [0-100% Solvent B, 20 min]: R_t = 17.5 min, purity (220 nm) = 98%.

^{1}H NMR (300 MHz, Chloroform-*d*) (mixture of rotamers) δ 8.66 (s, 1H), 7.80 (s, 1H), 7.35 (d, *J* = 1.9 Hz, 5H), 7.16 – 6.95 (m, 2H), 6.85 (dd, *J* = 8.0, 2.2 Hz, 1H), 6.79 – 6.73 (m, 2H), 6.71 – 6.59 (m, 2H), 6.52 – 6.40 (m, 3H), 5.58 (dd, *J* = 7.8, 6.0 Hz, 1H), 5.46 (d, *J* = 4.0 Hz, 1H), 5.18 (s, 0.5H), 5.14 (s, 1.5H), 4.72 (t, *J* = 7.9 Hz, 1H), 4.62 – 4.43 (m, 6H), 4.34 (dd, *J* = 14.9, 5.3 Hz, 1H), 4.12 – 4.05 (m, 1H), 3.99 (d, *J* = 6.3 Hz, 2H), 3.89 – 3.80 (m, 11H), 3.76 (s, 2H), 3.70 (s, 4H), 3.66 – 3.63 (m, 4H), 3.62 – 3.56 (m, 13H), 3.38 (d, *J* = 9.9 Hz, 1H), 2.86 – 2.69 (m, 1H), 2.56 (dd, *J* = 8.0, 5.0 Hz, 2H), 2.51 (s, 3H), 2.49 – 2.23 (m, 3H), 2.14 – 1.83 (m, 5H), 1.68 – 1.56 (m, 5H), 1.37 – 1.30 (m, 2H), 1.21 – 1.02 (m, 4H), 0.95 (s, 9H), 0.88 (s, 2H).

HRMS: m/z: found 1461.72591 $[M+H]^+$, calculated 1461.72621 $[M+H]^+$

(2S)-(1R)-3-(3,4-Dimethoxyphenyl)-1-(3-((1-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-2-oxoethoxy)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)propyl 1-((S)-2-cyclohexyl-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2-carboxylate (MTQ204)



MTQ204

MTQ204 was synthesized from **44a** (5 mg, 0. 0137 mmol) according to the General Method G. Purification by flash column chromatography (0% to 4% EtOH in DCM) gave a light yellow solid (10 mg, 0.0096 mmol, 70%).

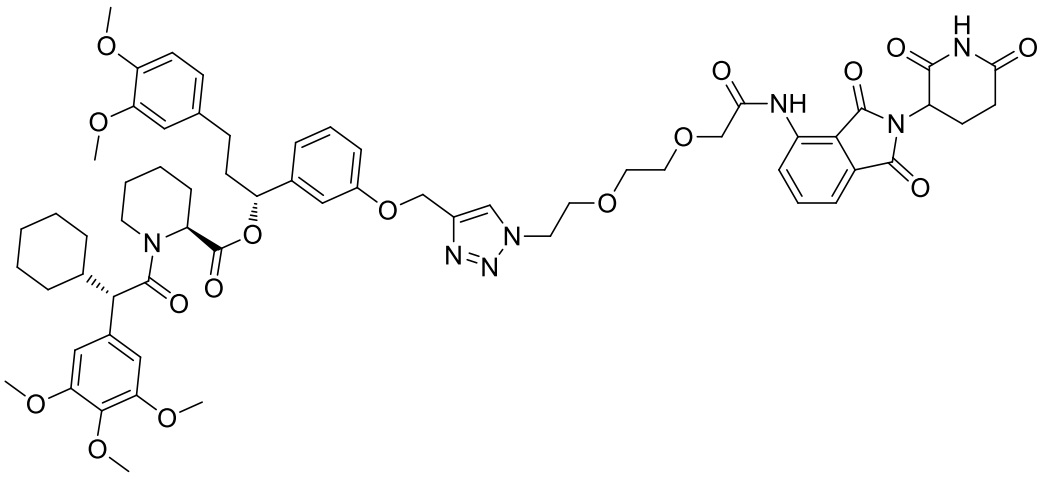
TLC [DCM:MeOH 95:5]: R_f = 0.57.

HPLC [0-100% Solvent B, 20 min]: R_t = 18.5 min, purity (220 nm) = 99%.

^{1}H NMR (500 MHz, Chloroform-*d*) (mixture of rotamers) δ 10.40 – 10.28 (m, 1H), 8.82 – 8.71 (m, 1H), 8.64 – 8.37 (m, 1H), 8.02 (d, *J* = 9.2 Hz, 1H), 7.73 – 7.63 (m, 1H), 7.57 – 7.47 (m, 1H), 7.07 – 6.86 (m, 1.5H), 6.83 – 6.74 (m, 1.5H), 6.73 – 6.66 (m, 1H), 6.63 – 6.52 (m, 2H), 6.47 – 6.34 (m, 3H), 5.74 (t, *J* = 7.2 Hz, 0.25H), 5.53 – 5.45 (m, 0.75H), 5.42 – 5.33 (m, 0.75H), 5.22 – 5.03 (m, 2H), 4.93 – 4.84 (m, 1H), 4.75 – 4.63 (m, 2H), 4.53 – 4.46 (m, 0.25H), 4.06 (d, *J* = 4.2 Hz, 2H), 3.99 – 3.94 (m, 2H), 3.90 – 3.84 (m, 1H), 3.81 – 3.71 (m, 9H), 3.69 (s, 2H), 3.62 (s, 2H), 3.61 (s, 2H), 3.30 (d, *J* = 9.8 Hz, 0.75H), 2.99 (dd, *J* = 9.7, 3.3 Hz, 0.25H), 2.81 – 2.56 (m, 4H), 2.46 – 2.36 (m, 1H), 2.33 – 2.15 (m, 2H), 2.07 – 1.98 (m, 2H), 1.93 – 1.72 (m, 3H), 1.68 – 1.56 (m, 4H), 1.48 – 1.44 (m, 1H), 1.38 – 1.30 (m, 1H), 1.25 – 1.20 (m, 2H), 1.14 – 1.00 (m, 3H), 0.92 – 0.75 (m, 2H), 0.73 – 0.62 (m, 1H).

HRMS: m/z: found 1128.49272 $[M+H]^+$, calculated 1128.49244 $[M+H]^+$

(2S)-(1R)-3-(3,4-Dimethoxyphenyl)-1-(3-((1-(2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-2-oxoethoxy)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)propyl 1-((S)-2-cyclohexyl-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2-carboxylate (MTQ205)



MTQ205

MTQ205 was synthesized from **44b** (6 mg, 0. 0137 mmol) according to the General Method G. Purification by flash column chromatography (0% to 4% EtOH in DCM) gave a light yellow solid (11 mg, 0.0093 mmol, 68%).

TLC [DCM:MeOH 95:5]: R_f = 0.52.

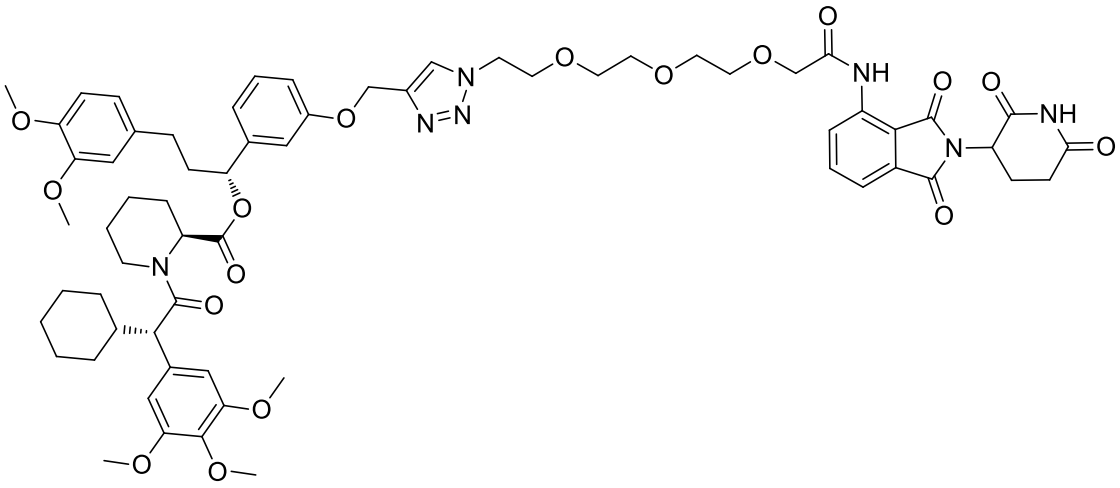
HPLC [30-100% Solvent B, 20 min]: R_t = 15.8 min, purity (220 nm) ≥ 99%.

^{1}H NMR (500 MHz, Chloroform-*d*) (mixture of rotamers) δ 10.42 (s, 1H), 8.84 (d, *J* = 8.4 Hz,

1H), 8.56 – 8.29 (m, 1H), 7.80 – 7.75 (m, 1H), 7.69 (t, J = 7.9 Hz, 1H), 7.55 (d, J = 7.1 Hz, 1H), 7.09 (t, J = 7.8 Hz, 0.75H), 7.00 – 6.89 (m, 0.75H), 6.85 – 6.58 (m, 4.75H), 6.50 (s, 1.5H), 6.47 – 6.39 (m, 1.25H), 5.80 (t, J = 6.5 Hz, 0.25H), 5.56 (t, J = 7.0 Hz, 0.75H), 5.48 (d, J = 5.0 Hz, 0.75H), 5.12 (s, 0.5H), 5.08 (s, 1.5H), 5.01 – 4.93 (m, 1H), 4.74 (d, J = 5.1 Hz, 0.25H), 4.63 – 4.50 (m, 2H), 4.15 (s, 2H), 4.02 – 3.92 (m, 3H), 3.89 – 3.79 (m, 9H), 3.77 (s, 6H), 3.70 (s, 4H), 3.38 (d, J = 9.9 Hz, 0.75H), 3.00 (d, J = 9.7 Hz, 0.25H), 2.93 – 2.69 (m, 4H), 2.57 – 2.33 (m, 2H), 2.32 – 2.25 (m, 1H), 2.19 – 2.04 (m, 2H), 1.99 – 1.76 (m, 3H), 1.75 – 1.61 (m, 5H), 1.45 – 1.28 (m, 3H), 1.20 – 1.08 (m, 3H), 0.97 – 0.68 (m, 3H).

HRMS: m/z: found 1172.51994 $[M+H]^+$, calculated 1172.51866 $[M+H]^+$

(2S)-(1R)-3-(3,4-Dimethoxyphenyl)-1-(3-((1-(2-(2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-2-oxoethoxy)ethoxy)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)propyl 1-((S)-2-cyclohexyl-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2-carboxylate (MTQ206)



MTQ206

MTQ206 was synthesized from **44c** (7 mg, 0. 0137 mmol) according to the General Method G. Purification by flash column chromatography (0% to 5% MeOH in DCM) gave a white solid (13 mg, 0.011 mmol, 78%).

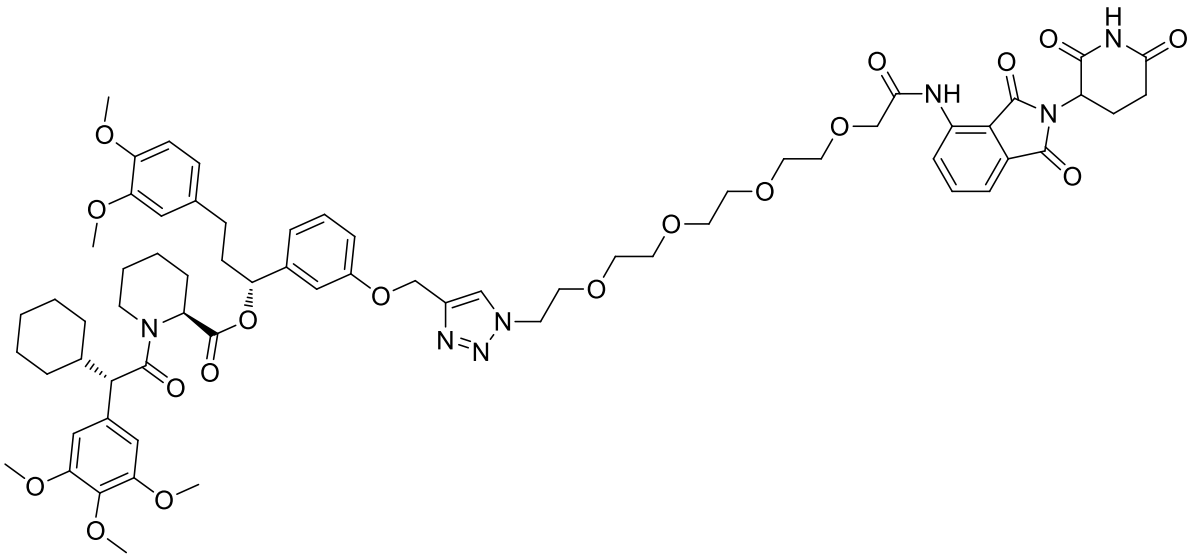
TLC [DCM:MeOH 95:5]: R_f = 0.50.

HPLC [0-100% Solvent B, 20 min]: R_t = 18.6 min, purity (220 nm) = 98%.

^{1}H NMR (300 MHz, Chloroform-*d*) (mixture of rotamers) δ 10.43 (s, 1H), 8.84 (d, J = 8.4 Hz, 1H), 8.39 (d, J = 4.6 Hz, 1H), 7.78 (s, 1H), 7.70 (t, J = 7.9 Hz, 1H), 7.56 (d, J = 7.3 Hz, 1H), 7.10 (t, J = 7.9 Hz, 0.75H), 7.04 – 6.93 (m, 0.75H), 6.88 – 6.71 (m, 2.5H), 6.71 – 6.59 (m, 2H), 6.46 (d, J = 20.6 Hz, 3H), 5.80 (t, J = 6.9 Hz, 0.25H), 5.63 – 5.51 (m, 0.75H), 5.47 (d, J = 3.7 Hz, 0.75H), 5.18 (s, 0.5H), 5.14 (s, 1.5H), 5.00 – 4.89 (m, 1H), 4.76 – 4.71 (m, 0.25H), 4.52 (t, J = 5.1 Hz, 2H), 4.18 (s, 2H), 3.88 (d, J = 5.2 Hz, 2H), 3.84 (dt, J = 5.8, 2.9 Hz, 9H), 3.80 – 3.71 (m, 7H), 3.70 (s, 4H), 3.64 – 3.57 (m, 4H), 3.38 (d, J = 9.9 Hz, 0.75H), 3.00 (d, J = 9.8 Hz, 0.25H), 2.93 – 2.66 (m, 4H), 2.59 – 2.24 (m, 3H), 2.17 – 1.81 (m, 5H), 1.73 – 1.65 (m, 2H), 1.56 – 1.51 (m, 1H), 1.46 – 1.02 (m, 8H), 0.98 – 0.50 (m, 3H).

HRMS: m/z: found 1216.54470 $[M+H]^+$, calculated 1216.54487 $[M+H]^+$

(2S)-(1R)-3-(3,4-Dimethoxyphenyl)-1-(3-((1-(14-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-14-oxo-3,6,9,12-tetraoxatetradecyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)propyl 1-((S)-2-cyclohexyl-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2-carboxylate (MTQ207)



MTQ207

MTQ207 was synthesized from **44d** (10 mg, 0. 0137 mmol) according to the General Method G. Purification by flash column chromatography (0% to 5% MeOH in DCM) gave a white solid (12 mg, 0.0097 mmol, 71%).

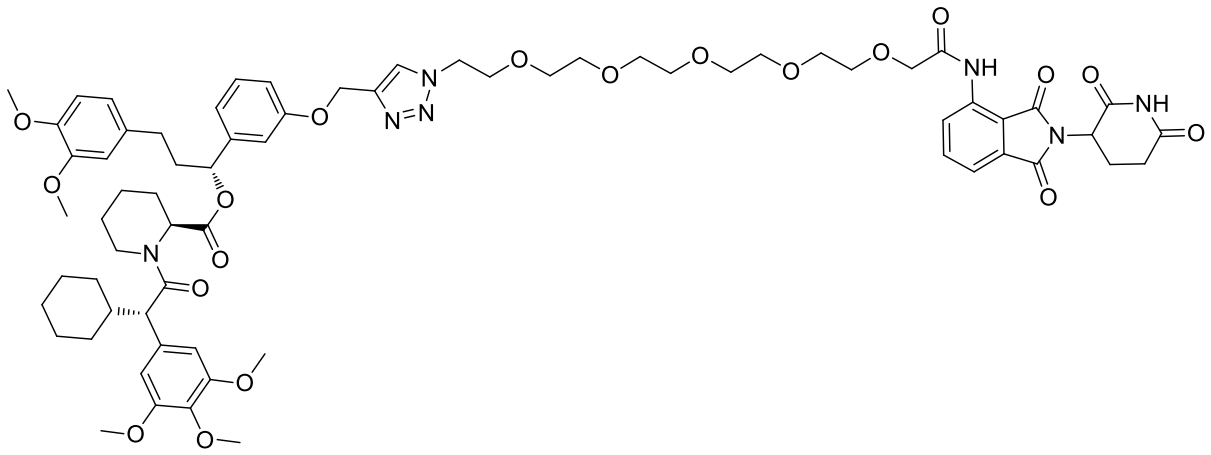
TLC [DCM:MeOH 95:5]: R_f = 0.50.

HPLC [0-100% Solvent B, 20 min]: R_t = 18.5 min, purity (220 nm) = 99%.

^{1}H NMR (300 MHz, Chloroform-*d*) (mixture of rotamers) δ 10.41 (s, 1H), 8.77 (d, *J* = 8.4 Hz, 1H), 8.51 (s, 1H), 7.73 (s, 1H), 7.68 – 7.58 (m, 1H), 7.50 (d, *J* = 7.3 Hz, 1H), 7.04 (t, *J* = 7.9 Hz, 0.75H), 6.97 – 6.85 (m, 1H), 6.79 (dd, *J* = 8.1, 2.0 Hz, 0.75H), 6.74 – 6.66 (m, 1.75H), 6.64 – 6.51 (m, 2H), 6.43 (s, 1.5H), 6.36 (t, *J* = 3.8 Hz, 1.25H), 5.80 – 5.68 (m, 0.25H), 5.51 (dd, *J* = 7.6, 5.8 Hz, 0.75H), 5.40 (d, *J* = 4.3 Hz, 0.75H), 5.12 (s, 0.5H), 5.08 (s, 1.5H), 4.92 – 4.83 (m, 1H), 4.70 – 4.63 (m, 0.25H), 4.47 (t, *J* = 5.1 Hz, 2H), 4.10 (d, *J* = 1.4 Hz, 2H), 3.94 – 3.85 (m, 1H), 3.82 (t, *J* = 5.2 Hz, 2H), 3.79 – 3.74 (m, 8H), 3.74 – 3.67 (m, 7H), 3.64 (s, 4H), 3.62 – 3.57 (m, 2H), 3.52 (s, 6H), 3.31 (d, *J* = 9.9 Hz, 0.75H), 2.94 (d, *J* = 9.8 Hz, 0.25H), 2.86 – 2.55 (m, 4H), 2.53 – 2.18 (m, 3H), 2.12 – 1.71 (m, 5H), 1.68 – 1.55 (m, 4H), 1.48 – 1.21 (m, 4H), 1.16 – 0.99 (m, 3H), 0.93 – 0.40 (m, 3H).

HRMS: m/z: found 1260.57118 $[M+H]^+$, calculated 1260.57109 $[M+H]^+$

(2S)-(1R)-3-(3,4-Dimethoxyphenyl)-1-(3-((1-(17-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-17-oxo-3,6,9,12,15-pentaoxaheptadecyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)propyl 1-((S)-2-cyclohexyl-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2-carboxylate (MTQ208)



MTQ208

MTQ208 was synthesized from **44e** (10 mg, 0. 0137 mmol) according to the General Method G. Purification by flash column chromatography (0% to 5% MeOH in DCM) gave a white solid (13 mg, 0.0097 mmol, 72%).

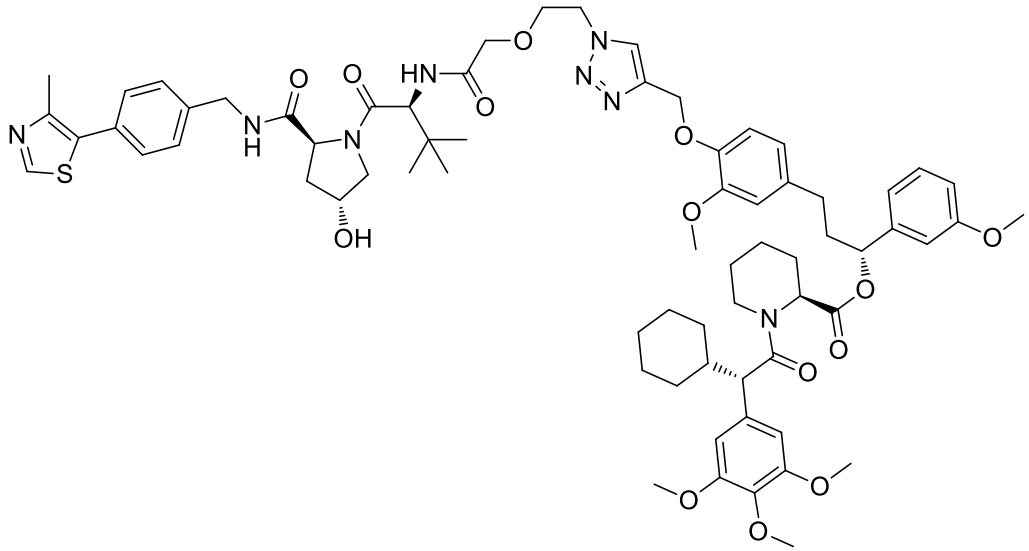
TLC [DCM:MeOH 95:5]: R_f = 0.31.

HPLC [0-100% Solvent B, 20 min]: R_t = 18.5 min, purity (220 nm) = 99%.

^{1}H NMR (300 MHz, Chloroform-*d*) (mixture of rotamers) δ 10.48 (s, 1H), 8.85 (d, *J* = 8.4 Hz, 1H), 8.70 (s, 1H), 7.80 (s, 1H), 7.76 – 7.67 (m, 1H), 7.56 (dd, *J* = 7.3, 0.7 Hz, 1H), 7.11 (t, *J* = 7.9 Hz, 0.75H), 7.04 – 6.93 (m, 0.75H), 6.86 (dd, *J* = 7.9, 2.2 Hz, 0.75H), 6.83 – 6.70 (m, 2H), 6.70 – 6.60 (m, 2H), 6.50 (s, 1.5H), 6.43 (t, *J* = 3.8 Hz, 1.25H), 5.86 – 5.76 (m, 0.25H), 5.58 (dd, *J* = 7.7, 5.9 Hz, 0.75H), 5.47 (d, *J* = 4.1 Hz, 0.75H), 5.19 (s, 0.5H), 5.15 (s, 1.5H), 5.01 – 4.90 (m, 1H), 4.76 – 4.70 (m, 0.25H), 4.53 (t, *J* = 5.1 Hz, 2H), 4.18 (d, *J* = 1.4 Hz, 2H), 4.02 – 3.91 (m, 1H), 3.89 – 3.81 (m, 10H), 3.81 – 3.75 (m, 7H), 3.71 (s, 4H), 3.69 – 3.66 (m, 2H), 3.66 – 3.58 (m, 10H), 3.38 (d, *J* = 9.8 Hz, 0.75H), 3.00 (d, *J* = 9.8 Hz, 0.25H), 2.91 – 2.61 (m, 4H), 2.60 – 2.23 (m, 3H), 2.18 – 1.80 (m, 5H), 1.77 – 1.62 (m, 4H), 1.54 – 1.28 (m, 4H), 1.24 – 1.08 (m, 3H), 1.05 – 0.48 (m, 3H).

HRMS: m/z: found 1304.59712 $[M+H]^+$, calculated 1304.59730 $[M+H]^+$

(S)-(R)-3-(4-((1-(2-(2-(((S)-1-((2S,4R)-4-Hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-2-oxoethoxy)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)-3-methoxyphenyl)-1-(3-methoxyphenyl)propyl 1-((S)-2-cyclohexyl-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2-carboxylate (MTQ328)



MTQ328

MTQ328 was synthesized from **31a** (10 mg, 0.0137 mmol) and **43a** (8 mg, 0. 0137 mmol) according to the General Method G. Purification by flash column chromatography (0% to 6% MeOH in DCM) gave a white solid (16 mg, 91%).

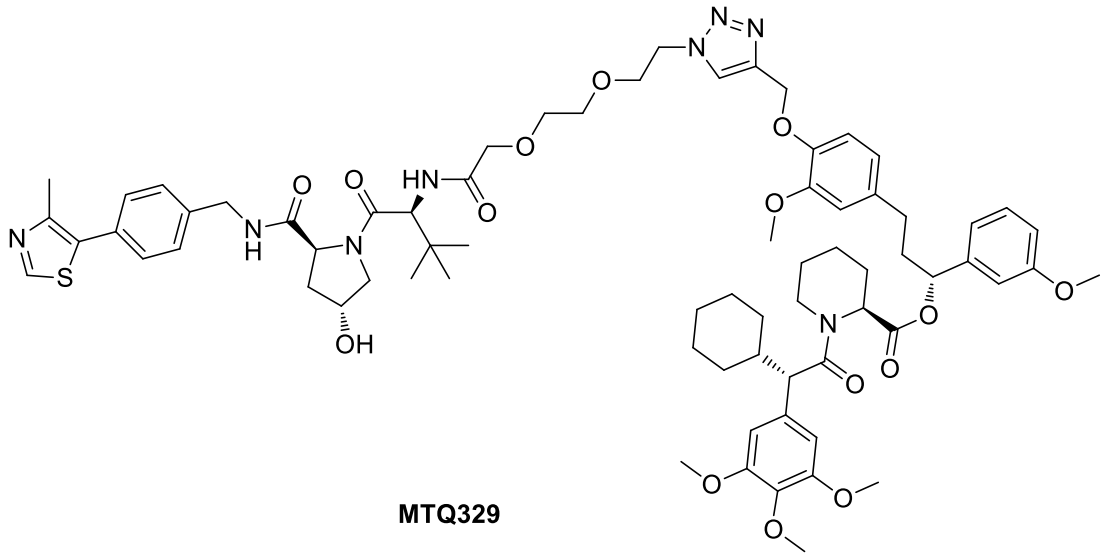
TLC [DCM:MeOH 90:10]: R_f = 0.49.

HPLC [0-100% Solvent B, 20 min]: R_t = 17.73 min, purity (220 nm) = 97%.

^{1}H NMR (500 MHz, Chloroform-*d*) (mixture of rotamers) δ 8.65 (s, 1H), 7.79 (s, 1H), 7.41 – 7.27 (m, 5.5H), 7.13 – 6.86 (m, 3.5H), 6.76 – 6.49 (m, 5H), 6.43 – 6.38 (m, 1H), 5.51 (dd, *J* = 8.1, 5.7 Hz, 1H), 5.48 (d, *J* = 5.0 Hz, 1H), 5.20 (d, *J* = 3.8 Hz, 2H), 4.75 – 4.68 (m, 1H), 4.63 – 4.44 (m, 6H), 4.33 (dd, *J* = 15.0, 5.6 Hz, 1H), 4.00 – 3.90 (m, 4H), 3.88 – 3.85 (m, 2H), 3.83 (d, *J* = 1.7 Hz, 2H), 3.80 (d, *J* = 6.9 Hz, 2H), 3.78 (s, 2H), 3.75 (d, *J* = 2.8 Hz, 4H), 3.71 (s, 4H), 3.64 (dd, *J* = 11.3, 3.6 Hz, 1H), 3.37 (s, 1H), 2.77 (td, *J* = 13.5, 2.7 Hz, 1H), 2.62 – 2.51 (m, 1H), 2.49 (s, 3H), 2.48 – 2.31 (m, 2H), 2.30 – 2.24 (m, 1H), 2.13 – 2.06 (m, 2H), 1.94 – 1.76 (m, 3H), 1.71 – 1.63 (m, 3H), 1.62 – 1.51 (m, 3H), 1.45 – 1.36 (m, 1H), 1.35 – 1.27 (m, 2H), 1.22 – 1.09 (m, 3H), 0.93 (s, 9H), 0.87 – 0.50 (m, 2H).

HRMS: m/z: found 1285.6204 $[M+H]^+$, calculated 1285.62135 $[M+H]^+$

(S)-(R)-3-(4-((1-(2-(2-(2-(((S)-1-((2S,4R)-4-Hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-2-oxoethoxy)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)-3-methoxyphenyl)-1-(3-methoxyphenyl)propyl 1-((S)-2-cyclohexyl-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2-carboxylate (MTQ329)



MTQ329

MTQ329 was synthesized from **31a** (10 mg, 0.0137 mmol) and **43b** (8 mg, 0. 0137 mmol) according to the General Method G. Purification by flash column chromatography (0% to 6% MeOH in DCM) gave a white solid (10 mg, 55%).

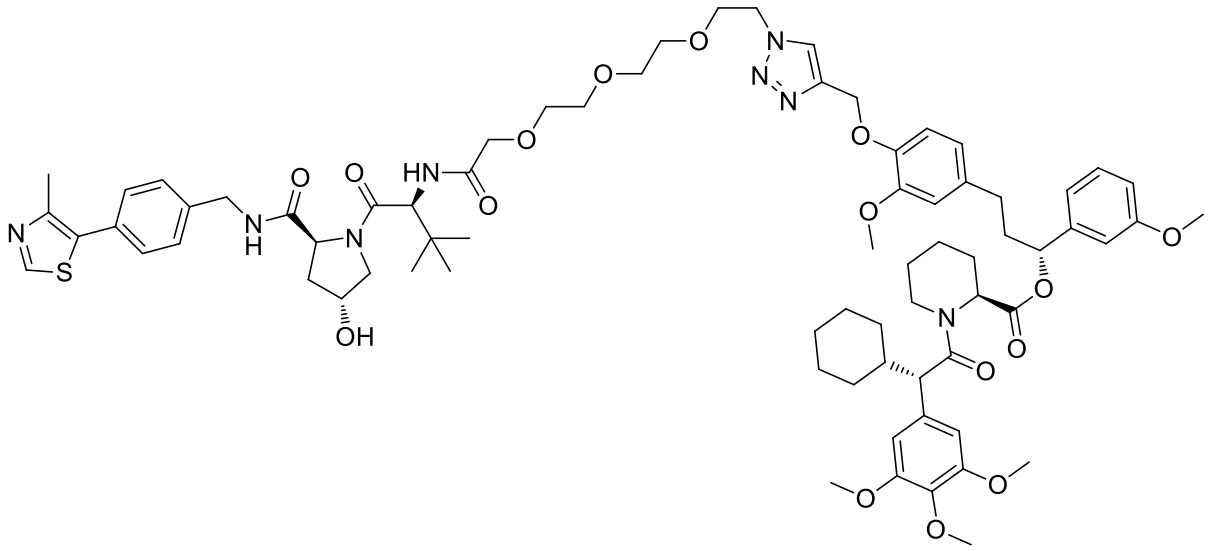
TLC [DCM:MeOH 90:10]: R_f = 0.57.

HPLC [0-100% Solvent B, 20 min]: R_t = 17.85 min, purity (220 nm) = 99%.

^{1}H NMR (500 MHz, Chloroform-*d*) (mixture of rotamers) δ 8.66 (s, 1H), 8.03 (s, 1H), 7.38 – 7.27 (m, 5H), 7.25 (d, *J* = 6.0 Hz, 1H), 7.00 – 6.85 (m, 2H), 6.78 – 6.55 (m, 4H), 6.51 (s, 2H), 6.43 – 6.36 (m, 1H), 5.51 (dd, *J* = 8.0, 5.7 Hz, 1H), 5.47 (d, *J* = 4.8 Hz, 1H), 5.24 – 5.16 (m, 2H), 4.66 – 4.40 (m, 7H), 4.26 (dt, *J* = 14.8, 5.2 Hz, 1H), 4.04 – 3.98 (m, 2H), 3.91 – 3.86 (m, 2H), 3.85 – 3.82 (m, 3H), 3.81 – 3.79 (m, 2H), 3.76 (s, 4H), 3.75 (s, 2H), 3.72 (s, 4H), 3.68 – 3.52 (m, 6H), 3.39 (d, *J* = 10.0 Hz, 1H), 2.78 (td, *J* = 13.5, 2.6 Hz, 1H), 2.64 – 2.52 (m, 1H), 2.50 (s, 3H), 2.48 – 2.32 (m, 3H), 2.30 – 2.22 (m, 1H), 2.13 – 1.86 (m, 4H), 1.83 – 1.70 (m, 2H), 1.62 – 1.53 (m, 3H), 1.47 – 1.37 (m, 1H), 1.36 – 1.28 (m, 2H), 1.23 – 1.11 (m, 3H), 0.97 (s, 9H), 0.95 – 0.71 (m, 3H).

HRMS: m/z: found 1329.64624 $[M+H]^+$, calculated 1329.64756 $[M+H]^+$

(S)-(R)-3-(4-((1-((S)-13-((2S,4R)-4-Hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidine-1-carbonyl)-14,14-dimethyl-11-oxo-3,6,9-trioxa-12-azapentadecyl)-1H-1,2,3-triazol-4-yl)methoxy)-3-methoxyphenyl)-1-(3-methoxyphenyl)propyl 1-((S)-2-cyclohexyl-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2-carboxylate (MTQ330)



MTQ330

MTQ330 was synthesized from **31a** (10 mg, 0.0137 mmol) and **43c** (9 mg, 0. 0137 mmol) according to the General Method G. Purification by flash column chromatography (0% to 6% MeOH in DCM) gave a white solid (17 mg, 90%).

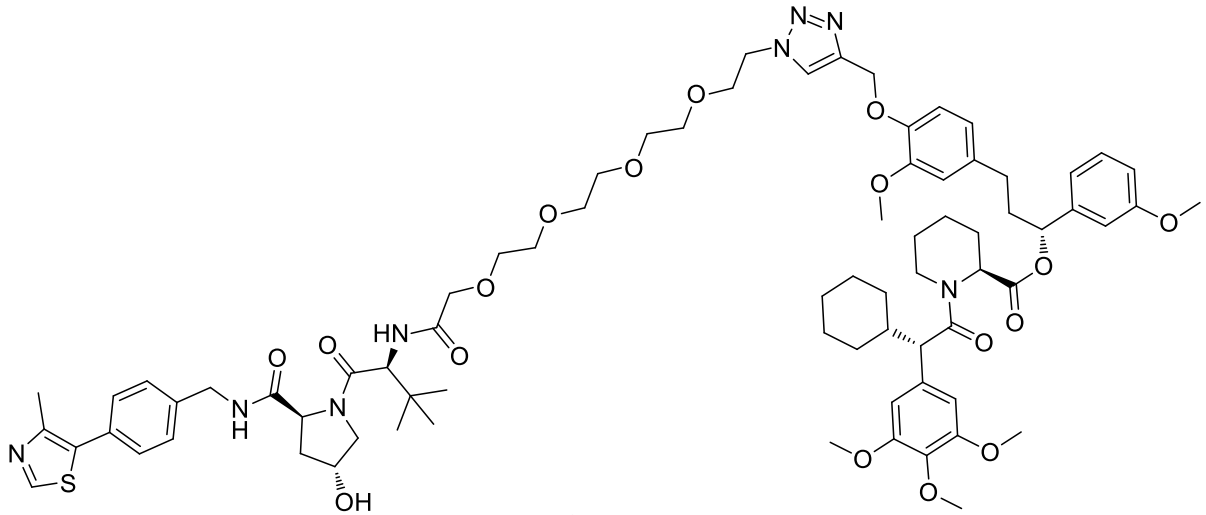
TLC [DCM:MeOH 90:10]: R_f = 0.57.

HPLC [0-100% Solvent B, 20 min]: R_t = 17.91 min, purity (220 nm) = 96%.

^{1}H NMR (500 MHz, Chloroform-*d*) (mixture of rotamers) δ 8.84 (s, 1H), 7.86 (s, 1H), 7.41 – 7.27 (m, 6H), 7.10 (t, *J* = 7.9 Hz, 1H), 7.00 – 6.82 (m, 2H), 6.74 (dd, *J* = 7.8, 2.0 Hz, 1H), 6.69 – 6.57 (m, 3H), 6.50 (s, 1H), 6.44 – 6.38 (m, 1H), 5.59 – 5.51 (m, 1H), 5.47 (d, *J* = 5.0 Hz, 1H), 5.19 (s, 2H), 4.74 – 4.44 (m, 7H), 4.34 (dd, *J* = 15.1, 5.4 Hz, 1H), 4.07 – 3.93 (m, 4H), 3.90 – 3.82 (m, 5H), 3.81 – 3.78 (m, 4H), 3.75 (d, *J* = 3.0 Hz, 4H), 3.71 (s, 4H), 3.64 (d, *J* = 3.8 Hz, 2H), 3.62 – 3.57 (m, 6H), 3.38 (d, *J* = 9.8 Hz, 1H), 2.82 – 2.73 (m, 1H), 2.62 – 2.57 (m, 1H), 2.55 (s, 3H), 2.54 – 2.49 (m, 1H), 2.48 – 2.32 (m, 4H), 2.31 – 2.24 (m, 1H), 2.14 – 2.06 (m, 2H), 1.94 – 1.71 (m, 3H), 1.62 – 1.53 (m, 3H), 1.47 – 1.29 (m, 3H), 1.22 – 1.08 (m, 3H), 0.96 (s, 9H), 0.92 – 0.69 (m, 2H).

HRMS: m/z: found 1373.67136 $[M+H]^+$, calculated 1373.67378 $[M+H]^+$

(S)-(R)-3-(4-((1-((S)-16-((2S,4R)-4-Hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidine-1-carbonyl)-17,17-dimethyl-14-oxo-3,6,9,12-tetraoxa-15-azaoctadecyl)-1H-1,2,3-triazol-4-yl)methoxy)-3-methoxyphenyl)-1-(3-methoxyphenyl)propyl 1-((S)-2-cyclohexyl-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2-carboxylate (MTQ331)



MTQ331

MTQ331 was synthesized from **31a** (10 mg, 0.0137 mmol) and **43d** (9 mg, 0. 0137 mmol) according to the General Method G. Purification by flash column chromatography (0% to 6% MeOH in DCM) gave a white solid (12 mg, 62%).

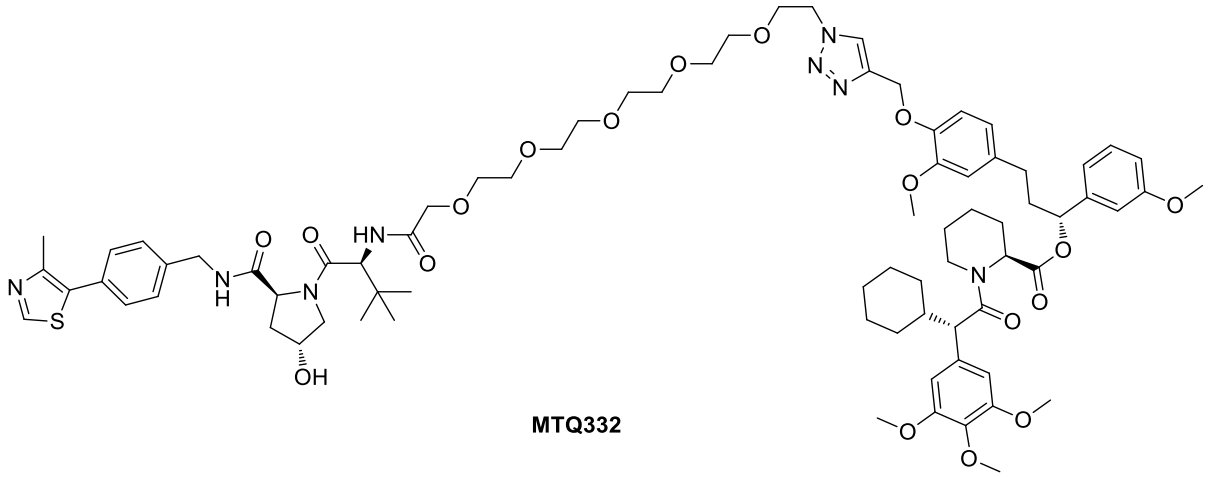
TLC [DCM:MeOH 90:10]: R_f = 0.61.

HPLC [0-100% Solvent B, 20 min]: R_t = 17.84 min, purity (220 nm) ≥ 99%.

^{1}H NMR (500 MHz, Chloroform-*d*) (mixture of rotamers) δ 8.90 (s, 1H), 7.84 (s, 1H), 7.43 – 7.27 (m, 6H), 7.25 – 7.22 (m, 1H), 6.99 – 6.83 (m, 2H), 6.75 (dd, *J* = 8.2, 2.5 Hz, 1H), 6.69 – 6.58 (m, 3H), 6.50 (s, 1H), 6.43 – 6.40 (m, 1H), 5.56 (dd, *J* = 7.7, 5.8 Hz, 1H), 5.47 (d, *J* = 5.3 Hz, 1H), 5.21 (s, 2H), 4.74 – 4.66 (m, 1H), 4.62 – 4.41 (m, 6H), 4.36 (dd, *J* = 15.1, 5.2 Hz, 1H), 4.09 (d, *J* = 11.2 Hz, 1H), 3.99 (d, *J* = 5.5 Hz, 2H), 3.86 (t, *J* = 5.1 Hz, 2H), 3.84 – 3.80 (m, 6H), 3.76 (d, *J* = 2.8 Hz, 4H), 3.71 (s, 4H), 3.66 – 3.55 (m, 14H), 3.38 (d, *J* = 9.8 Hz, 1H), 2.78 (td, *J* = 13.4, 2.5 Hz, 1H), 2.57 (s, 3H), 2.55 – 2.41 (m, 4H), 2.39 – 2.34 (m, 1H), 2.29 – 2.26 (m, 1H), 2.14 – 2.06 (m, 2H), 1.98 – 1.77 (m, 3H), 1.72 – 1.63 (m, 3H), 1.60 – 1.52 (m, 2H), 1.46 – 1.37 (m, 1H), 1.36 – 1.27 (m, 2H), 1.18 – 1.11 (m, 2H), 0.96 (s, 9H), 0.90 – 0.54 (m, 2H).

HRMS: m/z: found 1417.70127 $[M+H]^+$, calculated 1417.69999 $[M+H]^+$

(S)-(R)-3-(4-((1-((S)-19-((2S,4R)-4-Hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidine-1-carbonyl)-20,20-dimethyl-17-oxo-3,6,9,12,15-pentaoxa-18-azahenicosyl)-1H-1,2,3-triazol-4-yl)methoxy)-3-methoxyphenyl)-1-(3-methoxyphenyl)propyl 1-((S)-2-cyclohexyl-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2-carboxylate (MTQ332)



MTQ332

MTQ332 was synthesized from **31a** (10 mg, 0.0137 mmol) and **43e** (10 mg, 0. 0137 mmol) according to the General Method G. Purification by flash column chromatography (0% to 6% MeOH in DCM) gave a white solid (13 mg, 65%).

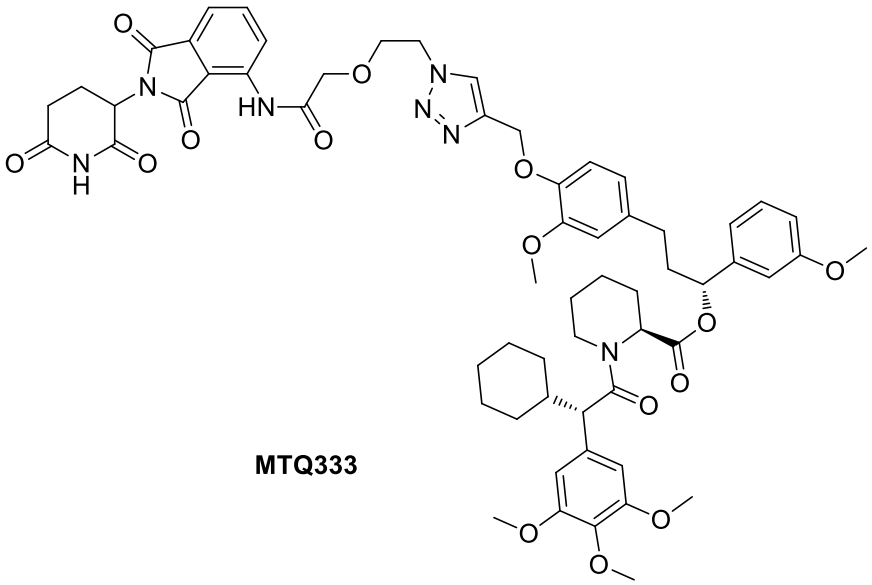
TLC [DCM:MeOH 90:10]: R_f = 0.58.

HPLC [0-100% Solvent B, 20 min]: R_t = 17.82 min, purity (220 nm) ≥ 99%.

^{1}H NMR (300 MHz, Chloroform-*d*) (mixture of rotamers) δ 8.89 (s, 1H), 7.82 (s, 1H), 7.36 (s, 6H), 7.24 – 7.04 (m, 1H), 7.00 – 6.80 (m, 2H), 6.79 – 6.59 (m, 3H), 6.59 – 6.37 (m, 3H), 5.56 (t, *J* = 6.1 Hz, 1H), 5.50 – 5.42 (m, 1H), 5.21 (s, 2H), 4.72 (t, *J* = 7.7 Hz, 1H), 4.60 – 4.46 (m, 5H), 4.41 – 4.28 (m, 1H), 4.13 – 3.91 (m, 4H), 3.90 – 3.78 (m, 9H), 3.75 (s, 4H), 3.71 (s, 4H), 3.67 – 3.55 (m, 17H), 3.37 (d, *J* = 9.4 Hz, 1H), 2.86 – 2.60 (m, 4H), 2.56 (s, 4H), 2.50 – 2.21 (m, 4H), 2.17 – 2.05 (m, 2H), 1.95 – 1.78 (m, 2H), 1.73 – 1.60 (m, 4H), 1.49 – 1.27 (m, 3H), 1.20 – 1.04 (m, 3H), 0.95 (s, 9H), 0.89 – 0.50 (m, 2H).

HRMS: m/z: found 1461.72733 $[M+H]^+$, calculated 1461.72621 $[M+H]^+$

(2S)-(1R)-3-(4-((1-(2-(2-((2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-2-oxoethoxy)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)-3-methoxyphenyl)-1-(3-methoxyphenyl)propyl 1-((S)-2-cyclohexyl-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2-carboxylate (MTQ333)



MTQ333

MTQ333 was synthesized from **31a** (10 mg, 0.0137 mmol) and **44a** (5 mg, 0. 0137 mmol) according to the General Method G. Purification by flash column chromatography (CH → EA:MeCN = 90:10) gave a white solid (12 mg, 78%).

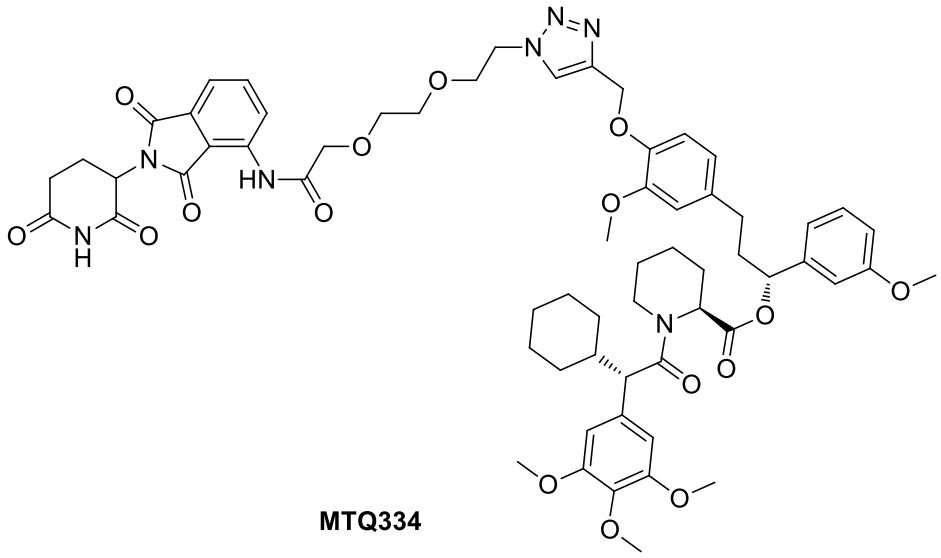
TLC [DCM:MeOH 95:5]: R_f = 0.42.

HPLC [0-100% Solvent B, 20 min]: R_t = 18.36 min, purity (220 nm) = 97%.

^{1}H NMR (500 MHz, Chloroform-*d*) (mixture of rotamers) δ 10.51 – 10.33 (m, 1H), 8.83 (dd, *J* = 8.5, 2.0 Hz, 1H), 8.75 – 8.53 (m, 1H), 8.15 (d, *J* = 7.0 Hz, 1H), 7.77 – 7.68 (m, 1H), 7.68 – 7.53 (m, 1H), 7.31 – 7.28 (m, 0.25H), 7.18 – 7.08 (m, 0.75H), 7.07 – 6.96 (m, 1H), 6.95 – 6.83 (m, 1H), 6.75 (dd, *J* = 8.2, 2.5 Hz, 0.75H), 6.66 – 6.56 (m, 2.75H), 6.52 – 6.40 (m, 2.5H), 5.81 – 5.75 (m, 0.25H), 5.56 – 5.49 (m, 0.75H), 5.47 (t, *J* = 4.4 Hz, 0.75H), 5.34 – 5.14 (m, 2H), 5.01 – 4.89 (m, 1H), 4.80 – 4.69 (m, 2H), 4.59 – 4.52 (m, 0.25H), 4.07 – 3.92 (m, 3H), 3.90 – 3.80 (m, 4H), 3.80 – 3.74 (m, 8H), 3.71 (d, *J* = 3.8 Hz, 5H), 3.38 (d, *J* = 9.7 Hz, 0.75H), 2.97 (d, *J* = 9.7 Hz, 0.25H), 2.88 – 2.60 (m, 4H), 2.58 – 2.33 (m, 2H), 2.31 – 2.22 (m, 1H), 2.16 – 2.06 (m, 2H), 1.96 – 1.84 (m, 2H), 1.67 – 1.55 (m, 5H), 1.47 – 1.37 (m, 1H), 1.34 – 1.10 (m, 6H), 1.01 – 0.53 (m, 3H).

HRMS: m/z: found 1128.49125 $[M+H]^+$, calculated 1128.49244 $[M+H]^+$

(2S)-(1R)-3-(4-((1-(2-(2-(2-((2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-2-oxoethoxy)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)-3-methoxyphenyl)-1-(3-methoxyphenyl)propyl 1-((S)-2-cyclohexyl-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2-carboxylate (MTQ334)



MTQ334

MTQ334 was synthesized from **31a** (10 mg, 0.0137 mmol) and **44b** (6 mg, 0. 0137 mmol) according to the General Method G. Purification by flash column chromatography (CH → EA:MeCN = 90:10) gave a white solid (10 mg, 63%).

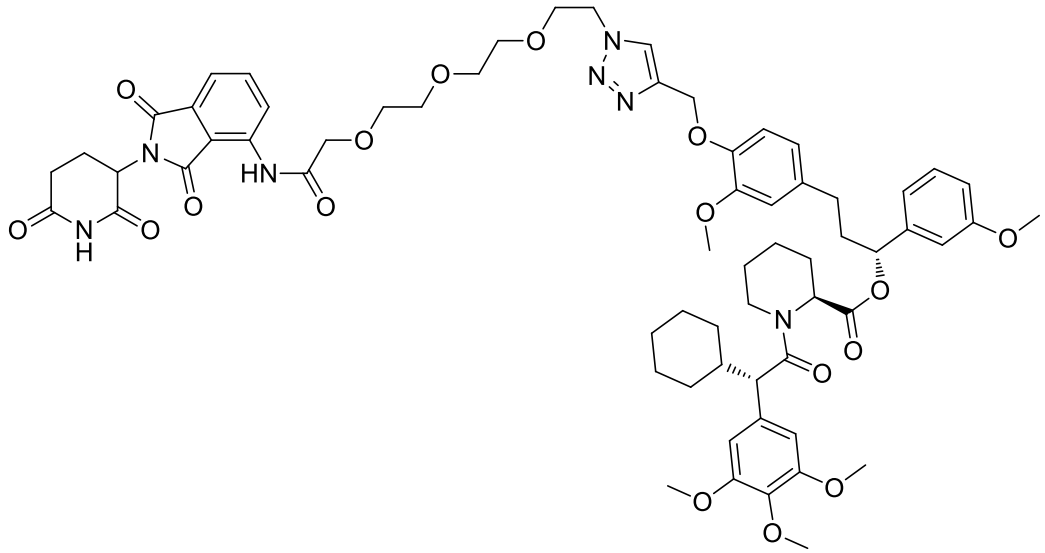
TLC [DCM:MeOH 95:5]: R_f = 0.31.

HPLC [0-100% Solvent B, 20 min]: R_t = 18.48 min, purity (220 nm) ≥ 99%.

^{1}H NMR (500 MHz, Chloroform-*d*) (mixture of rotamers) δ 10.41 (s, 1H), 8.83 (d, *J* = 8.4 Hz, 1H), 8.46 – 8.36 (m, 1H), 7.83 (s, 1H), 7.74 – 7.65 (m, 1H), 7.54 (d, *J* = 7.3 Hz, 1H), 7.30 (t, *J* = 7.9 Hz, 0.25H), 7.11 (t, *J* = 7.9 Hz, 0.75H), 6.96 – 6.83 (m, 2H), 6.75 (dd, *J* = 8.0, 2.2 Hz, 0.75H), 6.68 – 6.54 (m, 3H), 6.50 (s, 1.25H), 6.45 – 6.40 (m, 1H), 5.80 (t, *J* = 7.0 Hz, 0.25H), 5.55 (dd, *J* = 8.0, 5.7 Hz, 0.75H), 5.48 (d, *J* = 4.8 Hz, 0.75H), 5.17 (s, 2H), 4.95 (dd, *J* = 12.4, 5.4 Hz, 1H), 4.72 (d, *J* = 4.9 Hz, 0.25H), 4.56 (q, *J* = 4.7 Hz, 2H), 4.14 (s, 2H), 4.01 – 3.92 (m, 3H), 3.85 – 3.79 (m, 7H), 3.77 – 3.75 (m, 8H), 3.71 (s, 4H), 3.38 (d, *J* = 9.8 Hz, 0.75H), 2.97 (d, *J* = 9.8 Hz, 0.25H), 2.92 – 2.86 (m, 1H), 2.85 – 2.69 (m, 3H), 2.61 – 2.18 (m, 4H), 2.10 – 2.06 (m, 1H), 1.93 – 1.82 (m, 2H), 1.66 – 1.54 (m, 5H), 1.47 – 1.36 (m, 1H), 1.34 – 1.09 (m, 6H), 1.03 – 0.52 (m, 3H).

HRMS: m/z: found 1172.51505 $[M+H]^+$, calculated 1128.49244 $[M+H]^+$

(2S)-(1R)-3-(4-((1-(2-(2-(2-(2-((2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-2-oxoethoxy)ethoxy)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)-3-methoxyphenyl)-1-(3-methoxyphenyl)propyl 1-((S)-2-cyclohexyl-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2-carboxylate (MTQ335)



MTQ335

MTQ335 was synthesized from **31a** (10 mg, 0.0137 mmol) and **44c** (7 mg, 0. 0137 mmol) according to the General Method G. Purification by flash column chromatography (CH → EA:MeCN = 90:10) gave a white solid (12 mg, 72%).

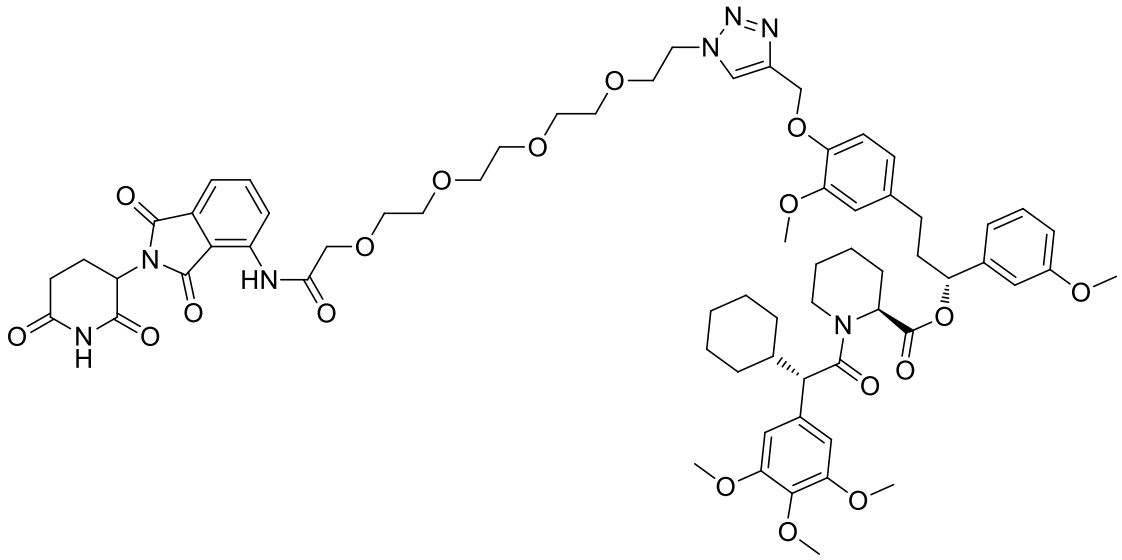
TLC [DCM:MeOH 95:5]: R_f = 0.36.

HPLC [0-100% Solvent B, 20 min]: R_t = 18.44 min, purity (220 nm) = 98%.

^{1}H NMR (300 MHz, Chloroform-*d*) (mixture of rotamers) δ 10.42 (s, 1H), 8.83 (d, *J* = 8.4 Hz, 1H), 8.51 – 8.33 (m, 1H), 7.83 (s, 1H), 7.76 – 7.65 (m, 1H), 7.56 (d, *J* = 7.3 Hz, 1H), 7.33 – 7.27 (m, 0.25H), 7.10 (t, *J* = 7.9 Hz, 0.75H), 6.99 – 6.81 (m, 2H), 6.74 (dd, *J* = 8.1, 2.5 Hz, 0.75H), 6.67 – 6.47 (m, 4.25H), 6.43 – 6.38 (m, 1H), 5.85 – 5.74 (m, 0.25H), 5.55 (dd, *J* = 7.7, 6.0 Hz, 0.75H), 5.48 (d, *J* = 4.0 Hz, 0.75H), 5.23 (s, 2H), 5.00 – 4.90 (m, 1H), 4.74 – 4.69 (m, 0.25H), 4.52 (t, *J* = 4.9 Hz, 2H), 4.18 (s, 2H), 4.00 – 3.85 (m, 3H), 3.84 – 3.76 (m, 9H), 3.75 (s, 6H), 3.71 (s, 4H), 3.64 – 3.57 (m, 4H), 3.37 (d, *J* = 9.9 Hz, 0.75H), 2.96 (d, *J* = 9.6 Hz, 0.25H), 2.92 – 2.67 (m, 4H), 2.45 – 2.05 (m, 5H), 1.94 – 1.84 (m, 2H), 1.69 – 1.52 (m, 6H), 1.45 – 1.27 (m, 3H), 1.22 – 1.07 (m, 3H), 1.04 – 0.49 (m, 3H).

HRMS: m/z: found 1216.54480 $[M+H]^+$, calculated 1216.54487 $[M+H]^+$

(2*S*)-(1*R*)-3-(4-((1-(14-((2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-14-oxo-3,6,9,12-tetraoxatetradecyl)-1H-1,2,3-triazol-4-yl)methoxy)-3-methoxyphenyl)-1-(3-methoxyphenyl)propyl 1-((*S*)-2-cyclohexyl-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2-carboxylate (MTQ336)



MTQ336

MTQ336 was synthesized from **31a** (10 mg, 0.0137 mmol) and **44d** (7 mg, 0. 0137 mmol) according to the General Method G. Purification by flash column chromatography (CH → EA:MeCN = 90:10) gave a white solid (13 mg, 76%).

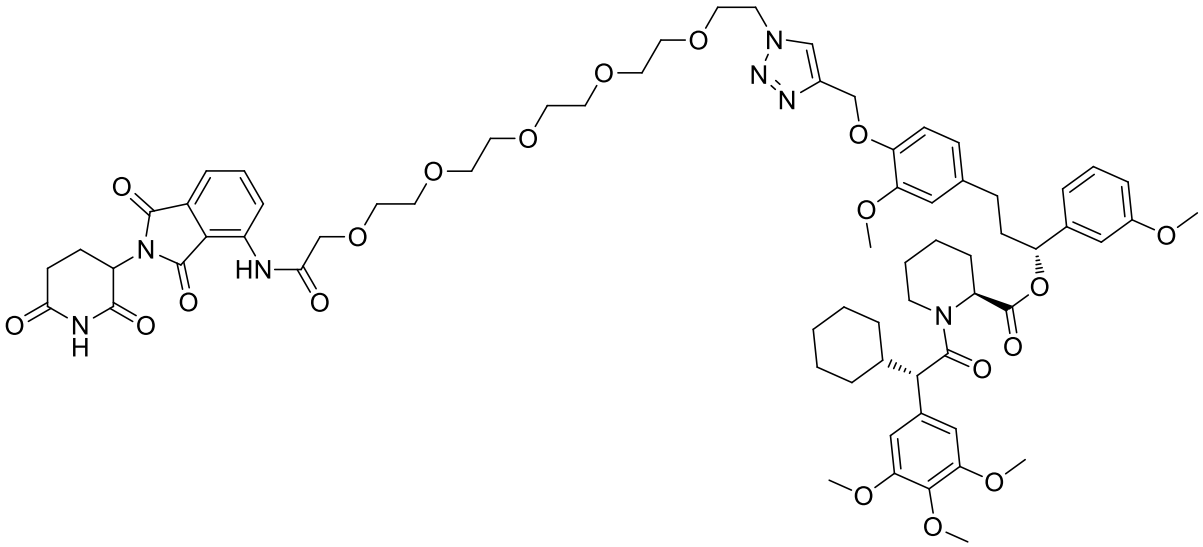
TLC [DCM:MeOH 95:5]: R_f = 0.35.

HPLC [0-100% Solvent B, 20 min]: R_t = 18.39 min, purity (220 nm) ≥ 99%.

^{1}H NMR (300 MHz, Chloroform-*d*) (mixture of rotamers) δ 10.48 (s, 1H), 8.84 (d, *J* = 8.5 Hz, 1H), 8.69 – 8.47 (m, 1H), 7.83 (s, 1H), 7.71 (t, *J* = 7.8 Hz, 1H), 7.56 (d, *J* = 7.3 Hz, 1H), 7.34 – 7.28 (m, 0.25H), 7.11 (t, *J* = 7.7 Hz, 0.75H), 7.00 – 6.83 (m, 2H), 6.75 (d, *J* = 7.4 Hz, 0.75H), 6.69 – 6.52 (m, 3H), 6.50 (s, 1.25H), 6.44 – 6.40 (m, 1H), 5.80 (t, *J* = 6.5 Hz, 0.25H), 5.56 (t, *J* = 6.5 Hz, 0.75H), 5.51 – 5.40 (m, 0.75H), 5.23 (s, 2H), 5.00 – 4.89 (m, 1H), 4.75 – 4.68 (m, 0.25H), 4.53 (s, 2H), 4.18 (s, 2H), 3.99 – 3.85 (m, 3H), 3.83 (d, *J* = 5.5 Hz, 7H), 3.77 (d, *J* = 7.5 Hz, 8H), 3.71 (s, 4H), 3.68 – 3.63 (m, 2H), 3.58 (s, 6H), 3.37 (d, *J* = 9.4 Hz, 0.75H), 3.00 – 2.93 (m, 0.25H), 2.90 – 2.60 (m, 4H), 2.58 – 2.22 (m, 3H), 2.20 – 1.83 (m, 5H), 1.65 (d, *J* = 9.8 Hz, 3H), 1.56 (d, *J* = 10.4 Hz, 2H), 1.38 – 1.10 (m, 6H), 1.05 – 0.49 (m, 3H).

HRMS: m/z: found 1260.57131 $[M+H]^+$, calculated 1260.57109 $[M+H]^+$

(2S)-(1R)-3-(4-((1-(17-((2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-17-oxo-3,6,9,12,15-pentaoxaheptadecyl)-1H-1,2,3-triazol-4-yl)methoxy)-3-methoxyphenyl)-1-(3-methoxyphenyl)propyl 1-((*S*)-2-cyclohexyl-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2-carboxylate (MTQ337)



MTQ337

MTQ337 was synthesized from **31a** (10 mg, 0.0137 mmol) and **44e** (8 mg, 0. 0137 mmol) according to the General Method G. Purification by flash column chromatography (CH → EA:MeCN = 90:10) gave a white solid (16 mg, 90%).

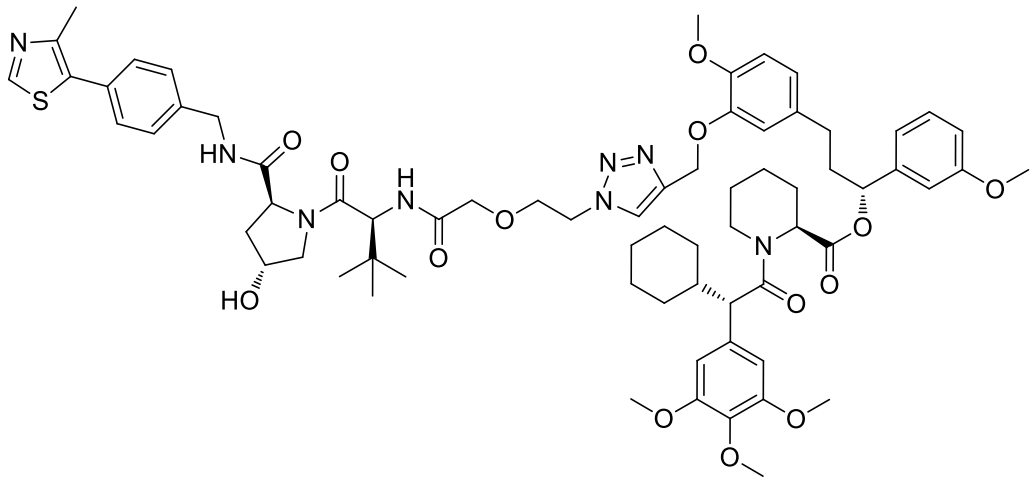
TLC [DCM:MeOH 95:5]: R_f = 0.33.

HPLC [0-100% Solvent B, 20 min]: R_t = 18.41 min, purity (220 nm) = 97%.

^{1}H NMR (300 MHz, Chloroform-*d*) (mixture of rotamers) δ 10.48 (s, 1H), 8.84 (d, *J* = 8.4 Hz, 1H), 8.76 (s, 1H), 7.84 (s, 1H), 7.75 – 7.66 (m, 1H), 7.57 (dd, *J* = 7.3, 0.7 Hz, 1H), 7.31 (d, *J* = 7.8 Hz, 0.25H), 7.10 (t, *J* = 7.9 Hz, 0.75H), 7.00 – 6.81 (m, 2H), 6.77 – 6.72 (m, 0.75H), 6.69 – 6.54 (m, 3H), 6.49 (s, 1.25H), 6.43 – 6.39 (m, 1H), 5.80 (t, *J* = 7.1 Hz, 0.25H), 5.56 (dd, *J* = 7.8, 5.7 Hz, 0.75H), 5.48 (d, *J* = 4.8 Hz, 0.75H), 5.24 (s, 2H), 5.04 – 4.89 (m, 1H), 4.75 – 4.69 (m, 0.25H), 4.52 (t, *J* = 5.1 Hz, 2H), 4.18 (d, *J* = 1.4 Hz, 2H), 4.00 – 3.89 (m, 1H), 3.90 – 3.85 (m, 2H), 3.85 – 3.80 (m, 7H), 3.80 – 3.73 (m, 9H), 3.71 (s, 4H), 3.69 – 3.62 (m, 4H), 3.60 (s, 4H), 3.59 (s, 3H), 3.37 (d, *J* = 9.8 Hz, 0.75H), 2.96 (d, *J* = 9.6 Hz, 0.25H), 2.91 – 2.69 (m, 4H), 2.60 – 2.24 (m, 3H), 2.19 – 1.99 (m, 3H), 1.88 – 1.73 (m, 2H), 1.70 – 1.61 (m, 3H), 1.59 – 1.51 (m, 2H), 1.48 – 1.27 (m, 3H), 1.22 – 1.06 (m, 3H), 1.02 – 0.50 (m, 3H).

HRMS: m/z: found 1304.5975 $[M+H]^+$, calculated 1304.5973 $[M+H]^+$

(S)-(R)-3-(3-((1-(2-(2-(((S)-1-((2S,4R)-4-Hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-2-oxoethoxy)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)-4-methoxyphenyl)-1-(3-methoxyphenyl)propyl 1-((S)-2-cyclohexyl-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2-carboxylate (MTQ338)



MTQ338

MTQ338 was synthesized from **31b** (12 mg, 0.0165 mmol) and **43a** (8 mg, 0. 0137 mmol) according to the General Method G. Purification by flash column chromatography (0% to 6% MeOH in DCM) gave a white solid (16 mg, 91%).

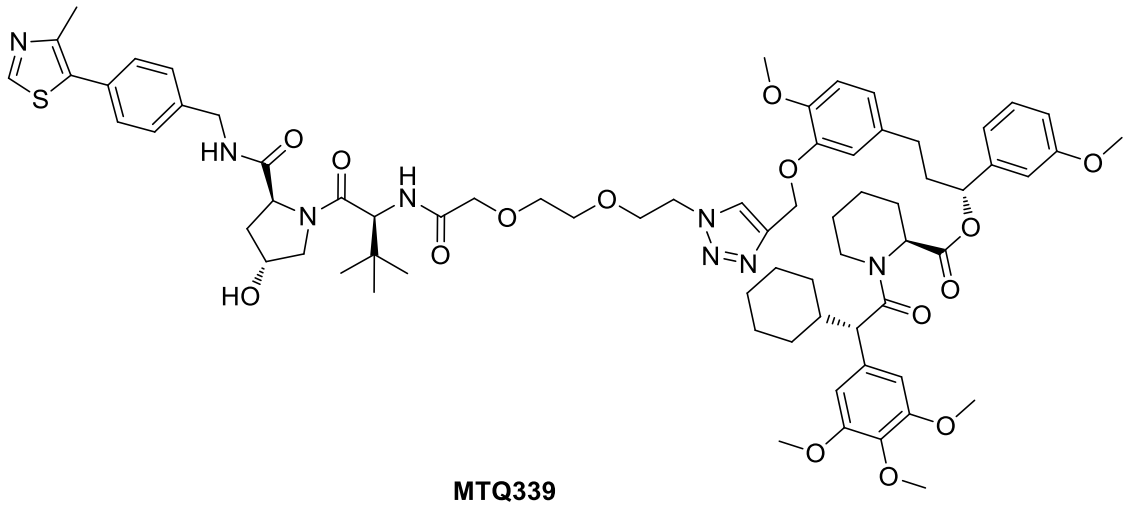
TLC [DCM:MeOH 93:7]: R_f = 0.38.

HPLC [0-100% Solvent B, 20 min]: R_t = 17.73 min, purity (220 nm) = 99%.

^{1}H NMR (300 MHz, Chloroform-*d*) (mixture of rotamers) δ 8.84 (s, 1H), 7.83 (s, 1H), 7.33 (s, 5H), 7.14 – 7.00 (m, 2H), 6.95 – 6.78 (m, 2H), 6.78 – 6.70 (m, 2H), 6.68 – 6.47 (m, 3H), 6.45 – 6.36 (m, 1H), 5.51 (t, *J* = 6.7 Hz, 1H), 5.45 (s, 1H), 5.20 (s, 2H), 4.78 – 4.43 (m, 7H), 4.31 (dd, *J* = 15.4, 3.9 Hz, 1H), 4.05 – 3.89 (m, 4H), 3.88 (d, *J* = 5.6 Hz, 1H), 3.83 (s, 2H), 3.79 (d, *J* = 7.7 Hz, 2H), 3.75 (s, 7H), 3.71 (s, 4H), 3.68 – 3.60 (m, 1H), 3.38 (d, *J* = 9.7 Hz, 1H), 3.00 – 2.64 (m, 4H), 2.54 (s, 3H), 2.49 – 2.33 (m, 3H), 2.30 – 2.23 (m, 1H), 2.17 – 1.66 (m, 7H), 1.59 – 1.49 (m, 2H), 1.42 – 1.28 (m, 2H), 1.21 – 1.06 (m, 3H), 0.94 (s, 9H), 0.88 – 0.52 (m, 2H).

HRMS: m/z: found 1285.62041 $[M+H]^+$, calculated 1285.62135 $[M+H]^+$

(S)-(R)-3-(3-((1-(2-(2-(2-(((S)-1-((2S,4R)-4-Hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-2-oxoethoxy)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)-4-methoxyphenyl)-1-(3-methoxyphenyl)propyl 1-((S)-2-cyclohexyl-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2-carboxylate (MTQ339)



MTQ339

MTQ339 was synthesized from **31b** (12 mg, 0.0165 mmol) and **43b** (8 mg, 0. 0137 mmol) according to the General Method G. Purification by flash column chromatography (0% to 6% MeOH in DCM) gave a white solid (13 mg, 71%).

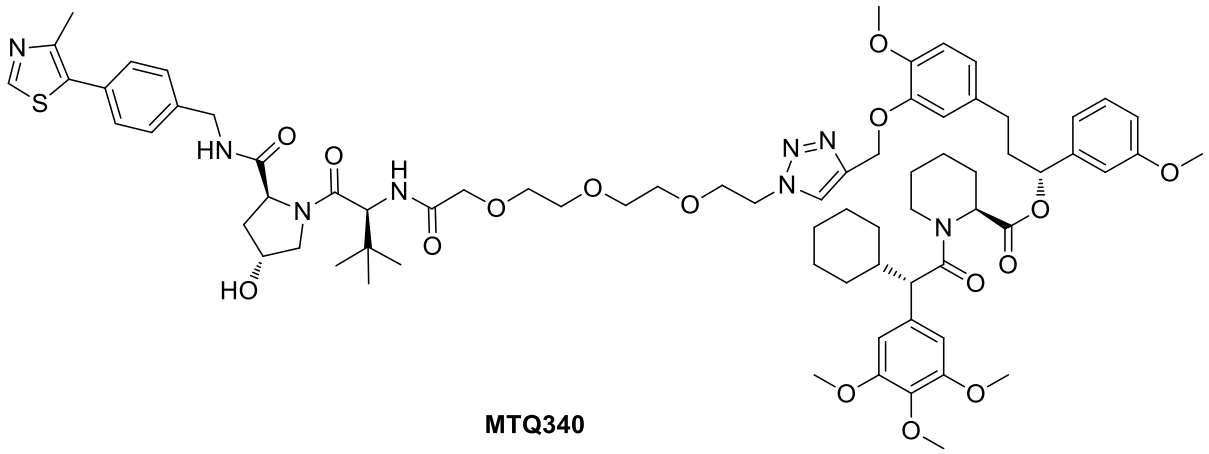
TLC [DCM:MeOH 93:7]: R_f = 0.30.

HPLC [0-100% Solvent B, 20 min]: R_t = 17.91 min, purity (220 nm) = 97%.

^{1}H NMR (300 MHz, Chloroform-*d*) (mixture of rotamers) δ 8.89 (d, *J* = 7.6 Hz, 1H), 8.11 (s, 1H), 7.42 – 7.27 (m, 6H), 7.09 (t, *J* = 7.9 Hz, 1H), 6.96 – 6.64 (m, 5H), 6.61 – 6.49 (m, 2H), 6.45 – 6.30 (m, 1H), 5.58 – 5.50 (m, 1H), 5.48 – 5.40 (m, 1H), 5.19 (s, 2H), 4.78 – 4.35 (m, 7H), 4.33 – 4.17 (m, 1H), 4.08 – 3.92 (m, 3H), 3.91 – 3.86 (m, 2H), 3.84 – 3.82 (m, 2H), 3.81 – 3.79 (m, 2H), 3.78 – 3.74 (m, 7H), 3.71 (s, 4H), 3.69 – 3.56 (m, 5H), 3.38 (d, *J* = 10.0 Hz, 1H), 2.81 – 2.59 (m, 3H), 2.57 (s, 3H), 2.47 – 2.33 (m, 3H), 2.32 – 2.22 (m, 2H), 2.14 – 1.94 (m, 3H), 1.93 – 1.67 (m, 4H), 1.59 – 1.51 (m, 2H), 1.43 – 1.29 (m, 2H), 1.20 – 1.07 (m, 3H), 0.98 (s, 9H), 0.93 – 0.52 (m, 3H).

HRMS: m/z: found 1329.64738 $[M+H]^+$, calculated 1329.64756 $[M+H]^+$

(S)-(R)-3-(3-((1-((S)-13-((2S,4R)-4-Hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidine-1-carbonyl)-14,14-dimethyl-11-oxo-3,6,9-trioxa-12-azapentadecyl)-1H-1,2,3-triazol-4-yl)methoxy)-4-methoxyphenyl)-1-(3-methoxyphenyl)propyl 1-((S)-2-cyclohexyl-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2-carboxylate (MTQ340)



MTQ340

MTQ340 was synthesized from **31b** (12 mg, 0.0165 mmol) and **43c** (9 mg, 0. 0137 mmol) according to the General Method G. Purification by flash column chromatography (0% to 6% MeOH in DCM) gave a white solid (15 mg, 80%).

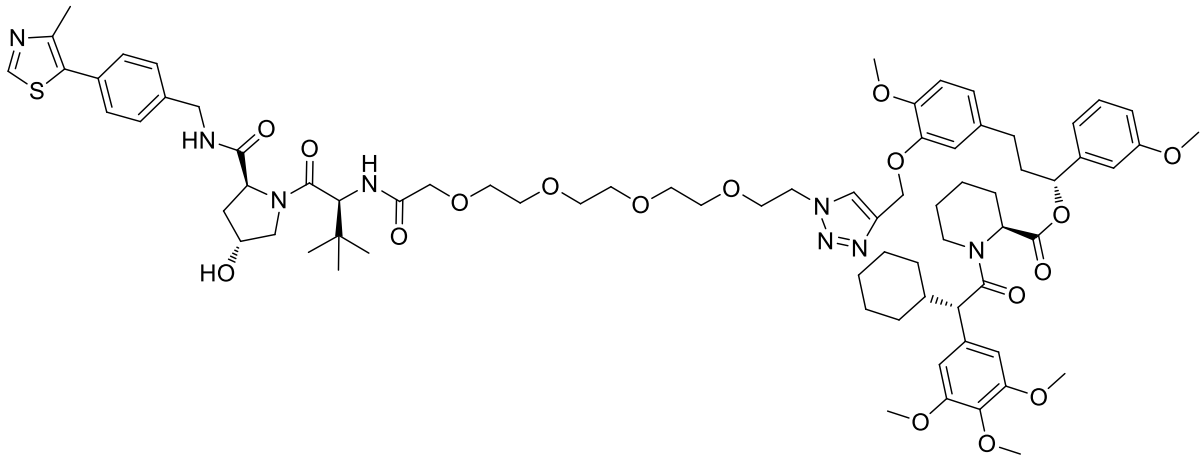
TLC [DCM:MeOH 93:7]: R_f = 0.30.

HPLC [0-100% Solvent B, 20 min]: R_t = 17.96 min, purity (220 nm) = 97%.

^{1}H NMR (300 MHz, Chloroform-*d*) (mixture of rotamers) δ 8.85 (d, *J* = 4.3 Hz, 1H), 7.85 (d, *J* = 2.8 Hz, 1H), 7.44 – 7.32 (m, 6H), 7.30 – 7.27 (m, 1H), 6.96 – 6.77 (m, 3H), 6.76 – 6.60 (m, 3H), 6.50 (s, 1H), 6.44 – 6.39 (m, 1H), 5.54 (dd, *J* = 7.8, 6.0 Hz, 1H), 5.47 (d, *J* = 4.1 Hz, 1H), 5.19 (s, 2H), 4.70 – 4.45 (m, 7H), 4.33 (dd, *J* = 15.1, 5.4 Hz, 1H), 4.08 – 4.03 (m, 1H), 3.97 (d, *J* = 3.2 Hz, 2H), 3.87 – 3.82 (m, 5H), 3.81 (d, *J* = 1.9 Hz, 2H), 3.79 (s, 2H), 3.76 (s, 4H), 3.71 (s, 4H), 3.66 – 3.60 (m, 5H), 3.59 (s, 4H), 3.38 (d, *J* = 9.8 Hz, 1H), 2.84 – 2.72 (m, 1H), 2.64 – 2.58 (m, 1H), 2.56 (s, 3H), 2.51 – 2.35 (m, 5H), 2.31 – 2.24 (m, 1H), 2.18 – 2.03 (m, 3H), 1.94 – 1.70 (m, 3H), 1.60 – 1.51 (m, 2H), 1.45 – 1.27 (m, 3H), 1.21 – 1.08 (m, 3H), 0.96 (s, 9H), 0.92 – 0.53 (m, 3H).

HRMS: m/z: found 1373.67431 $[M+H]^+$, calculated 1373.67378 $[M+H]^+$

(S)-(R)-3-(3-((1-((S)-16-((2S,4R)-4-Hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidine-1-carbonyl)-17,17-dimethyl-14-oxo-3,6,9,12-tetraoxa-15-azaoctadecyl)-1H-1,2,3-triazol-4-yl)methoxy)-4-methoxyphenyl)-1-(3-methoxyphenyl)propyl 1-((S)-2-cyclohexyl-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2-carboxylate (MTQ341)



MTQ341

MTQ341 was synthesized from **31b** (12 mg, 0.0165 mmol) and **43d** (9 mg, 0. 0137 mmol) according to the General Method G. Purification by flash column chromatography (0% to 6% MeOH in DCM) gave a white solid (13 mg, 67%).

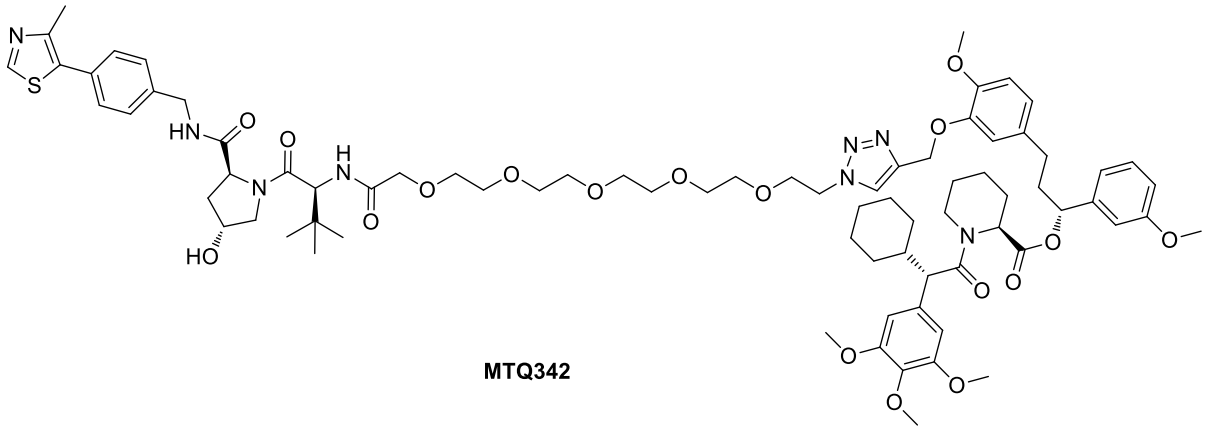
TLC [DCM:MeOH 93:7]: R_f = 0.28.

HPLC [0-100% Solvent B, 20 min]: R_t = 17.89 min, purity (220 nm) = 99%.

^{1}H NMR (300 MHz, Chloroform-*d*) (mixture of rotamers) δ 8.89 (s, 1H), 7.83 (s, 1H), 7.45 – 7.28 (m, 6H), 7.11 (t, *J* = 7.9 Hz, 1H), 6.96 – 6.71 (m, 4H), 6.70 – 6.47 (m, 3H), 6.45 – 6.39 (m, 1H), 5.55 (t, *J* = 6.5 Hz, 1H), 5.50 – 5.42 (m, 1H), 5.21 (s, 2H), 4.76 – 4.56 (m, 2H), 4.55 – 4.45 (m, 5H), 4.35 (dd, *J* = 14.4, 5.1 Hz, 1H), 4.14 – 4.03 (m, 1H), 3.99 (s, 2H), 3.90 – 3.78 (m, 9H), 3.76 (s, 4H), 3.71 (s, 4H), 3.65 – 3.55 (m, 13H), 3.37 (d, *J* = 9.7 Hz, 1H), 2.84 – 2.73 (m, 1H), 2.69 – 2.61 (m, 1H), 2.57 (s, 3H), 2.50 – 2.35 (m, 3H), 2.32 – 2.25 (m, 1H), 2.18 – 2.01 (m, 3H), 1.95 – 1.81 (m, 2H), 1.75 – 1.60 (m, 4H), 1.54 (s, 1H), 1.47 – 1.27 (m, 3H), 1.20 – 1.02 (m, 3H), 0.96 (s, 9H), 0.89 – 0.63 (m, 2H).

HRMS: m/z: found 1417.69865 $[M+H]^+$, calculated 1417.69999 $[M+H]^+$

(S)-(R)-3-(3-((1-((S)-19-((2S,4R)-4-Hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidine-1-carbonyl)-20,20-dimethyl-17-oxo-3,6,9,12,15-pentaoxa-18-azahenicosyl)-1H-1,2,3-triazol-4-yl)methoxy)-4-methoxyphenyl)-1-(3-methoxyphenyl)propyl 1-((S)-2-cyclohexyl-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2-carboxylate (MTQ342)



MTQ342

MTQ342 was synthesized from **31b** (12 mg, 0.0165 mmol**)** and **43e** (10 mg, 0. 0137 mmol) according to the General Method G. Purification by flash column chromatography (0% to 6% MeOH in DCM) gave a white solid (15 mg, 75%).

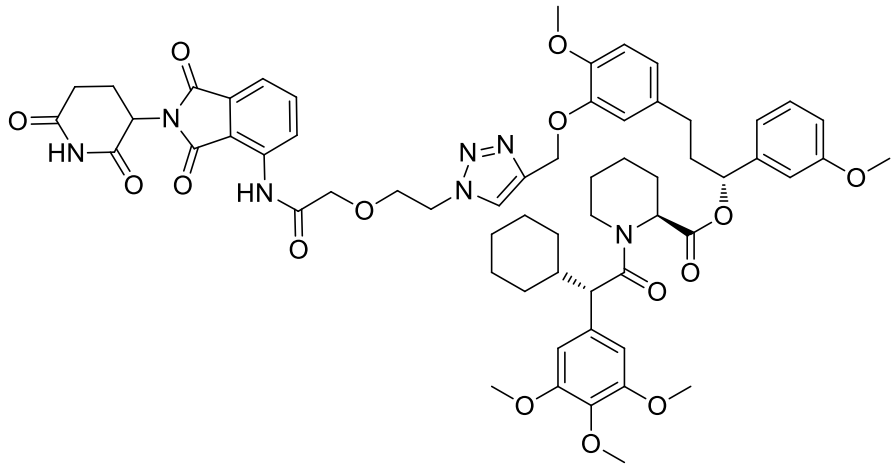
TLC [DCM:MeOH 90:10]: R_f = 0.56.

HPLC [0-100% Solvent B, 20 min]: R_t = 17.88 min, purity (220 nm) = 95%.

^{1}H NMR (300 MHz, Chloroform-*d*) (mixture of rotamers) δ 8.87 (s, 1H), 7.82 (s, 1H), 7.36 (s, 5H), 7.30 – 7.27 (m, 1H), 7.11 (t, *J* = 7.9 Hz, 1H), 6.96 – 6.86 (m, 1H), 6.86 – 6.60 (m, 5H), 6.49 (s, 1H), 6.44 – 6.40 (m, 1H), 5.54 (dd, *J* = 7.9, 5.7 Hz, 1H), 5.47 (d, *J* = 3.5 Hz, 1H), 5.21 (s, 2H), 4.76 – 4.56 (m, 2H), 4.55 – 4.46 (m, 5H), 4.35 (dd, *J* = 15.1, 5.4 Hz, 1H), 4.13 – 4.04 (m, 1H), 3.99 (d, *J* = 6.3 Hz, 2H), 3.88 – 3.79 (m, 9H), 3.76 (d, *J* = 0.7 Hz, 4H), 3.71 (s, 4H), 3.65 (s, 4H), 3.62 – 3.55 (m, 13H), 3.37 (d, *J* = 9.9 Hz, 1H), 2.84 – 2.69 (m, 2H), 2.56 (s, 3H), 2.52 – 2.24 (m, 5H), 2.18 – 2.01 (m, 3H), 1.94 – 1.79 (m, 2H), 1.75 – 1.60 (m, 4H), 1.55 – 1.27 (m, 4H), 1.20 – 1.05 (m, 3H), 0.95 (s, 9H), 0.88 – 0.49 (m, 3H).

HRMS: m/z: found 1461.72644 $[M+H]^+$, calculated 1461.72621 $[M+H]^+$

(2S)-(1R)-3-(3-((1-(2-(2-((2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-2-oxoethoxy)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)-4-methoxyphenyl)-1-(3-methoxyphenyl)propyl 1-((S)-2-cyclohexyl-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2-carboxylate (MTQ343)



MTQ343

MTQ343 was synthesized from **31b** (12 mg, 0.0165 mmol) and **44a** (5 mg, 0. 0137 mmol) according to the General Method G. Purification by flash column chromatography (CH → EA:MeCN = 90:10) gave a white solid (6 mg, 40%).

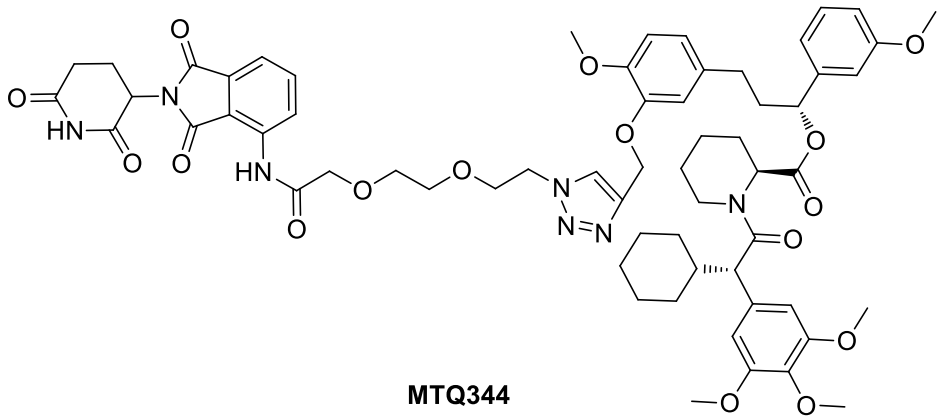
TLC [DCM:MeOH 95:5]: R_f = 0.40.

HPLC [0-100% Solvent B, 20 min]: R_t = 18.42 min, purity (220 nm) = 95%.

^{1}H NMR (300 MHz, Chloroform-*d*) (mixture of rotamers) δ 10.44 (s, 1H), 8.85 (d, *J* = 8.4 Hz, 1H), 8.80 – 8.69 (m, 1H), 8.23 – 8.08 (m, 1H), 7.82 – 7.69 (m, 1H), 7.60 (dd, *J* = 7.2, 2.6 Hz, 1H), 7.34 – 7.29 (m, 0.25H), 7.12 (t, *J* = 7.9 Hz, 0.75H), 7.00 – 6.60 (m, 6H), 6.53 – 6.43 (m, 2H), 5.82 – 5.75 (m, 0.25H), 5.55 (dd, *J* = 7.9, 5.7 Hz, 0.75H), 5.49 (d, *J* = 3.9 Hz, 0.75H), 5.34 – 5.20 (m, 2H), 5.03 – 4.94 (m, 1H), 4.80 – 4.69 (m, 2H), 4.62 – 4.53 (m, 0.25H), 4.14 (d, *J* = 2.7 Hz, 2H), 4.10 – 3.90 (m, 4H), 3.87 – 3.80 (m, 5H), 3.80 – 3.76 (m, 6H), 3.74 (d, *J* = 1.8 Hz, 3H), 3.39 (d, *J* = 9.9 Hz, 0.75H), 3.07 – 2.99 (m, 0.25H), 2.90 – 2.67 (m, 4H), 2.58 – 2.25 (m, 3H), 2.20 – 1.73 (m, 6H), 1.62 – 1.50 (m, 3H), 1.48 – 1.30 (m, 3H), 1.26 – 1.05 (m, 4H), 1.01 – 0.50 (m, 3H).

HRMS: m/z: found 1128.49199 $[M+H]^+$, calculated 1128.49244 $[M+H]^+$

(2S)-(1R)-3-(3-((1-(2-(2-(2-((2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-2-oxoethoxy)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)-4-methoxyphenyl)-1-(3-methoxyphenyl)propyl 1-((S)-2-cyclohexyl-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2-carboxylate (MTQ344)



MTQ344

MTQ344 was synthesized from **31b** (12 mg, 0.0165 mmol) and **44b** (6 mg, 0. 0137 mmol) according to the General Method G. Purification by flash column chromatography (CH → EA:MeCN = 90:10) gave a white solid (10 mg, 63%).

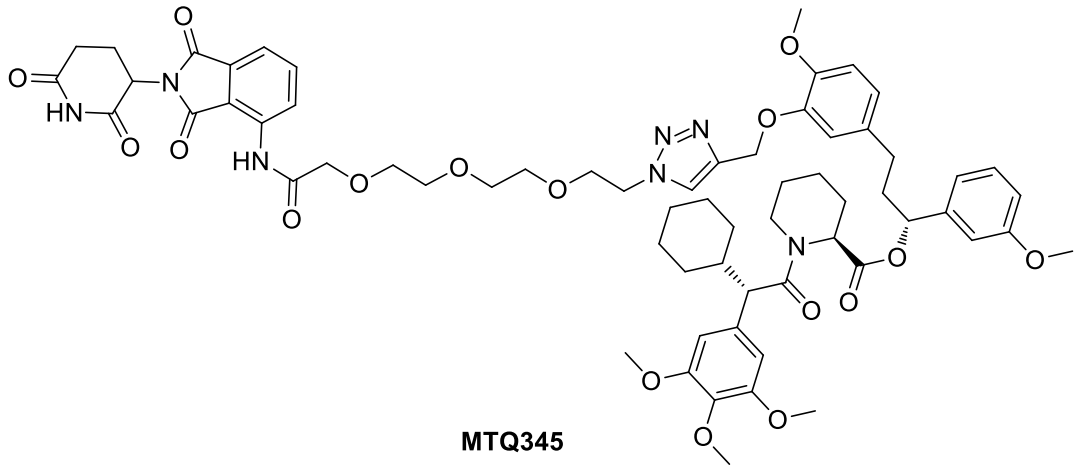
TLC [DCM:MeOH 95:5]: R_f = 0.42.

HPLC [0-100% Solvent B, 20 min]: R_t = 18.55 min, purity (220 nm) = 98%.

^{1}H NMR (300 MHz, Chloroform-*d*) (mixture of rotamers) δ 10.42 (s, 1H), 8.82 (d, *J* = 8.5 Hz, 1H), 8.61 – 8.43 (m, 1H), 7.84 (s, 1H), 7.68 (t, *J* = 7.9 Hz, 1H), 7.53 (d, *J* = 7.4 Hz, 1H), 7.33 – 7.28 (m, 0.25H), 7.11 (t, *J* = 7.9 Hz, 0.75H), 6.98 – 6.70 (m, 4H), 6.70 – 6.58 (m, 1.75H), 6.50 (s, 1.25H), 6.45 – 6.40 (m, 1H), 5.81 – 5.73 (m, 0.25H), 5.54 (dd, *J* = 7.9, 5.8 Hz, 0.75H), 5.48 (d, *J* = 5.0 Hz, 0.75H), 5.17 (s, 2H), 4.95 (dd, *J* = 11.9, 5.3 Hz, 1H), 4.78 – 4.71 (m, 0.25H), 4.55 (s, 2H), 4.14 (s, 2H), 3.99 – 3.87 (m, 3H), 3.85 – 3.78 (m, 7H), 3.75 (d, *J* = 1.9 Hz, 8H), 3.72 (s, 4H), 3.38 (d, *J* = 9.8 Hz, 0.75H), 3.05 – 2.94 (m, 0.25H), 2.93 – 2.60 (m, 4H), 2.59 – 2.03 (m, 6H), 1.94 – 1.83 (m, 2H), 1.71 – 1.62 (m, 3H), 1.60 – 1.51 (m, 2H), 1.35 – 1.22 (m, 3H), 1.20 – 1.03 (m, 3H), 1.02 – 0.48 (m, 3H).

HRMS: m/z: found 1172.51801 $[M+H]^+$, calculated 1172.51866 $[M+H]^+$

(2S)-(1R)-3-(3-((1-(2-(2-(2-(2-((2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-2-oxoethoxy)ethoxy)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)-4-methoxyphenyl)-1-(3-methoxyphenyl)propyl 1-((S)-2-cyclohexyl-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2-carboxylate (MTQ345)



MTQ345

MTQ345 was synthesized from **31b** (12 mg, 0.0165 mmol) and **44c** (7 mg, 0. 0137 mmol) according to the General Method G. Purification by flash column chromatography (CH → EA:MeCN = 90:10) gave a white solid (13 mg, 78%).

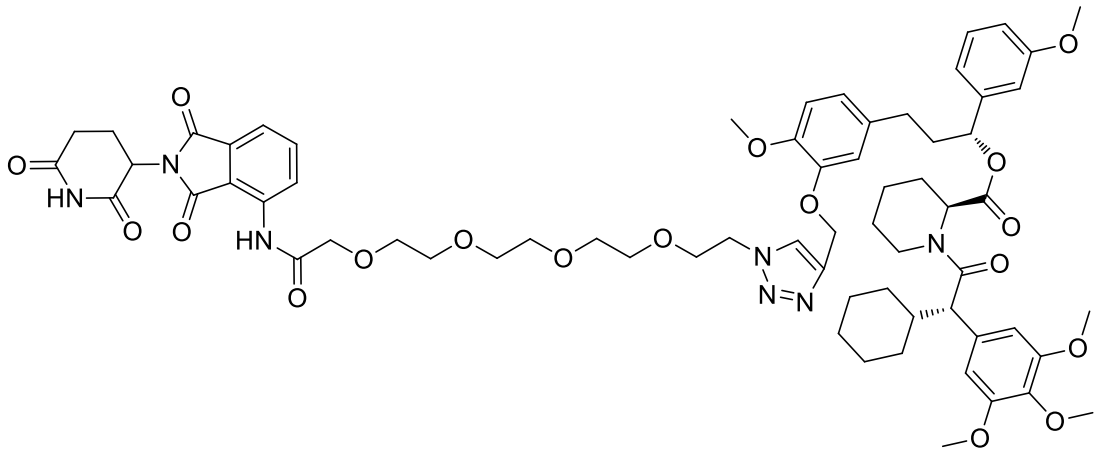
TLC [DCM:MeOH 95:5]: R_f = 0.30.

HPLC [0-100% Solvent B, 20 min]: R_t = 18.49 min, purity (220 nm) = 98%.

^{1}H NMR (500 MHz, Chloroform-*d*) (mixture of rotamers) δ 10.42 (s, 1H), 8.83 (d, *J* = 8.5 Hz, 1H), 8.52 – 8.34 (m, 1H), 7.88 – 7.81 (m, 1H), 7.70 (t, *J* = 7.9 Hz, 1H), 7.56 (d, *J* = 8.1 Hz, 1H), 7.30 (d, *J* = 7.9 Hz, 0.25H), 7.11 (t, *J* = 7.9 Hz, 0.75H), 6.95 – 6.73 (m, 4H), 6.70 – 6.62 (m, 1.75H), 6.49 (s, 1.25H), 6.45 – 6.41 (m, 1H), 5.80 – 5.73 (m, 0.25H), 5.54 (dd, *J* = 8.0, 5.7 Hz, 0.75H), 5.48 (d, *J* = 4.9 Hz, 0.75H), 5.24 (s, 0.5H), 5.23 (s, 1.5H), 4.99 – 4.90 (m, 1H), 4.75 (d, *J* = 5.4 Hz, 0.25H), 4.51 (q, *J* = 5.2 Hz, 2H), 4.18 (s, 2H), 3.95 (d, *J* = 13.8 Hz, 1H), 3.85 (dt, *J* = 12.9, 3.5 Hz, 5H), 3.82 – 3.80 (m, 4H), 3.79 – 3.77 (m, 2H), 3.76 (d, *J* = 1.7 Hz, 4H), 3.74 (dd, *J* = 6.1, 3.1 Hz, 2H), 3.71 (s, 4H), 3.63 – 3.57 (m, 4H), 3.37 (d, *J* = 9.8 Hz, 0.75H), 2.99 (d, *J* = 9.7 Hz, 0.25H), 2.91 – 2.73 (m, 4H), 2.58 – 2.25 (m, 3H), 2.18 – 2.02 (m, 3H), 1.93 (dd, *J* = 14.0, 5.4 Hz, 1H), 1.73 – 1.63 (m, 3H), 1.59 – 1.52 (m, 2H), 1.46 – 1.39 (m, 1H), 1.34 – 1.24 (m, 3H), 1.19 – 1.06 (m, 3H), 1.02 – 0.52 (m, 3H).

HRMS: m/z: found 1216.54526 $[M+H]^+$, calculated 1216.54487 $[M+H]^+$

(2*S*)-(1*R*)-3-(3-((1-(14-((2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-14-oxo-3,6,9,12-tetraoxatetradecyl)-1H-1,2,3-triazol-4-yl)methoxy)-4-methoxyphenyl)-1-(3-methoxyphenyl)propyl 1-((*S*)-2-cyclohexyl-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2-carboxylate (MTQ346)



MTQ346

MTQ346 was synthesized from **31b** (12 mg, 0.0165 mmol) and **44d** (7 mg, 0. 0137 mmol) according to the General Method G. Purification by flash column chromatography (CH → EA:MeCN = 90:10) gave a white solid (13 mg, 76%).

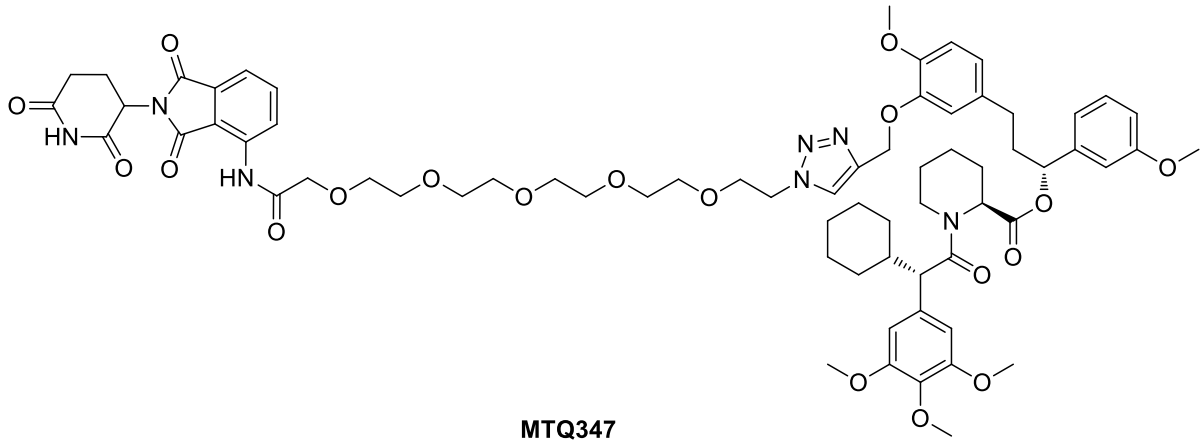
TLC [DCM:MeOH 95:5]: R_f = 0.27.

HPLC [30-100% Solvent B, 20 min]: R_t = 15.86 min, purity (220 nm) ≥ 99%.

^{1}H NMR (300 MHz, Chloroform-*d*) (mixture of rotamers) δ 10.48 (s, 1H), 8.84 (d, *J* = 8.4 Hz, 1H), 8.71 – 8.53 (m, 1H), 7.84 (s, 1H), 7.77 – 7.66 (m, 1H), 7.56 (d, *J* = 7.3 Hz, 1H), 7.34 – 7.28 (m, 0.25H), 7.11 (t, *J* = 7.9 Hz, 0.75H), 6.95 – 6.59 (m, 6H), 6.49 (s, 1H), 6.45 – 6.41 (m, 1H), 5.82 – 5.74 (m, 0.25H), 5.54 (dd, *J* = 7.9, 5.8 Hz, 0.75H), 5.47 (d, *J* = 4.3 Hz, 0.75H), 5.25 (s, 0.5H), 5.23 (s, 1.5H), 5.00 – 4.89 (m, 1H), 4.77 – 4.71 (m, 0.25H), 4.57 – 4.47 (m, 2H), 4.17 (d, *J* = 1.4 Hz, 2H), 3.96 – 3.85 (m, 3H), 3.84 – 3.79 (m, 7H), 3.78 (s, 3H), 3.76 (s, 4H), 3.73 – 3.62 (m, 7H), 3.61 – 3.55 (m, 6H), 3.37 (d, *J* = 9.7 Hz, 0.75H), 2.99 (d, *J* = 9.5 Hz, 0.25H), 2.90 – 2.66 (m, 4H), 2.62 – 2.26 (m, 3H), 2.18 – 1.92 (m, 4H), 1.71 – 1.51 (m, 6H), 1.46 – 1.26 (m, 3H), 1.22 – 1.07 (m, 3H), 1.03 – 0.50 (m, 3H).

HRMS: m/z: found 1260.57145 $[M+H]^+$, calculated 1260.57109 $[M+H]^+$

(2S)-(1R)-3-(3-((1-(17-((2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-17-oxo-3,6,9,12,15-pentaoxaheptadecyl)-1H-1,2,3-triazol-4-yl)methoxy)-4-methoxyphenyl)-1-(3-methoxyphenyl)propyl 1-((*S*)-2-cyclohexyl-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2-carboxylate (MTQ347)



MTQ347

MTQ347 was synthesized from **31b** (12 mg, 0.0165 mmol) and **44e** (8 mg, 0. 0137 mmol) according to the General Method G. Purification by flash column chromatography (CH → EA:MeCN = 90:10) gave a white solid (11 mg, 62%).

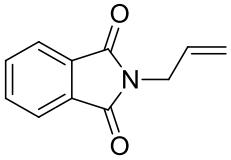
TLC [DCM:MeOH 95:5]: R_f = 0.41.

HPLC [0-100% Solvent B, 20 min]: R_t = 18.47 min, purity (220 nm) = 98%.

^{1}H NMR (500 MHz, Chloroform-*d*) (mixture of rotamers) δ 10.49 (s, 1H), 8.85 (d, *J* = 8.5 Hz, 1H), 8.73 (s, 1H), 7.85 (d, *J* = 5.0 Hz, 1H), 7.71 (t, *J* = 7.9 Hz, 1H), 7.64 – 7.52 (m, 1H), 7.31 – 7.29 (m, 0.25H), 7.11 (t, *J* = 7.9 Hz, 0.75H), 6.95 – 6.62 (m, 5.75H), 6.49 (s, 1.25H), 6.46 – 6.42 (m, 1H), 5.80 – 5.74 (m, 0.25H) 5.54 (dd, *J* = 8.0, 5.7 Hz, 0.75H), 5.47 (d, *J* = 5.2 Hz, 0.75H), 5.26 (s, 0.5H), 5.24 (s, 1.5H), 4.97 (dd, *J* = 11.6, 4.8 Hz, 1H), 4.75 (d, *J* = 5.0 Hz, 0.25H), 4.55 – 4.49 (m, 2H), 4.18 (d, *J* = 4.1 Hz, 2H), 3.97 – 3.86 (m, 3H), 3.85 – 3.82 (m, 4H), 3.81 (s, 3H), 3.79 (s, 4H), 3.76 (d, *J* = 1.4 Hz, 4H), 3.71 (s, 4H), 3.70 – 3.66 (m, 2H), 3.64 – 3.60 (m, 6H), 3.59 (s, 4H), 3.37 (d, *J* = 9.8 Hz, 0.75H), 2.99 (d, *J* = 9.8 Hz, 0.25H), 2.90 – 2.71 (m, 4H), 2.60 – 2.34 (m, 2H), 2.31 – 2.24 (m, 1H), 2.19 – 1.99 (m, 3H), 1.97 – 1.90 (m, 1H), 1.67 – 1.54 (m, 5H), 1.47 – 1.35 (m, 1H), 1.35 – 1.22 (m, 3H), 1.21 – 1.06 (m, 3H), 1.03 – 0.51 (m, 3H).

HRMS: m/z: found 1304.59619 $[M+H]^+$, calculated 1304.59730 $[M+H]^+$

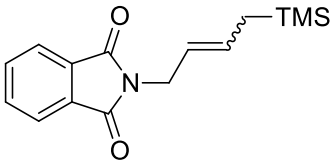
2-Allylisoindoline-1,3-dione (55)



55

K_2CO_3 (85.0 g, 611.7 mmol) and allylbromide (74.0 g, 53.0 mL, 611.7 mmol) were added to a solution of phtalimide (90.0 g, 611.7 mmol) in DMF (250 mL). After stirring at room temperature for 3h, EtO_2 (900 mL) was added and the mixture was washed with sat. aq. NaCl solution (3 × 200 mL). The solvent was removed under reduced pressure to give a white solid which was used for the next step without further purification.

2-(4-(Trimethylsilyl)but-2-en-1-yl)isoindoline-1,3-dione (56)



56

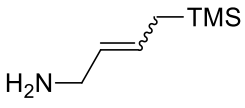
Grubbs I generation catalyst (9% loading, 5.1g) and allyltrimethylsilane (79.3 g, 110 mL, 694 mmol) were added to a solution of **55** (13.0 g, 69.4 mmol) in CH_2Cl_2 (500 mL). After refluxing at 60°C for 4h, tris(hydrodxymethyl)phosphine (1 M solution in i-PrOH, 58 mL) was added and the mixture was stirred under reflux for 12 h. Then sat. aq. NaCl solution (100 mL) was added and the aqueous phase was extracted with CH_2Cl_2 (3 × 200 mL). The combined organic phase was dried over $MgSO_4$ and the solvent was evaporated under reduced pressure. The crude product was purified by flash column chromatography (CH → CH:EA=85:15) gave a light yellow solid (12.5 g, 45.8 mmol, 66%).

TLC [CH:EA 8:2]: R_f = 0.46.

^{1}H NMR: (300 MHz, Chloroform-*d*) δ 7.84 (dd, J = 5.4, 3.1 Hz, 2H), 7.70 (dd, J = 5.5, 3.1 Hz, 2H), 5.87 (ddt, J = 17.1, 10.1, 5.7 Hz, 1H), 5.30 – 5.12 (m, 2H), 4.28 (dt, J = 5.7, 1.5 Hz, 2H).

^{13}C NMR: (75 MHz, Chloroform-*d*) δ 167.98, 134.06, 132.23, 131.65, 123.39, 117.84, 40.15.

4-(Trimethylsilyl)but-2-en-1-aminen (57)

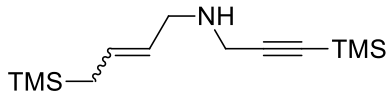


57

Hydrazine hydrate (50-60%, 1.14 mL, 18.29 mmol) was added to a solution of **56** (2.5 g, 9.14 mmol) in MeOH (90 mL). After stirring at 75°C for 24h, DCM (200 mL) was added and the mixture was washed with NaOH (1 M solution, 3 x 10 mL). The organic phase was dried over

$MgSO_4$ and the solvent was removed under reduced pressure. The given yellow residue was used in the next step without further purification.

4-(Trimethylsilyl)-N-(3-(trimethylsilyl)prop-2-yn-1-yl)but-2-en-1-amine (59a)



59a

57 (5.27 mmol) in 20 ml EtOH was added into a solution of 3-(trimethylsilyl)propiolaldehyde (1.00 g, 7.91 mmol) and Ti(O*i*Pr)$_4$ (2.34 mL, 7.91 mmol) in 32 ml EtOH and stirred at -78°C. After 4h stirring at -78°C, sodium borohydride (590 mg, 15.81 mmol) was added to the solution at that temperature, which was stirred until no gas evolution was observed. H_2O was added to the reaction mixture, which was extracted with CH_2Cl_2 (200 mL). The organic layer was dried over $MgSO_4$ and the solvent was removed under reduced pressure. Purification by flash column chromatography (DCM + 0.4% DIPEA → [(DCM:Et_2O)=95:5] + 0.4% DIPEA) gave **59a** as a light yellow oil (903 mg, 3.57 mmol, 68%).

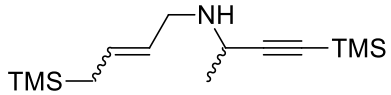
TLC [DCM:MeOH 95:5]: R_f = 0.5.

MS (ESI+): m/z: found 254.07 $[M+H]^+$, calculated 254.18 $[M+H]^+$

^{1}H NMR: (300 MHz, Chloroform-*d*) (mixture of cis/trans isomers) δ 5.66 – 5.49 (m, 1H), 5.40 – 5.29 (m, 1H), 3.43 – 3.38 (m, 2H), 3.33 – 3.22 (m, 2H), 1.57 – 1.42 (m, 2H), 1.31 (s, 1H), 0.16 (s, 9H), 0.03 – -0.03 (m, 9H).

^{13}C NMR: (75 MHz, Chloroform-*d*) (major) δ 130.22, 126.03, 104.68, 87.95, 50.66, 38.21, 22.91, 0.16, -1.82.

4-(Trimethylsilyl)-N-(4-(trimethylsilyl)but-3-yn-2-yl)but-2-en-1-amine (59b)



59b

57 (16.56 mmol) in 20 ml EtOH was added into a solution of 4-(trimethylsilyl)-3-butyn-2-one (4.1 mL, 24.85 mmol) and Ti(O*i*Pr)$_4$ (7.3 mL, 24.85 mmol) in 146 ml EtOH and stirred at -78°C. After 3h stirring at -78°C, sodium borohydride (1.88 g, 49.7 mmol) was added to the solution at that temperature, which was stirred until no gas evolution was observed. H_2O was added to the reaction mixture, which was extracted with CH_2Cl_2 (300 mL). The organic layer was dried over $MgSO_4$ and the solvent was removed under reduced pressure. Purification by flash column chromatography (DCM + 0.4% DIPEA → [(DCM:Et_2O)=95:5] + 0.4% DIPEA) gave **59b** as a light yellow oil (2.83 g, 10.6 mmol, 64%).

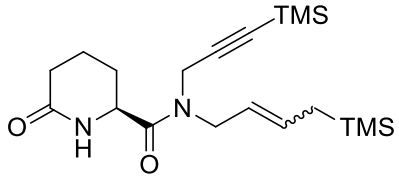
TLC [DCM:MeOH 95:5]: R_f = 0.32.

MS (ESI+): m/z: found 268.00 $[M+H]^+$, calculated 268.19 $[M+H]^+$

^{1}H NMR: (500 MHz, Chloroform-*d*) (mixture of cis/trans isomers) δ 5.49 – 5.35 (m, 1H), 5.26 – 5.15 (m, 1H), 3.36 – 3.17 (m, 2H), 3.11 – 3.00 (m, 1H), 1.31 – 1.14 (m, 5H), 0.99 (s, 1H), 0.00 (s, 9H), -0.14 – -0.18 (m, 9H).

^{13}C NMR: (126 MHz, Chloroform-*d*) (major) δ 129.77, 126.42, 108.91, 86.85, 49.70, 44.86, 22.86, 22.36, 0.26, -1.84.

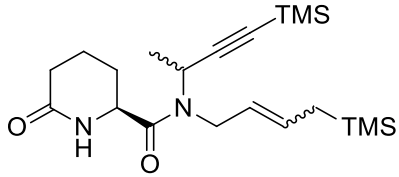
(S)-6-Oxo-N-(4-(trimethylsilyl)but-2-en-1-yl)-N-(3-(trimethylsilyl)prop-2-yn-1-yl)piperidine-2-carboxamide (60a)



60a

To a solution of (S)-6-oxo-2-piperidinecarboxylic acid (0.91 g, 6.39 mmol) in DMF (53 mL) were added **59a** (1.35 g, 15.33 mmol), DIPEA (2.2 mL, 12.79 mmol) and HATU (2.43 g, 6.39 mmol). After 2h stirring at room temperature Et_2O (200 mL) was added to the reaction and washed with sat. aq. NaCl solution (3 × 30 mL). The organic layer was dried over $MgSO_4$, the solvent was removed under reduced pressure and the crude title compound was used for the next step without further purification.

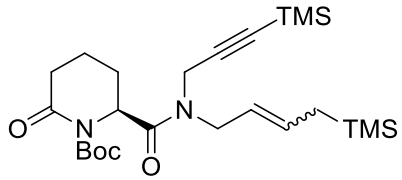
(2S)-6-Oxo-N-(4-(trimethylsilyl)but-2-en-1-yl)-N-(4-(trimethylsilyl)but-3-yn-2-yl)piperidine-2-carboxamide (60b)



60b

To a solution of (S)-6-oxo-2-piperidinecarboxylic acid (1.79 g, 12.56 mmol) in DMF (104 mL) were added HATU (4.77 g, 12.56 mmol), DIPEA (4.38 mL, 25.12 mmol) and **59b** (2.8 g, 10.47 mmol), DIPEA. After 2h stirring at room temperature Et_2O (500 mL) was added to the reaction and washed with sat. aq. NaCl solution (3 × 70 mL). The organic layer was dried over $MgSO_4$, the solvent was removed under reduced pressure and the crude title compound was used for the next step without further purification.

(S)-tert-Butyl 2-oxo-6-((4-(trimethylsilyl)but-2-en-1-yl)(3-(trimethylsilyl)prop-2-yn-1-yl)carbamoyl)piperidine-1-carboxylate (61a)



61a

DIPEA (1.84 mL, 10.66 mmol), Boc_2O (4.64 g, 21.32 mmol) and DMAP (0.65 g, 5.33 mmol) were added into a solution of **60a** in DCM (53 mL). After 16 h stirring at room temperature sat. aq. NaCl solution (25 mL) was added to the reaction and the organic phase was separated. The aqueous phase was extracted with CH_2Cl_2 (3 × 50 mL), the combined organic layers were dried over $MgSO_4$ and the solvent was removed under reduced pressure. Purification by flash column chromatography (CH → CH:EA=7:3]) gave a yellow oil (1.55 g, 3.24 mmol, 61% for 2 steps).

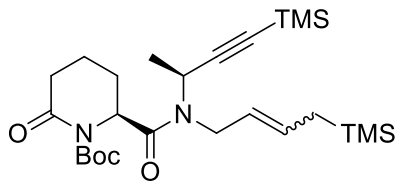
TLC [CH:EA 6:4]: R_f = 0.47.

MS (ESI+): m/z: found 501.45 $[M+Na]^+$, calculated 501.26 $[M+Na]^+$

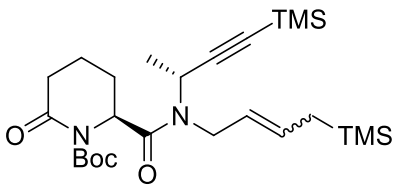
^{1}H NMR: (500 MHz, Chloroform-*d*) (mixture of cis/trans isomers) δ 5.76 – 5.60 (m, 1H), 5.40 – 5.14 (m, 1H), 5.03 – 4.91 (m, 1H), 4.35 – 3.88 (m, 4H), 2.64 – 2.55 (m, 1H), 2.49 – 2.39 (m, 1H), 2.02 – 1.66 (m, 4H), 1.51 – 1.43 (m, 11H), 0.15 (d, J = 9.4 Hz, 9H), 0.05 – -0.01 (m, 9H).

^{13}C NMR: (major) (126 MHz, Chloroform-*d*) δ 171.35, 171.29, 152.83, 132.90, 121.88, 100.68, 88.95, 83.21, 55.95, 48.62, 36.98, 34.54, 28.15, 25.88, 23.11, 18.23, 0.00, -1.78.

(S)-tert-Butyl 2-oxo-6-((4-(trimethylsilyl)but-2-en-1-yl)((S)-4-(trimethylsilyl)but-3-yn-2-yl)carbamoyl)piperidine-1-carboxylate (61b) and **(S)-tert-butyl 2-oxo-6-((4-(trimethylsilyl)but-2-en-1-yl)((R)-4-(trimethylsilyl)but-3-yn-2-yl)carbamoyl)piperidine-1-carboxylate (61c)**



61b



61c

DIPEA (3.65 mL, 20.94 mmol), Boc_2O (9.13 g, 41.88 mmol) and DMAP (1.28 g, 10.47 mmol) were added into a solution of **60b** in DCM (104 mL). After 16 h stirring at room temperature sat. aq. NaCl solution (150 mL) was added to the reaction and the organic phase was separated. The aqueous phase was extracted with CH_2Cl_2 (3 × 100 mL), the combined organic layers were dried over $MgSO_4$ and the solvent was removed under reduced pressure. Purification by flash column chromatography (CH → CH:EA = 8:2]) gave **61b** and **61c** as yellow oil (**61b**: 1.50 g, 3.1 mmol; **61c**:1.59 g, 3.2 mmol. Yield in total: 61% for 2 steps).

61b:

TLC [CH:EA 6:4]: R_f= 0.43.

MS (ESI+): m/z: found 515.24 $[M+Na]^+$, calculated 515.27 $[M+Na]^+$

^{1}H NMR: (500 MHz, Chloroform-*d*) (mixture of cis/trans isomers) δ 5.71 – 5.28 (m, 3H), 4.83 – 4.61 (m, 1H), 4.44 – 3.80 (m, 2H), 2.56 (dt, J = 17.3, 5.0 Hz, 1H), 2.46 – 2.33 (m, 1H), 1.97 – 1.86 (m, 3H), 1.69 – 1.59 (m, 1H), 1.49 – 1.44 (m, 11H), 1.32 – 1.26 (m, 3H), 0.16 – 0.13 (m, 9H), 0.06 – -0.03 (m, 9H).

^{13}C NMR: (major) (126 MHz, Chloroform-*d*) (major) δ 171.50, 171.21, 153.90, 130.32, 125.81, 105.25, 89.13, 83.11, 56.25, 46.80, 43.82, 41.52, 34.69, 28.12, 26.17, 22.95, 20.30, 18.41, -0.01, -1.72.

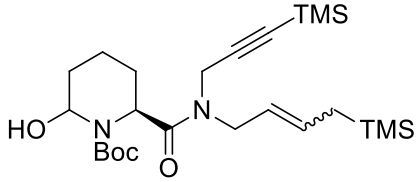
61c:
TLC [CH:EA 6:4]: R_f = 0.57.

MS (ESI+): m/z: found 515.28 $[M+Na]^+$, calculated 515.27 $[M+Na]^+$

^{1}H NMR: (500 MHz, Chloroform-*d*) (mixture of cis/trans isomers) δ 5.83 – 5.13 (m, 3H), 4.96 – 4.63 (m, 1H), 4.23 – 3.83 (m, 2H), 2.65 – 2.50 (m, 1H), 2.49 – 2.35 (m, 1H), 1.99 – 1.62 (m, 4H), 1.51 – 1.43 (m, 11H), 1.41 – 1.27 (m, 3H), 0.20 – 0.08 (m, 9H), 0.04 – -0.07 (m, 9H).

^{13}C NMR: (major) (126 MHz, Chloroform-*d*) (major) δ 171.41, 170.75, 152.65, 130.96, 125.25, 104.59, 89.22, 83.17, 56.10, 47.02, 43.50, 34.55, 28.14, 26.15, 23.01, 21.27, 18.19, -0.01, -1.72.

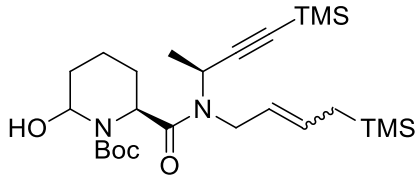
(6S)-tert-Butyl 2-hydroxy-6-((4-(trimethylsilyl)but-2-en-1-yl)(3-(trimethylsilyl)prop-2-yn-1-yl)carbamoyl)piperidine-1-carboxylate (62a)



62a

DIBAL-H (4.8 ml, 4.8 mmol) (1 M in DCM) was added into a solution of **61a** (1.55 g, 3.24 mmol) in THF (extra dry, 32 mL) dropwise at -78°C under Ar for 10 min. Then 2 spoons of Na_2SO_4-$10H_2O$ were added into the solution and stirred at -78°C. After raising to RT, another 1 spoon of Na_2SO_4 were added and stirred for 15 min. Then the mixture was filtered through celite and the solvent was removed under reduced pressure. The resulting residue was used in the next step without purification.

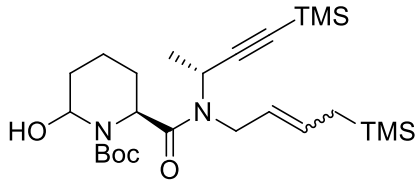
(6S)-tert-Butyl 2-hydroxy-6-((4-(trimethylsilyl)but-2-en-1-yl)((S)-4-(trimethylsilyl)but-3-yn-2-yl)carbamoyl)piperidine-1-carboxylate (62b)



62b

DIBAL-H (4.05 ml, 4.05 mmol) (1 M in DCM) was added into a solution of **61b** (1.33 g, 2.70 mmol) in THF (extra dry, 27 mL) dropwise at -78°C under Ar for 10 min. Then 2 spoons of $Na_2SO_4.10H_2O$ were added into the solution and stirred at -78°C. After raising to RT, another 1 spoon of Na_2SO_4 were added and stirred for 15 min. Then the mixture was filtered through celite and the solvent was removed under reduced pressure. The resulting residue was used in the next step without purification.

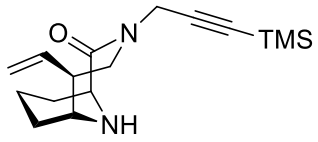
(6S)-tert-Butyl 2-hydroxy-6-((4-(trimethylsilyl)but-2-en-1-yl)((R)-4-(trimethylsilyl)but-3-yn-2-yl)carbamoyl)piperidine-1-carboxylate (62c)



62c

DIBAL-H (4.50 ml, 4.50 mmol) (1 M in DCM) was added into a solution of **61c** (1.48 g, 3.00 mmol) in THF (extra dry, 30 mL) dropwise at -78°C under Ar for 10 min. Then 2 spoons of $Na_2SO_4.10H_2O$ were added into the solution and stirred at -78°C. After raising to RT, another 1 spoon of Na_2SO_4 were added and stirred for 15 min. Then the mixture was filtered through celite and the solvent was removed under reduced pressure. The resulting residue was used in the next step without purification.

(1S,5R,6R)-3-(3-(Trimethylsilyl)prop-2-yn-1-yl)-5-vinyl-3,10-diazabicyclo[4.3.1]decan-2-one (63a)



63a

62a (1.62 mmol) was dissolved in DCM (84 mL) and cooled down to -78°C. Then a solution of HF in pyridine (70% in pyridine) (5% of DCM, 6.4 mL) was added into the solution at -78°C and warmed to 0°C. The reaction mixture was stirred for 1h at 0°C and then sat. $CaCO_3$ (30 mL) and NaOH (10 M, 30 ml) were added. The product was extracted with DCM. Purification by flash column chromatography (EA + 2% TEA → EA + 2% TEA + 2% MeOH) gave yellow solid (303 mg, 1.0 mmol, 64% for 2 steps).

TLC [DCM:MeOH 95:5]: $R_f = 0.39$.

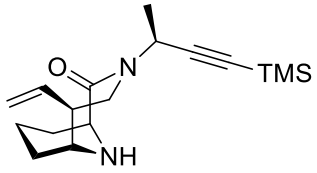
MS (ESI+): m/z: found 291.17 $[M+H]^+$, calculated 291.19 $[M+H]^+$

^{1}H NMR (500 MHz, Chloroform-*d*) δ 5.68 (ddd, J = 17.2, 10.2, 8.4 Hz, 1H), 5.08 – 4.97 (m, 2H), 4.55 (d, J = 17.4 Hz, 1H), 4.04 (d, J = 17.4 Hz, 1H), 3.96 (dd, J = 13.8, 10.7 Hz, 1H), 3.75 (d, J = 3.4 Hz, 1H), 3.23 (dd, J = 13.8, 1.8 Hz, 1H), 2.86 – 2.80 (m, 1H), 2.69 (q, J = 8.5 Hz, 1H), 2.28 – 2.20 (m, 1H), 2.04 (s, 1H), 1.70 – 1.49 (m, 5H), 0.14 (s, 9H).

^{13}C NMR (126 MHz, Chloroform-*d*) δ 174.44, 139.17, 115.24, 101.22, 88.29, 57.83, 52.78, 49.98, 49.72, 39.73, 29.57, 28.19, 16.85, 0.00.

HPLC [0-100% Solvent B, 20 min]: R_t = 10.86 min, purity (220 nm) ≥ 99%.

(1S,5R,6R)-3-((S)-4-(Trimethylsilyl)but-3-yn-2-yl)-5-vinyl-3,10-diazabicyclo[4.3.1]decan-2-one (63b)



63b

62b (2.70 mmol) was dissolved in DCM (140 mL) and cooled down to -78°C. Then a solution of HF in pyridine (70% in pyridine) (5% of DCM, 10.7 mL) was added into the solution at -78°C and warmed to 0°C. The reaction mixture was stirred for 1h at 0°C and then sat. $CaCO_3$ (82 mL) and NaOH (10 M, 82 ml) were added. The product was extracted with DCM. Purification by flash column chromatography (EA + 2% TEA → EA + 2% TEA + 2% MeOH) gave yellow solid (481 mg, 1.58 mmol, 50% for 2 steps).

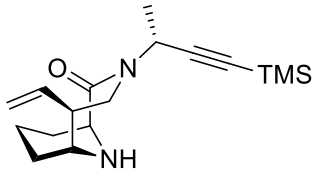
TLC [DCM:MeOH 95:5]: R_f = 0.53.

MS (ESI+): m/z: found 305.26 $[M+H]^+$, calculated 305.20 $[M+H]^+$

^{1}H NMR (500 MHz, Chloroform-*d*) δ 5.76 – 5.67 (m, 1H), 5.61 (q, J = 6.9 Hz, 1H), 5.11 – 4.98 (m, 2H), 3.88 – 3.71 (m, 2H), 3.27 (dd, J = 14.5, 1.7 Hz, 1H), 2.88 – 2.77 (m, 1H), 2.48 (q, J = 8.5 Hz, 1H), 2.23 (d, J = 12.0 Hz, 1H), 2.17 (s, 1H), 1.72 – 1.47 (m, 5H), 1.31 (d, J = 6.9 Hz, 3H), 0.14 (s, 9H).

^{13}C NMR (126 MHz, Chloroform-*d*) δ 173.78, 139.16, 115.14, 105.10, 88.22, 57.90, 52.97, 50.47, 45.83, 44.30, 29.49, 27.97, 19.82, 16.94, 0.04.

(1S,5R,6R)-3-((R)-4-(Trimethylsilyl)but-3-yn-2-yl)-5-vinyl-3,10-diazabicyclo[4.3.1]decan-2-one (63c)



63c

62c (3.00 mmol) was dissolved in DCM (155 mL) and cooled down to -78°C. Then a solution

of HF in pyridine (70% in pyridine) (5% of DCM, 11.9 mL) was added into the solution at -78°C and warmed to 0°C. The reaction mixture was stirred for 1h at 0°C and then sat. $CaCO_3$ (91 mL) and NaOH (10 M, 91 ml) were added. The product was extracted with DCM. Purification by flash column chromatography (EA + 2% TEA → EA + 2% TEA + 2% MeOH) gave yellow solid (303 mg, 1.0 mmol, 53% for 2 steps).

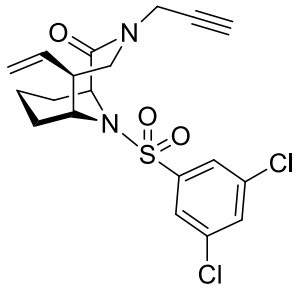
TLC [DCM:MeOH 95:5]: R_f = 0.42.

MS (ESI+): m/z: found 305.29 $[M+H]^+$, calculated 305.20 $[M+H]^+$

^{1}H NMR (500 MHz, Chloroform-*d*) δ 5.79 – 5.62 (m, 2H), 5.08 – 4.96 (m, 2H), 3.81 – 3.66 (m, 2H), 3.41 (dd, J = 13.5, 1.8 Hz, 1H), 2.83 (dd, J = 6.7, 4.2 Hz, 1H), 2.61 (q, J = 8.5 Hz, 1H), 2.35 (s, 1H), 2.26 – 2.19 (m, 1H), 1.69 – 1.49 (m, 5H), 1.28 (d, J = 7.0 Hz, 3H), 0.12 (s, 9H).

^{13}C NMR (126 MHz, Chloroform-*d*) δ 173.47, 139.22, 115.04, 105.30, 87.81, 58.00, 52.82, 50.10, 45.15, 43.95, 29.80, 28.28, 20.14, 16.83, 0.06.

(1S,5R,6R)-10-((3,5-Dichlorophenyl)sulfonyl)-3-(prop-2-yn-1-yl)-5-vinyl-3,10-diazabicyclo[4.3.1]decan-2-one (45a)



45a

3,5-Dichlorobenzenesulfonyl chloride (108 mg, 0.44 mmol) and DIPEA (67 mg, 0.52 mmol) were added into a solution of **63a** (77 mg, 0.26 mmol) in MeCN (3 mL) and stirred at RT for 16h. After removing solvent under reduced pressure, the residue was dissolved in MeOH (1 mL) and stirred at RT for 1.5 h. Purification by flash column chromatography (CH → CH:EA = 8:2) gave light yellow solid (65 mg, 0.15 mmol, 58% for 2 steps).

TLC [CH:EA 8:2]: R_f = 0.27.

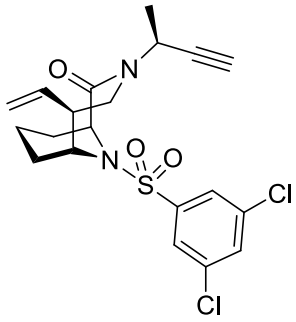
^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.74 – 7.65 (m, 2H), 7.61 – 7.52 (m, 1H), 5.81 (ddd, J = 17.0, 10.0, 8.9 Hz, 1H), 5.21 – 5.11 (m, 2H), 4.70 (d, J = 6.0 Hz, 1H), 4.64 (dd, J = 17.2, 2.5 Hz, 1H), 4.04 – 3.94 (m, 2H), 3.91 (dd, J = 17.2, 2.5 Hz, 1H), 3.20 (dd, J = 14.2, 1.9 Hz, 1H), 2.76 – 2.66 (m, 1H), 2.28 (d, J = 13.2 Hz, 1H), 2.21 (t, J = 2.4 Hz, 1H), 1.62 – 1.47 (m, 3H), 1.36 – 1.16 (m, 2H).

^{13}C NMR (126 MHz, Chloroform-*d*) δ 170.07, 144.14, 137.19, 136.51, 132.87, 125.04, 117.27, 78.68, 71.85, 57.01, 55.08, 51.00, 49.44, 39.45, 27.68, 26.38, 15.56.

HPLC [0-100% Solvent B, 20 min]: R_t = 16.90 min, purity (220 nm) ≥ 99%.

HRMS: m/z: found 427.06484 $[M+H]^+$, calculated 427.06445 $[M+H]^+$

(1S,5R,6R)-3-((S)-but-3-yn-2-yl)-10-((3,5-dichlorophenyl)sulfonyl)-5-vinyl-3,10-diazabicyclo[4.3.1]decan-2-one (45b)



45b

3,5-Dichlorobenzenesulfonyl chloride (452 mg, 1.84 mmol) and DIPEA (170 mg, 1.31 mmol) were added into a solution of **63b** (200 mg, 0.66 mmol) in MeCN (6 mL) and stirred at RT for 16h. After removing solvent under reduced pressure, the residue was dissolved in MeOH (2 mL) and stirred at RT for 1.5 h. Purification by flash column chromatography (CH → CH:EA = 8:2) gave light yellow solid (150 mg, 0.34 mmol, 52% for 2 steps).

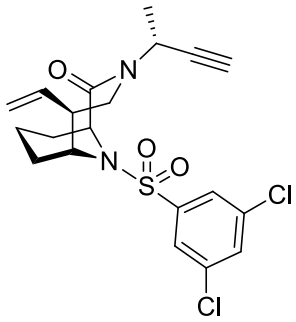
TLC [CH:EA 8:2]: R_f = 0.33.

^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.70 (d, J = 1.8 Hz, 2H), 7.56 (t, J = 1.8 Hz, 1H), 5.83 (ddd, J = 16.8, 10.2, 9.0 Hz, 1H), 5.61 (qd, J = 6.8, 2.3 Hz, 1H), 5.19 – 5.10 (m, 2H), 4.74 (d, J = 5.9 Hz, 1H), 3.98 (t, J = 5.8 Hz, 1H), 3.80 (dd, J = 14.9, 10.8 Hz, 1H), 3.32 – 3.24 (m, 1H), 2.45 (q, J = 9.1 Hz, 1H), 2.32 – 2.23 (m, 2H), 1.56 – 1.45 (m, 3H), 1.34 (d, J = 6.9 Hz, 3H), 1.32 – 1.19 (m, 2H).

^{13}C NMR (126 MHz, Chloroform-*d*) δ 169.28, 144.18, 137.31, 136.54, 132.87, 125.09, 117.09, 82.20, 72.55, 57.05, 55.12, 50.31, 46.59, 44.10, 27.78, 26.26, 19.79, 15.67.

HRMS: m/z: found 441.08061 $[M+H]^+$, calculated 441.08010 $[M+H]^+$

(1S,5R,6R)-3-((R)-But-3-yn-2-yl)-10-((3,5-dichlorophenyl)sulfonyl)-5-vinyl-3,10-diazabicyclo[4.3.1]decan-2-one (45c)



45c

3,5-Dichlorobenzenesulfonyl chloride (452 mg, 1.84 mmol) and DIPEA (170 mg, 1.31 mmol) were added into a solution of **63c** (200 mg, 0.66 mmol) in MeCN (6 mL) and stirred at RT for 16h. After removing solvent under reduced pressure, the residue was dissolved in MeOH (2 mL) and stirred at RT for 1.5 h. Purification by flash column chromatography (CH → CH:EA = 8:2) gave light yellow solid (147 mg, 0.33 mmol, 50% for 2 steps).

TLC [CH:EA 8:2]: R_f = 0.41.

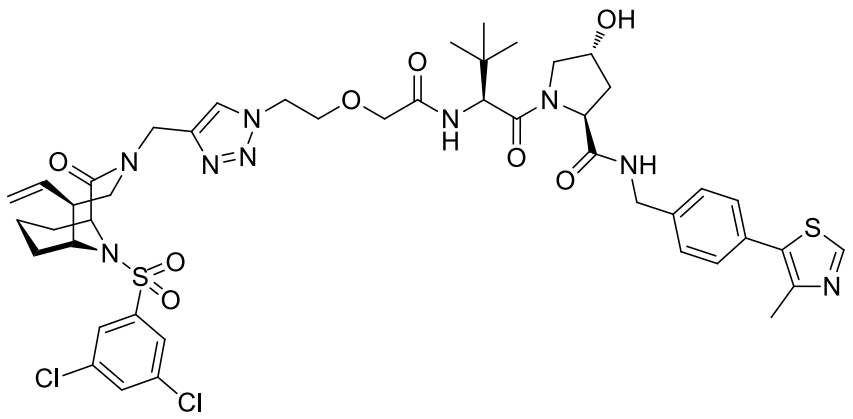
^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.69 (d, J = 1.8 Hz, 2H), 7.56 (t, J = 1.8 Hz, 1H), 5.88 – 5.78 (m, 1H), 5.69 (qd, J = 7.0, 2.2 Hz, 1H), 5.20 – 5.09 (m, 2H), 4.69 (d, J = 6.0 Hz, 1H), 4.02 (t, J = 5.7 Hz, 1H), 3.70 (dd, J = 14.1, 10.6 Hz, 1H), 3.38 (dd, J = 14.1, 1.5 Hz, 1H), 2.63 (q, J = 8.7 Hz, 1H), 2.30 – 2.23 (m, 2H), 1.60 – 1.49 (m, 3H), 1.33 (d, J = 7.0 Hz, 3H), 1.32 – 1.17 (m, 2H).

^{13}C NMR (126 MHz, Chloroform-*d*) δ 169.26, 144.20, 137.39, 136.51, 132.84, 125.04, 117.11, 82.83, 71.87, 57.21, 55.22, 49.77, 46.03, 43.68, 27.96, 26.59, 19.98, 15.59.

HPLC [0-100% Solvent B, 20 min]: R_t = 17.81 min, purity (220 nm) ≥ 99%.

HRMS: m/z: found 441.08022 $[M+H]^+$, calculated 441.08010 $[M+H]^+$

(2S,4R)-1-((S)-2-(2-(2-(4-(((1S,5R,6R)-10-((3,5-dichlorophenyl)sulfonyl)-2-oxo-5-vinyl-3,10-diazabicyclo[4.3.1]decan-3-yl)methyl)-1H-1,2,3-triazol-1-yl)ethoxy)acetamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (MTQ498)



MTQ498

MTQ498 was synthesized from **45a** (4 mg, 0.01 mmol) and **43a** (6 mg, 0. 010 mmol) according to the General Method G. Purification by flash column chromatography (0% to 7% MeOH in DCM) gave a white solid (6 mg, 61%).

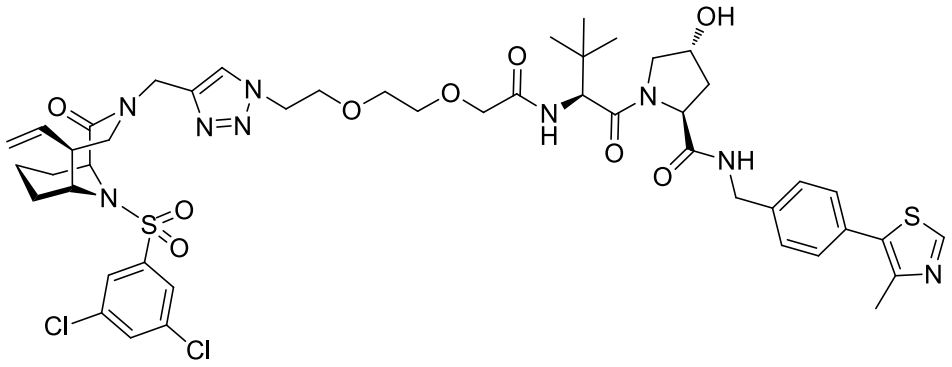
TLC [DCM:MeOH 93:7]: R_f = 0.29.

HPLC [0-100% Solvent B, 20 min]: R_t = 14.74 min, purity (254 nm) ≥ 99%.

^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.60 (s, 1H), 7.67 (s, 1H), 7.63 – 7.59 (m, 3H), 7.49 (t, J = 1.8 Hz, 1H), 7.33 – 7.28 (m, 4H), 7.02 (d, J = 9.2 Hz, 1H), 5.66 (ddd, J = 16.8, 10.3, 8.7 Hz, 1H), 5.04 – 4.96 (m, 2H), 4.81 (d, J = 14.9 Hz, 1H), 4.73 (t, J = 8.0 Hz, 1H), 4.60 – 4.53 (m, 3H), 4.51 – 4.45 (m, 3H), 4.26 (dd, J = 15.0, 5.2 Hz, 1H), 4.21 (d, J = 14.8 Hz, 1H), 3.97 (d, J = 14.7 Hz, 1H), 3.91 – 3.84 (m, 4H), 3.76 – 3.69 (m, 2H), 3.61 (dd, J = 11.2, 3.6 Hz, 1H), 3.28 – 3.23 (m, 1H), 2.90 (d, J = 4.6 Hz, 1H), 2.55 (q, J = 9.7, 8.6 Hz, 1H), 2.47 – 2.41 (m, 4H), 2.13 – 2.05 (m, 2H), 1.42 – 1.32 (m, 3H), 1.16 – 1.06 (m, 2H), 0.89 (s, 9H).

HRMS: m/z: found 984.3058 $[M+H]^+$, calculated 984.3058 $[M+H]^+$

(2S,4R)-1-((S)-2-(2-(2-(2-(4-(((1S,5R,6R)-10-((3,5-dichlorophenyl)sulfonyl)-2-oxo-5-vinyl-3,10-diazabicyclo[4.3.1]decan-3-yl)methyl)-1H-1,2,3-triazol-1-yl)ethoxy)ethoxy)acetamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (MTQ499)



MTQ499

MTQ499 was synthesized from **45a** (4 mg, 0.01 mmol) and **43b** (6 mg, 0. 010 mmol) according to the General Method G. Purification by flash column chromatography (0% to 7% MeOH in DCM) gave a white solid (6 mg, 56%).

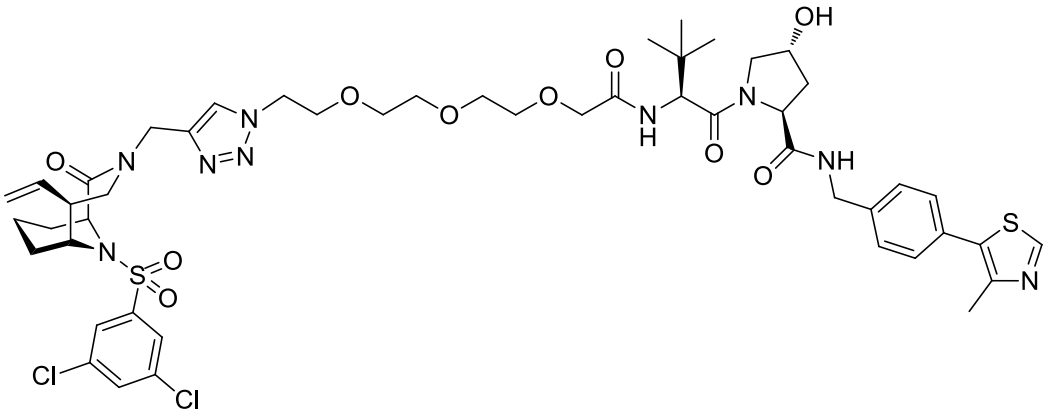
TLC [DCM:MeOH 93:7]: R_f = 0.33.

HPLC [0-100% Solvent B, 20 min]: R_t = 14.81 min, purity (254 nm) ≥ 99%.

^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.67 (s, 1H), 8.05 (s, 1H), 7.70 (t, J = 5.5 Hz, 1H), 7.66 – 7.64 (m, 2H), 7.57 – 7.53 (m, 1H), 7.42 (d, J = 9.4 Hz, 1H), 7.30 (s, 4H), 5.68 (ddd, J = 17.2, 10.0, 8.8 Hz, 1H), 5.07 – 4.92 (m, 2H), 4.75 – 4.70 (m, 2H), 4.67 – 4.56 (m, 4H), 4.49 – 4.42 (m, 3H), 4.35 (dd, J = 15.0, 5.6 Hz, 1H), 4.05 – 3.96 (m, 3H), 3.90 (ddd, J = 13.1, 7.3, 3.2 Hz, 3H), 3.83 (d, J = 16.0 Hz, 1H), 3.71 (dd, J = 11.3, 3.6 Hz, 1H), 3.68 – 3.63 (m, 1H), 3.61 – 3.57 (m, 1H), 3.51 (ddt, J = 15.0, 8.8, 2.9 Hz, 2H), 3.28 – 3.21 (m, 1H), 2.50 (s, 3H), 2.42 (ddd, J = 13.1, 8.8, 4.4 Hz, 2H), 2.23 (dd, J = 13.2, 7.7 Hz, 2H), 1.40 (t, J = 12.4 Hz, 3H), 1.24 – 1.12 (m, 2H), 1.01 (s, 9H).

HRMS: m/z: found 1028.3323 $[M+H]^+$, calculated 1028.3327 $[M+H]^+$

(2S,4R)-1-((S)-2-(tert-butyl)-14-(4-(((1S,5R,6R)-10-((3,5-dichlorophenyl)sulfonyl)-2-oxo-5-vinyl-3,10-diazabicyclo[4.3.1]decan-3-yl)methyl)-1H-1,2,3-triazol-1-yl)-4-oxo-6,9,12-trioxa-3-azatetradecan-1-oyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (MTQ500)



MTQ500

MTQ500 was synthesized from **45a** (4 mg, 0.01 mmol) and **43c** (7 mg, 0. 010 mmol) according to the General Method G. Purification by flash column chromatography (0% to 7% MeOH in DCM) gave a white solid (8 mg, 75%).

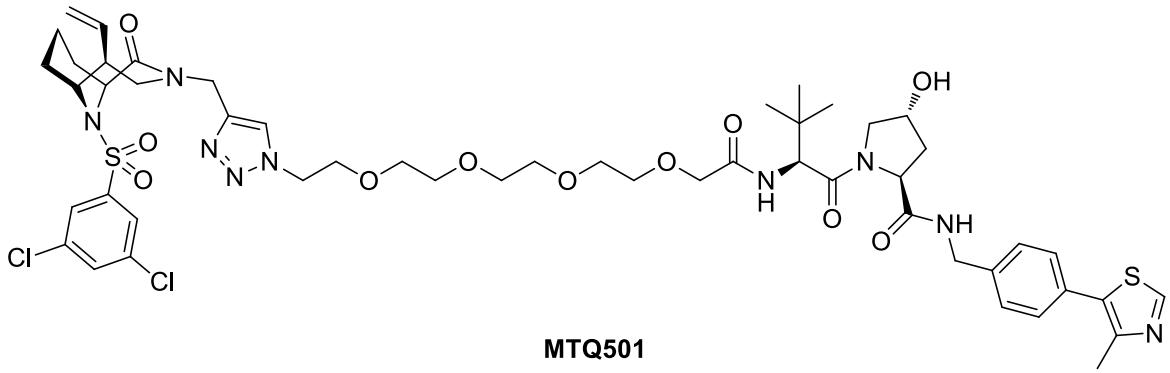
TLC [DCM:MeOH 93:7]: R_f = 0.26.

HPLC [0-100% Solvent B, 20 min]: R_t = 14.79 min, purity (254 nm) ≥ 99%.

^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.67 (s, 1H), 7.75 (s, 1H), 7.67 (d, J = 1.8 Hz, 2H), 7.55 (t, J = 1.8 Hz, 1H), 7.43 (t, J = 5.9 Hz, 1H), 7.37 – 7.32 (m, 4H), 7.27 – 7.26 (m, 1H), 5.73 (ddd, J = 17.0, 10.1, 8.8 Hz, 1H), 5.11 – 5.03 (m, 2H), 4.73 – 4.64 (m, 4H), 4.52 (ddd, J = 18.3, 8.1, 5.2 Hz, 5H), 4.35 (dd, J = 15.0, 5.4 Hz, 1H), 4.02 – 3.99 (m, 2H), 3.99 – 3.94 (m, 2H), 3.85 (t, J = 5.3 Hz, 2H), 3.71 – 3.56 (m, 10H), 3.32 – 3.25 (m, 1H), 3.20 (d, J = 4.0 Hz, 1H), 2.63 – 2.57 (m, 1H), 2.54 – 2.48 (m, 4H), 2.24 – 2.12 (m, 2H), 1.51 – 1.44 (m, 3H), 1.26 – 1.16 (m, 2H), 0.96 (s, 9H).

HRMS: m/z: found 1072.3588 $[M+H]^+$, calculated 1072.3589 $[M+H]^+$

(2S,4R)-1-((S)-2-(tert-butyl)-17-(4-(((1S,5R,6R)-10-((3,5-dichlorophenyl)sulfonyl)-2-oxo-5-vinyl-3,10-diazabicyclo[4.3.1]decan-3-yl)methyl)-1H-1,2,3-triazol-1-yl)-4-oxo-6,9,12,15-tetraoxa-3-azaheptadecan-1-oyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (MTQ501)



MTQ501

MTQ501 was synthesized from **45a** (4 mg, 0.01 mmol) and **43d** (7 mg, 0. 010 mmol) according to the General Method G. Purification by flash column chromatography (0% to 7% MeOH in DCM) gave a white solid (8 mg, 72%).

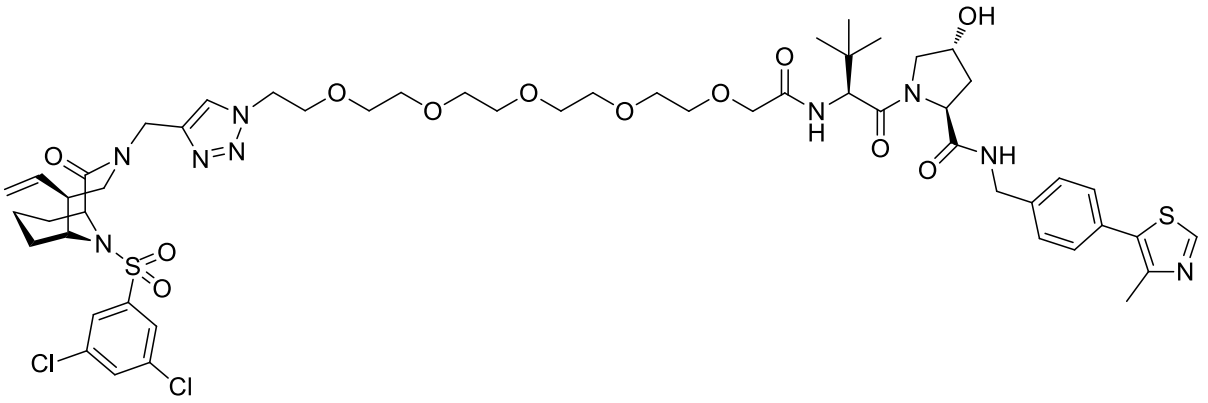
TLC [DCM:MeOH 93:7]: R_f = 0.26.

HPLC [0-100% Solvent B, 20 min]: R_t = 14.79 min, purity (254 nm) ≥ 99%.

^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.67 (s, 1H), 7.75 (s, 1H), 7.68 (d, J = 1.7 Hz, 2H), 7.55 (t, J = 1.7 Hz, 1H), 7.44 (t, J = 6.1 Hz, 1H), 7.36 (d, J = 3.2 Hz, 4H), 7.24 (d, J = 8.6 Hz, 1H), 5.73 (ddd, J = 17.0, 10.1, 8.8 Hz, 1H), 5.11 – 5.01 (m, 2H), 4.81 – 4.72 (m, 2H), 4.68 (d, J = 5.9 Hz, 1H), 4.61 (d, J = 14.8 Hz, 1H), 4.58 – 4.47 (m, 5H), 4.37 (dd, J = 15.0, 5.4 Hz, 1H), 4.03 – 3.93 (m, 4H), 3.89 – 3.81 (m, 2H), 3.72 – 3.52 (m, 14H), 3.36 (d, J = 4.1 Hz, 1H), 3.27 (dd, J = 14.2, 1.7 Hz, 1H), 2.62 – 2.49 (m, 5H), 2.23 (d, J = 13.3 Hz, 1H), 2.18 – 2.11 (m, 1H), 1.47 (td, J = 6.6, 6.0, 2.3 Hz, 3H), 1.27 – 1.18 (m, 2H), 0.95 (s, 9H).

HRMS: m/z: found 1116.3859 $[M+H]^+$, calculated 1116.3851 $[M+H]^+$

(2S,4R)-1-((S)-2-(tert-butyl)-20-(4-(((1S,5R,6R)-10-((3,5-dichlorophenyl)sulfonyl)-2-oxo-5-vinyl-3,10-diazabicyclo[4.3.1]decan-3-yl)methyl)-1H-1,2,3-triazol-1-yl)-4-oxo-6,9,12,15,18-pentaoxa-3-azaicosan-1-oyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (MTQ502)



MTQ502

MTQ502 was synthesized from **45a** (4 mg, 0.01 mmol) and **43e** (7 mg, 0. 010 mmol) according to the General Method G. Purification by flash column chromatography (0% to 7% MeOH in DCM) gave a white solid (7 mg, 64%).

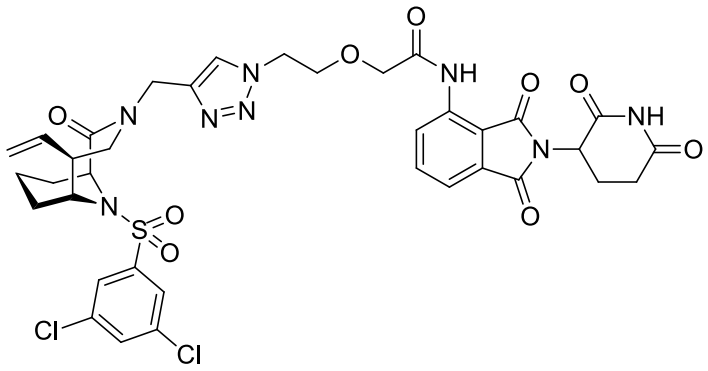
TLC [DCM:MeOH 93:7]: R_f = 0.21.

HPLC [0-100% Solvent B, 20 min]: R_t = 14.78 min, purity (254 nm) ≥ 99%.

^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.67 (s, 1H), 7.72 (s, 1H), 7.70 – 7.66 (m, 2H), 7.57 – 7.53 (m, 1H), 7.41 (t, J = 5.9 Hz, 1H), 7.38 – 7.32 (m, 4H), 7.24 (s, 1H), 5.74 (ddd, J = 17.1, 10.1, 8.8 Hz, 1H), 5.12 – 5.03 (m, 2H), 4.82 – 4.71 (m, 2H), 4.68 (d, J = 5.9 Hz, 1H), 4.63 (d, J = 14.9 Hz, 1H), 4.58 – 4.48 (m, 5H), 4.36 (dd, J = 14.9, 5.3 Hz, 1H), 4.05 – 3.94 (m, 4H), 3.88 – 3.82 (m, 2H), 3.69 – 3.56 (m, 18H), 3.28 (dd, J = 14.1, 1.5 Hz, 1H), 3.19 (d, J = 4.0 Hz, 1H), 2.64 – 2.52 (m, 2H), 2.51 (s, 3H), 2.23 (d, J = 13.5 Hz, 1H), 2.17 – 2.09 (m, 1H), 1.52 – 1.43 (m, 3H), 1.26 – 1.17 (m, 2H), 0.95 (s, 9H).

HRMS: m/z: found 1160.4113 $[M+H]^+$, calculated 1160.4113 $[M+H]^+$

2-(2-(4-(((1S,5R,6R)-10-((3,5-dichlorophenyl)sulfonyl)-2-oxo-5-vinyl-3,10-diazabicyclo[4.3.1]decan-3-yl)methyl)-1H-1,2,3-triazol-1-yl)ethoxy)-N-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)acetamide (MTQ503)



MTQ503

MTQ503 was synthesized from **45a** (4 mg, 0.01 mmol) and **44a** (4 mg, 0. 010 mmol) according to the General Method G. Purification by flash column chromatography (CH → EA:MeCN = 90:10) gave a white solid (5 mg, 60%).

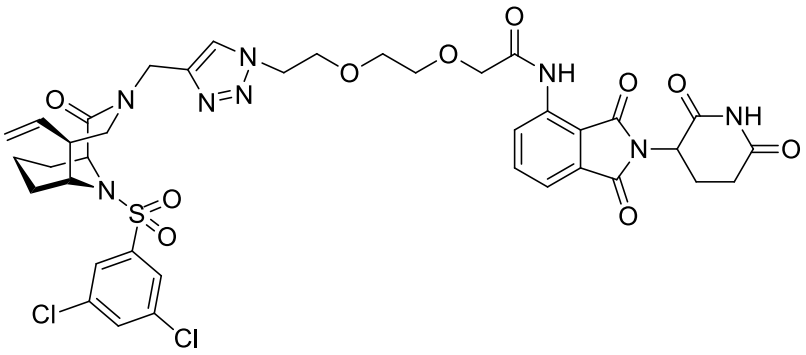
TLC [DCM:MeOH 97:3]: R_f = 0.36.

HPLC [50-100% Solvent B, 20 min]: R_t = 6.49 min, purity (254 nm) = 96%.

^{1}H NMR (500 MHz, Chloroform-*d*) δ 10.51 (d, J = 28.2 Hz, 1H), 8.83 (dd, J = 11.6, 8.4 Hz, 1H), 7.97 (d, J = 4.6 Hz, 1H), 7.86 – 7.63 (m, 4H), 7.59 (dd, J = 7.3, 3.4 Hz, 1H), 7.53 (dt, J = 4.5, 1.8 Hz, 1H), 5.89 – 5.71 (m, 1H), 5.19 – 5.07 (m, 3H), 4.82 – 4.69 (m, 3H), 4.34 – 4.24 (m, 1H), 4.16 – 4.08 (m, 3H), 4.07 – 3.90 (m, 4H), 3.65 – 3.42 (m, 1H), 2.94 – 2.66 (m, 4H), 2.29 – 2.13 (m, 2H), 1.52 – 1.37 (m, 3H), 1.31 – 1.18 (m, 2H).

HRMS: m/z: found 827.17767 $[M+H]^+$, calculated 827.17758 $[M+H]^+$

2-(2-(2-(4-(((1S,5R,6R)-10-((3,5-dichlorophenyl)sulfonyl)-2-oxo-5-vinyl-3,10-diazabicyclo[4.3.1]decan-3-yl)methyl)-1H-1,2,3-triazol-1-yl)ethoxy)ethoxy)-N-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)acetamide (MTQ504)



MTQ504

MTQ504 was synthesized from **45a** (4 mg, 0.01 mmol) and **44b** (4 mg, 0. 010 mmol) according to the General Method G. Purification by flash column chromatography (CH → EA:MeCN = 90:10) gave a white solid (7 mg, 77%).

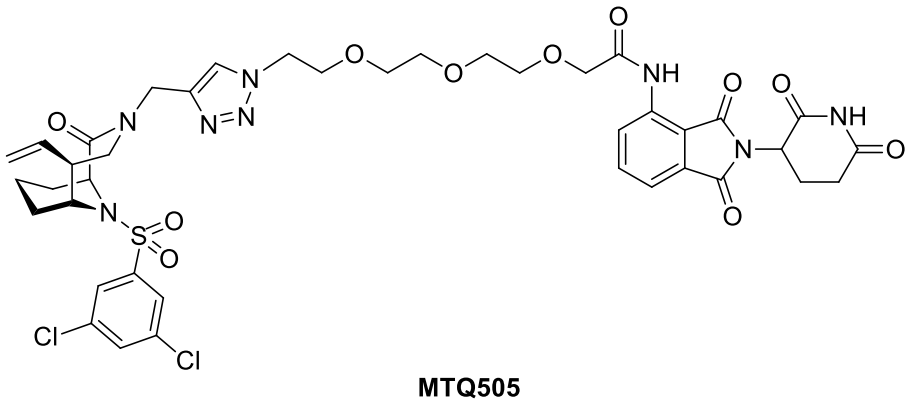
TLC [DCM:MeOH 97:3]: R_f = 0.32.

HPLC [0-100% Solvent B, 20 min]: R_t = 15.67 min, purity (254 nm) ≥ 99%.

^{1}H NMR (300 MHz, Chloroform-*d*) δ 10.45 (s, 1H), 9.01 – 8.76 (m, 2H), 7.76 – 7.69 (m, 2H), 7.67 (dd, J = 5.3, 1.9 Hz, 2H), 7.58 (dd, J = 7.3, 0.7 Hz, 1H), 7.54 (dt, J = 3.8, 1.9 Hz, 1H), 5.74 (dddd, J = 17.3, 10.1, 8.7, 1.7 Hz, 1H), 5.15 – 5.04 (m, 2H), 5.04 – 4.94 (m, 1H), 4.78 (dd, J = 14.9, 7.7 Hz, 1H), 4.69 (dt, J = 4.1, 1.7 Hz, 1H), 4.64 – 4.49 (m, 3H), 4.23 – 4.10 (m, 2H), 4.04 – 3.90 (m, 4H), 3.82 – 3.70 (m, 4H), 3.28 (dt, J = 14.3, 2.2 Hz, 1H), 2.97 – 2.72 (m, 3H), 2.71 – 2.57 (m, 1H), 2.28 – 2.10 (m, 2H), 1.52 – 1.42 (m, 3H), 1.31 – 1.15 (m, 2H).

HRMS: m/z: found 871.2043 $[M+H]^+$, calculated 871.2038 $[M+H]^+$

2-(2-(2-(2-(4-(((1S,5R,6R)-10-((3,5-dichlorophenyl)sulfonyl)-2-oxo-5-vinyl-3,10-diazabicyclo[4.3.1]decan-3-yl)methyl)-1H-1,2,3-triazol-1-yl)ethoxy)ethoxy)ethoxy)-N-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)acetamide (MTQ505)



MTQ505

MTQ505 was synthesized from **45a** (4 mg, 0.01 mmol) and **44c** (5 mg, 0. 010 mmol) according to the General Method G. Purification by flash column chromatography (CH → EA:MeCN = 90:10) gave a white solid (7 mg, 80%).

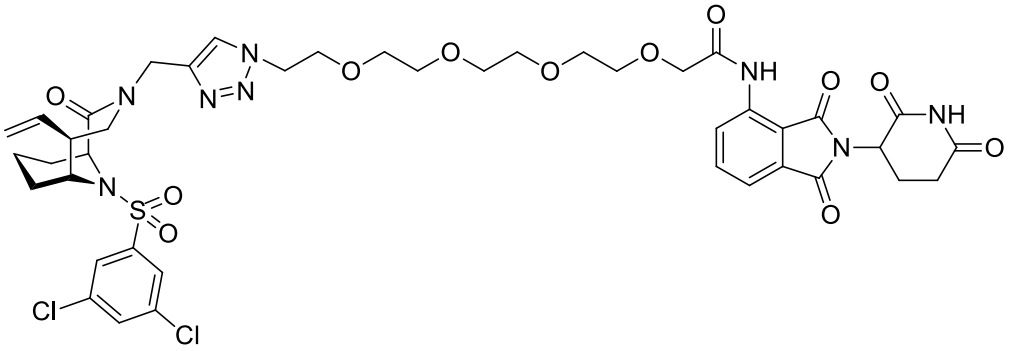
TLC [DCM:MeOH 97:3]: R_f = 0.30.

HPLC [0-100% Solvent B, 20 min]: R_t = 15.57 min, purity (254 nm) ≥ 99%.

^{1}H NMR (300 MHz, Chloroform-*d*) δ 10.46 (s, 1H), 8.92 – 8.67 (m, 2H), 7.76 – 7.66 (m, 4H), 7.58 (dd, J = 7.3, 0.7 Hz, 1H), 7.54 (dt, J = 3.2, 1.9 Hz, 1H), 5.84 – 5.64 (m, 1H), 5.10 (s, 1H), 5.06 (d, J = 5.9 Hz, 1H), 5.02 – 4.93 (m, 1H), 4.79 – 4.63 (m, 3H), 4.55 – 4.46 (m, 2H), 4.20 (d, J = 1.7 Hz, 2H), 4.03 – 3.92 (m, 2H), 3.85 (t, J = 5.2 Hz, 2H), 3.83 – 3.75 (m, 4H), 3.67 – 3.62 (m, 2H), 3.60 – 3.55 (m, 2H), 3.30 (dd, J = 14.2, 1.6 Hz, 1H), 2.96 – 2.74 (m, 3H), 2.67 – 2.55 (m, 1H), 2.29 – 2.11 (m, 2H), 1.53 – 1.42 (m, 3H), 1.31 – 1.17 (m, 2H).

HRMS: m/z: found 915.22996 $[M+H]^+$, calculated 915.23001 $[M+H]^+$

14-(4-(((1S,5R,6R)-10-((3,5-dichlorophenyl)sulfonyl)-2-oxo-5-vinyl-3,10-diazabicyclo[4.3.1]decan-3-yl)methyl)-1H-1,2,3-triazol-1-yl)-N-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)-3,6,9,12-tetraoxatetradecan-1-amide (MTQ506)



MTQ506

MTQ506 was synthesized from **45a** (4 mg, 0.01 mmol) and **44d** (5 mg, 0. 010 mmol) according to the General Method G. Purification by flash column chromatography (CH → EA:MeCN = 90:10) gave a white solid (7 mg, 76%).

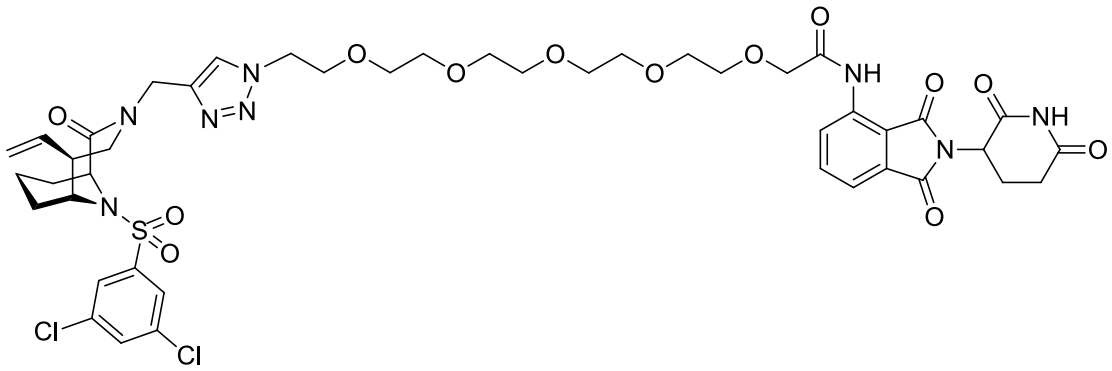
TLC [DCM:MeOH 97:3]: R_f = 0.24.

HPLC [0-100% Solvent B, 20 min]: R_t = 15.61 min, purity (254 nm) ≥ 99%.

^{1}H NMR (300 MHz, Chloroform-*d*) δ 10.50 (s, 1H), 8.98 – 8.78 (m, 2H), 7.76 – 7.68 (m, 4H), 7.58 (dd, J = 7.3, 0.7 Hz, 1H), 7.54 (t, J = 1.8 Hz, 1H), 5.82 – 5.67 (m, 1H), 5.12 – 5.08 (m, 1H), 5.07 – 5.03 (m, 1H), 5.02 – 4.94 (m, 1H), 4.79 – 4.62 (m, 3H), 4.57 – 4.47 (m, 2H), 4.19 (d, J = 1.4 Hz, 2H), 4.03 – 3.92 (m, 2H), 3.90 – 3.84 (m, 2H), 3.80 (s, 4H), 3.72 – 3.66 (m, 2H), 3.64 – 3.55 (m, 6H), 3.30 (d, J = 14.2 Hz, 1H), 2.94 – 2.73 (m, 3H), 2.61 (q, J = 8.9 Hz, 1H), 2.29 – 2.11 (m, 2H), 1.52 – 1.41 (m, 3H), 1.31 – 1.16 (m, 2H).

HRMS: m/z: found 959.25696 $[M+H]^+$, calculated 959.25622 $[M+H]^+$

17-(4-(((1S,5R,6R)-10-((3,5-dichlorophenyl)sulfonyl)-2-oxo-5-vinyl-3,10-diazabicyclo[4.3.1]decan-3-yl)methyl)-1H-1,2,3-triazol-1-yl)-N-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)-3,6,9,12,15-pentaoxaheptadecan-1-amide (MTQ507)



MTQ507

MTQ507 was synthesized from **45a** (4 mg, 0.01 mmol) and **44e** (6 mg, 0. 010 mmol) according to the General Method G. Purification by flash column chromatography (CH → EA:MeCN = 90:10) gave a white solid (8 mg, 82%).

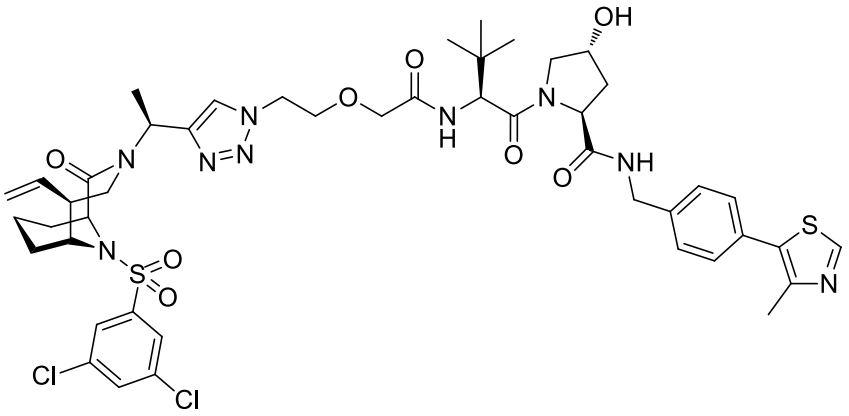
TLC [DCM:MeOH 97:3]: R_f = 0.26.

HPLC [0-100% Solvent B, 20 min]: R_t = 15.51 min, purity (254 nm) ≥ 99%.

^{1}H NMR (300 MHz, Chloroform-*d*) δ 10.49 (s, 1H), 9.00 – 8.75 (m, 2H), 7.70 (dd, J = 10.0, 1.6 Hz, 4H), 7.61 – 7.52 (m, 2H), 5.74 (ddd, J = 17.4, 10.6, 8.9 Hz, 1H), 5.15 – 4.92 (m, 3H), 4.78 (dd, J = 14.8, 4.0 Hz, 1H), 4.73 – 4.59 (m, 2H), 4.56 – 4.46 (m, 2H), 4.19 (d, J = 1.2 Hz, 2H), 4.03 – 3.91 (m, 2H), 3.86 (t, J = 5.2 Hz, 2H), 3.80 (s, 4H), 3.73 – 3.68 (m, 2H), 3.67 – 3.57 (m, 10H), 3.30 (d, J = 14.0 Hz, 1H), 2.94 – 2.75 (m, 3H), 2.61 (q, J = 8.6 Hz, 1H), 2.30 – 2.07 (m, 2H), 1.52 – 1.42 (m, 3H), 1.34 – 1.17 (m, 2H).

HRMS: m/z: found 1003.28266 $[M+H]^+$, calculated 1003.28244 $[M+H]^+$

(2S,4R)-1-((S)-2-(2-(2-(4-((S)-1-((1S,5R,6R)-10-((3,5-dichlorophenyl)sulfonyl)-2-oxo-5-vinyl-3,10-diazabicyclo[4.3.1]decan-3-yl)ethyl)-1H-1,2,3-triazol-1-yl)ethoxy)acetamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (MTQ508)



MTQ508

MTQ508 was synthesized from **45b** (4 mg, 0.01 mmol) and **43a** (6 mg, 0. 010 mmol) according to the General Method G. Purification by flash column chromatography (0% to 7% MeOH in DCM) gave a white solid (7 mg, 67%).

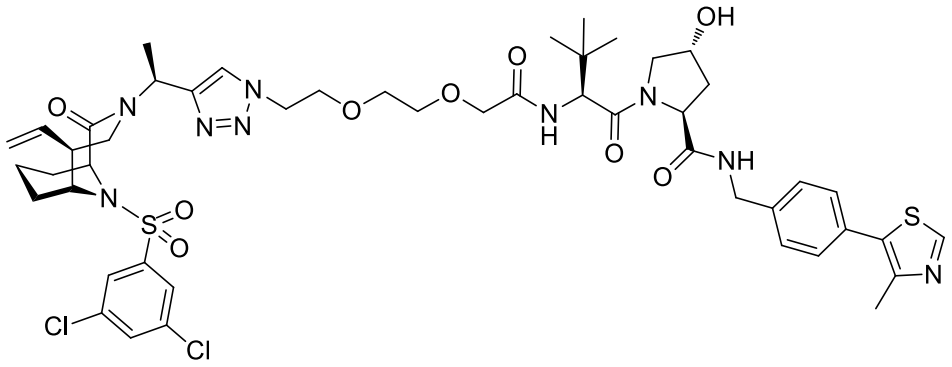
TLC [DCM:MeOH 93:7]: R_f = 0.29.

HPLC [0-100% Solvent B, 20 min]: R_t = 14.94 min, purity (254 nm) ≥ 99%.

^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.66 (s, 1H), 7.90 (t, J = 5.9 Hz, 1H), 7.68 (s, 1H), 7.59 (d, J = 1.8 Hz, 2H), 7.54 – 7.52 (m, 1H), 7.37 (s, 4H), 7.19 (d, J = 8.8 Hz, 1H), 6.13 (q, J = 7.0 Hz, 1H), 5.72 (dt, J = 17.1, 9.5 Hz, 1H), 5.15 – 5.08 (m, 2H), 4.84 (t, J = 7.9 Hz, 1H), 4.70 (dt, J = 14.4, 3.7 Hz, 1H), 4.58 (s, 1H), 4.55 – 4.48 (m, 5H), 4.06 (d, J = 14.6 Hz, 1H), 3.99 – 3.92 (m, 2H), 3.91 – 3.86 (m, 1H), 3.85 – 3.80 (m, 1H), 3.74 – 3.67 (m, 2H), 3.36 (dd, J = 14.9, 10.5 Hz, 1H), 3.30 – 3.23 (m, 1H), 2.77 (s, 1H), 2.54 – 2.46 (m, 5H), 2.24 – 2.18 (m, 1H), 2.11 (d, J = 13.1 Hz, 1H), 1.61 (d, J = 7.0 Hz, 3H), 1.54 – 1.48 (m, 3H), 1.17 – 1.04 (m, 2H), 0.98 (s, 9H).

HRMS: m/z: found 998.3224 $[M+H]^+$, calculated 998.3221 $[M+H]^+$

(2S,4R)-1-((S)-2-(2-(2-(2-(4-((S)-1-((1S,5R,6R)-10-((3,5-dichlorophenyl)sulfonyl)-2-oxo-5-vinyl-3,10-diazabicyclo[4.3.1]decan-3-yl)ethyl)-1H-1,2,3-triazol-1-yl)ethoxy)ethoxy)acetamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (MTQ509)



MTQ509

MTQ509 was synthesized from **45b** (4 mg, 0.01 mmol) and **43b** (6 mg, 0. 010 mmol) according to the General Method G. Purification by flash column chromatography (0% to 7% MeOH in DCM) gave a white solid (7 mg, 73%).

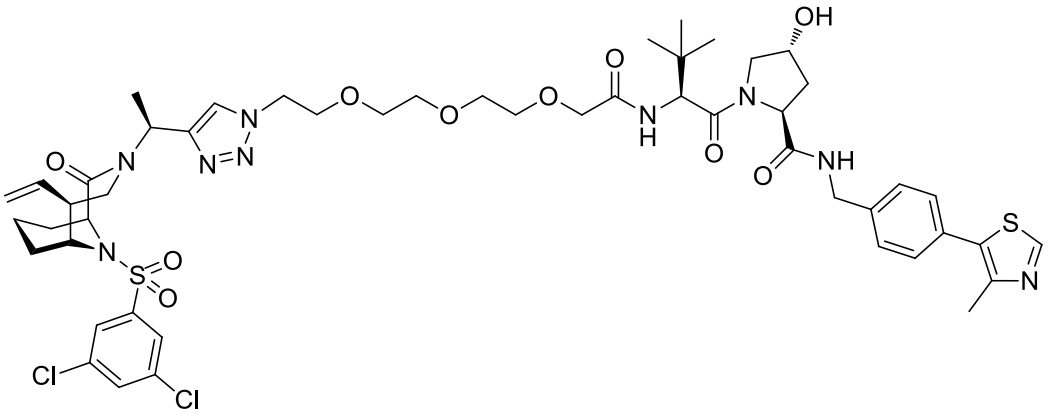
TLC [DCM:MeOH 93:7]: R_f = 0.30.

HPLC [0-100% Solvent B, 20 min]: R_t = 15.05 min, purity (254 nm) ≥ 99%.

^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.67 (s, 1H), 7.72 (s, 1H), 7.65 (dd, J = 1.8, 0.6 Hz, 2H), 7.58 – 7.53 (m, 1H), 7.39 (t, J = 5.9 Hz, 1H), 7.34 (s, 4H), 7.31 (d, J = 9.0 Hz, 1H), 6.10 (q, J = 7.0 Hz, 1H), 5.72 (dt, J = 17.1, 9.4 Hz, 1H), 5.16 – 5.06 (m, 2H), 4.72 – 4.64 (m, 2H), 4.62 – 4.53 (m, 4H), 4.47 (ddd, J = 14.3, 5.5, 3.8 Hz, 1H), 4.36 (dd, J = 15.0, 5.4 Hz, 1H), 4.08 (d, J = 11.3 Hz, 1H), 3.98 (d, J = 15.7 Hz, 1H), 3.96 – 3.84 (m, 4H), 3.68 – 3.56 (m, 4H), 3.55 – 3.50 (m, 1H), 3.45 (dd, J = 14.8, 10.8 Hz, 1H), 3.31 (s, 1H), 3.14 (d, J = 14.1 Hz, 1H), 2.57 – 2.47 (m, 5H), 2.26 (d, J = 12.9 Hz, 1H), 2.17 (dd, J = 13.5, 7.9 Hz, 1H), 1.60 (d, J = 7.0 Hz, 3H), 1.57 – 1.51 (m, 3H), 1.31 – 1.21 (m, 2H), 0.97 (s, 9H).

HRMS: m/z: found 1042.3485 $[M+H]^+$, calculated 1042.3483 $[M+H]^+$

(2S,4R)-1-((S)-2-(tert-butyl)-14-(4-((S)-1-((1S,5R,6R)-10-((3,5-dichlorophenyl)sulfonyl)-2-oxo-5-vinyl-3,10-diazabicyclo[4.3.1]decan-3-yl)ethyl)-1H-1,2,3-triazol-1-yl)-4-oxo-6,9,12-trioxa-3-azatetradecan-1-oyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (MTQ510)



MTQ510

MTQ510 was synthesized from **45b** (4 mg, 0.01 mmol) and **43c** (7 mg, 0. 010 mmol) according to the General Method G. Purification by flash column chromatography (0% to 7% MeOH in DCM) gave a white solid (10 mg, 88%).

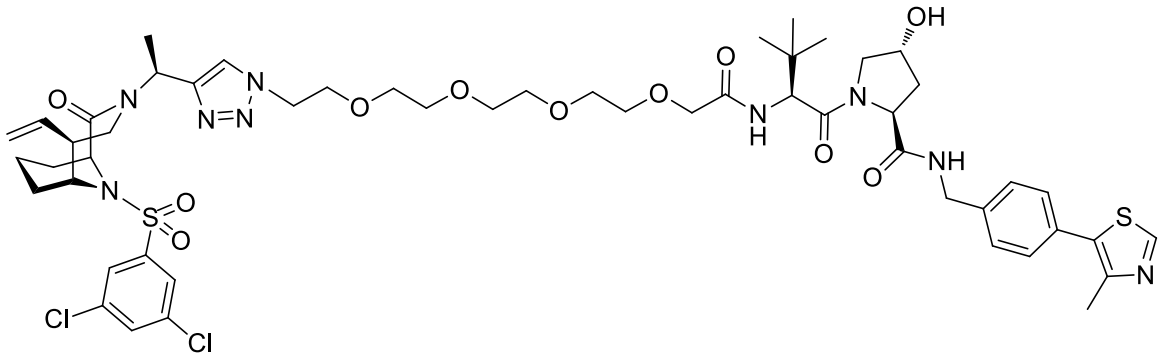
TLC [DCM:MeOH 93:7]: R_f = 0.23.

HPLC [0-100% Solvent B, 20 min]: R_t = 15.12min, purity (254 nm) ≥ 99%.

^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.67 (s, 1H), 7.72 – 7.63 (m, 3H), 7.59 – 7.53 (m, 1H), 7.43 (t, J = 5.9 Hz, 1H), 7.39 – 7.32 (m, 4H), 7.28 (s, 1H), 6.10 (q, J = 6.9 Hz, 1H), 5.73 (dt, J = 17.0, 9.5 Hz, 1H), 5.16 – 5.05 (m, 2H), 4.74 – 4.65 (m, 2H), 4.60 – 4.47 (m, 5H), 4.35 (dd, J = 15.0, 5.3 Hz, 1H), 4.09 (d, J = 11.4 Hz, 1H), 4.01 (d, J = 4.1 Hz, 2H), 3.97 – 3.93 (m, 1H), 3.84 (t, J = 5.3 Hz, 2H), 3.63 (tdd, J = 14.0, 10.4, 6.2 Hz, 9H), 3.46 (dd, J = 14.8, 10.8 Hz, 1H), 3.23 (d, J = 4.3 Hz, 1H), 3.11 – 3.05 (m, 1H), 2.56 – 2.47 (m, 5H), 2.28 (d, J = 13.4 Hz, 1H), 2.18 – 2.10 (m, 1H), 1.61 (d, J = 7.0 Hz, 3H), 1.54 (d, J = 11.1 Hz, 3H), 1.32 – 1.20 (m, 2H), 0.96 (s, 9H).

HRMS: m/z: found 1086.3753 $[M+H]^+$, calculated 1086.3746 $[M+H]^+$

(2S,4R)-1-((S)-2-(tert-butyl)-17-(4-((S)-1-((1S,5R,6R)-10-((3,5-dichlorophenyl)sulfonyl)-2-oxo-5-vinyl-3,10-diazabicyclo[4.3.1]decan-3-yl)ethyl)-1H-1,2,3-triazol-1-yl)-4-oxo-6,9,12,15-tetraoxa-3-azaheptadecan-1-oyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (MTQ511)



MTQ511

MTQ511 was synthesized from **45b** (4 mg, 0.01 mmol) and **43d** (7 mg, 0. 010 mmol) according to the General Method G. Purification by flash column chromatography (0% to 7% MeOH in DCM) gave a white solid (10 mg, 86%).

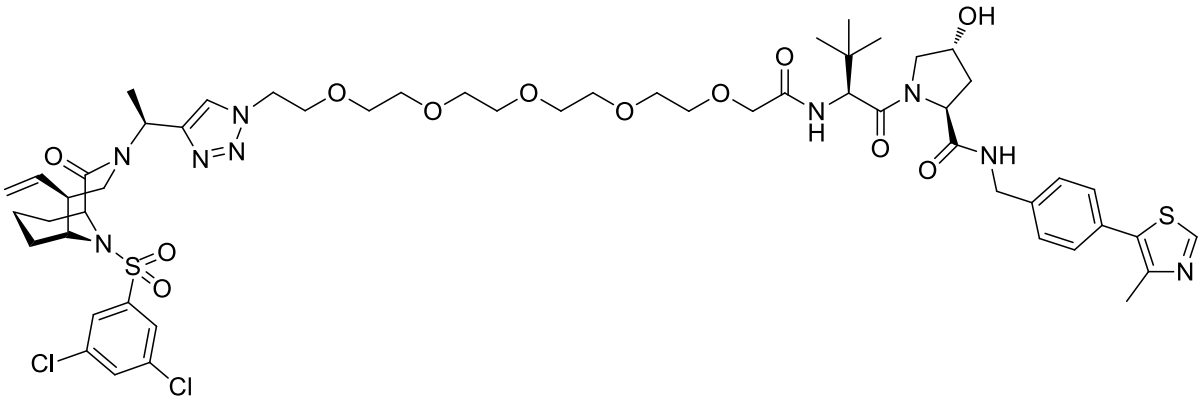
TLC [DCM:MeOH 93:7]: R_f = 0.17.

HPLC [0-100% Solvent B, 20 min]: R_t = 15.07 min, purity (254 nm) ≥ 99%.

^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.67 (s, 1H), 7.70 – 7.65 (m, 2H), 7.63 (s, 1H), 7.57 – 7.54 (m, 1H), 7.41 (t, J = 5.8 Hz, 1H), 7.38 – 7.32 (m, 4H), 7.26 – 7.22 (m, 1H), 6.12 (q, J = 6.9 Hz, 1H), 5.73 (dt, J = 17.1, 9.5 Hz, 1H), 5.18 – 5.03 (m, 2H), 4.73 (t, J = 7.9 Hz, 1H), 4.68 (d, J = 5.9 Hz, 1H), 4.59 – 4.47 (m, 5H), 4.35 (dd, J = 14.9, 5.3 Hz, 1H), 4.10 (d, J = 11.3 Hz, 1H), 4.07 – 3.98 (m, 2H), 3.97 – 3.94 (m, 1H), 3.86 (t, J = 5.2 Hz, 2H), 3.68 – 3.58 (m, 13H), 3.46 (dd, J = 14.8, 10.7 Hz, 1H), 3.30 – 3.25 (m, 1H), 3.07 (d, J = 13.8 Hz, 1H), 2.58 – 2.48 (m, 5H), 2.29 (d, J = 13.5 Hz, 1H), 2.19 – 2.09 (m, 1H), 1.62 (d, J = 7.0 Hz, 3H), 1.58 – 1.51 (m, 3H), 1.34 – 1.22 (m, 2H), 0.95 (s, 9H).

HRMS: m/z: found 1130.4008 $[M+H]^+$, calculated 1130.4008 $[M+H]^+$

(2S,4R)-1-((S)-2-(tert-butyl)-20-(4-((S)-1-((1S,5R,6R)-10-((3,5-dichlorophenyl)sulfonyl)-2-oxo-5-vinyl-3,10-diazabicyclo[4.3.1]decan-3-yl)ethyl)-1H-1,2,3-triazol-1-yl)-4-oxo-6,9,12,15,18-pentaoxa-3-azaicosan-1-oyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (MTQ512)



MTQ512

MTQ512 was synthesized from **45b** (4 mg, 0.01 mmol) and **43e** (7 mg, 0. 010 mmol) according to the General Method G. Purification by flash column chromatography (0% to 7% MeOH in DCM) gave a white solid (10 mg, 73%).

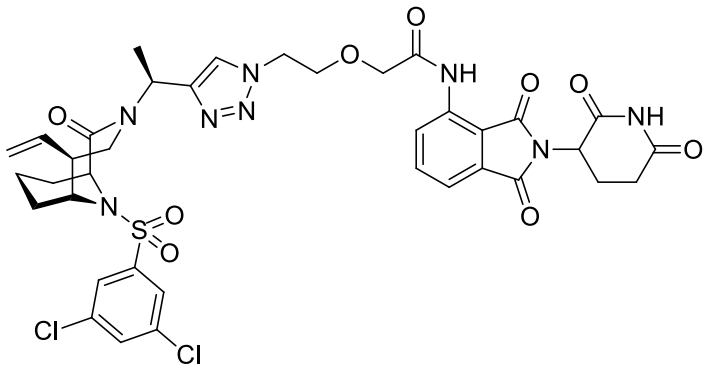
TLC [DCM:MeOH 93:7]: R_f = 0.21.

HPLC [0-100% Solvent B, 20 min]: R_t = 15.08 min, purity (254 nm) ≥ 99%.

^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.67 (s, 1H), 7.71 – 7.65 (m, 2H), 7.62 (s, 1H), 7.58 – 7.53 (m, 1H), 7.43 – 7.32 (m, 5H), 7.29 – 7.27 (m, 1H), 6.12 (q, J = 6.9 Hz, 1H), 5.73 (dt, J = 17.1, 9.5 Hz, 1H), 5.17 – 5.06 (m, 2H), 4.74 (t, J = 7.9 Hz, 1H), 4.68 (d, J = 5.9 Hz, 1H), 4.60 – 4.47 (m, 5H), 4.35 (dd, J = 14.9, 5.3 Hz, 1H), 4.10 (d, J = 11.5 Hz, 1H), 4.04 – 3.93 (m, 3H), 3.87 (t, J = 5.2 Hz, 2H), 3.70 – 3.59 (m, 17H), 3.46 (dd, J = 14.8, 10.7 Hz, 1H), 3.19 (s, 1H), 3.08 (d, J = 14.5 Hz, 1H), 2.60 – 2.49 (m, 5H), 2.30 (d, J = 13.2 Hz, 1H), 2.17 – 2.08 (m, 1H), 1.62 (d, J = 7.0 Hz, 3H), 1.59 – 1.51 (m, 3H), 1.36 – 1.28 (m, 1H), 1.25 – 1.16 (m, 1H), 0.95 (s, 9H).

HRMS: m/z: found 1174.4276 $[M+H]^+$, calculated 1174.4270 $[M+H]^+$

2-(2-(4-((S)-1-((1S,5R,6R)-10-((3,5-dichlorophenyl)sulfonyl)-2-oxo-5-vinyl-3,10-diazabicyclo[4.3.1]decan-3-yl)ethyl)-1H-1,2,3-triazol-1-yl)ethoxy)-N-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)acetamide (MTQ513)



MTQ513

MTQ513 was synthesized from **45b** (4 mg, 0.01 mmol) and **44a** (4 mg, 0. 010 mmol) according to the General Method G. Purification by flash column chromatography (CH → EA:MeCN = 90:10) gave a white solid (6 mg, 74%).

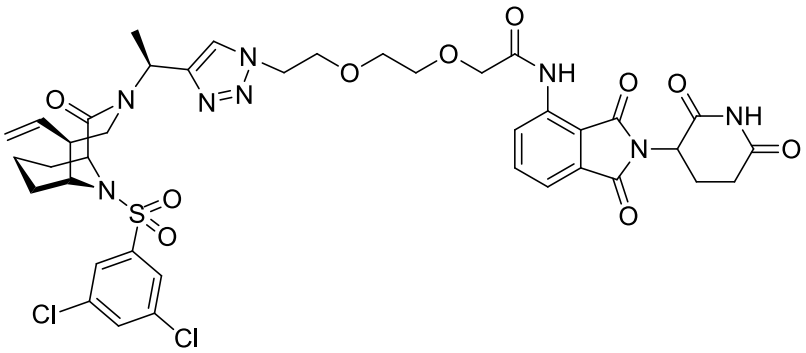
TLC [DCM:MeOH 97:3]: R_f = 0.36.

HPLC [0-100% Solvent B, 20 min]: R_t = 15.56 min, purity (254 nm) ≥ 99%.

^{1}H NMR (500 MHz, Chloroform-*d*) diastereomers mixture δ 10.44 (d, J = 7.9 Hz, 1H), 8.81 (dd, J = 21.3, 8.4 Hz, 1H), 8.68 (s, 1H), 7.99 (s, 0.5H), 7.79 (s, 0.5H), 7.73 – 7.63 (m, 2H), 7.60 – 7.53 (m, 2H), 7.52 – 7.48 (m, 1H), 6.23 (q, J = 6.9 Hz, 0.5H), 6.10 (q, J = 7.0 Hz, 0.5H), 5.74 (ddt, J = 23.3, 17.3, 9.5 Hz, 1H).

HRMS: m/z: found 841.19396 $[M+H]^+$, calculated 841.19323 $[M+H]^+$

2-(2-(2-(4-((S)-1-((1S,5R,6R)-10-((3,5-dichlorophenyl)sulfonyl)-2-oxo-5-vinyl-3,10-diazabicyclo[4.3.1]decan-3-yl)ethyl)-1H-1,2,3-triazol-1-yl)ethoxy)ethoxy)-N-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)acetamide (MTQ514)



MTQ514

MTQ514 was synthesized from **45b** (4 mg, 0.01 mmol) and **44b** (4 mg, 0. 010 mmol) according to the General Method G. Purification by flash column chromatography (CH → EA:MeCN = 90:10) gave a white solid (8 mg, 84%).

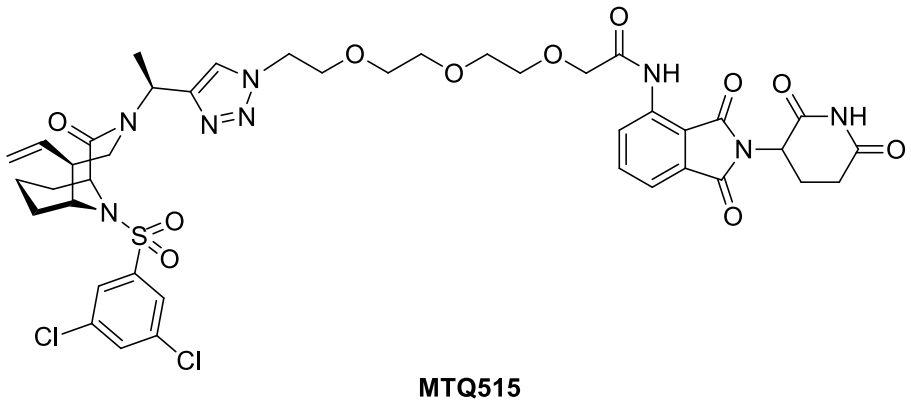
TLC [DCM:MeOH 97:3]: R_f = 0.26.

HPLC [0-100% Solvent B, 20 min]: R_t = 15.83 min, purity (254 nm) ≥ 99%.

^{1}H NMR (500 MHz, Chloroform-*d*) δ 10.45 (d, J = 4.2 Hz, 1H), 8.85 (dd, J = 8.5, 3.9 Hz, 1H), 8.67 (d, J = 42.6 Hz, 1H), 7.73 – 7.69 (m, 1H), 7.68 – 7.61 (m, 3H), 7.59 – 7.50 (m, 2H), 6.11 (q, J = 7.0 Hz, 1H), 5.71 (dtd, J = 16.6, 9.5, 6.4 Hz, 1H), 5.15 – 5.04 (m, 2H), 5.00 (dd, J = 12.2, 5.4 Hz, 1H), 4.73 – 4.66 (m, 1H), 4.61 – 4.49 (m, 2H), 4.22 – 4.12 (m, 2H), 3.99 – 3.91 (m, 3H), 3.83 – 3.73 (m, 4H), 3.46 (ddd, J = 14.5, 10.7, 3.5 Hz, 1H), 3.05 (d, J = 14.7 Hz, 1H), 2.94 – 2.73 (m, 3H), 2.56 – 2.47 (m, 1H), 2.28 (d, J = 13.1 Hz, 1H), 2.20 – 2.13 (m, 1H), 1.63 – 1.60 (m, 3H), 1.56 – 1.50 (m, 3H), 1.33 – 1.18 (m, 2H).

HRMS: m/z: found 885.21874 $[M+H]^+$, calculated 885.21944 $[M+H]^+$

2-(2-(2-(2-(4-((S)-1-((1S,5R,6R)-10-((3,5-dichlorophenyl)sulfonyl)-2-oxo-5-vinyl-3,10-diazabicyclo[4.3.1]decan-3-yl)ethyl)-1H-1,2,3-triazol-1-yl)ethoxy)ethoxy)ethoxy)-N-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)acetamide (MTQ515)



MTQ515

MTQ515 was synthesized from **45b** (4 mg, 0.01 mmol) and **44c** (5 mg, 0. 010 mmol) according to the General Method G. Purification by flash column chromatography (CH → EA:MeCN = 90:10) gave a white solid (9 mg, 97%).

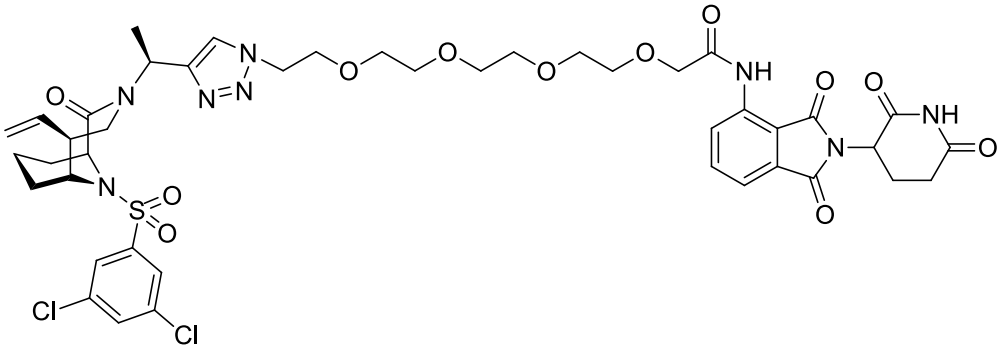
TLC [DCM:MeOH 97:3]: R_f = 0.30.

HPLC [0-100% Solvent B, 20 min]: R_t = 15.81 min, purity (254 nm) ≥ 99%.

^{1}H NMR (500 MHz, Chloroform-*d*) δ 10.46 (d, J = 2.7 Hz, 1H), 8.92 – 8.79 (m, 1H), 8.70 (d, J = 36.0 Hz, 1H), 7.75 – 7.69 (m, 1H), 7.68 (ddd, J = 7.9, 1.8, 0.7 Hz, 2H), 7.64 (d, J = 6.2 Hz, 1H), 7.62 – 7.49 (m, 2H), 6.12 (p, J = 7.0 Hz, 1H), 5.80 – 5.65 (m, 1H), 5.15 – 5.05 (m, 2H), 5.01 – 4.94 (m, 1H), 4.77 – 4.69 (m, 1H), 4.57 – 4.46 (m, 2H), 4.25 – 4.14 (m, 2H), 3.95 (t, J = 5.3 Hz, 1H), 3.88 – 3.84 (m, 2H), 3.83 – 3.76 (m, 4H), 3.68 – 3.56 (m, 4H), 3.46 (dd, J = 14.8, 10.8 Hz, 1H), 3.08 (d, J = 14.6 Hz, 1H), 2.93 – 2.74 (m, 3H), 2.52 (q, J = 9.2 Hz, 1H), 2.29 (d, J = 12.8 Hz, 1H), 2.20 – 2.12 (m, 1H), 1.62 (dd, J = 7.0, 1.9 Hz, 3H), 1.54 (d, J = 10.5 Hz, 3H), 1.32 – 1.18 (m, 2H).

HRMS: m/z: found 929.24548 $[M+H]^+$, calculated 929.24566 $[M+H]^+$

14-(4-((S)-1-((1S,5R,6R)-10-((3,5-dichlorophenyl)sulfonyl)-2-oxo-5-vinyl-3,10-diazabicyclo[4.3.1]decan-3-yl)ethyl)-1H-1,2,3-triazol-1-yl)-N-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)-3,6,9,12-tetraoxatetradecan-1-amide (MTQ516)



MTQ516

MTQ516 was synthesized from **45b** (4 mg, 0.01 mmol) and **44d** (5 mg, 0. 010 mmol) according to the General Method G. Purification by flash column chromatography (CH → EA:MeCN = 90:10) gave a white solid (9 mg, 88%).

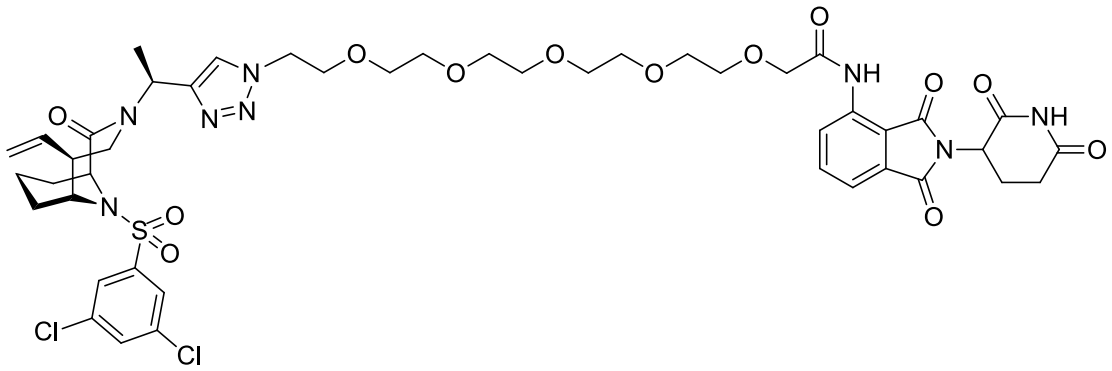
TLC [DCM:MeOH 97:3]: R_f = 0.24.

HPLC [0-100% Solvent B, 20 min]: R_t = 15.82 min, purity (254 nm) ≥ 99%.

^{1}H NMR (500 MHz, Chloroform-*d*) δ 10.51 (s, 1H), 8.94 – 8.70 (m, 2H), 7.74 – 7.70 (m, 1H), 7.69 – 7.67 (m, 2H), 7.64 (d, J = 6.5 Hz, 1H), 7.59 – 7.56 (m, 1H), 7.55 – 7.52 (m, 1H), 6.12 (p, J = 7.1 Hz, 1H), 5.73 (dtd, J = 17.1, 9.4, 4.2 Hz, 1H), 5.16 – 5.03 (m, 2H), 5.02 – 4.95 (m, 1H), 4.71 (t, J = 5.2 Hz, 1H), 4.60 – 4.47 (m, 2H), 4.24 – 4.14 (m, 2H), 3.96 (t, J = 5.4 Hz, 1H), 3.88 (t, J = 4.9 Hz, 2H), 3.80 (s, 4H), 3.71 – 3.67 (m, 2H), 3.65 – 3.57 (m, 6H), 3.45 (ddd, J = 15.1, 10.7, 4.6 Hz, 1H), 3.08 (t, J = 13.4 Hz, 1H), 2.93 – 2.76 (m, 3H), 2.53 (q, J = 8.9 Hz, 1H), 2.30 (d, J = 13.4 Hz, 1H), 2.21 – 2.11 (m, 1H), 1.63 (d, J = 7.0 Hz, 3H), 1.57 – 1.51 (m, 3H), 1.35 – 1.28 (m, 1H), 1.25 – 1.18 (m, 1H).

HRMS: m/z: found 973.27224 $[M+H]^+$, calculated 973.27187 $[M+H]^+$

17-(4-((S)-1-((1S,5R,6R)-10-((3,5-dichlorophenyl)sulfonyl)-2-oxo-5-vinyl-3,10-diazabicyclo[4.3.1]decan-3-yl)ethyl)-1H-1,2,3-triazol-1-yl)-N-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)-3,6,9,12,15-pentaoxaheptadecan-1-amide (MTQ517)



MTQ517

MTQ517 was synthesized from **45b** (4 mg, 0.01 mmol) and **44e** (6 mg, 0. 010 mmol) according to the General Method G. Purification by flash column chromatography (CH → EA:MeCN = 90:10) gave a white solid (9 mg, 86%).

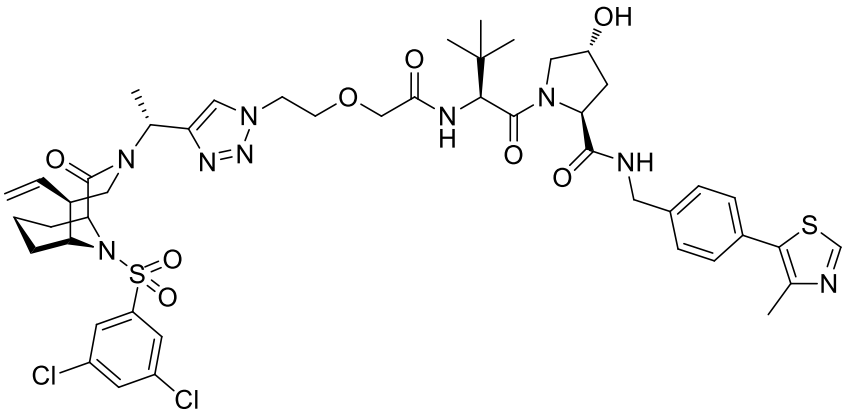
TLC [DCM:MeOH 97:3]: R_f = 0.24.

HPLC [0-100% Solvent B, 20 min]: R_t = 15.87 min, purity (254 nm) ≥ 99%.

^{1}H NMR (500 MHz, Chloroform-*d*) δ 10.50 (s, 1H), 8.85 (d, J = 8.5 Hz, 2H), 7.74 – 7.70 (m, 1H), 7.68 (d, J = 1.8 Hz, 2H), 7.64 (s, 1H), 7.59 – 7.55 (m, 1H), 7.54 (q, J = 1.8 Hz, 1H), 6.12 (q, J = 7.0 Hz, 1H), 5.80 – 5.66 (m, 1H), 5.16 – 5.05 (m, 2H), 5.03 – 4.94 (m, 1H), 4.75 – 4.67 (m, 1H), 4.59 – 4.46 (m, 2H), 4.19 (d, J = 3.7 Hz, 2H), 3.96 (t, J = 5.3 Hz, 1H), 3.87 (t, J = 5.2 Hz, 2H), 3.80 (s, 4H), 3.71 – 3.58 (m, 12H), 3.49 – 3.40 (m, 1H), 3.11 – 3.05 (m, 1H), 2.93 – 2.74 (m, 3H), 2.53 (q, J = 9.5 Hz, 1H), 2.31 (d, J = 13.4 Hz, 1H), 2.20 – 2.11 (m, 1H), 1.64 – 1.62 (m, 3H), 1.57 – 1.51 (m, 3H), 1.35 – 1.29 (m, 1H), 1.25 – 1.19 (m, 1H).

HRMS: m/z: found 1017.29857 $[M+H]^+$, calculated 1017.29809 $[M+H]^+$

(2S,4R)-1-((S)-2-(2-(2-(4-((R)-1-((1S,5R,6R)-10-((3,5-dichlorophenyl)sulfonyl)-2-oxo-5-vinyl-3,10-diazabicyclo[4.3.1]decan-3-yl)ethyl)-1H-1,2,3-triazol-1-yl)ethoxy)acetamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (MTQ518)



MTQ518

MTQ518 was synthesized from **45c** (4 mg, 0.01 mmol) and **43a** (6 mg, 0. 010 mmol) according to the General Method G. Purification by flash column chromatography (0% to 7% MeOH in DCM) gave a white solid (6 mg, 64%).

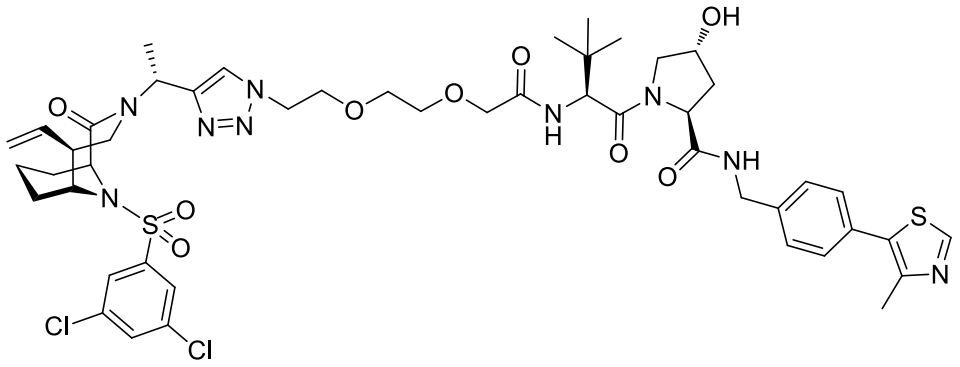
TLC [DCM:MeOH 93:7]: R_f = 0.29.

HPLC [0-100% Solvent B, 20 min]: R_t = 15.04 min, purity (254 nm) ≥ 99%.

^{1}H NMR (300 MHz, Chloroform-*d*) δ 8.67 (s, 1H), 7.76 (t, J = 6.1 Hz, 1H), 7.68 (d, J = 1.8 Hz, 2H), 7.63 (s, 1H), 7.56 (t, J = 1.8 Hz, 1H), 7.38 (s, 4H), 6.96 (d, J = 9.2 Hz, 1H), 6.23 (q, J = 7.0 Hz, 1H), 5.66 (ddd, J = 17.0, 10.1, 8.6 Hz, 1H), 4.98 (dd, J = 10.1, 1.1 Hz, 1H), 4.89 (d, J = 17.0 Hz, 1H), 4.77 (t, J = 7.9 Hz, 1H), 4.72 (d, J = 6.0 Hz, 1H), 4.68 – 4.48 (m, 5H), 4.46 – 4.37 (m, 1H), 4.06 (d, J = 14.7 Hz, 1H), 3.98 – 3.77 (m, 5H), 3.75 – 3.65 (m, 2H), 3.13 (dd, J = 14.1, 1.5 Hz, 1H), 2.84 (s, 1H), 2.57 – 2.39 (m, 5H), 2.32 – 2.12 (m, 2H), 1.54 – 1.43 (m, 3H), 1.41 (d, J = 7.1 Hz, 3H), 1.34 – 1.26 (m, 2H), 0.97 (s, 9H).

HRMS: m/z: found 998.32293 $[M+H]^+$, calculated 998.32213 $[M+H]^+$

(2S,4R)-1-((S)-2-(2-(2-(2-(4-((R)-1-((1S,5R,6R)-10-((3,5-dichlorophenyl)sulfonyl)-2-oxo-5-vinyl-3,10-diazabicyclo[4.3.1]decan-3-yl)ethyl)-1H-1,2,3-triazol-1-yl)ethoxy)ethoxy)acetamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (MTQ519)



MTQ519

MTQ519 was synthesized from **45c** (4 mg, 0.01 mmol) and **43b** (6 mg, 0. 010 mmol) according to the General Method G. Purification by flash column chromatography (0% to 7% MeOH in DCM) gave a white solid (5 mg, 47%).

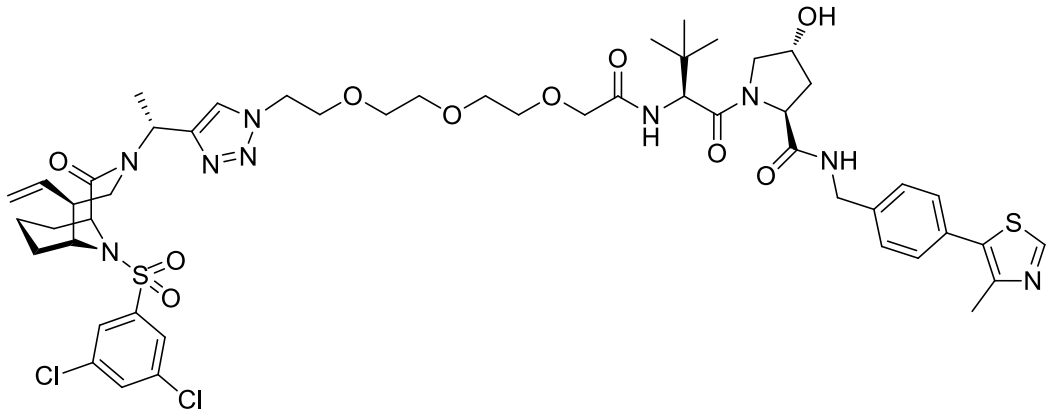
TLC [DCM:MeOH 93:7]: R_f = 0.33.

HPLC [0-100% Solvent B, 20 min]: R_t = 15.11 min, purity (254 nm) ≥ 99%.

^{1}H NMR (300 MHz, Chloroform-*d*) δ 8.66 (s, 1H), 8.16 (s, 1H), 7.92 (t, J = 5.9 Hz, 1H), 7.66 (d, J = 1.9 Hz, 2H), 7.55 (t, J = 1.8 Hz, 1H), 7.45 – 7.35 (m, 1H), 7.27 (d, J = 1.2 Hz, 4H), 5.85 (q, J = 7.0 Hz, 1H), 5.57 (ddd, J = 17.8, 10.2, 8.4 Hz, 1H), 4.92 (d, J = 10.3 Hz, 1H), 4.77 – 4.61 (m, 4H), 4.60 – 4.42 (m, 4H), 4.34 (dd, J = 15.0, 5.6 Hz, 1H), 4.04 – 3.83 (m, 5H), 3.80 – 3.47 (m, 7H), 3.03 (d, J = 14.4 Hz, 1H), 2.49 (s, 3H), 2.42 – 2.23 (m, 3H), 2.15 – 2.04 (m, 1H), 1.46 (d, J = 7.1 Hz, 3H), 1.40 – 1.25 (m, 5H), 1.05 (s, 9H).

HRMS: m/z: found 1042.34904 $[M+H]^+$, calculated 1042.34835 $[M+H]^+$

(2S,4R)-1-((S)-2-(tert-butyl)-14-(4-((R)-1-((1S,5R,6R)-10-((3,5-dichlorophenyl)sulfonyl)-2-oxo-5-vinyl-3,10-diazabicyclo[4.3.1]decan-3-yl)ethyl)-1H-1,2,3-triazol-1-yl)-4-oxo-6,9,12-trioxa-3-azatetradecan-1-oyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (MTQ520)



MTQ520

MTQ520 was synthesized from **45c** (4 mg, 0.01 mmol) and **43c** (7 mg, 0. 010 mmol) according to the General Method G. Purification by flash column chromatography (0% to 7% MeOH in DCM) gave a white solid (7 mg, 63%).

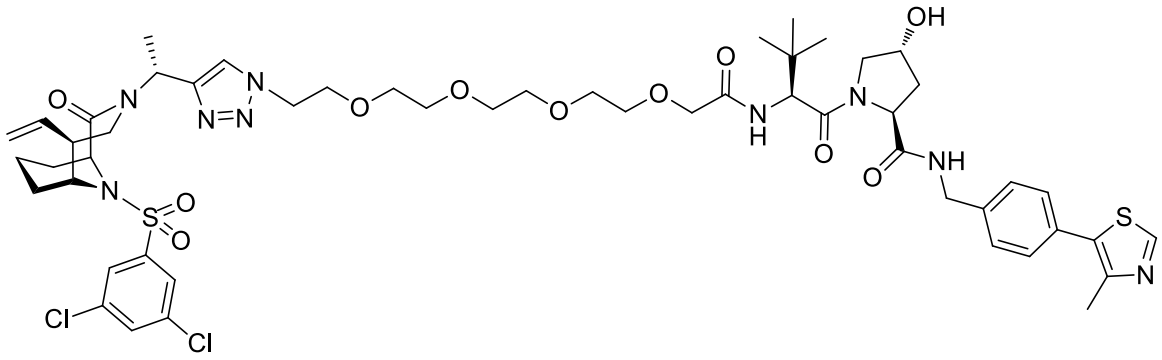
TLC [DCM:MeOH 93:7]: R_f = 0.29.

HPLC [0-100% Solvent B, 20 min]: R_t = 15.12 min, purity (254 nm) ≥ 99%.

^{1}H NMR (300 MHz, Chloroform-*d*) δ 8.67 (s, 1H), 7.71 (s, 1H), 7.68 (d, J = 1.8 Hz, 2H), 7.56 (t, J = 1.8 Hz, 1H), 7.47 (t, J = 5.7 Hz, 1H), 7.39 – 7.29 (m, 4H), 7.20 (d, J = 9.0 Hz, 1H), 6.10 (q, J = 7.2 Hz, 1H), 5.62 (ddd, J = 17.1, 10.2, 8.9 Hz, 1H), 4.98 – 4.90 (m, 1H), 4.80 (d, J = 16.9 Hz, 1H), 4.76 – 4.67 (m, 2H), 4.60 – 4.45 (m, 5H), 4.43 – 4.33 (m, 1H), 4.10 – 3.99 (m, 3H), 3.96 – 3.78 (m, 3H), 3.71 – 3.58 (m, 10H), 3.14 (s, 1H), 2.98 (dd, J = 14.3, 1.4 Hz, 1H), 2.56 – 2.43 (m, 4H), 2.40 – 2.25 (m, 2H), 2.21 – 2.09 (m, 1H), 1.55 – 1.38 (m, 6H), 1.30 – 1.23 (m, 2H), 0.96 (s, 9H).

HRMS: m/z: found 1086.37535 $[M+H]^+$, calculated 1086.37456 $[M+H]^+$

(2S,4R)-1-((S)-2-(tert-butyl)-17-(4-((R)-1-((1S,5R,6R)-10-((3,5-dichlorophenyl)sulfonyl)-2-oxo-5-vinyl-3,10-diazabicyclo[4.3.1]decan-3-yl)ethyl)-1H-1,2,3-triazol-1-yl)-4-oxo-6,9,12,15-tetraoxa-3-azaheptadecan-1-oyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (MTQ521)



MTQ521

MTQ521 was synthesized from **45c** (4 mg, 0.01 mmol) and **43d** (7 mg, 0. 010 mmol) according to the General Method G. Purification by flash column chromatography (0% to 7% MeOH in DCM) gave a white solid (8 mg, 68%).

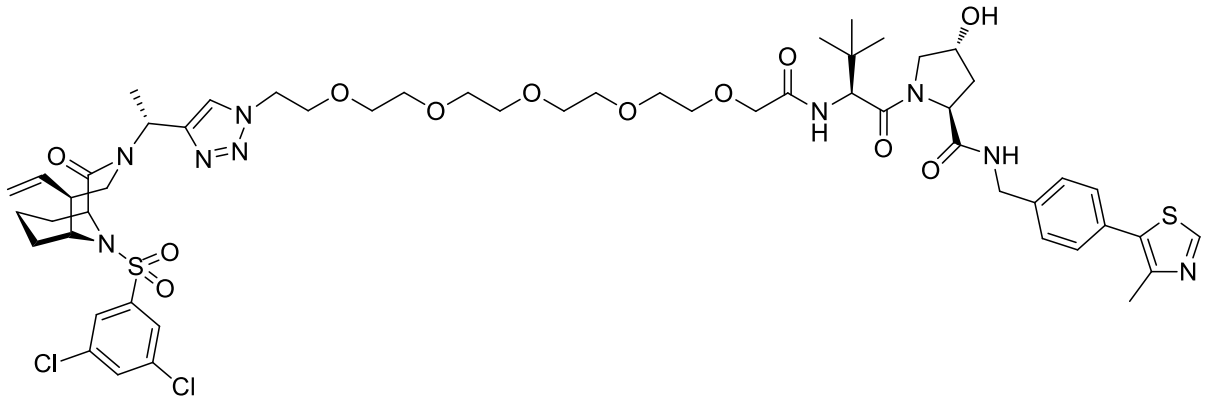
TLC [DCM:MeOH 93:7]: R_f = 0.21.

HPLC [0-100% Solvent B, 20 min]: R_t = 15.12 min, purity (254 nm) ≥ 99%.

^{1}H NMR (300 MHz, Chloroform-*d*) δ 8.67 (s, 1H), 7.75 (s, 1H), 7.69 (d, J = 1.8 Hz, 2H), 7.60 (t, J = 5.9 Hz, 1H), 7.55 (t, J = 1.8 Hz, 1H), 7.37 (s, 4H), 7.17 (d, J = 8.8 Hz, 1H), 6.19 (q, J = 7.0 Hz, 1H), 5.67 – 5.53 (m, 1H), 4.92 (dd, J = 10.0, 1.1 Hz, 1H), 4.84 – 4.69 (m, 3H), 4.60 – 4.36 (m, 6H), 4.07 – 3.81 (m, 5H), 3.80 – 3.70 (m, 2H), 3.69 – 3.56 (m, 13H), 2.90 (dd, J = 14.3, 1.5 Hz, 1H), 2.58 – 2.40 (m, 4H), 2.39 – 2.22 (m, 2H), 2.20 – 2.06 (m, 1H), 1.57 – 1.33 (m, 6H), 1.32 – 1.24 (m, 2H), 0.95 (s, 9H).

HRMS: m/z: found 1130.39944 $[M+H]^+$, calculated 1130.40078 $[M+H]^+$

(2S,4R)-1-((S)-2-(tert-butyl)-20-(4-((R)-1-((1S,5R,6R)-10-((3,5-dichlorophenyl)sulfonyl)-2-oxo-5-vinyl-3,10-diazabicyclo[4.3.1]decan-3-yl)ethyl)-1H-1,2,3-triazol-1-yl)-4-oxo-6,9,12,15,18-pentaoxa-3-azaicosan-1-oyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (MTQ522)



MTQ522

MTQ522 was synthesized from **45c** (4 mg, 0.01 mmol) and **43e** (7 mg, 0. 010 mmol) according to the General Method G. Purification by flash column chromatography (0% to 7% MeOH in DCM) gave a white solid (7 mg, 62%).

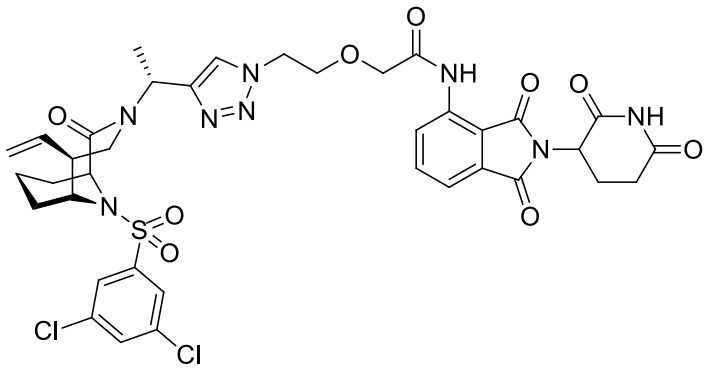
TLC [DCM:MeOH 93:7]: R_f = 0.24.

HPLC [0-100% Solvent B, 20 min]: R_t = 15.10 min, purity (254 nm) ≥ 99%.

^{1}H NMR (300 MHz, Chloroform-*d*) δ 8.70 (s, 1H), 7.72 (d, J = 1.8 Hz, 2H), 7.69 (s, 1H), 7.58 (t, J = 1.8 Hz, 1H), 7.50 – 7.33 (m, 5H), 7.24 (d, J = 8.3 Hz, 1H), 6.18 (q, J = 7.0 Hz, 1H), 5.64 (ddd, J = 16.9, 10.0, 8.7 Hz, 1H), 4.96 (dd, J = 10.2, 1.1 Hz, 1H), 4.90 – 4.71 (m, 3H), 4.63 – 4.46 (m, 5H), 4.46 – 4.34 (m, 1H), 4.13 – 4.06 (m, 1H), 4.05 – 3.92 (m, 3H), 3.90 – 3.78 (m, 2H), 3.73 – 3.56 (m, 18H), 3.27 (d, J = 4.3 Hz, 1H), 3.06 – 2.90 (m, 1H), 2.67 – 2.48 (m, 4H), 2.47 – 2.29 (m, 2H), 2.20 – 2.09 (m, 1H), 1.62 – 1.42 (m, 6H), 1.37 – 1.28 (m, 2H), 0.98 (s, 9H).

HRMS: m/z: found 1174.42451 $[M+H]^+$, calculated 1174.42699 $[M+H]^+$

2-(2-(4-((R)-1-((1S,5R,6R)-10-((3,5-dichlorophenyl)sulfonyl)-2-oxo-5-vinyl-3,10-diazabicyclo[4.3.1]decan-3-yl)ethyl)-1H-1,2,3-triazol-1-yl)ethoxy)-N-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)acetamide (MTQ523)



MTQ523

MTQ523 was synthesized from **45c** (4 mg, 0.01 mmol) and **44a** (4 mg, 0. 010 mmol) according to the General Method G. Purification by flash column chromatography (CH → EA:MeCN = 90:10) gave a white solid (7 mg, 80%).

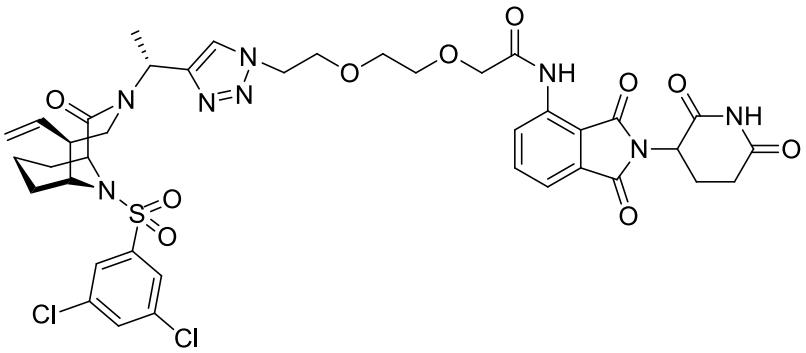
TLC [DCM:MeOH 97:3]: R_f = 0.39.

HPLC [0-100% Solvent B, 20 min]: R_t = 15.71 min, purity (254 nm) ≥ 99%.

^{1}H NMR (300 MHz, Chloroform-*d*) (diastereomers mixture) δ 10.49 (s, 0.5H), 10.35 (s, 0.5H), 8.83 (dd, J = 8.4, 2.3 Hz, 1H), 8.41 (s, 1H, br), 8.01 (s, 0.5H), 7.82 (s, 0.5H), 7.77 – 7.68 (m, 3H), 7.61 (d, J = 7.3 Hz, 1H), 7.59 – 7.51 (m, 1H), 6.12 (q, J = 7.2 Hz, 0.5H), 5.99 (q, J = 7.4 Hz, 0.5H), 5.83 – 5.59 (m, 1H), 5.22 – 4.82 (m, 3H), 4.77 – 4.63 (m, 3H), 4.14 – 3.90 (m, 5H), 3.79 – 3.62 (m, 1.5H), 3.28 (dd, J = 14.6, 1.2 Hz, 0.5H), 2.97 – 2.66 (m, 3H), 2.42 – 2.15 (m, 3H), 1.54 – 1.48 (m, 3H), 1.47 – 1.34 (m, 3H), 1.29 – 1.19 (m, 2H).

HRMS: m/z: found 841.19364 $[M+H]^+$, calculated 841.19323 $[M+H]^+$

2-(2-(2-(4-((R)-1-((1S,5R,6R)-10-((3,5-dichlorophenyl)sulfonyl)-2-oxo-5-vinyl-3,10-diazabicyclo[4.3.1]decan-3-yl)ethyl)-1H-1,2,3-triazol-1-yl)ethoxy)ethoxy)-N-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)acetamide (MTQ524)



MTQ524

MTQ524 was synthesized from **45c** (4 mg, 0.01 mmol) and **44b** (4 mg, 0. 010 mmol) according to the General Method G. Purification by flash column chromatography (CH → EA:MeCN = 90:10) gave a white solid (4 mg, 50%).

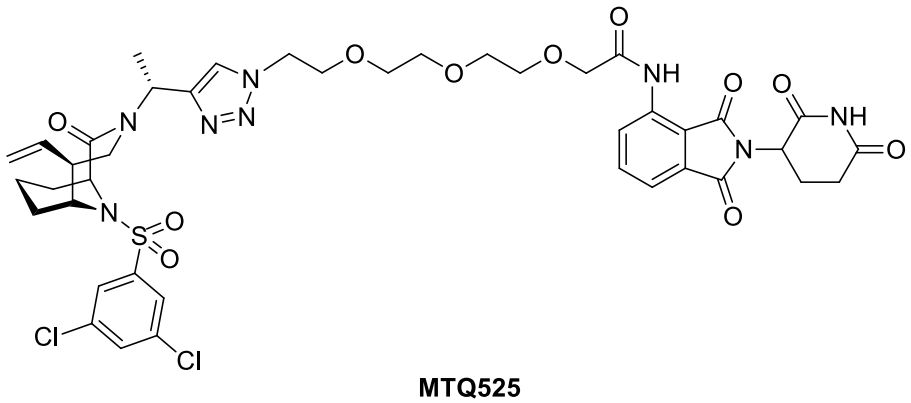
TLC [DCM:MeOH 97:3]: R_f = 0.32.

HPLC [0-100% Solvent B, 20 min]: R_t = 15.93 min, purity (254 nm) ≥ 99%.

^{1}H NMR (300 MHz, Chloroform-*d*) δ 10.43 (s, 1H), 8.87 (d, J = 8.3 Hz, 1H), 8.62 (s, 1H), 7.77 – 7.71 (m, 1H), 7.70 (t, J = 1.9 Hz, 2H), 7.63 – 7.57 (m, 2H), 7.56 (t, J = 1.8 Hz, 1H), 6.15 – 6.00 (m, 1H), 5.72 – 5.55 (m, 1H), 5.03 – 4.91 (m, 2H), 4.84 (d, J = 17.0 Hz, 1H), 4.74 (d, J = 5.4 Hz, 1H), 4.60 – 4.48 (m, 2H), 4.20 – 4.12 (m, 2H), 3.98 – 3.89 (m, 3H), 3.81 – 3.73 (m, 4H), 3.66 (dd, J = 14.2, 10.6 Hz, 1H), 3.03 (d, J = 14.0 Hz, 1H), 2.93 – 2.73 (m, 3H), 2.47 – 2.27 (m, 2H), 2.22 – 2.12 (m, 1H), 1.53 – 1.40 (m, 6H), 1.29 – 1.19 (m, 2H).

HRMS: m/z: found 885.21980 $[M+H]^+$, calculated 885.21944 $[M+H]^+$

2-(2-(2-(2-(4-((R)-1-((1S,5R,6R)-10-((3,5-dichlorophenyl)sulfonyl)-2-oxo-5-vinyl-3,10-diazabicyclo[4.3.1]decan-3-yl)ethyl)-1H-1,2,3-triazol-1-yl)ethoxy)ethoxy)ethoxy)-N-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)acetamide (MTQ525)



MTQ525

MTQ525 was synthesized from **45c** (4 mg, 0.01 mmol) and **44c** (5 mg, 0. 010 mmol) according to the General Method G. Purification by flash column chromatography (CH → EA:MeCN = 90:10) gave a white solid (7 mg, 72%).

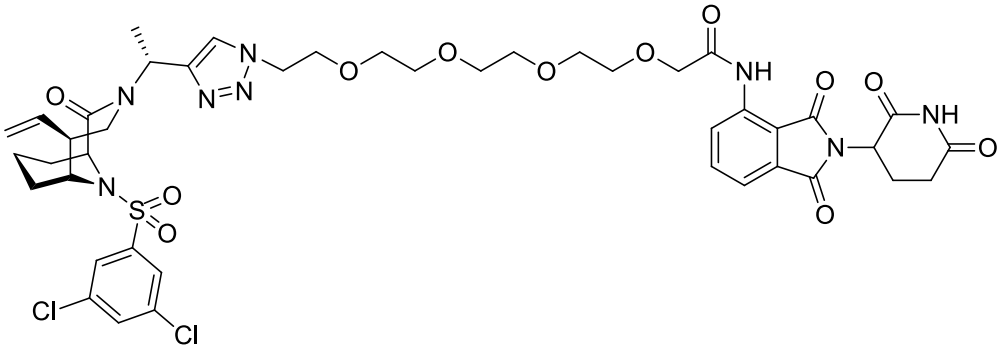
TLC [DCM:MeOH 97:3]: R_f = 0.33.

HPLC [0-100% Solvent B, 20 min]: R_t = 15.90 min, purity (254 nm) ≥ 99%.

^{1}H NMR (300 MHz, Chloroform-*d*) δ 10.44 (s, 1H), 8.86 (d, J = 8.4 Hz, 1H), 8.51 (d, J = 10.7 Hz, 1H), 7.77 – 7.68 (m, 3H), 7.65 (d, J = 1.6 Hz, 1H), 7.61 – 7.52 (m, 2H), 6.17 – 6.06 (m, 1H), 5.71 – 5.55 (m, 1H), 5.01 – 4.91 (m, 2H), 4.83 (d, J = 17.2 Hz, 1H), 4.78 – 4.72 (m, 1H), 4.55 – 4.43 (m, 2H), 4.21 (s, 2H), 3.97 – 3.90 (m, 1H), 3.87 – 3.80 (m, 4H), 3.78 – 3.73 (m, 2H), 3.68 – 3.55 (m, 5H), 3.03 (d, J = 14.1 Hz, 1H), 2.95 – 2.75 (m, 3H), 2.45 – 2.27 (m, 2H), 2.21 – 2.12 (m, 1H), 1.55 – 1.39 (m, 6H), 1.31 – 1.21 (m, 2H).

HRMS: m/z: found 929.24586 $[M+H]^+$, calculated 929.24566 $[M+H]^+$

14-(4-((R)-1-((1S,5R,6R)-10-((3,5-dichlorophenyl)sulfonyl)-2-oxo-5-vinyl-3,10-diazabicyclo[4.3.1]decan-3-yl)ethyl)-1H-1,2,3-triazol-1-yl)-N-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)-3,6,9,12-tetraoxatetradecan-1-amide (MTQ526)



MTQ526

MTQ526 was synthesized from **45c** (4 mg, 0.01 mmol) and **44d** (5 mg, 0. 010 mmol) according to the General Method G. Purification by flash column chromatography (CH → EA:MeCN = 90:10) gave a white solid (6 mg, 61%).

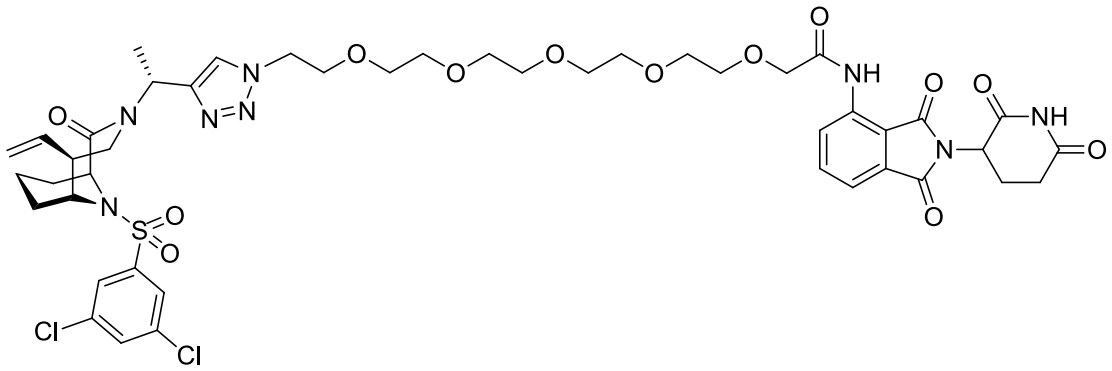
TLC [DCM:MeOH 97:3]: R_f = 0.27.

HPLC [0-100% Solvent B, 20 min]: R_t = 15.92 min, purity (254 nm) ≥ 99%.

^{1}H NMR (300 MHz, Chloroform-*d*) δ 10.48 (s, 1H), 8.85 (d, J = 7.9 Hz, 1H), 8.65 (d, J = 9.1 Hz, 1H), 7.76 – 7.72 (m, 1H), 7.70 (d, J = 1.8 Hz, 2H), 7.66 (s, 1H), 7.58 (dd, J = 7.3, 0.8 Hz, 1H), 7.55 (td, J = 1.8, 1.2 Hz, 1H), 6.13 (q, J = 7.1 Hz, 1H), 5.71 – 5.55 (m, 1H), 5.00 – 4.91 (m, 2H), 4.87 – 4.74 (m, 2H), 4.51 (t, J = 5.0 Hz, 2H), 4.20 (d, J = 1.0 Hz, 2H), 3.97 – 3.90 (m, 1H), 3.88 – 3.77 (m, 6H), 3.71 – 3.65 (m, 3H), 3.64 – 3.57 (m, 6H), 3.07 – 2.97 (m, 1H), 2.94 – 2.73 (m, 3H), 2.44 – 2.28 (m, 2H), 2.22 – 2.13 (m, 1H), 1.55 – 1.39 (m, 6H), 1.29 – 1.20 (m, 2H).

HRMS: m/z: found 973.27295 $[M+H]^+$, calculated 973.27187 $[M+H]^+$

17-(4-((R)-1-((1S,5R,6R)-10-((3,5-dichlorophenyl)sulfonyl)-2-oxo-5-vinyl-3,10-diazabicyclo[4.3.1]decan-3-yl)ethyl)-1H-1,2,3-triazol-1-yl)-N-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)-3,6,9,12,15-pentaoxaheptadecan-1-amide (MTQ527)



MTQ527

MTQ527 was synthesized from **45c** (4 mg, 0.01 mmol) and **44e** (6 mg, 0. 010 mmol) according to the General Method G. Purification by flash column chromatography (CH → EA:MeCN = 90:10) gave a white solid (6 mg, 58%).

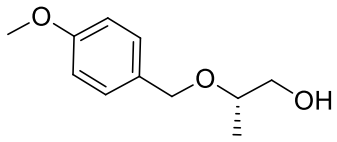
TLC [DCM:MeOH 97:3]: R_f = 0.27.

HPLC [0-100% Solvent B, 20 min]: R_t = 15.88 min, purity (254 nm) ≥ 99%.

^{1}H NMR (300 MHz, Chloroform-*d*) δ 10.49 (s, 1H), 8.86 (d, J = 8.4 Hz, 1H), 8.73 (d, J = 9.9 Hz, 1H), 7.76 – 7.68 (m, 3H), 7.65 (s, 1H), 7.60 – 7.53 (m, 2H), 6.14 (q, J = 6.9 Hz, 1H), 5.71 – 5.54 (m, 1H), 5.00 – 4.89 (m, 2H), 4.86 – 4.74 (m, 2H), 4.54 – 4.46 (m, 2H), 4.19 (d, J = 1.0 Hz, 2H), 3.97 – 3.90 (m, 1H), 3.86 – 3.77 (m, 6H), 3.70 – 3.56 (m, 13H), 3.04 – 2.71 (m, 4H), 2.42 – 2.27 (m, 2H), 2.20 – 2.11 (m, 1H), 1.54 – 1.41 (m, 6H), 1.30 – 1.21 (m, 2H).

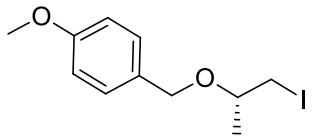
HRMS: m/z: found 1017.29876 $[M+H]^+$, calculated 1017.29809 $[M+H]^+$

(S)-2-((4-methoxybenzyl)oxy)propan-1-ol (98)



The preparation of **98** followed a procedure described in literatures[109, 110].

(S)-1-(((1-iodopropan-2-yl)oxy)methyl)-4-methoxybenzene(99)



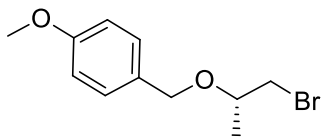
99

To a stirred solution of **98** (0.50 g, 2.55 mmol) in DCM (25 mL) was added imidazole (502 mg, 7.65 mmol) and Ph_3P (802 mg, 3.06 mmol). After cooling to 0°C, iodine (842 mg, 3.32 mmol) was added and the mixture was allowed to warm to room temperature. The mixture was stirred at rt in the dark for 24h. Then 20 ml 1.75M Na_2SO_3 aqueous solution was added and the mixture was stirred for 30 min at RT. The aqueous phase was extracted with DCM (20 ml x 3) and the combined organic phase was dried over $MgSO_4$. After filteration, the filtrate was concentrated under reduced pressure and the residue was purified by flash column chromatography (CH → CH:EA = 90:10) to give **99** (602 mg, 1.9 mmol, 77%) as yellow oil.

^{1}H NMR (300 MHz, Chloroform-*d*) δ 7.27 – 7.17 (m, 2H), 6.86 – 6.75 (m, 2H), 4.49 – 4.32 (m, 2H), 3.72 (s, 3H), 3.42 (m, 1H), 3.24 – 3.10 (m, 2H), 1.21 (d, J = 6.1 Hz, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 159.40, 130.33, 129.46, 113.96, 73.76, 70.70, 55.41, 20.46, 11.63.

(S)-1-(((1-bromopropan-2-yl)oxy)methyl)-4-methoxybenzene(100)



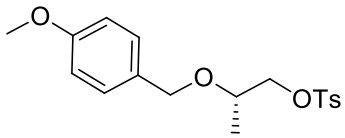
100

Carbon tetrabromide (1.0 g, 3.1 mmol) was added to a solution of **98** (0.5 g, 2.55 mmol) in DCM (25 mL). The resulting solution was cooled to 0°C and Ph_3P (802 mg, 3.1 mmol) was added. The reaction mixture was stirred at room temperature for 24h. The solvent was removed under reduced pressure and the residue was purified by flash column chromatography (CH → CH:EA = 90:10) to give **100** (460 mg, 1.8 mmol, 70%) as yellow oil.

^{1}H NMR (300 MHz, Chloroform-*d*) δ 7.32 (d, *J* = 7.9 Hz, 2H), 6.91 (d, *J* = 7.9 Hz, 2H), 3.83 (s, 3H), 3.75 (q, *J* = 5.6 Hz, 1H), 3.53 – 3.33 (m, 2H), 1.33 (d, *J* = 6.0 Hz, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 159.38, 130.33, 129.40, 113.94, 73.96, 70.87, 55.37, 36.61, 19.02.

(S)-1-(((1-bromopropan-2-yl)oxy)methyl)-4-methoxybenzene (101)



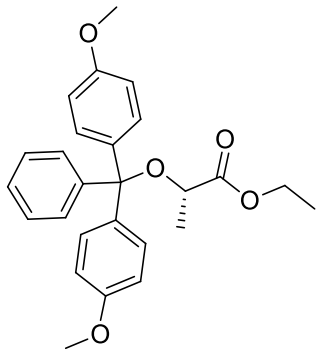
101

To a solution of **98** (161 mg, 0.82 mmol), Et_3N (342 μL, 2.46 mmol), and DMAP (20 mg, 0.16 mmol) in DCM (4 mL) was added p-toluenesulfonyl chloride (234 mg, 1.23 mmol) at 0°C and the reaction mixture was stirred at RT for 24h. Then 5 ml water was added and the mixture was extracted with DCM (15 ml x 3) and the combined organic phase was dried over $MgSO_4$. After filteration, the filtrate was concentrated under reduced pressure and the residue was purified by flash column chromatography (CH → CH:EA = 90:10) to give **101** (205 mg, 0.59 mmol, 71%) as yellow oil.

^{1}H NMR (300 MHz, Chloroform-*d*) δ 7.78 (d, *J* = 8.3 Hz, 2H), 7.31 (d, *J* = 8.2 Hz, 2H), 7.19 (d, *J* = 8.5 Hz, 2H), 6.85 (d, *J* = 8.6 Hz, 2H), 4.51 – 4.29 (m, 2H), 4.05 – 3.92 (m, 2H), 3.79 (s, 3H), 3.74 (td, *J* = 6.2, 4.7 Hz, 1H), 2.43 (s, 3H), 1.14 (d, *J* = 6.4 Hz, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 159.28, 144.82, 133.03, 130.21, 129.86, 129.26, 127.96, 113.82, 72.77, 72.03, 71.00, 55.31, 21.66, 16.82.

(S)-ethyl 2-(bis(4-methoxyphenyl)(phenyl)methoxy)propanoate (DMT-1)



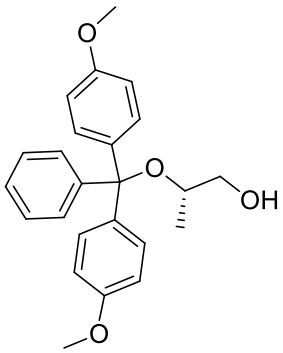
20 mL Pyridine was added to a mixture of 4,4′-dimethoxytrityl chloride (3.3 g, 1 mmol) and (−)-ethyl L-lactate (11.8 g, 10 mmol) at RT. The mixture was stirred at RT for 16h. Then 20 ml sat. $NaHCO_3$ and 300 ml EA were added and the mixture was washed with brine (30 ml x 3). The organic phase was dried over MgSO4 and the solvent was removed under reduced pressure. The residue was purified by flash column chromatography (CH → CH:EA = 90:10) to give **DMT-1** (3.96 g, 95%) as yellow oil.

^{1}H NMR (300 MHz, Chloroform-*d*) δ 7.40 (d, *J* = 7.6 Hz, 2H), 7.28 (d, *J* = 8.6 Hz, 4H), 7.22 – 7.11 (m, 3H), 6.73 (dd, *J* = 8.7, 2.6 Hz, 4H), 4.12 – 4.05 (m, 1H), 3.70 (s, 6H), 3.62 (q, *J* = 7.1 Hz, 2H), 1.27 (d, *J* = 6.7 Hz, 3H), 0.98 (t, *J* = 7.1 Hz, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 173.69, 158.59, 145.02, 136.25, 136.07, 130.52, 130.46, 128.47, 127.72, 126.86, 113.01, 112.97, 69.47, 60.21, 55.20, 26.92, 20.07, 13.90.

MS (ESI+): m/z: found 442.97 $[M+Na]^+$, calculated 443.18 $[M+Na]^+$

(S)-2-(bis(4-methoxyphenyl)(phenyl)methoxy)propan-1-ol (DMT-2)

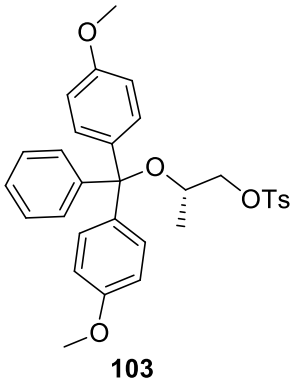


$LiAlH_4$ solution (1M in THF, 11.1 ml, 11.1 mmol) was added slowly to a stirred solution of **DMT-1** (3.9 g, 9.28 mmol) in 50 mL of THF at 0°C under argon. After 30min, the reaction mixture was allowed to warm to room temperature and the mixture was stirred at rt for 1h. After it was cooled back to 0°C, the reaction was quenched by the sequential addition of water (0.46 mL), 15% NaOH aqueous solution (0.46 mL) and water (1.4 mL). After allowing the mixture to warm to room temperature, the white solid was removed by filtration while washing with Et_2O. The filtrate was washed with saturated aqueous NaCl, dried, and concentrated. The crude product was purified by flash chromatography on silica gel with EtOAc/CH (4/1) as eluents to give **DMT-2** as a yellow oil (3.42 g, 98%).

^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.38 – 7.34 (m, 2H), 7.24 – 7.10 (m, 7H), 6.75 (d, J = 8.9 Hz, 4H), 3.92 – 3.84 (m, 1H), 3.71 (s, 6H), 3.70 – 3.67 (m, 1H), 3.05 (dd, J = 9.3, 3.4 Hz, 1H), 2.92 (dd, J = 9.2, 7.8 Hz, 1H), 1.02 (d, J = 6.4 Hz, 3H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 158.66, 145.02, 136.25, 130.19, 128.30, 127.96, 126.94, 113.28, 69.00, 67.25, 55.34, 27.07, 19.15.

(S)-2-(bis(4-methoxyphenyl)(phenyl)methoxy)propyl 4-methylbenzenesulfonate (103)



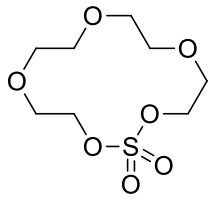
103

To a solution of **DMT-2** (2.18 g, 5.8 mmol), Et3N (2.4 mL, 17.3 mmol), and DMAP (0.14 g, 1.2 mmol) in DCM (50 mL) was added p-toluenesulfonyl chloride (1.7 g, 8.7 mmol) at 0°C and the reaction mixture was stirred at rt overnight. Then 10 ml water was added and the mixture was extracted with DCM (15 ml x 3) and the combined organic phase was dried over $MgSO_4$. After filteration, the filtrate was concentrated under reduced pressure and the residue was purified by flash column chromatography (CH → CH:EA = 85:15) to give **103** (2.1 g, 67%) as yellow oil.

^{1}H NMR (300 MHz, Chloroform-*d*) δ 7.83 (d, *J* = 8.4 Hz, 1H), 7.68 (d, *J* = 8.3 Hz, 1H), 7.42 – 7.19 (m, 11H), 6.87 – 6.77 (m, 4H), 4.07 – 3.99 (m, 1H), 3.81 (m, 7H), 3.46 (d, *J* = 5.1 Hz, 1H), 2.47 (d, *J* = 3.4 Hz, 3H), 1.23 – 0.96 (m, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 158.58, 147.36, 145.53, 139.49, 136.56, 130.20, 129.68, 129.13, 127.91, 127.77, 127.71, 113.17, 86.57, 74.79, 67.08, 55.25, 21.64, 18.75.

1,3,6,9,12-Pentaoxa-2-thiacyclotetradecane 2,2-dioxide (87)



87

Under the atmosphere of Ar, $SOCl_2$ (2.3 mL, 31.20 mmol, in 50 mL DCM) was added over 1 h to a stirring solution of tetraethylene glycol (3.03 g, 15.60 mmol), DIPEA (13.0 mL, 74.88 mmol) and DMAP (0.10 g, 0.78 mmol) in DCM (300 mL) at 0°C. After the addition, the mixture was stirred at 0°C for 1 h and quenched with cold brine (100 mL). The organic layer was collected and the aqueous layer was extracted with DCM (50 mL, twice). The combined organic layer was dried over anhydrous Na_2SO_4, concentrated, purified by flash chromatography on silica gel with EtOAc/CH (1/1) as eluents to give macrocyclic sulfite 1,3,6,9,12-pentaoxa-2-thiacyclotetradecane 2-oxide (2.15 g, 9.2 mmol, 59%) as brown oil.
1,3,6,9,12-pentaoxa-2-thiacyclotetradecane 2-oxide (2.10 g, 8.74 mmol) was dissolved in a mixture of CH_3CN (80 mL), DCM (80 mL) and water (120 mL) at 0°C. $NaIO_4$ (2.29 g, 10.76 mmol) and $RuCl_3{\cdot}3H_2O$ (0.01 g, 0.04 mmol) were sequentially added to the reaction mixture and the resulting mixture was stirred at 0°C for 1 h. The organic layer was collected and the aqueous layer was extracted with DCM (100 mL, twice). The combined organic layer was dried over anhydrous Na_2SO_4, filtrated through a pad of celite, concentrated, purified by flash chromatography on silica gel with EtOAc/CH (1/1) as eluents to give **87** as a white solid (1.78 g, 7.1 mmol, 81%).

TLC [CH/EtOAc=4:6]: Rf= 0.39.

^{1}H NMR (500 MHz, Chloroform-*d*) δ 4.47 – 4.41 (m, 4H), 3.84 – 3.79 (m, 4H), 3.69 – 3.60 (m, 8H).

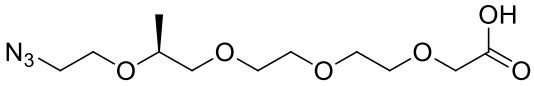
^{13}C NMR (126 MHz, $CDCl_3$) δ 72.28, 70.82, 70.78, 68.53.

General Method I for Solid Phase Synthesis of PEG linkers

Wang resin (1 eq.) was swelled with THF, then tBuOK (1.5 eq.) was added. After stirring for 30 min, a cyclic sulfite (2 eq.) was added and stirred overnight, after which the resin was washed with water and THF. A solution of TsOH/dioxane/H_2O (1 mmol/19 ml/ 1 ml) was added to the resin and the mixture was stirred at 60°C for 4h followed by washing with THF, DCM and dioxane. The resin was reacted with tBuOK (2 eq.) in dioxane for 30 min, then **103** (2.2 eq.) was added and stirred overnight at 60°C. This step was repeated for another 2 times before the DMTr protection group was removed by TsOH/DCM/THF solution. The resulting alcohol was then coupled with cyclic sulfite (2 eq.) in the presence of tBuOK (1.5 eq.) in DMF

and deprotected by TsOH/dioxane/H_2O at 60°C. The resin was swelled with DCM and reacted with TsCl (1.5 eq.), TEA (3 eq.) and DMAP (0.2 eq.) at RT for 9h. Then the resin was washed with THF, DCM, DMF, and reacted with NaN_3 (5 eq.) in DMF) in the presence of 18-crown-6 (cat.) at 60°C. Then the resin was washed with water, DMF, THF and DCM. The azido alcohol was cleaved from the resin by treating with 50% TFA in DCM, followed by the oxidation with Jones reagent (4 eq.) in aceton at 0°C for 4h. The purification by preparative HPLC gave the desired methyl PEG linker.

(S)-14-azido-11-methyl-3,6,9,12-tetraoxatetradecan-1-oic acid (114)



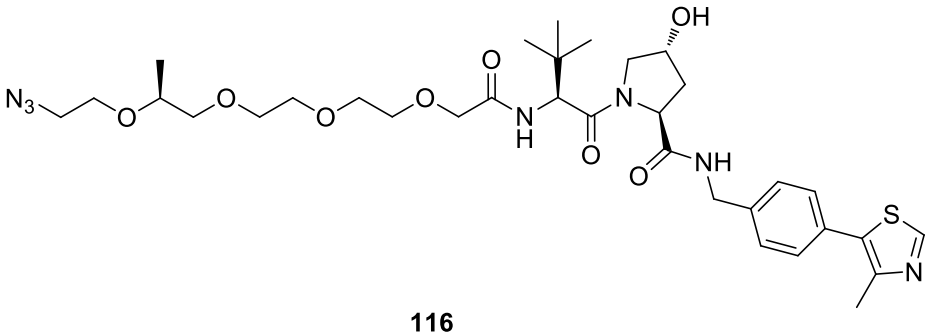
114 (11 mg, 7% for 9 steps) was synthesized from **106**, **103** and **85** sequentially according to the General Method I.

TLC [DCM + 5% MeOH + 1% HCOOH]: R_f= 0.36.

^{1}H NMR (500 MHz, Chloroform-*d*) δ 4.15 (s, 2H), 3.76 (dd, *J* = 5.7, 2.9 Hz, 2H), 3.73 – 3.64 (m, 9H), 3.52 – 3.44 (m, 2H), 3.42 – 3.31 (m, 2H), 1.18 (d, *J* = 6.4 Hz, 3H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 171.68, 75.39, 75.20, 71.49, 70.71, 70.47, 70.10, 69.09, 68.01, 50.96, 16.74.

(2S,4R)-1-((2S,14S)-17-azido-2-(tert-butyl)-14-methyl-4-oxo-6,9,12,15-tetraoxa-3-azaheptadecan-1-oyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (116)



116

(2S,4R)-1-((S)-2-amino-3,3-dimethylbutanoyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide hydrochloride (11 mg, 24 μmol) was added to a solution of PEG-linker **114** (7mg, 24 μmol) in DCM (4 ml). HATU (14 mg, 36 μmol) was added and the pH adjusted to >9 by addition of DIPEA (21 μL, 120 μmol). After stirring overnight at RT the reaction mixture was extracted with water. The organic phase was dried over magnesium sulfate and evaporated to dryness. The crude product was purified by preparative HPLC (30-80% MeCN in H_2O) to afford 116 as a colorless oil (7 mg, 10 μmol, 41%).

TLC [DCM + 5% MeOH]: R_f= 0.22.

^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.84 (s, 1H), 7.30 (s, 5H), 7.22 (d, J = 6.4 Hz, 1H), 4.68 – 4.64 (m, 1H), 4.53 – 4.40 (m, 3H), 4.30 (dd, J = 15.1, 5.2 Hz, 1H), 4.11 – 3.87 (m, 3H), 3.71 – 3.52 (m, 12H), 3.46 – 3.35 (m, 2H), 3.34 – 3.22 (m, 2H), 2.54 – 2.38 (m, 4H), 2.08 (dd, J = 12.6, 7.9 Hz, 1H), 1.09 (d, J = 6.3 Hz, 3H), 0.90 (s, 9H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 171.43, 170.89, 170.82, 151.32, 146.97, 138.77, 129.91, 129.50, 128.25, 75.27, 75.17, 71.00, 70.62, 70.44, 70.29, 70.20, 70.09, 67.98, 58.53, 57.44, 56.77, 50.96, 43.22, 35.95, 34.82, 26.38, 16.75, 15.16.

HPLC [0-100% Solvent B, 20 min]: R_t = 11.61 min, purity (220 nm) ≥ 99%.

MS (ESI+): m/z: found 704.39 $[M+H]^+$, calculated 704.34 $[M+H]^+$

7 References

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