New Approaches toward the Syntheses of Hsp70 Agonists

by

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The thesis presented new syntheses of Hsp70 agonists. Chapter 1 overviewed neurological disorders, the current therapeutic and diagnostic approaches for the diseases. Chapter 2 touched upon heat shock protein (Hsp) and the molecular chaperone, Hsp70. Chapter 3, 4, and 5 presented efforts in the design for more potent analogues than MAL1-271, a Hsp70 agonist. While Chapter 3 showcased the synthesis of the new dihydropyrimidinone (DHPM) molecules, Chapter 4 focused on the preparation for the thiadiazine analogues. All of these analogues were evaluated in a cellular Huntington's disease model in the laboratory of Prof. J. Brodsky at the University of Pittsburgh and resulted in several lead structures. Chapter 5 reviewed the preliminary results on a synthesis of thiadiazine-containing macrocycles. Initial explorations involved the use of ruthenium-based catalysts to construct a 10-membered ring.
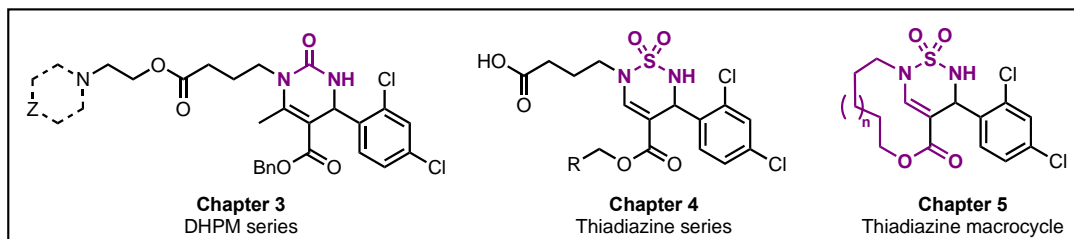
Chapter 3
DHPM series

Chapter 4
Thiadiazine series

Chapter 5
Thiadiazine macrocycle

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List of Abbreviations

4-DMAP...…............   4-Dimethylaminopyridine

ACN...….................   Acetonitrile

AcOH...…...............   Acetic acid

COSY…………...…   Correlation spectroscopy 2D NMR ($^{2\text{-}4}$ J_{HH})

DBAD ...….............   Di-tert-butyl azodicarboxylate

DCC ...…................   N,N'-Dicyclohexylcarbodiimide

DIAD ...…...............   Diisopropyl azodicarboxylate

DMF ...……….........   Dimethyl formamide

EDCl ...……….........   1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide

eq...……....................   Equivalence

Et_2O ...…................   Diethyl ether

Et_3N ...….................   Triethyl amine

EtOAc ...…...............   Ethyl acetate

HFIP ...….................   Hexafluoro-2-propanol

HOBt ...……….........   Hydroxybenzotriazole

HRMS ...…................   High-resolution mass spectrometry

IC_{50} …………….…..   Concentration causing 50% inhibition

KOTMS ...………........   Potassium trimethylsilanoate

LC/MS ...……….........   Liquid chromatography/mass spectrometry

LDA ...…...................   Lithium diisopropylamide

Mp.……....................	Melting point
MeI..…………...........	Idomethane
n-BuLi ...………..........	n-Butyllithium
NMR...……….............	Nuclear magnetic resonance
PE……………………..	Petroleum ether
p-TsOH ..….................	p-Toluene sulfonic acid
Pd(PPh₃)₄ ..……..............	Tetrakis(triphenylphosphine)palladium
PPh₃ ..…….....................	Triphenylphosphine
rt ..……..........................	Room temperature
Rf ……………………	Retention factor $\frac{a}{f}$
rxn ..……......................	Reaction
TFA ..……....................	Trifluoroacetic acid
THF ..…….....................	Tetrahydrofuran
TLC ..…….....................	Thin layer chromatography
TMSCHN₂ ..…….............	Trimethylsilyl diazomethane

1.0 Neurodegenerative Diseases-A Global Health Priority

"The discovery of new drugs is a monumental struggle with Nature"- Bruce Maryanoff (2006)

1.1 Overview of Neurodegeneration Diseases

Neurodegeneration can be considered as a vulnerability or weakness of certain neuronal regions in the brain, probably starting 10-20 years before cognitive symptoms manifest themselves.[1, 2] Among neurological disorders, Parkinson's (PD), Huntington's (HD) and Alzheimer's (AD) disease are the most widely known and studied pathogens. Due to their distinct markers, these diseases can be distinguishable at a molecular level.[3] Yet, a common feature shared among these disorders is the abnormal built-up of misfolded proteins or aggregates, which are formed via intermolecular interactions among misfolded polypeptides.

Aggregates or so-called plaques or tangles, in this context, are formed via a hydrogen bonding between β-sheet rich proteins like amyloids or tau.[2] Etiologies for many of the neurodegenerative disorders, unfortunately, remain inconclusive. At the same time, the appearance of uncontrollable toxic aggregates that are found in common among these diseases has been considered as the central hypothesis, upon which numerous investigations for causes and therapeutics have been built over the past 20 years.

Aging is considered as a leading risk in neurodegeneration.[4] Deficient clearance systems and defect protein quality control, both of which accelerate in aging cells, are considered as the

factors that exacerbate the diseases. As cells age, their capacities for controlling the quality of protein and tracking the misfolded proteins decline, making aging cells prone to inflammation, cellular dysfunction, and cell death. All of these consequences seem contribute to the hallmarks of neurological disorders. As a result, many have shifted their attention to the biology of aging and considered the area to hold the key to unlock causes and treatments for the cognitive decline.[4, 5]

1.2 Economic Burdens of Neurological Disorders

As the age of the world population increases due to increasing life expectancy, the number of people diagnosed with dementia rises as well. 75 million cases of people with dementia are expected by 2030 and 131 million by 2050.[6] The World Health Organization (WHO) found a similar trend in a study and described dementia or neurological diseases as a fast-growing epidemic that should be prioritized as a global health issue.

The social and economic burdens of neurodegenerative diseases are substantial.[6] The costs from medical and social care for people with dementia and AD are estimated as $480 billions dollars, accounting for 0.65% of the global gross domestic product (GDP). Compared to the separate costs from cancer, diabetes or cardiovascular diseases, the current costs from neurological disorders are formidable. Yet, the number is considerable for a single pathology.

1.3 Current Therapeutic and Diagnostic Approaches for Neurodegenerative Diseases

Many neurogenerative disorders are currently incurable.[6, 7] No drug so far has been shown to either halt, slow, or reverse these disorders. In a market analysis, only 1% of the cost of neurodegenerative therapies is actually spent on the treatment and diagnosis due to lack of effective drugs and accessible diagnostic tools.[6] For compensation, most of the remaining cost are spent on accommodation and primary care for the patients. The lack of an effective treatment could be explained by the fact that cognitive impairments involved complicated pathologies and we, as scientists, are still trying to understand the underlying causes for many of these neurological disorders.

As a consequence, there is an unmet need for effective, safe, and affordable neurotherapeutics and neural diagnostics.[5, 6] During the past 20 years, tremendous efforts and resources were invested in designing therapeutics and testing them in clinical studies. These therapeutics include 1/ disease-modifying agents which aim to slow, halt, or reverse the disease courses, 2/ symptomatic drugs that improve symptoms and thus, quality of life of the patients, and 3/ drugs that aim for preventing the avert cognitive decline in the high-risk population before the symptoms appear.

Under the umbrella of disease-modifying agents, small molecules, natural products, and biologics have been developed in pre-clinical process and tested in clinical trials.[5] Targets of these molecules are broad, covering neuroprotection, inflammation, synaptic activity, misfolded proteins, epigenetics, and others. Current approaches for combating cognitive decline also include the search for an improved diagnostic tool, ideally one with a more affordable price and ease of applications, as an alternative to the current spinal tap or PET scan.

2.0 Protein Homeostasis, Molecular Chaperones, and Hsp70

"If progress in important fields such as medicine, biochemistry, and materials science is to continue, it is essential that we be able to synthesize literally any structure that the imagination can conceive... Our textbooks are filled with hundreds of synthetic methods, all of which have limitations that will never be discovered unless the methods are tested in the challenging arena of [synthesis]. Although our approaches to problems have matured, we need even more mature strategies of synthesis. There is no reason that organic chemists should not be able to surpass nature's virtuosity in the synthesis of complex organic structures. In fact, we are still very far from this goal in most cases."-Clayton Heathcock (1992)

2.1 Overview of Protein Homeostasis

Proteins are essential for the majority of cellular functions.[8] To function properly, most of the proteins are required to maintain several factors, which are included but not limited to well-defined structures, correct timings of production, and meaningful cellular locations. The state all of these processes are in harmony is now referred as protein homeostasis or in short, proteostasis.

Proteins fold and unfold continuously, and errors occur during the folding cycles.[9] Speaking the language of mathematics and computational chemistry, one can say that the functional proteins adopt specific 3D structures, which are considered to be thermodynamically stable. However, during the process of folding, proteins fold along multiple energetic pathways, including those that are kinetically preferred but thermodynamically unstable. This navigation

risks the integrity of the protein structures. As a consequence, proteins may adopt non-native structures, presumably the kinetically favorable states, which tend to form unwanted aggregation. To detangle or remove these undesired misfolded polypeptides, a machinery such as the molecular chaperone is required.

2.2 Overview of Molecular Chaperone and Hsp

The term chaperone was coined in 1987 by John Ellis for any protein that binds and aids in the folding and stabilization of another protein without being part of the final structure.[10, 11] Functions of the chaperone include but not limit to decide the fate of otherwise unstable proteins. Accordingly, chaperones control a/ the binding and releasing of newly synthesized proteins and b/ the transport and degradation of unstable proteins.

Heat shock protein (Hsp) was one of the early-studied proteins that function as a chaperone although at the time, molecular chaperone wasn't known yet.[12, 13] Hsps are found in most prokaryotes and eukaryotes, and they are grouped according to their molecular weights. Among these Hsps, Hsp70 and Hsp90 are the two that carry chaperoning activities and have been considered as the therapeutic targets for diseases that are related to the defects of protein folding such as cystic fibrosis, cancer, and neurodegeneration.[11, 14-16]

2.3 Hsp70

Hsp70s are 70kDa molecular chaperones.[14] Genomes from bacteria to human were shown to encode a variety of isoforms of Hsp70. Each of them is expressed and regulated at a different level depending on a specific need at a specific compartment. Yet, many of them are believed to share similar roles.[17]

2.3.1 General Structure and Mechanism of Hsp70

The architecture of Hsp70 contains three major domains: the nucleotide-binding domain (NBD), the substrate-binding domain (SBD), and the 'lid' region.[14] The NBD is a highly conserved 45 kDa N-terminal, which contains an ATPase active site, while the SBD is a 15 kDa flexible linker, which interacts with hydrophobic amino acids in peptides. The "lid" or the 10kDa α-helical C-terminal domain is believed to hold or release a given substrate and also to mediate the binding of Hsp70 and its co-chaperone.

During a catalytic cycle, Hsp70s adopt two conformations, an open (ATP-bound) and a closed (ADP-bound).[14] When ATP binds to the NBD, a given substrate is loosely bound to the SBD. It is only upon the hydrolysis of ATP to ADP that the lid locks the substrate in place and recruits other protein machineries to decide the fate of the substrate. Once the lid re-opens, the product is released, followed by the displacement of ADP. Due to the higher concentration of ATP over ADP at the local site, the NBD regains ATP, and the protein restores its open conformation.

2.3.2 Hsp70 and Co-chaperone, Hsp40

Hsp70s typically require auxiliary proteins or co-chaperones for the ATP hydrolysis.[18] The rate of these ATPases is normally low, 3×10^{-4} to 1.6×10^{-2} s^{-1}, depending of the methods of measurement. Binding of a substrate at the SBD improves the basal activity but only by a factor of two to ten. Thus, a co-chaperone known as J-protein or Hsp40, is often found binding to Hsp70 to help accelerate the hydrolysis activity. E.coli's DnaK, the prokaryotic analogue of Hsp70, was shown to gain over 1,000-fold in ATPase activity in the presence of DnaJ, its co-chaperone.[19]

2.3.3 Functions of Hsp70

Hsp70s help maintain the integrity and quality of protein structures.[20, 21] Their functions include but not limit to 1/ monitoring the folding and the transport of newly synthesized polypeptides to mitochondria, cytoplasm, or the endoplasmic reticulum (ER) and 2/ promoting native conformation of nascent proteins 3/ dissembling misfolded proteins followed by disposal.

Given their direct participation in proteostasis, it has been hypothesized and demonstrated that Hsp70s have the neuroprotective effects and potentially suppress certain types of neurodegeneration.[22, 23] Evidence suggested that an over-expression of Hsp70s could benefit an anti-amyloid therapeutic approach in treating neurodegeneration, although precise mechanisms remain elusive. Research focused on Hsp70s as the potential therapeutic targets, thus, have enabled the syntheses of drug-like chaperone modulators, which targeted the protein-protein interaction (PPI) between the Hsp70-Hsp40 complex.[21, 24, 25] Among these modulators, MAL1-271, a DHPM scaffold, was shown to mirror the co-chaperone activity of Hsp40 and to active the ATP-dependent

activity of Hsp70. Thus, MAL1-271 has served as the starting point for my project and inspired the chemistry in this thesis.

3.0 DHPM Analogues as the Potential Hsp70 Agonists

"Science is not just about seeing, it's about measuring, preferably with something that's not your own eyes, which are inextricably conjoined with the baggage of your brain. That baggage is more often than not a satchel of preconceived ideas, post-conceived notions, and outright bias."- Neil deGrasse Tyson (2017)

3.1 Importance of the Urea Group in Chemistry

The urea functional group has a strong impact in medicinal chemistry.[26] As illustrated in Figure 1, one of the first urea-containing substance is the sleeping aid Veronal or diethylacetylurea, a potent hypnotic agent, which was discovered in the 90s. Nexavar® (cancer), Novir® (AIDS), Dopergin® (PD) are some of the other urea-containing pharmaceuticals. Various forms of urea derivatives were developed as lead molecules against a variety of pathologies. As drawn in Figure 1, acyclic urea-containing molecules were shown to display promising activities on different enzymes such as glutaminyl cyclase (QC),[27] α-7 nicotinic acetylcholine receptor, β2-adrenergic receptor,[28, 29] and acetylcholinesterase,[29] all of which are considered critical to the etiologies of neurological disorders. On the other hand, some cyclic urea-containing compounds have demonstrated activities against neuroinin-1 receptor (NK1)[30] and ATPase of yeast Hsp70.[31] For the latter case, NSC 630668-R/1 (R/1), a dimeric pyrimidinone, was identified as an inhibitor of the ATPase Hsp70 with and without the Hsp40. More recently, another similar dihydropyrimidinone (DHPM) scaffold (IC$_{50}$= 0.06 µM) was demonstrated as the first multitarget-

directed ligand that simultaneously addressed several enzymes involved in the AD pathological

cascade.[32]
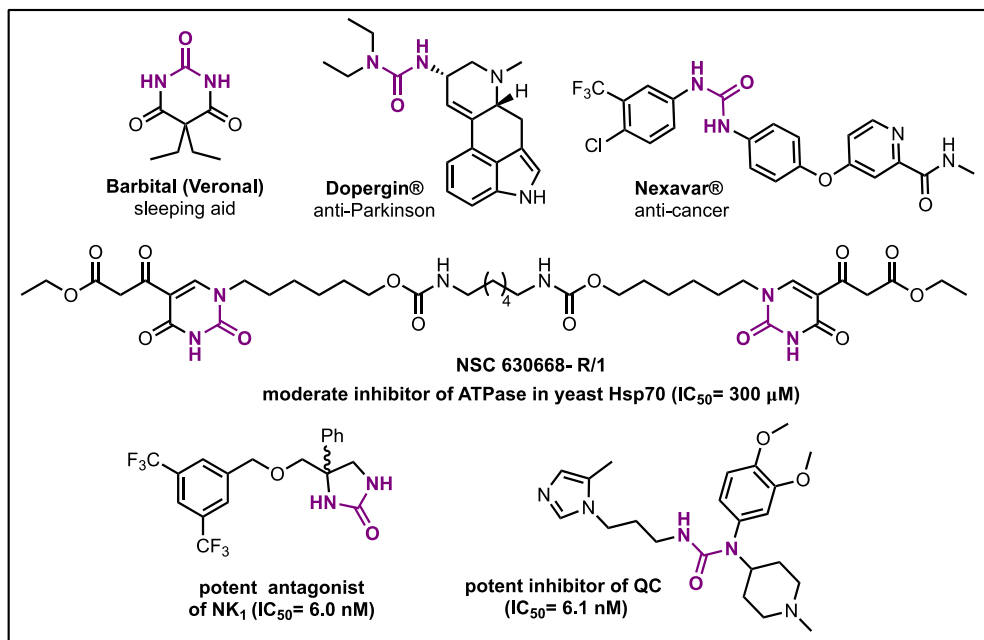
Figure 1. Urea-containing pharmaceuticals and lead molecules

3.2 Wipf Group Methodology for the Construction DHPM Scaffolds

Based on a long-term interest in heterocycles and an inspiration from R/1, the Wipf group

started a project for designing the DHPM scaffold as superior Hsp70 modulators. Specifically, we

envisioned that the new scaffold would carry a similar cyclic urea moiety to R/1 and different

functionalized positions.

As of today, the Wipf and UPCMLD labs have produced around 500 DHPM analogues.[25,]

[33-36] The syntheses for these molecules were given in Scheme 1. Key transformations included a

3-component Biginelli followed by a 4-component Ugi reactions were utilized to create the highly

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functionalized heterocycle, DHPM. Among these structures, MAL3-101, as shown in Figure 2, was demonstrated to inhibit the ATPase activity of Hsp70 in the presence of T-antigen. Since then, MAL3-101 has been further derivatized and severed as a lead molecule for new analogues, some of which were shown to be active in a variety of disease models.[33, 35-37]
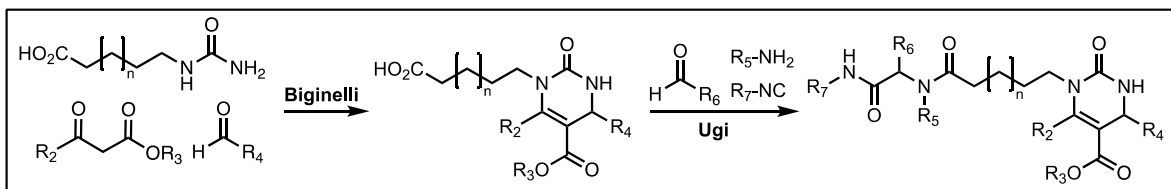
Scheme 1. A Rapid Biginelli-Ugi sequence for the preparation of DHPM analogues

In contrast to MAL3-101, MAL1-271 (Figure 2) was another hit that were found to increase the ATP-dependent activity of bacterial Hsp70 in the presence of bacterial Hsp40.[38] Further study showed that MAL1-271 seemed mirror the co-chaperone activity of Hsp40 and modulate the chaperone as an agonist.
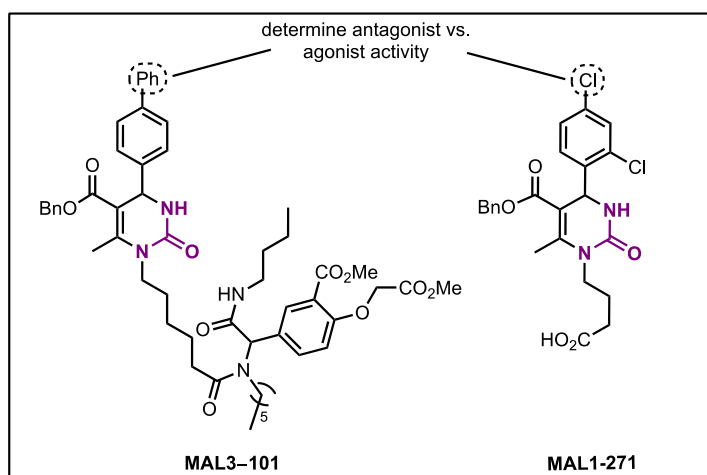
Figure 2. Discovery of the two lead structures, MAL3-101 and MAL1-271

Another collaboration among the Wipf, Brodsky, and Segatori groups showed that the treatment of MAL1-271 and some other analogues decreased an α-syn aggregations in the human neuroglioma cells.[24, 39] These Hsp70 agonists, thus, may represent a new class of compounds for treating diseases characterized by the α-syn misfolding and aggregation. Despite its promising in vivo activity, MAL1-271 is not an ideal lead structure for the clinical studies. Since MAL1-271 has a low potency (IC_{50}=100 µM) in vitro, high dosing will be required especially in vertebrate animal studies.[38] Therefore, a development of more potent analogues of MAL1-271 became a priority for the current studies.

3.3 Rationales behind the Design of 3.3a-f

In order to gain more insight to the previously reported work, we decided to continue the focus on diversifying the ester in zone 1 of MAL1-271.[24] Given the frequent incorporation (top 3) in many FDA-approved drugs, 6-membered aza-heterocycles including piperazine (Figure 3, **Z**= N), thiomorpholine (**Z**= S), and thiomorpholine 1,1-dioxide (**Z**= SO_2) were chosen as substituents in zone 1.[40]

However, we were also concerned about the potential hydrolysis of the ester group in zone 1 to the corresponding carboxylic acid under the assay condition and developed **3.3f**. The analogue (Figure 3, **R**= CH_3, **Z**= O) was designed to contain a gem-dimethyl substituent at the α-C to increase the steric bulk around the ester. This strategy was demonstrated in the literature, some of which often resulted in molecules with either enhancing potency or stability.[40-42] Another modification taken place in zone 1 was the replacement of the ester with an amide. Compared to its bio-isoteric ester analogue, the amide, (Figure 3, **X**= NH, **Z**= O) is known to create an additional

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hydrogen bond with protein residues.[43, 44] Accordingly, we performed the substitution to aim for a

better engagement between the analogue and the targeted Hsp70-Hsp40 complex.
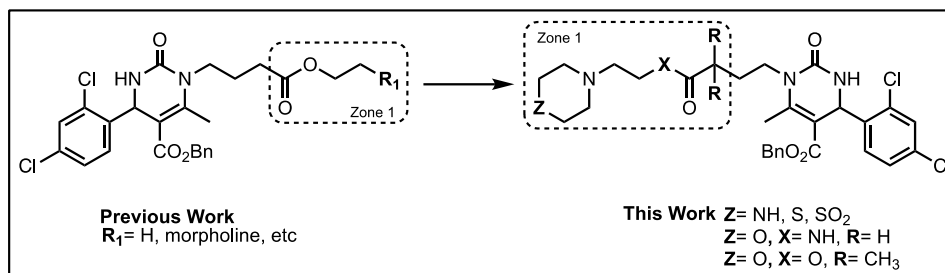
Figure 3. Reported work for the preparation of DHPM analogues

3.4 Syntheses of the DHPM Analogues

3.4.1 Syntheses of 3.3a-e

As shown in Scheme 2, the established Biginelli reaction was utilized to create the core

DHPM in (±) **3.2** from **3.1a**, 2,4-dichlorobenzaldehyde, and benzyl acetoacetate. The heterocycle

was then coupled with a series of different alcohols and amines via a Steglich reaction to rapidly

create 4 new analogues with the desired modifications at zone 1. An analogue with the morpholine

substituent (Scheme 2, **3.3a**) was re-synthesized for re-evaluation, which showed the expected

improvement in efficiency compared to previous work: 67% yield (Steglich, this work) versus

28% yield (S$_N$2, past work).[24]
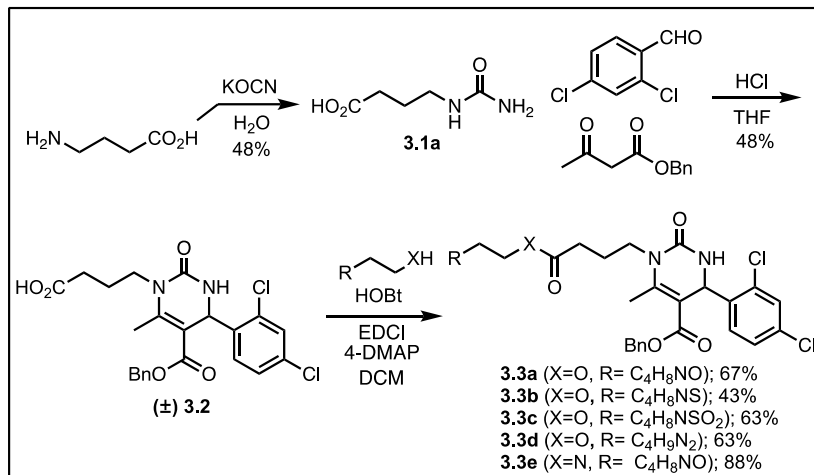
Scheme 2. Syntheses of 3.3a-e

3.4.2 Synthesis of 3.3f

Applying the earlier Biginelli-Steglich sequence, however, failed to deliver **3.3f.** As drawn in Figure 4, the synthesis of the gem-dimethyl ureido acid **3.15** became challenging. Due to the Thorpe-Ingold effect, a subjection of **3.14** to KOCN at elevated temperature resulted in a lactam **3.16** as the major product instead of **3.15**.[45] Alternative strategies such as S_N2 (Scheme 6, section 3.4.3) and alkylation (Scheme 5, section 3.4.3) were tried and proved to be unfruitful.
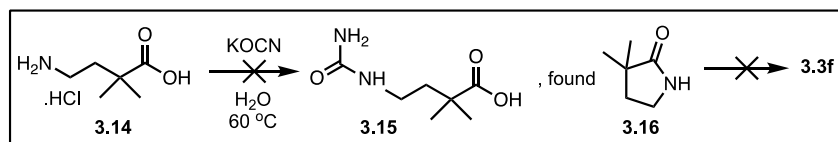
Figure 4. Unsuccessful formation of 3.15 and 3.3f

Another option, which was illustrated in Scheme 3**,** was to perform a series of a Biginelli and a Mitsunobu reactions.[46] The sequence was enabled with formation of the heterocycle (±) **3.4**

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as a racemic mixture followed by the acetyl protection of (±) **3.4** using acetic anhydride to form

3.6 in 80%. Interestingly, without the acetyl protecting step, the Mitsunobu reaction of (±) **3.4** only

resulted in the starting material. The regioselectivity for the protection step of (±) **3.4** was

confirmed with the NMR analysis, which indicated no COSY crosspeak in **3.6** and a COSY

crosspeak in **3.12**.[47] Compound **3.6** was then subjected to the Mitsunobu reaction in CHCl₃ to form

3.10 in 32%. The intermediate **3.10** was then acetyl deprotected with K_2CO_3 and chemo-selectively

hydrolyzed at the methyl ester with KOTMS to give **3.13** in 73% over 2 steps. For the final step,

the acid **3.13** was coupled with the morpholine alcohol under the Steglich condition to yield the

desired **3.3f** in 19% yield.
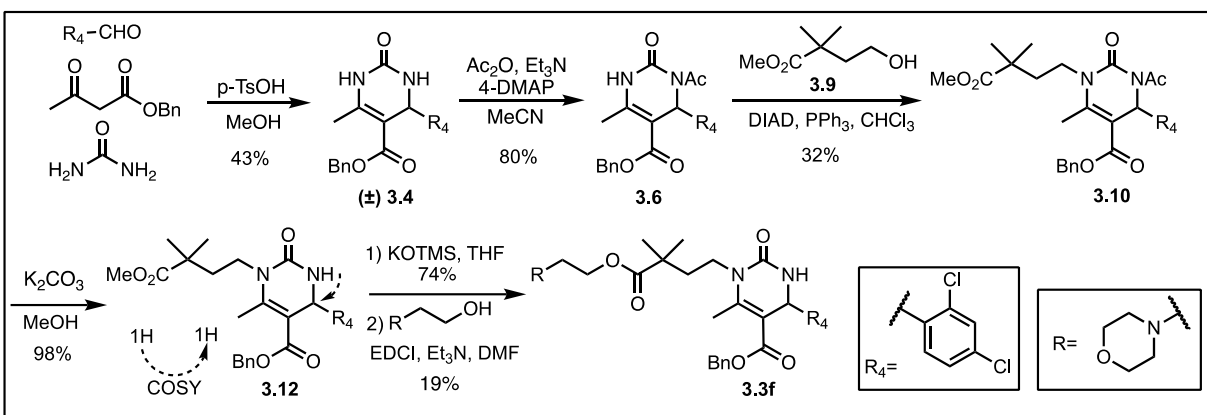
Scheme 3. Successful synthesis of 3.3f

As drawn in Scheme 4, the Mitsunobu alkylation between the alcohol **3.9** and the

heterocycle **3.6** was found to occur at both O and N atoms of the urea, which was not observed in

the precedence.[46] Compound **3.11** was found to be stable to chromatography on SiO₂, and the

chromatographic separation of **3.10** and **3.11** was a challenge. Aiming to improve the selective

alkylation at the desired N atom, we tried switching THF to chloroform and isolated **3.10** in 32%

yield and found traces of **3.11** on the crude NMR.[48] However, the Mitsunobu reaction in CHCl$_3$

could not go to completion despite stirring for 72 h.

Solvent	Isolated yield (%)
THF	**3.10**: 6.0; **3.11**: 21
CHCl$_3$	**3.10**: 32; **3.11**: not found
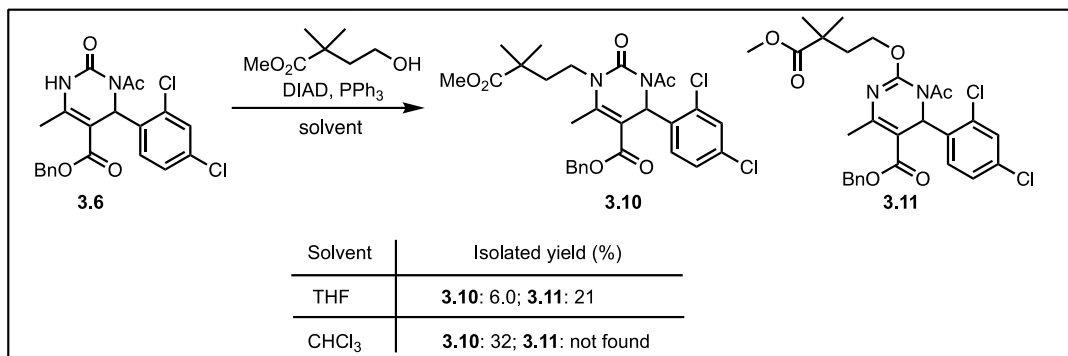
Scheme 4. The challenging Mitsunobu reaction for the formation of 3.10

3.4.3 Other Unsuccessful Attempts toward 3.3f

Other unsuccessful attempts toward the formation of 3.3f were given in schemes 5 and 6. While the direct alkylation using the lithium bases resulted in messy TLC, S_N2 using NaH,[49] LiHMDS, and DIPEA found only starting material.

Condition	Result
LDA (1.1 eq) Mei (1.1 eq	complex TLC
LiHMDS (1.2 eq) Mei (5.0 eq)	complex TLC
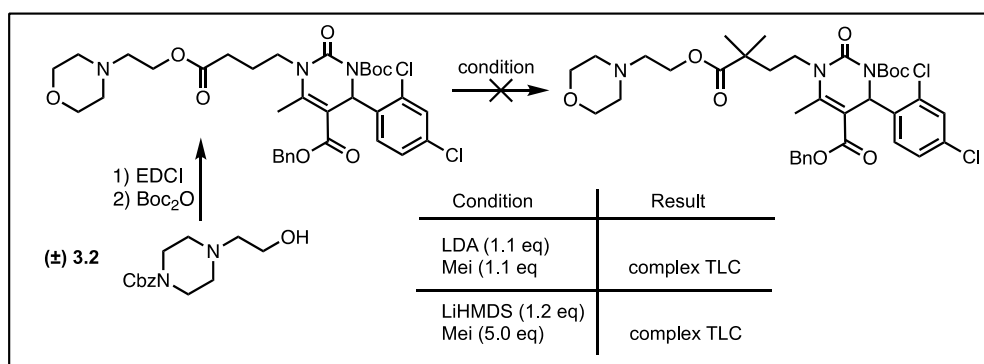
Scheme 5. An unsuccessful attempt toward 3.3f via the direct alkylation
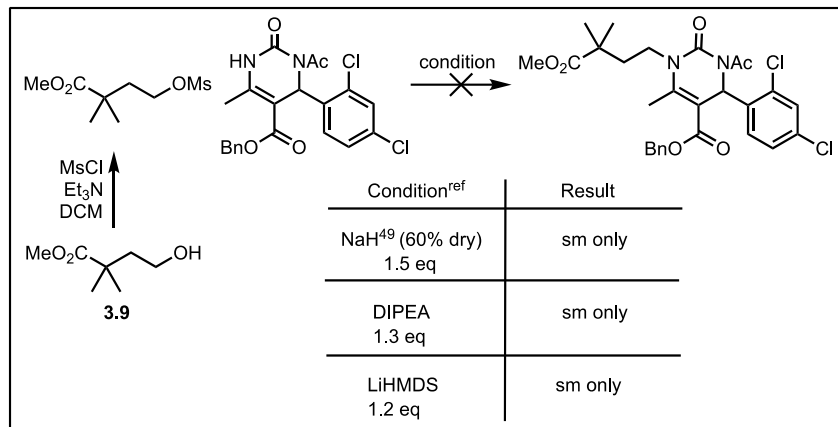
Scheme 6. An unsuccessful attempt toward 3.3f via S$_N$2

3.5 Biological Evaluation of the DHPM Analogues

As part of the long-term collaboration between the Wipf and Brodsky labs, the biological evaluation of some of these new compounds were achieved. The assay was performed by using HEK293H cells to express a huntingtin (HTT) exon containing 17 repeats of Glutamine (Q) that was fused with mCherry fluorescence.[50, 51] After 24 h, cells with these mCherry tagged-polyQ expanded HTT were treated with 10 μL DMSO, MAL1-271, and **3.3a-f**. DMSO and MAL1-271 were used a negative and a positive controls, respectively. After another 6 h of transfection, the cells were harvested and stained with 4′,6-diamidino-2-phenylindole (DAPI) for confocal microscope imaging. A bright spot detection tool was used to identify and quantify the number of protein aggregates ("dots") per cell. The effectiveness of these analogues was evaluated based on the number of toxic aggregates (puncta) that were formed in the presence of the analogues. Thus, depending on the number of puncta that were counted in the presences of **3.3b-f** compared to that in the presence of MAL1-271, the analogues would be evaluated to be more effective or not than

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MAL1-271. However, since the expression level that is different from cells to cells, all of these results were nonquantitative.

The qualitative result, as given in Figure 5, showed that the replacement of an ester with an amide resulted in **3.3e**, which showed a similar activity to MAL1-271. The addition of a piperazine or a thiomorpholine resulted in **3.3d** and **3.3b,** which has similar activities as MAL1-271. However, the switch to 1,1-dioxide morpholine resulted in **3.3c** with the loss of activity.
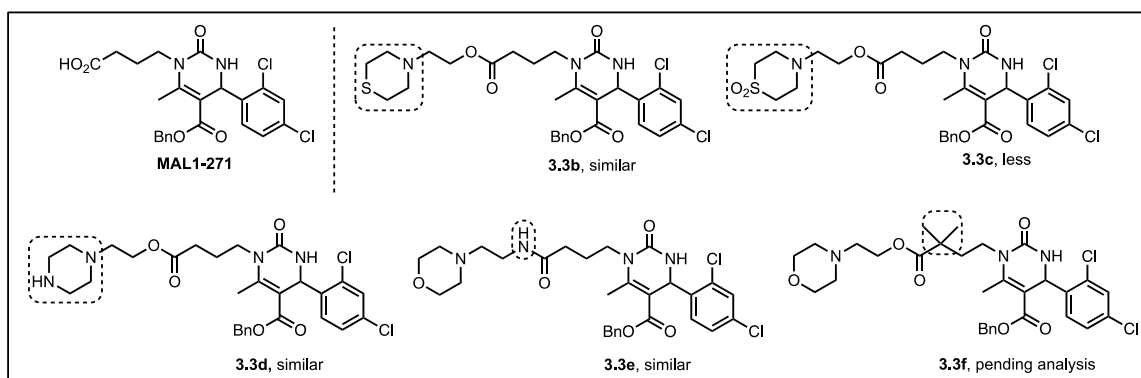
Figure 5. Comparison of the effectiveness between 3.3b-f to MAL1-271

In conclusion, we were able to synthesize another 5 DHPM analogues via the key 3-component Biginelli reaction. Modification was mainly achieved at zone 1 of MAL1-271 resulting in analogues with a variety of the saturated aza-heterocycles, with the amide bond in place of the ester bond, or with the gem-dimethyl group in place of the hydrogens. Since numerous efforts have been spent on investigating modifications at the remaining zones of MAL1-271, it was deemed more worthwhile to investigate an alternative approach, a scaffold-hopping approach. As drawn in Figure 6, we proposed to replace the urea group with its bio-isosteric equivalence, the sulfamide, and test whether the new scaffold elicit any agonist activity on Hsp70.

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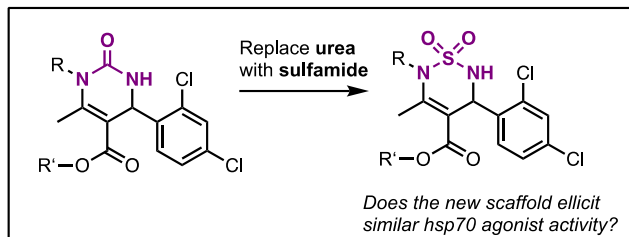
Figure 6. Future directions for designing more potent analogues

3.6 Experimentals

3.6.1 General

All reactions were carried out under a positive pressure of N_2 in a well-ventilated fume hood unless otherwise noted. Hexanes (ACS grade), ethyl acetate (ACS grade), toluene (ACS grade), diethyl ether (ACS grade), dichloromethane (ACS grade), acetonitrile (ACS grade), and chloroform (ACS grade) were purchased from Fisher Chemical and used without further purification. Anhydrous tetrahydrofuran was distilled from sodium (10% w/v) under a positive pressure of N_2. Anhydrous dichloromethane and chloroform were distilled from calcium hydride (10% w/v) under a positive pressure of N_2. Commercially available reagents were used without further purification unless otherwise noted. Reactions were monitored by TLC analysis (pre-coated silica gel 60 F254 plates, 250 µm layer thickness) and visualization was accomplished with a 254 nm UV light and by staining with a $KMnO_4$ solution (1.5 g of $KMnO_4$ and 1.5 g of K_2CO_3 in 100 mL of a 0.1% NaOH solution). Flash column chromatography was performed over Silica gel 60 (particle size 0.04- 0.063 mm) from EMD chemicals. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker 300, 400, and 500 (equipped with cryoprobe) spectrometers using residual

solvent peaks as internal standard (CDCl$_3$ @ 7.26 ppm ^{1}H NMR, 77.00 ppm ^{13}C NMR; DMSO-d$_6$ @ 2.50 ppm ^{1}H NMR, 39.52 ppm ^{13}C NMR). Low- resolution mass spectra were recorded on an Agilent Technologies 1260 Infinity II LCMS. High-resolution mass spectra were obtained on a Micromass UK Limited, Q-TOF Ultima API or a Thermo Scientific Exactive Orbitrap LCMS.

3.6.2 Experimentals
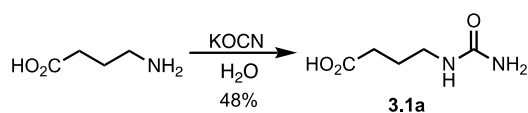
4-Ureidobutanoic acid (3.1a).[52] A solution of 4-aminobutanoic acid (1.00 g, 9.6 mmol, 1.0 eq) in H$_2$O (20 mL, [rxn]= 0.5 M) was treated with potassium cyanate (2.40 g, 29 mmol, 3.0 eq). The mixture was stirred at 60 °C for 4 h and at rt for 15 h, cooled to 0 °C and acidified to pH=1 with 12 M HCl. The resulting milky white mixture was filtered. The precipitate was washed with H$_2$O to afford 3.1a as fine white solid (0.670 g, 4.6 mmol, 48%); **Mp.** 181-184 °C; [1]**H NMR (400 MHz, DMSO-d$_6$)** δ 12.09 (1 H, brs), 5.97 (1 H, brs), 5.38 (2 H, brs), 2.94 (2 H, q, J = 7.0 Hz), 2.19 (2 H, t, J = 7.0 Hz), 1.57 (2 H, q, J = 7.0 Hz); [13]**C NMR (100 MHz, DMSO-d$_6$)** δ 174.4, 158.8, 31.1, 25.6; HRMS **ESI**[+] m/z calcd for C$_5$H$_{10}$N$_2$O$_3$ [M+1] 147.0764, found 147.0762; **IR** (neat) 3411, 3339, 3199, 2958, 2887, 2478, 1699, 1659 cm^{-1}.
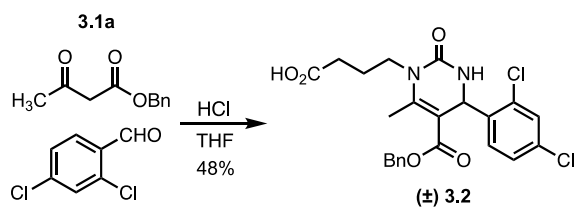
4-(5-((Benzyloxy)carbonyl)-4-(2,4-dichlorophenyl)-6-methyl-2-oxo-3,4-dihydropyrimidin-1(2H)-yl)butanoic acid (± 3.2). To a suspension of **3.1a** (0.200 g, 1.4 mmol, 1.0 eq) and 2,4-dichlorobenzaldehyde (0.593 g, 3.4 mmol, 2.5 eq) in THF (35.0 mL, [rxn]= 0.2 M) was added benzyl acetoacetate (0.662 g, 3.4 mmol, 2.5 eq). After stirring at rt for 5 mins, the suspension was treated dropwise with 12 M HCl (0.5 mL) and heated at reflux for 45 h. The reaction was concentrated and resuspended with 20% acetone in hexane and filtered to afford (**±) 3.2** (0.320 g, 0.67 mmol, 48%) as a beige chunk solid: **Mp.** 207-212 °C; **^{1}H NMR (500 MHz, DMSO-d$_6$)** δ 12.14 (1 H, brs), 7.96 (1 H, d, J = 3.0 Hz), 7.52 (1 H, d, J = 2.0 Hz), 7.34 (1 H, dd, J = 8.0, 2.0 Hz), 7.25-7.21 (4 H, m), 7.01-6.99 (2 H, m), 5.59 (1 H, d, J = 3.0 Hz), 5.03 (1 H, d, J = 13 Hz), 4.93 (1 H, d, J = 12 Hz), 3.83-3.78 (1 H, m), 3.61-3.51 (1 H, m), 2.58 (3 H, s), 1.81-1.77 (1 H, m), 1.67-1.64 (1 H, m), 1.22 (2 H, t, J = 7.0 Hz) 1.81-1.77 (1 H, m), 1.67-1.64 (1 H, m); **^{13}C NMR (125 MHz, DMSO-d$_6$)** δ 173.9, 164.8, 151.7, 151.6, 139.7, 136.2, 133.0, 132.9, 129.8, 129.0, 128.2, 127.8, 127.4, 127.1, 100.4, 65.0, 50.0, 41.1, 30.8, 24.6, 15.6; HRMS **ESI$^-$** m/z calcd for C$_{23}$H$_{21}$N$_2$O$_5$Cl$_2$ [M-H] 475.0822, found 475.0839; **IR** (neat) 3171, 2181, 1665, 1636 cm^{-1}.
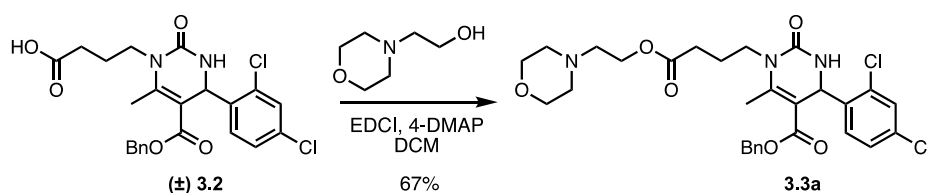
Benzyl 4-(2,4-dichlorophenyl)-6-methyl-1-(4-(2-morpholinoethoxy)-4-oxobutyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (3.3a). To a suspension of (**±) 3.2** (0.310 g, 0.65 mmol, 1.0 eq) in CH$_2$Cl$_2$ (14 mL, [rxn]= 0.06 M) were added N-(-2-hydroxyethyl)morpholine (0.100 mL, 0.78 mmol, 1.2 eq), HOBt (0.108 g, 0.78 mmol, 1.2 eq), 4-DMAP (0.042 g, 0.32 mmol, 0.5 eq), and EDCI (0.151 g, 0.78 mmol, 1.2 eq). The solution was stirred for 16 h until the

conversion was completed by TLC analysis. The mixture was quenched with sat. NH$_4$Cl and extracted with EtOAc (5x). The combined organic layers were washed with water, brine, dried (Na$_2$SO$_4$), and triturated with 30% acetone in hexane to afford **3.3a** (0.254 g, 0.43 mmol, 67%) as a white chunk solid: **Mp.** 142-145 $^{\circ}$C; **^{1}H NMR (500 MHz, CDCl$_3$)** δ 7.35 (1 H, d, J = 2.0 Hz), 7.26-7.14 (2 H, m), 7.13 (1 H, d, J = 2.0 Hz), 7.04 (1 H, d, J = 8.0 Hz), 7.01-6.98 (3 H, m), 5.76 (1 H, d, J = 3.0 Hz), 5.61 (1 H, d, J = 3.0 Hz), 5.07 (1 H, d, J = 12 Hz), 4.95 (1 H, d, J = 12 Hz), 4.12 (2 H, t, J = 5.0 Hz), 3.92-3.89 (1 H, m), 3.70-3.64 (5 H, m), 2.69 (3 H, s), 2.61 (2 H, t, J = 5.0 Hz), 2.49 (4 H, s), 2.35-2.16 (2 H, m), 1.93-1.81 (1 H, m), 1.79-1.78 (1 H, m); **^{13}C NMR (125 MHz, CDCl$_3$)** δ 172.8, 165.2, 152.6, 151.6, 137.9, 136.0, 134.6, 133.7, 130.0, 128.6, 128.5, 128.1, 127.8, 127.8, 101.4, 70.0, 66.1, 61.7, 57.2, 53.9, 50.8, 42.0, 31.2, 25.0, 16.2; HRMS **ESI$^+$** m/z calcd for C$_{29}$H$_{34}$N$_3$O$_6$Cl$_2$ [M+H] 590.1824, found 590.1852; **IR** (neat) 3340, 3078, 3011, 2951, 2799, 1742, 1702, 1640, 1609, 1560 cm^{-1}.
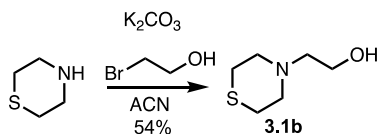
2-Thiomorpholinoethan-1-ol (3.1b).[53] To a suspension of 2-bromoethanol (0.340 mL, 6.06 mmol, 6.0 eq) and K$_2$CO$_3$ (0.305 g, 2.22 mmol, 3.0 eq) in ACN (2.1 mL, [rxn]= 0.35 M) was added thiomorpholine (0.480 mL, 4.74 mmol, 1.0 eq). The mixture was stirred at rt for 3 h, heated at reflux for another 14 h, and filtered to give a red solid crude. The crude was resuspended in CH$_2$Cl$_2$ and filtered to afford red crystalline salt, which was redissolved in water and CH$_2$Cl$_2$. The mixture was extracted vigorously with Et$_3$N/CH$_2$Cl$_2$ (5x). The combined organic layers were dried (MgSO$_4$) to afford **3.1b** (0.383 g, 2.57 mmol, 54%) as light, yellow oil, which crystalized upon

freezing: ^{1}**H (500 MHz, CDCl$_3$)** δ 4.39 (1 H, brs), 3.47 (2 H, 1, J = 5.0 Hz), 2.67 (4 H, d, J = 5.0 Hz), 2.57 (2 H, t, J = 5.0 Hz), 2.50-2.39 (2 H, m); ^{13}**C (125 MHz, CDCl$_3$)** δ 60.8, 58.2, 54.9, 27.0.
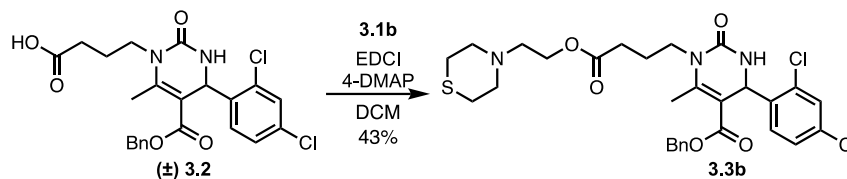

Benzyl 4-(2,4-dichlorophenyl)-6-methyl-2-oxo-1-(4-oxo-4-(2-thiomorpholinoethoxy) butyl)-1,2,3,4-tetrahydropyrimidine-5-carboxylate (3.3b). To a suspension of (**±) 3.2** (0.160 g, 0.34 mmol, 1.0 eq) in CH$_2$Cl$_2$ (5.2 mL, [rxn]= 0.06 M) were added EDCI (0.077 g, 0.40 mmol, 1.2 eq), HOBt (0.054 g, 0.40 mmol, 1.2 eq), and 4-DMAP (0.021 g, 0.17 mmol, 0.5 eq). After 15 min of activation, the mixture was treated with **3.1b** (1.0 M solution in CH$_2$Cl$_2$, 0.4 mL, 0.40 mmol, 1.2 eq), stirred for 45 h, washed with 1.0 M HCl, sat. NaHCO$_3$, H$_2$O (3x), brine, and dried (Na$_2$SO$_4$) to form a white solid crude, which was triturated in 20% acetone in hexane to afford **3.3b** (0.087 g, 0.14 mmol, 43%): **Mp**. 145-150 °C; ^{1}**H NMR (500 MHz, CDCl$_3$)** δ 7.36 (1 H, d, J = 2.0 Hz), 7.24-7.21 (4 H, m, overlapping with CDCl$_3$), 7.14 (1 H, dd, J = 8.0, 2.0 Hz), 7.04 (1 H, d, J = 2.0 Hz), 6.99-6.98 (2H, m), 5.75 (1 H, d, J = 3.0 Hz), 5.51 (1 H, d, J = 3.0 Hz), 5.07 (1 H, d, J = 12 Hz), 4.95 (1 H, d, J = 12 Hz), 4.17 (2 H, t, J = 6.0 Hz), 3.92-3.89 (1H, m), 3.68-3.64 (1H, m), 2.76-2.74 (4 H, m), 2.69 (3 H, s), 2.66-2.62 (6 H, m), 2.33-2.29 (2 H, m), 1.93-1.90 (1 H, m), 1.81-1.76 (1 H, m); ^{13}**C NMR (125 MHz, CDCl$_3$)** δ 172.8, 165.2, 152.6, 151.7, 137.9, 135.9, 134.6, 133.7, 130.0, 128.5, 128.51, 128.1, 127.9, 127.8, 101.4, 66.1, 61.8, 57.4, 52.2, 50.8, 43.1, 31.2, 28.1, 25.0, 16.2; HRMS **ESI$^+$** m/z calcd for C$_{29}$H$_{34}$N$_3$O$_5$N$_4$Cl$_2$S [M+H] 606.1590, found 606.1561; **IR** (neat) 3339, 2953, 2798, 1741, 1702, 1676, 1608, 1559 cm^{-1}.
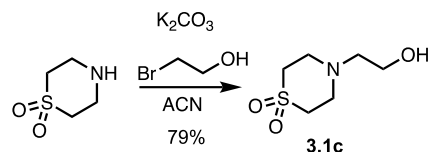
4-(2-Hydroxyethyl)thiomorpholine 1,1-dioxide (3.1c).[53] To a suspension of 2-bromoethanol (1.90 mL, 6.9 mmol, 1.0 eq) and K_2CO_3 (1.53 g, 11 mmol, 3.0 eq) in ACN (11 mL, [rxn]= 0.35 M) was treated with morpholine dioxide (0.501 g, 3.7 mmol, 6.9 eq). The mixture was stirred for 3 h, heated at reflux for another 15 h, and filtered. The filtrate was purified by chromatography on SiO_2 (10% MeOH in CH_2Cl_2; **Rf:** 0.45) to afford **3.1c** as yellow oil (0.520 g, 2.93 mmol, 79%): **^{1}H NMR (500 MHz, DMSO-d6)** δ 4.48 (1 H, m), 3.49-3.48 (2 H, m), 3.06-3.05 (4 H, m), 2.95-2.94 (4 H, m), 2.56 (2 H, t, $J = 6.0$ Hz); **^{13}C NMR (125 MHz, DMSO-d6)** δ 58.8, 57.8, 50.8, 50.4.
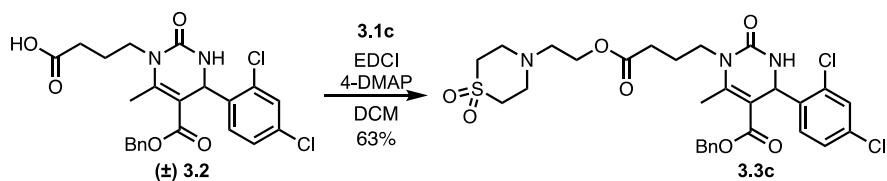
Benzyl 4-(2,4-dichlorophenyl)-1-(4-(2-(1,1-dioxidothiomorpholino)ethoxy)-4-oxobutyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (3.3c). To a suspension of (±) **3.2** (0.150 g, 0.31 mmol, 1.0 eq) in CH_2Cl_2 (5.2 mL, [rxn]= 0.06 M) were added EDCI (0.072 g, 0.38 mmol, 1.2 eq), HOBt (0.051 g, 0.38 mmol, 1.2 eq), and 4-DMAP (0.019 g, 0.16 mmol, 0.5 eq). After 30 min of activation, the mixture was treated with **3.1c** (0.068 g, 0.38 mmol, 1.2 eq), stirred for 38 h, and washed with 1.0 M HCl, sat. $NaHCO_3$, water (3x), and brine. The combined organic layers were dried ($MgSO_4$) to afford a yellow crude, which was triturated in 20% acetone in hexane to furnish **3.3c** (0.130 g, 0.19 mmol, 63% yield) as an off-white solid: **Mp.** 68-71 °C; **^{1}H NMR (500 MHz, CDCl3)** δ 7.37 (1 H, d, $J = 2.0$ Hz), 7.25-7.21 (3 H, m, overlapping with $CDCl_3$), 7.14 (1 H, dd, $J = 8.0, 2.0$ Hz), 7.05 (1 H, d, $J = 8.0$ Hz), 7.00 (1 H, dd, $J = 8.0, 1.6$ Hz), 5.77 (1 H, d, J

= 2.5 Hz), 5.56 (1 H, d, J = 2.5 Hz), 5.08 (1 H, d, J = 13 Hz), 4.96 (1 H, d, J = 13 Hz), 4.23-4.17

(2 H, m), 3.95-3.89 (1 H, m), 3.70-3.65 (1 H, m), 3.04 (2 H, d, J = 7.2 Hz), 2.79 (2 H, t, J = 5.5

Hz), 2.69 (3 H, s), 2.34-2.31 (2 H, m), 1.96-1.90 (1 H, m), 1.84-1.77 (1 H, m); **¹³C NMR (125**

MHz, CDCl₃) δ 172.5, 165.0, 152.4, 151.3, 137.8, 135.7, 134.4, 133.6, 129.9, 128.4, 128.3, 128.0,

127.6, 101.3, 66.0, 61.5, 55.3, 51.4, 50.8, 50.6, 41.7, 30.9, 24.8, 16.0; HRMS **ESI⁺** *m/z* calcd for

$C_{29}H_{34}N_3O_7N_3Cl_2S$ [M+H] 638.1489, found 638.1506; **IR** (neat) 2936, 1729, 1701, 1678, 1619,

1585 cm⁻¹.

(±) 3.2   EDCI, 4-DMAP / Et₃N, DCM / 63%   **3.14**
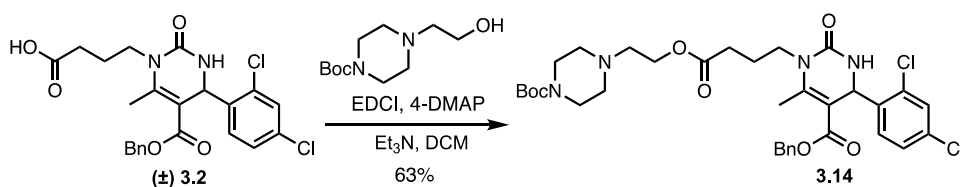
Benzyl **1-(4-(2-(4-(*tert*-butoxycarbonyl)piperazin-1-yl)ethoxy)-4-oxobutyl)-4-(2,4-**

dichlorophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (3.14). To a

suspension of **(±) 3.2** (1.00 g, 2.1 mmol, 1.0 eq) in CH_2Cl_2 (35 mL, [rxn]= 0.06 M) were added

EDCI (0.482 g, 2.5 mmol, 1.2 eq), HOBt (0.340 g, 2.5 mmol, 1.2 eq), and 4-DMAP (0.128 g, 1.0

mmol, 0.5 eq). After 30 min of activation, the mixture was treated with tert-butyl 4-(2-

hydroxyethyl) piperazine-1-carboxylate (0.591 g, 2.5 mmol, 1.2 eq), stirred for 29 h, and washed

with 1.0 M HCl, sat. NaHCO₃, water (3x), and brine. The combined organic layers were dried

(MgSO₄) and recrystallized with acetone (2x) to afford **3.14** (0.92 g, 1.32 mmol, 63% yield) as a

fluffy crystal: **¹H NMR (500 MHz, CDCl₃)** δ 7.37 (1 H, d, J = 2.0 Hz), 7.22-7.21 (3 H, m,

overlapping with CDCl₃), 7.15-7.13 (1 H, m), 7.05-7.03 (1 H, m), 7.00-6.99 (2 H, m), 5.76 (1 H,

d, J = 3.0 Hz), 5.51 (1 H, d, J = 3.0 Hz), 5.08 (1 H, d, J = 13 Hz), 4.96 (1 H, d, J = 13 Hz), 4.21 (2

H, t, J = 6.0 Hz), 3.92 (1 H, m), 3.60 (1 H, m), 3.42 (4 H, t, J = 3.0 Hz), 2.69 (3 H, s), 2.62 (2 H, t, J = 6.0 Hz), 2.43 (4 H, s), 2.35 (2 H, m), 1.99 (1 H, m), 1.80 (1 H, m), 1.46 (9 H, s).

3.14   TFA, DCM   *quant.* yield   **3.3d**
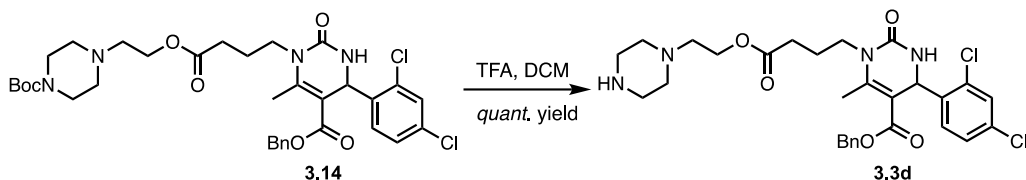
Benzyl 4-(2,4-dichlorophenyl)-6-methyl-2-oxo-1-(4-oxo-4-(2-(piperazin-1-yl)ethoxy)butyl)-1,2,3,4-tetrahydropyrimidine-5-carboxylate (3.3d). To a 0 °C solution of **3.14** (0.400 g, 0.58 mmol, 1.0 eq) in CH_2Cl_2 (5.5 mL, [rxn]= 0.11 M) was added TFA (0.689 mL, 15 mmol, 15 eq). The solution mixture was stirred at 0 °C for 2 h and rt for 4 h until the conversion was completed by TLC analysis. The mixture was diluted with EtOAc/sat. $NaHCO_3$ and extracted with EtOAc (2x). The organic layers were combined, washed with brine, dried (Na_2SO_4) to afford **3.3d** (quant.) as a white solid: **Mp**. 68-72 °C; **^{1}H NMR (500 MHz, CDCl₃)** δ 7.35 (1 H, s), 7.34-7.20 (3 H, m, overlapping with CDCl₃), 7.12 (1 H, dd, J = 8.0, 2.0 Hz), 7.04 (1 H, d, J = 8.0 Hz), 6.99-6.98 (2 H, m), 5.89 (1 H, d, J = 3.0 Hz), 5.76 (1 H, d, J = 3.0 Hz), 5.06 (1 H, d, J = 13 Hz), 4.94 (1 H, d, J = 13 Hz), 4.19-4.16 (2 H, m), 3.88 (1 H, m), 3.66 (1 H, m), 3.14 (4 H, t, J = 5.0 Hz), 2.77 (4 H, t, J = 4.0 Hz), 2.76-2.68 (5 H, m), 2.31 (2 H, t, J = 7.0 Hz), 1.94-1.81 (1 H, m), 1.80-1.70 (1 H, m); **^{13}C NMR (125 MHz, CDCl₃)** δ 172.7, 165.2, 152.7, 151.4, 138.0, 135.9, 134.5, 133.8, 130.0, 128.7, 128.5, 128.1, 127.9, 127.8, 101.6, 66.1, 61.4, 56.3, 50.9, 50.0, 43.9, 42.0, 31.2, 25.0, 16.2; HRMS **ESI⁺** *m/z* calcd for $C_{29}H_{35}N_4O_5N_3Cl_2$ [M+H] 589.1979, found 589.1957; **IR** (neat) 2949, 1727, 1673, 1619 cm⁻¹.
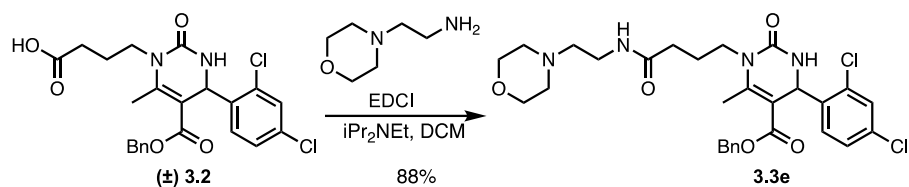
Benzyl 4-(2,4-dichlorophenyl)-6-methyl-1-(4-((2-morpholinoethyl)amino)-4-oxobutyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (3.3e). To a suspension of (**±**) **3.2** (0.150 g, 0.31 mmol, 1.0 eq) in CH$_2$Cl$_2$ (3.2 mL, [rxn]= 0.1 M) were added HOBt (0.048 g, 0.34 mmol, 1.1 eq), DIPEA (0.120 mL, 0.69 mmol, 2.2 eq), and EDCI (0.063 g, 0.31 mmol, 1.0 eq). After 30 min of activation, the mixture was treated with 4-(2-aminoethyl)-morpholine (0.042 mL, 0.31 mmol, 1.0 eq), stirred at rt for 21 h until the conversion was completed by TLC analysis. The mixture was quenched with sat. NH$_4$Cl and extracted with EtOAc (5x). The combined organic layers were washed with water, brine, dried (Na$_2$SO$_4$), and purified by chromatography SiO$_2$ (20% MeOH in EtAOc; **R$_f$:** 0.28) to afford **3.3e** (0.163 g, 0.28 mmol, 88%) as a colorless foam: **^{1}H NMR (500 MHz, CDCl$_3$)** δ 7.36 (1 H, d, J = 2.0 Hz), 7.24-7.21 (2 H, m), 7.13 (1 H, dd, J = 8.0, 2.0 Hz), 7.05 (1 H, d, J = 8.0 Hz), 7.02 (2 H, d, J = 2.0 Hz), 6.19 (1 H, brs), 5.77 (1 H, s), 5.55 (1 H, brs), 5.09 (1 H, d, J = 12 Hz), 4.96 (1 H, d, J = 12 Hz), 3.95-3.90 (1 H, m), 3.72-3.66 (5 H, m), 3.37 (2 H, s), 2.69 (3 H, s), 2.49 (5 H, brs), 2.18 (2 H, t, J = 7.0 Hz), 1.96-1.92 (1 H, m), 1.85-1.79 (1 H, m); **^{13}C NMR (125 MHz, CDCl$_3$)** δ 171.9, 165.2, 153.0, 151.6, 138.0, 135.8, 134.5, 133.7, 128.5, 130.0, 128.7, 128.1, 127.9, 127.8, 101.6, 66.9, 66.1, 57.3, 53.5, 50.8, 42.1, 35.8, 33.1, 25.6, 16.2; HRMS **ESI$^+$** *m/z* calcd for C$_{29}$H$_{35}$N$_2$O$_5$N$_4$Cl$_2$ [M+H] 589.1979, found 589.1997; **IR** (neat) 2946, 1681, 1622 cm^{-1}.
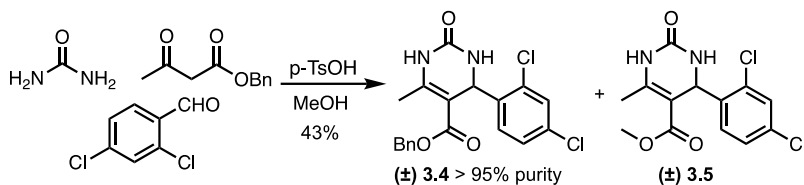
Benzyl 4-(2,4-dichlorophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (

± 3.4). To a solution of 2,4-dichlorobenzaldehyde (2.00 g, 11 mmol, 1.0 eq), urea (1.00 g, 17 mmol,

1.5 eq), and benzyl acetoacetate (3.00 mL, 17 mmol, 1.5 eq) in MeOH (15 mL, [rxn]= 0.8 M) was

added p-TsOH (0.340 g, 1.7 mmol, 0.15 eq). The reaction mixture was heat at reflux for 20 h and

filtered to afford a solid crude, which was washed with CH_2Cl_2 to yield (**±) 3.4** (> 95% purity, 2.00

g, 4.9 mmol, 43%) as a white solid: **^{1}H NMR (400 MHz; DMSO-d$_6$)** δ 9.37 (1 H, brs), 7.76 (1 H,

s), 7.49 (1 H, d, J = 2.4 Hz), 7.37 (1 H, dd, J = 8.4, 2.4 Hz), 7.27 (1 H, d, J = 8.4 Hz), 7.25-7.19 (3

H, m), 6.99-6.97 (2 H, m), 5.61 (1 H, d, J = 2.8 Hz), 5.03 (1 H, d, J = 13 Hz), 4.88 (1 H, d, J = 13

Hz), 2.3 (3 H, s).
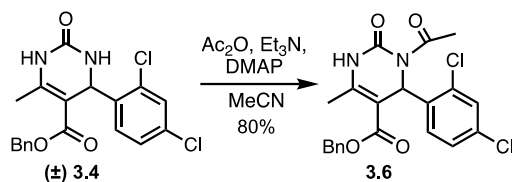
Benzyl 3-acetyl-4-(2,4-dichlorophenyl)-6-methyl-2-oxo-1,2,3,4 tetrahydropyrimidine-5-

carboxylate (3.6). To a solution of (**±) 3.4** (2.00 g, 1.4 mmol, 1.0 eq) and 4-DMAP (0.120 g, 1.0

mmol, 0.20 eq) in ACN (2.7 mL, [rxn]= 0.5 M) were added Et$_3$N (3.00 mL, 12 mmol, 2.6 eq), and

acetic anhydride (4.50 mL, 12 mmol, 2.6 eq). The mixture was stirred at 130 °C for 2 h until the

conversion was completed with TLC analysis. The resulting brown solution was cooled to rt, added

with celite, concentrated and purified by chromatography SiO$_2$ (15% EtOAc in CH_2Cl_2; **R$_f$**: 0.5)

to afford a light yellow solid, which was triturated in CH_2Cl_2 to yield **3.6** (1.77 g, 4.09 mmol, 80%)

as an off-white solid: **¹H NMR (400 MHz; DMSO-d₆)** δ 10.4 (1 h, s), 7.49 (1 H, s), 7.34-7.28 (5 H, m), 7.20-7.19 (2 H, m), 6.57 (1 H, s), 5.08 (2 H, s), 2.25 (3 H, s), 2.29 (3 H, s); **13C NMR (100 MHz; DMSO-d₆)** δ 170.7, 164.1, 150.4, 147.6, 137.4, 136.1, 133.3, 133.2, 131.1, 129.3, 128.3, 127.9, 127.8, 127.6, 101.8, 65.2, 51.7, 26.2, 17.2; HRMS **ESI+** *m/z* calcd for $C_{21}H_{19}N_2O_4Cl_2$ [M+H] 433.0716, found 433.0708.
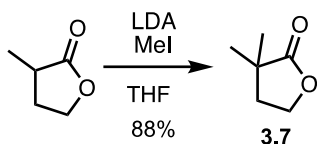
3,3-Dimethyldihydrofuran-2(3*H*)-one (3.7).[54] To a solution of diisopropylamine (5.0 mL) in 9.8 mL THF at -78 °C was added 2.48 M nBuLi in hexane (10 mL). The solution was stirred at -78 °C for 5 min then rt for 5 min to yield an LDA solution. To another solution of methyl-γ-butyrolactone (2.00 g, 19 mmol, 1.0 eq) in THF (20 mL) was treated dropwise the 1.0 M LDA solution (22 mL, 21 mmol, 1.1 eq) over 30 min via syringe at -78 °C. After stirring vigorously at the same temperature for an hour, the solution was treated with Me₃I (2.4 mL, 40 mmol, 2.0 eq), warmed to rt, stirred for 15 h and carefully quenched with sat. NH₄Cl. The aqueous layer was extracted with Et₂O (3x). The combined organic layers were dried (MgSO₄) and purified by chromatography on SiO₂ (100% Et₂O) to yield **3.7** (2.21 g, 17 mmol, 88%) as yellow oil. **¹H NMR (500 MHz; CHCl₃)** δ 4.26 (2 H, t, *J* = 6.5 Hz), 2.11 (2 H, t, *J* = 6.5 Hz), 1.27 (6 H, s).
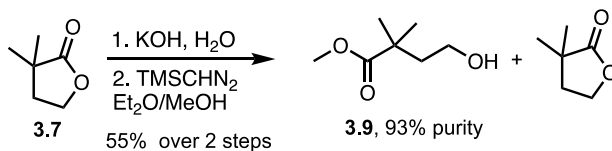
Methyl 4-hydroxy-2,2-dimethylbutanoate (3.9).[55] A mixture of **3.7** (1.47 g, 13 mmol, 1.0 eq),

KOH (0.72 g, 13 mmol, 1.0 eq) in H_2O (13 mL, [rxn]= 1.0 M) was stirred at 110 °C for 2 h, cooled

to 0 °C, and adjusted pH=3-4 with 5.0 M HCl, and extracted with EtOAc (3x). The combined

organic layers were washed with brine and dried (Na_2SO_4) to afford the crude acid as yellow oil,

which was used without further purification.

To a solution of the crude acid (1.20 g, 8.9 mmol, 1.0 eq) in 2/3 Et_2O/MeOH (22 mL,

[rxn]= 0.4 M) at 0° C was added a 2.0 M hexane solution of $TMSCHN_2$ (7.0 mL, 13 mmol, 1.5

eq). The reaction mixture was stirred at 0° C for 1 h and concentrated. The residue was redissolved

with EtOAc, washed with sat. Na_2CO_3 and dried ($MgSO_4$) to afford **3.9** (93% purity, 0.87 g, 5.58

mmol, 55% over 2 steps) as colorless oil: **¹H NMR (500 MHz; CHCl₃)** δ 3.70-3.67 (5 H, m), 1.83

(2 H, t *J* = 11 Hz), 1.22 (6 H, s); **¹³C NMR (125 MHz; CHCl₃)** δ 182.3, 64.6, 50.7, 38.5, 36.9,

24.1.
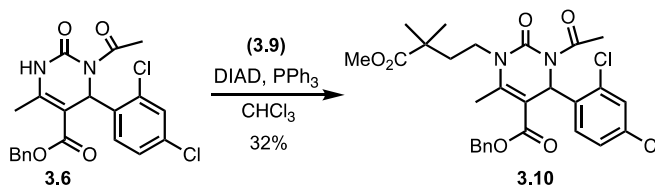
Benzyl 3-acetyl-4-(2,4-dichlorophenyl)-1-(4-methoxy-3,3-dimethyl-4-oxobutyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (3.10). To a solution of **3.6** (0.100 g, 0.23

mmol, 1.0 eq), **3.9** (0.910 g, 0.58 mmol, 2.5 eq), and PPh_3 (0.153 g, 0.58 mmol, 2.5 eq) in

chloroform (3.0 mL, [rxn]= 0.08 M) was treated dropwise DIAD (0.120 mL, 0.58 mmol, 2.5 eq).

The resulting yellow solution was stirred for 72 h, diluted with CH_2Cl_2 (5x), washed with brine,

dried ($MgSO_4$), and purified by chromatography on SiO_2 (25% EtOAc in hexane followed by a

second chromatography on SiO_2 with 5% EtOAc in CH_2Cl_2; **R_f:** 0.38) to afford **3.10** (0.041 g, 0.07

mmol, 32%) as a sticky foam: **^{1}H NMR (500 MHz; CDCl$_3$)** δ 7.31-7.28 (3 H, m), 7.22-7.21 (2 H, m), 7.10 (1 H, d, $J = 8.5$ Hz), 7.06 (1 H, dd, $J = 8.0$, 2.0 Hz), 6.76 (1H, s), 5.13 (2H, s), 3.87-3.81 (1 H, m), 3.72-3.66 (4 H, m), 2.52 (3 H, s), 2.45 (3 H, s), 1.92-1.86 (1 H, m), 1.77-1.71 (1 H, m), 1.26 (6 H, s); **^{13}C NMR (125 MHz; CDCl$_3$)** δ 177.6, 171.2, 164.9, 151.7, 148.0, 135.8, 135.7, 134.6, 134.4, 130.6, 130.5, 128.6, 128.4, 127.1, 107.9, 66.6, 52.2, 50.9, 41.3, 41.2, 38.9, 26.2, 25.4, 25.3, 15.9.
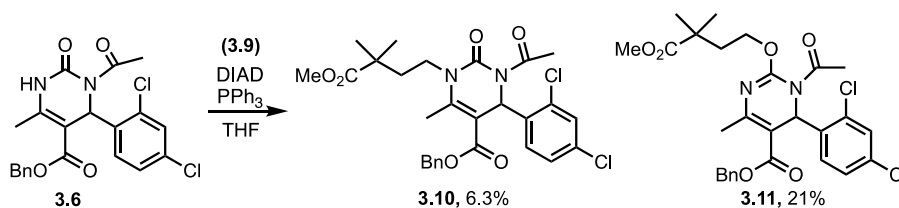
Benzyl 1-acetyl-6-(2,4-dichlorophenyl)-2-(4-methoxy-3,3-dimethyl-4-oxobutoxy)-4-methyl-1,6-dihydropyrimidine-5-carboxylate (3.11). To 0 °C solution of **3.6** (0.210 g, 0.48 mmol, 1.0 eq), **3.9** (0.110 g, 0.48 mmol, 1.0 eq) and PPh$_3$ (0.150 g, 0.58 mmol, 1.2 eq) in THF (2.3 mL, [rxn]=0.2 M) was treated dropwise DIAD (0.1 mL, 0.58 mmol, 1.2 eq). The mixture was stirred at rt for 43 h, diluted with CH$_2$Cl$_2$ (5x), and washed with brine. The combined organic layers were dried (MgSO$_4$) and purified by chromatography on SiO$_2$ (25% EtOAc in hexane followed by a second chromatography on SiO$_2$ with 15-20% acetone in hexane to isolate both **3.10** (0.008 g, 0.015 mmol, 6.3%) and **3.11** (0.030 g, 0.052 mmol, 21%). **3.11**: **^{1}H NMR (300 MHz; CDCl$_3$)** δ 7.31 (1 H, s, overlapping with CDCl$_3$), 7.18-7.17 (2 H, m), 7.10 (2 H, s), 6.82 (1 H, s), 5.1 (2 H, q, $J = 13$ Hz), 4.49-4.40 (1 H, m), 4.38-4.29 (1 H, m), 3.54 (3 H, s), 2.41 (3 H, s), 2.29 (3 H, s), 2.07-1.97 (1 H, m), 1.95-1.86 (1 H, m), 1.20 (6 H, s); **^{13}C NMR (75 MHz; CDCl$_3$)** δ 177.5, 168.8, 164.9, 154.7, 151.5, 136.1, 135.7, 134.6, 134.3, 130.1, 130.0, 128.5, 128.1, 127.3, 110.8, 66.1,

66.0, 52.0, 51.0, 40.8, 38.5, 25.7, 25.3, 25.2, 21.7; HRMS **ESI**$^+$ *m/z* calcd for $C_{28}H_{31}O_6N_2Cl_2$ [M+H] 561.1553, found 561.1551; **IR** (neat) 2951, 1708, 1622, 1568 cm^{-1}.

3.10   **3.12**

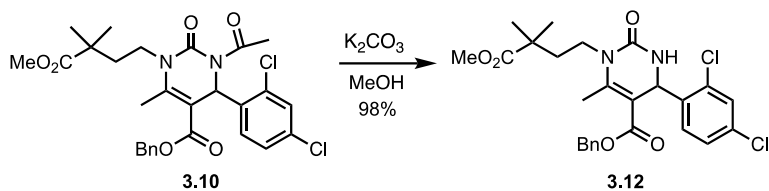**Benzyl 4-(2,4-dichlorophenyl)-1-(4-methoxy-3,3-dimethyl-4-oxobutyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (3.12)**. To a solution of **3.10** (0.100 g, 0.18 mmol, 1.0 eq) in MeOH (1.7 mL, [rxn]= 0.1 M) was added K_2CO_3 (finely ground, 0.049 g, 0.36 mmol, 2.0 eq). The mixture was stirred at rt for 1 h until the conversion was completed by TLC analysis. The mixture was quenched with water and extracted with CH_2Cl_2 (3x). The combined organic layers were washed with brine and dried (MgSO$_4$) to afford **3.12** (0.089 g, 0.17 mmol, 98%) as a white solid: **^{1}H NMR (500 MHz; DMSO-d$_6$)** δ 7.95 (1 H, d, *J* = 2.0 Hz), 7.52 (1 H, d, *J* = 2.5 Hz), 7.35 (1 H, d, *J* = 2.0 Hz), 7.25-7.21 (4 H, m), 7.01-6.99 (2 H, m), 5.58 (1 H, d, *J* = 3.0 Hz), 5.03 (1 H, d, *J* = 13 Hz), 4.93 (1 H, d, *J* = 13 Hz), 3.73 (1 H, dt, *J* = 13, 4.5 Hz), 3.58 (3 H, s), 3.52 (1 H, dt, *J* = 13, 4.5 Hz), 2.56 (3 H, s), 1.82 (1 H, dt, *J* = 13, 4.5 Hz), 1.64 (1 H, dt, *J* = 13, 4.5 Hz), 1.16 (6H, s); **^{13}C NMR (125 MHz; DMSO-d$_6$)** δ 176.9, 164.8, 151.5, 151.3, 140.0, 136.2, 132.9, 132.8, 129.9, 129.0 128.1, 127.8, 127.7, 127.4, 100.4, 65.0, 51.8, 50.0, 40.6, 24.9, 24.8, 15.4.

3.12   **3.13**
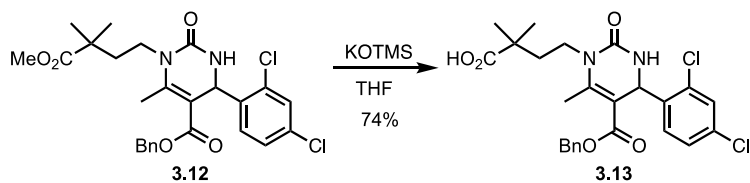
4-(5-((Benzyloxy)carbonyl)-4-(2,4-dichlorophenyl)-6-methyl-2-oxo-3,4-dihydropyrimidin-1(2H)-yl)-2,2-dimethylbutanoic acid (3.13). To a solution of **3.12** (0.111 g, 0.21 mmol, 1.0 eq) in THF (2.1 ml, [rxn]= 0.1 M) was added TMSOK (0.109 g, 0.84 mmol, 4.0 eq). The reaction mixture was stirred at rt for 6 h until the conversion was completed by TLC analysis. The mixture was quenched with 0.5 M citric acid (0.2 mL) and stirred for 5 min, diluted with water (5x), and extracted with Et_2O (3x). The combined organic layers were washed with brine, dried (Na_2SO_4), and purified by chromatography on SiO_2 (5% MeOH in CH_2Cl_2, spiked with 0.5% AcOH) to afford **3.13** (0.079 g, 0.16 mmol, 74%) as an off-white solid: **Mp**. 205-209 °C; **^{1}H NMR (500 MHz; DMSO-d$_6$)** δ 12.3 (1 H, brs), 7.93 (1 H, d, J = 3.0 Hz), 7.34 (1 H, dd, J = 8.5, 2.0 Hz), 7.23-7.21 (4 H, m), 6.99-6.98 (2 H, m), 5.57 (1 H, d, J = 3.0 Hz), 5.01 (1 H, d, J = 13 Hz), 4.91 (1 H, d, J = 13 Hz), 3.77 (1 H, m), 3.56 (1 H, m), 2.55 (3 H, s), 1.77 (1 H, td, J = 13, 5.0 Hz), 1.58 (1 H, td, J = 13, 5.0 Hz), 1.28-1.21 (6 H, d, J = 4.0 Hz); **^{13}C NMR (125 MHz; DMSO-d$_6$)** δ 178.3, 164.8, 151.6, 151.3, 139.2, 136.2, 132.9, 132.8, 129.9, 128.9, 128.1, 127.8, 127.7, 127.4, 100.3, 67.1, 64.9, 49.9, 40.2, 24.9, 24.8, 15.4; HRMS **ESI**$^-$ m/z calcd for $C_{25}H_{25}O_5N_5Cl_2$ [M-H] 503.1135, found 503.1142.
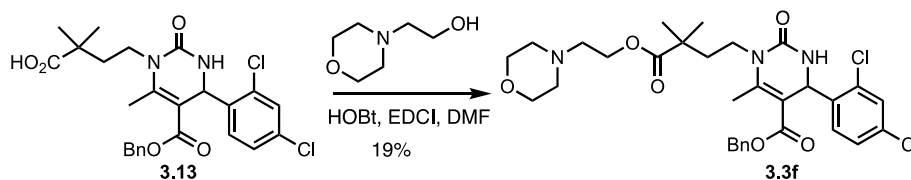
Benzyl 4-(2,4-dichlorophenyl)-6-methyl-1-(4-(2-morpholinoethoxy)-4-oxobutyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (3.3f). To a 0 °C solution of **3.13** (0.079 g, 0.16 mmol, 1.0 eq) in DMF (0.75 mL, [rxn]= 0.2 M) were treated EDCI (0.027 g, 0.017 mmol, 1.1 eq) and HOBt (0.024 g, 0.017 mmol, 1.1 eq). After 1 h of activation, the reaction mixture was treated

N-(2-hydroxyethyl) morpholine (2.0 M solution in DMF, 0.1 mL, 0.2 mmol, 1.3 eq), stirred for 15 h at rt, diluted with EtOAc (10x), washed with 0.1 M HCl (2x), water, and brine. The combined organic layers were dried (Na_2SO_4), and purified by chromatography on SiO_2 (gradient 1-10% MeOH in EtOAc; R_f= 0.19) to yield **3.3f** as a sticky foam (0.018 g, 0.029 mmol, 19%): **^{1}H NMR (400 MHz; CDCl$_3$)** δ 7.33 (1 H, d), 7.24-7.18 (3 H, m, overlapping with CDCl$_3$, 7.12 (1 H, dd, J = 8.0, 2.0 Hz), 7.02 (1 H, d,), 6.98 (1 H, dd, J = 8.0, 2.0 Hz), 5.74 (1 H, d, J = 3.0 Hz), 5.60 (1 H, d, J = 3.0 Hz), 5.05 (1 H, d, J = 13 Hz), 4.91 (1 H, d, J = 13 Hz), 4.23 (2 H, t, J = 6.0 Hz), 3.84 (1 H, td, J = 13, 4.6 Hz), 3.68-3.59 (5 H, m), 2.65-2.61 (5 H, m), 2.51 (4 H, s), 1.83 (1 H, td, J = 13, 4.6 Hz), 1.65 (1 H, td, J = 13, 4.6 Hz), 1.21 (6 H, s); **^{13}C NMR (100 MHz; CDCl$_3$)** δ 177.1, 165.3, 152.4, 151.7, 138.1, 135.9, 134.5, 133.6, 129.9, 128.6, 128.4, 128.1, 127.8, 101.2, 66.9, 66.1, 57.1, 53.8, 50.7, 41.2, 39.7, 39.4, 29.3, 25.5, 25.3, 16.1; HRMS **ESI$^+$** *m/z* calcd for $C_{31}H_{38}O_6N_3Cl_2$ [M+H] 618.2132, found 618.2130; **IR** (neat) 3046, 1709, 1636, 1524 cm^{-1}.

4.0 Thiadiazine Heterocycles as the Potential Hsp70 Agonists

"Synthesis-Including Organic Synthesis-Remains an Essential Part of Chemistry, and of Science and Technology. The trick will be to find classes of problems that return complex synthesis (Organic Synthesis, perhaps in an evolved and more expansive and ambitious form) to a central role in the future of the field, rather than having it become an extraordinary specialty or craft, admired but occupying an increasingly isolated place as the rest of synthesis moves on."-George M. Whitesides (2018)

4.1 Importance of the Sulfamide Group in Chemistry

The sulfamide is an important functional group in medicinal chemistry.[56-60] As drawn in Figure 7, the moiety presents in the ß-lactam antibiotic Doribax® and the histamine-2 blocker Pepcid®, which is used for treating heartburn and ulcer. Acyclic structures carried the sulfamide groups were considered as promising inhibitors against different classes of enzymes such as carbonic anhydrase,[61, 62] lipase,[63] c-Met kinase,[64] HIV-1 protease,[65-67] carboxypeptidase,[68, 69] and 11β-hydroxysteroid dehydrogenase type 1.[70] For some of these compounds, the sub nanomolar IC_{50} were found to come from the direct interactions between the sulfamide groups and the targeted proteins.[68, 69] As drawn in the same figure, cyclic structures carried the sulfamide group such as the 5-membered heterocyclic sulfamides were also found to have the moderate to good inhibition against γ-secretase and tyrosine phosphatase-1B.[71-72]
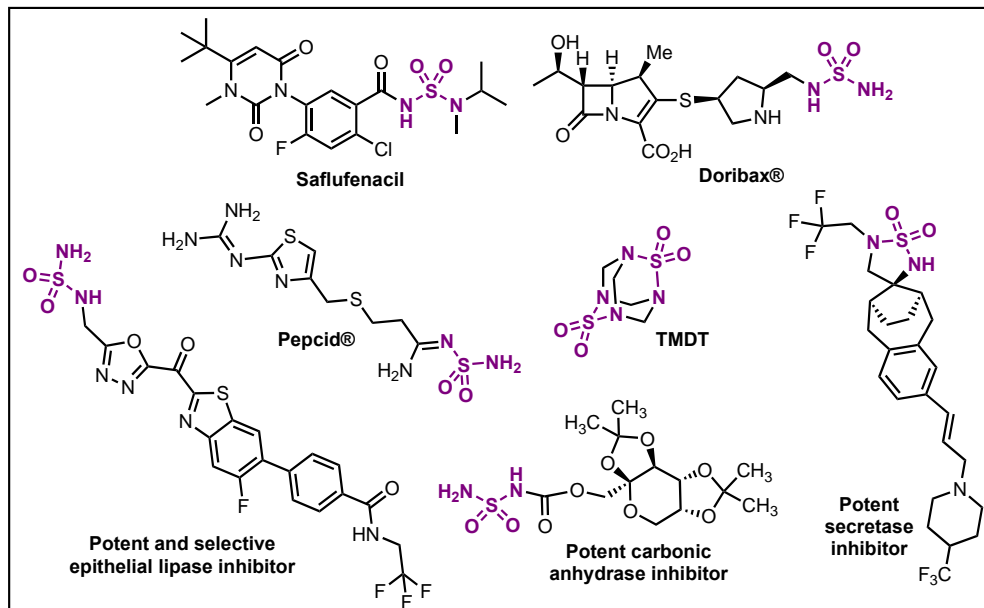
Figure 7. Examples of drugs, herbicides, and potent enzymatic inhibitors with the sulfamide groups

The sulfamide groups are also found in herbicides and pesticides. As provided in Figure 7, saflufenacil, which contains an acyclic sulfamide, is a regulated, mild and environmentally benign herbicide in China.[73, 74] On the other hands, tetramethylenedisulfontetramine (TMDT), which has a dimeric sulfamide, is highly toxic and currently banned as a pesticide in the same country. As an interesting side note, given TMDT's high potency with an estimated lethal dose of 0.1 mg/kg, this odorless and tasteless substance, unfortunately, is used as a common method of poisoning and considered as a potential chemical of agent or terror.

As part of our effort in improving the potency of MAL1-271, we were interested in using the sulfamide as a replacement for the urea group. Urea and sulfamide are bio-isosteres, yet the development of the sulfamide-containing pharmaceuticals lays far behind than that of the ureas. We envisioned that we could take advantage of the difference in H-bonding properties between the sulfonyl and the carbonyl moieties to engage new interaction between the small molecule and

the targeted protein complex. While the S=O groups of the sulfonyl can extend above and below

the plane of the heterocycle, the C=O in the carbonyl of the DHPM stays in the plane. This type

of replacement has been illustrated before in the search for HIV-protease inhibitors.[75, 76]

4.2 Wipf Group Methodology for the Synthesis of Thiadiazine Heterocycle

Early work from the Wipf group has established the preparation for a novel heterocycle,

1,2,6-thiadiazine 1,1-dioxides.[77-78] As drawn in Scheme 7, the thiadiazine heterocycle was

achieved via 2 steps from sulfamide and ethyl 3,3-diethoxypropionate. More recently, another

interesting thiadiazine scaffold was achieved via the acid-catalyzed 3-component Biginelli

reaction using mechanochemistry.[79]
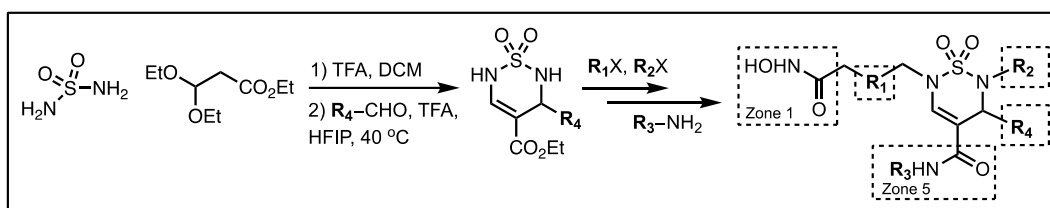
Scheme 7. Preparations for the thiadiazine heterocycle and analogues

Further chemical transformations developed by the Wipf group led to two series of

analogues containing the thiadiazine scaffold. Within the two series, one has an amide substituent

in zone 5, and the other has a hydroxamic group in zone 1 (Figure 8).[77-78] Based on the cellular

Huntington's disease model, which was conducted by the lab of Prof. J. L. Brodsky at Biological

Sciences (for further details, see Section 3.5), two compounds from the second series were showed

to be more active than MAL1-271.[78]
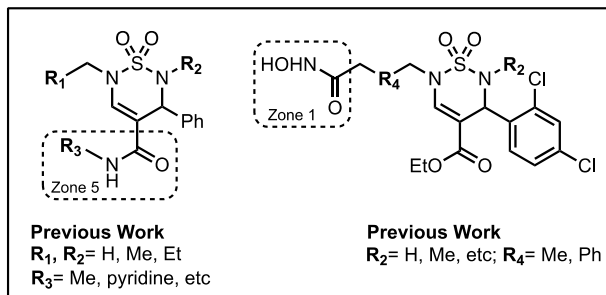
Figure 8. Previous modifications made on the thiadiazine scaffold

4.3 Rationales behind the Design of 4.8a-g

In order to evaluate the activity of the thiadizine series compared to the DHPM series, we focused on replacing the urea moiety in MAL1-271 with the sulfamide moiety while maintaining the carboxylic acid, the 3-carbon length linker, and the 2,4-dichlorobenzene (Figure 9). Based on the predicted key interactions from a docking study of MAL1-271 to the bacterial Hsp70, we learned that in MAL1-271, the benzyl ester, which corresponded to zone 5 in the thiadiazine series, was buried in a hydrophobic pocket.[38] Thus, a variety of hydrophobic groups was also placed as different ester substituents in zone 5.
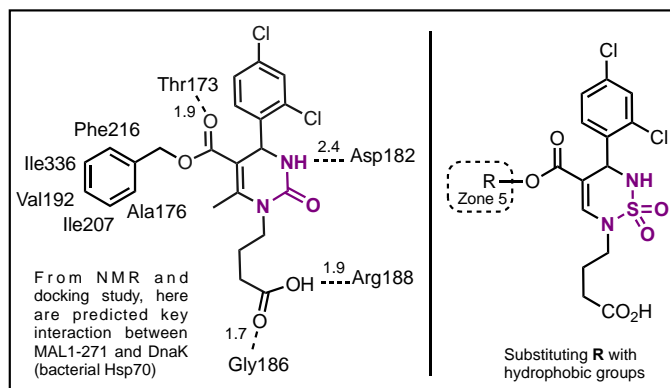
Figure 9. Rationale behind the design of 4.8a-c & 4.8e

The second modification we attempted was to use a different chlorine substituting pattern in zone 4 (Figure 10). We learned that by placing a phenyl, instead of the chlorine, in the solvent-exposed position (zone 4) resulted in an inhibitor.[38] Thus, we were curious about the agonist activity of the thiadiazine series when we carried subtle changes like replacing the 2,4- with the 3,4-dichlorobenzene and removing completely the two chlorine groups.
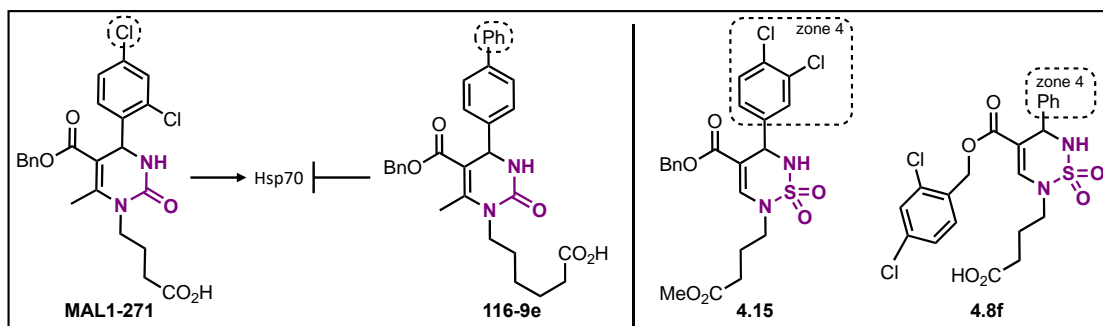
Figure 10. Rationale behind the design of 4.15 & 4.8f

4.4 Syntheses of 4.8a-g

4.4.1 1st Generation Route toward 4.8a-c

As drawn in Scheme 8, the 1st generation route, which involved 9 steps for the longest-linear sequence (LLS), started with a dimerization to form the known 8-membered thiadiazine **4.2,** which underwent an acid-mediated condensation with 2,4-dichlorobenzaldehyde to yield (±) **4.3** as a racemic mixture.[80] The product (±) **4.3** was subjected to a Mitsunobu alkylation to achieve **4.4** in 52% yield and **4.24** in 5% yield.[78] We found that the formation of the minor product **4.24**, which challenged the chromatography purification despite a small amount, was not reported in the past and could be avoided when all of the reagents were strictly used at the same equivalence.
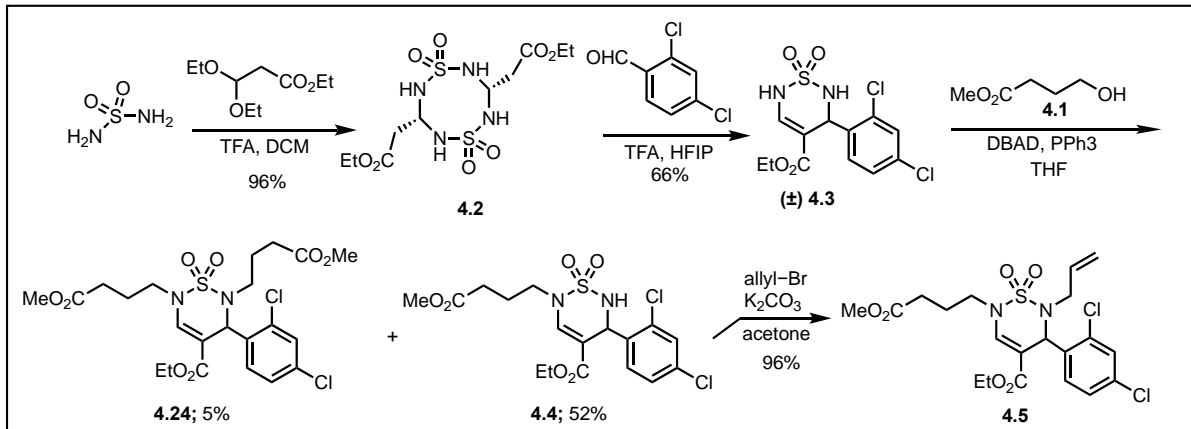
Scheme 8. 1st Generation route toward the common intermediate 4.5

Other efforts to study and eliminate the formation of **4.24** were switching from DBAD to DEAD and DIAD, running the reaction at higher concentration (4x), adding DBAD in several portions instead of one, and forming the complex between PPh₃ and DBAD before adding (±) **4.3**

and **4.1**. At this point, we think that the temperature at which the addition of DBAD took place to be an important parameter for the formation of **4.24.** In one of our protocol in which DBAD was added last to the reaction mixture at room temperature, **4.24** was not observed either on LC/MS or crude NMR. In contrast, the undesired product **4.24** was found when DBAD was added to the reaction mixture at 0 °C and leaved in the mixture for 5-30 min before warming up to rt.

We reasoned that due to the poor solubility of (±) **4.3** compared to **4.4** in the cooled THF, **4.4** continued to undergo the second Mitsunobu alkylation before (±) **4.3** was completely consumed. Although the visible precipitate of (±) **4.3** in 0 °C THF was not observed, further experiment to confirm its solubility in THF should be helpful. As a result, the regioselectivity of the Mitsunobu step could be completely achieved when all of the reagents were added at rt. At the same time, the quality of the alcohol **4.1** should also be considered as another important parameter for the formation of **4.24**. Several commercially available alcohols were tried at both 0 °C and rt and found with no formation of the corresponding bis-alkylated product. (see Section 5.5.2).
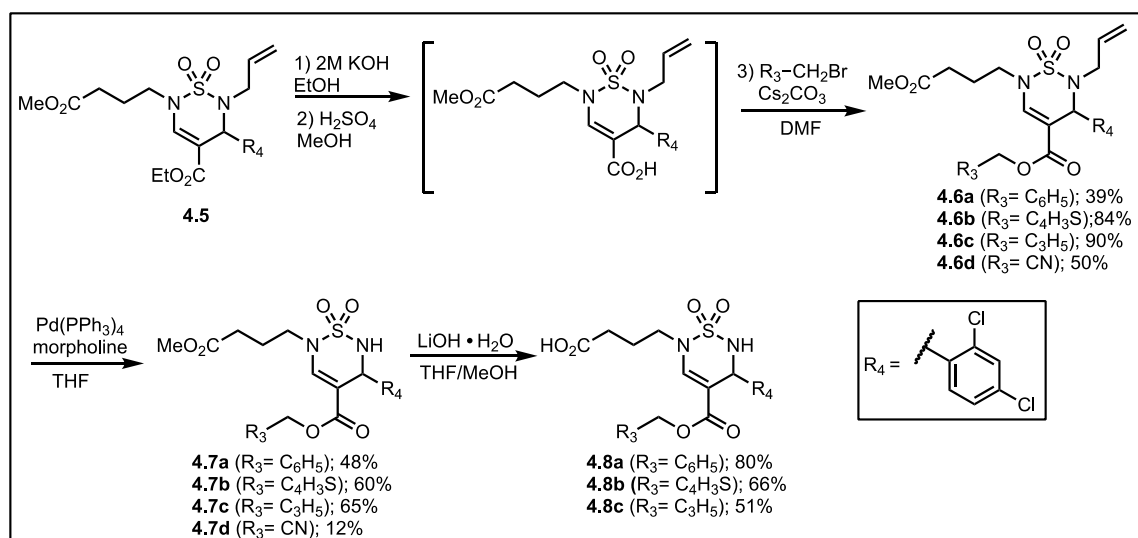
Scheme 9. 1ˢᵗ Generation route toward 4.8a-c

The formation of **4.24** raised a concern about the acidity and thus reactivity of the remaining N atom (ca. pKa= 9.5) in **4.4**, leading to the subsequent allylic protection of **4.4** to make **4.5**.[78] The common intermediate **4.5**, as drawn in Scheme 9, was then subjected to a sequence of a hydrolysis, a regioselective Fischer esterification, and a S_N2 with various bromine electrophiles such as benzyl bromide, (bromomethyl)cyclopropane, 3-(bromomethyl)thiophene, and bromo acetonitrile to form **4.6a-d**, with yields varied from 39-90% over 3 steps. Then, **4.6a-d** were subjected to a deprotection of the allylic group with $Pd(PPh_3)_4$ to yield **4.7a-d** in 12-65%. For the final step, the methyl esters of **4.7a-c** were chemo selective hydrolyzed under a mild hydrolysis condition using $LiOH\bullet H_2O$ to afford the final targets **4.8a-c** in 51-80%.

4.4.2 2nd Generation Route toward 4.8e & 4.15

With the aim to improve the overall efficiency of the synthesis, we designed the 2nd generation route, using a Steglich esterification instead of the S_N2. This effectively removed the series of protection and deprotection, scaling down the LLS from 9 to 7 steps. As drawn in Scheme 10, **4.4** and **4.14** were obtained in 52% and 54%, respectively via a series of similar steps from 1st route. The steps are the dimerization, the acid-mediated condensation, and the Mitsunobu alkylation.
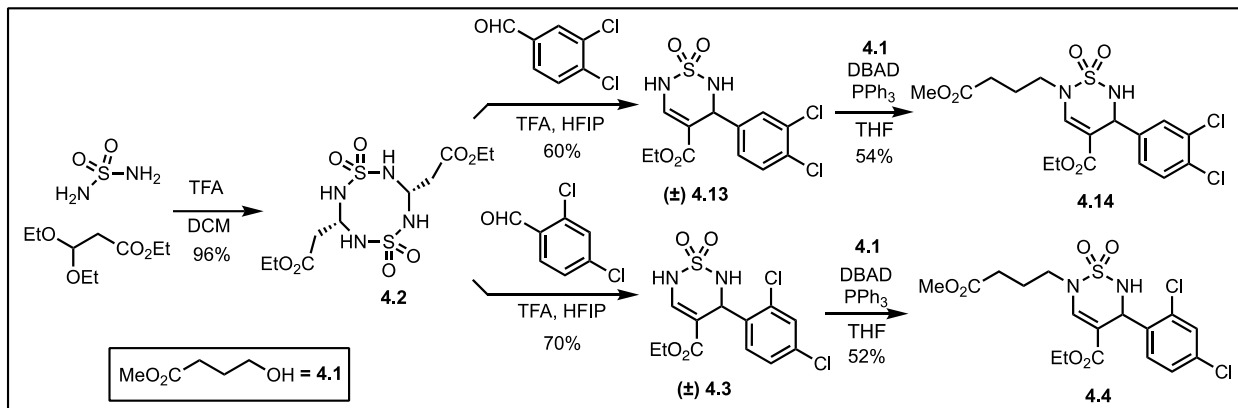
Scheme 10. 2nd Generation route toward 4.4 & 4.14

The two intermediates **4.4** and **4.14** were then underwent another series of a hydrolysis with KOH, a Fischer esterification, and a Steglich reaction with 2,4-dichlorobenzyl alcohol furnish **4.11** and **4.15** in 24% and 67% yields, respectively (Scheme 11). An addition of HOBt to the current Steglich condition may improve the efficiency of the step. An early Steglich protocol involved the use of HOBt (see Section 3.4.1) was applied to several DHPM analogues and resulted in good yields. For the final step, the similar mild and chemo-selectivity hydrolysis of the methyl ester with LiOH•H_2O was applied to **4.11** to obtain **4.8e** in 67% yield.
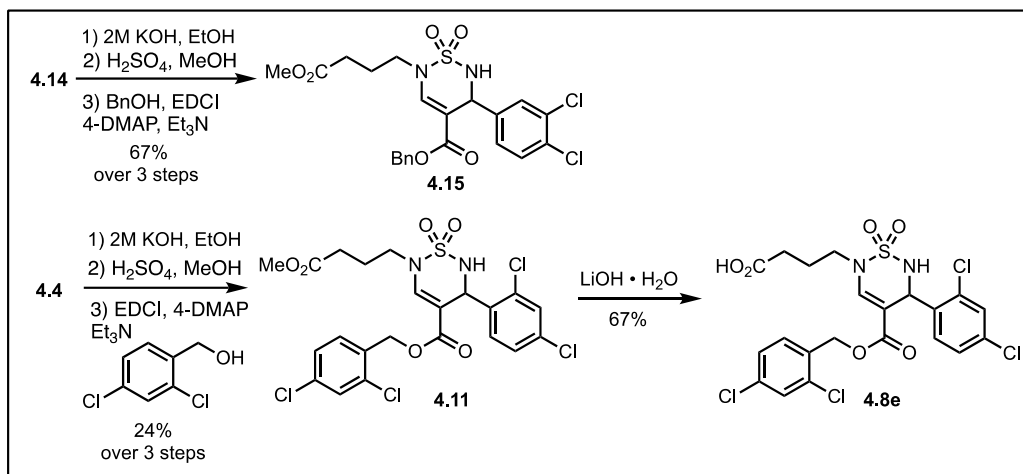
Scheme 11. 2ⁿᵈ Generation route toward 4.8e & 4.15

4.4.3 3ʳᵈ Generation Route toward 4.8f-g

A 3ʳᵈ generation route, with 6 steps for the LLS, was accomplished when the Fischer esterification was also eliminated.[81] The shortened route was enabled when ethyl 3,3-diethoxypropionate was found to successfully undergo a sequence of a hydrolysis and a Steglich esterification to provide **4.18** in 87% yield over 2 steps (Scheme 12).[81, 82] The resulting ester **4.18** underwent a dimerization with sulfamide to form the thiadiazine **4.19** in 90% yield.

Solubility of the dimer **4.19**, unfortunately, was a challenge under the acid-mediated condensation. An excess of TFA (20.0 eq) and a long reaction time (>20 hours) were needed to facilitate its solubility. Nonetheless, as seen in Scheme 10, the heterocycles (±) **4.20** and (±) **4.22** were formed in decent yields, 53% and 87%, respectively as racemic mixtures. The intermediate (±) **4.20** was subjected to the Mitsunobu protocol with alcohol **4.1** to form the semi-clean **4.21** in 75% yield, while (±) **4.22** was coupled with alcohol **3.9** to form **4.23** in 76% yield.

For the final step, compound **4.21** successfully underwent the same chemo selective hydrolysis at the methyl ester with LiOH•H₂O to form the corresponding acid **4.8f** in 51% yield.

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Compound **4.23**, in contrast, failed to hydrolyze under the same condition. A complex mixture of over-hydrolyzing product and the desired product was found when excess amounts of the base were used. Yet, using 2.0 eq of LiOH•H$_2$O either at rt or elevated temperature (60 $^{\circ}$C) did not facilitate any hydrolysis. Potassium trimethylsilanolate (KOTMS), thus, was used to selectively hydrolyze the methyl ester of **4.23** to form **4.8g** in 48% yield.[83]
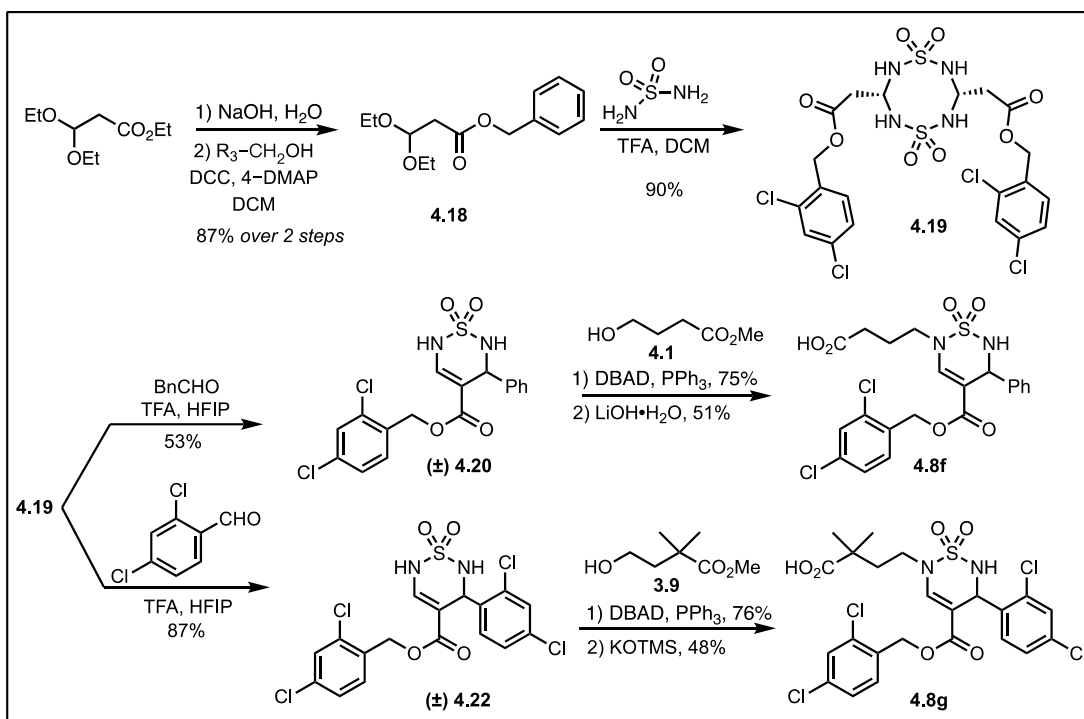
Scheme 12. 3rd Generation route toward 4.8f-g

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4.5 Biological Evaluation of the Thiadiazine Analogues

The bBiological evaluation was performed by the lab of Prof. Brodsky (for further details, see Section 3.5). The effectiveness of these analogues in the same cellular Huntington's disease model was evaluated qualitatively based on the number of toxic aggregates. The fewer toxic aggregates were observed between these analogues and MAL1-271, the more effective the analogues were compared to MAL1-271 While the biological evaluation is being analyzed for **4.8f-g**, some of the results for **4.8a-e** and related intermediates were reported in Figure 11.
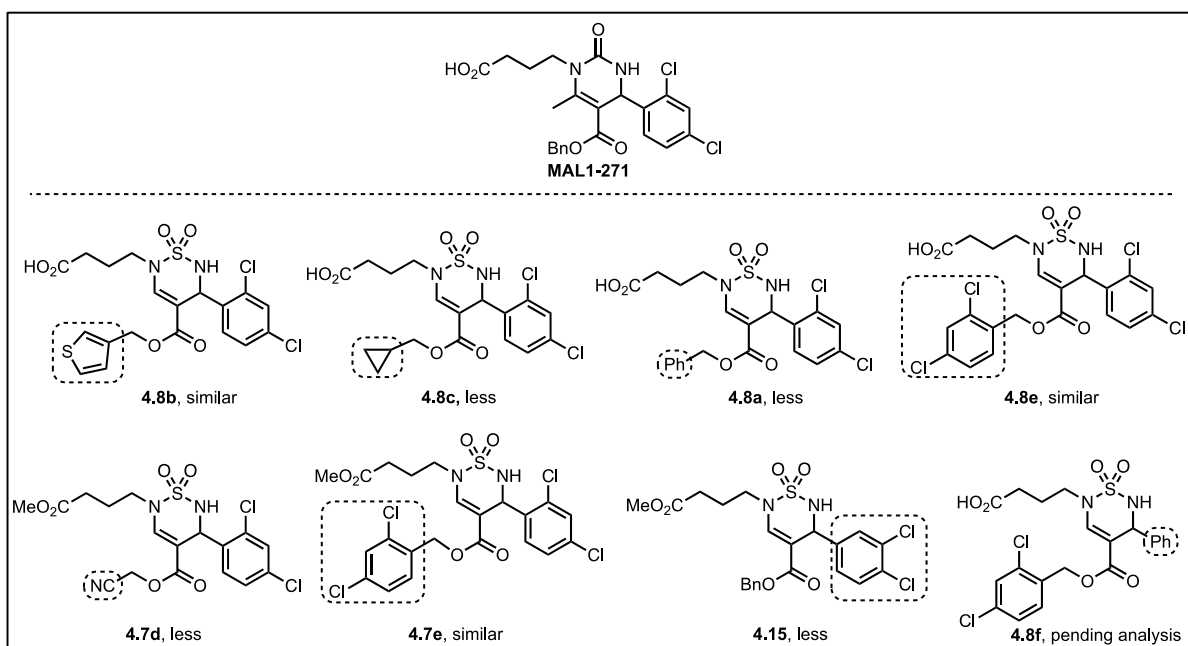
Figure 11. Comparison of the effectiveness between the 4.8a-g and MAL1-271

Specifically, the substitution in zone 5 with different hydrophobic groups resulted in **4.8b** and **4.8e** that retained the agonist activities of MAL1-271. On the other hand, the switch to the hydrophilic group such as methyl acetonitrile resulted in **4.7d** with a loss of the activity. The

replacement of a 2,4- with a 3,4-dichlorobenzene resulted in **4.15,** which also showed the loss of agonist activity. Thus, it would be interesting to perform the same evaluation on the carboxylic acid equivalence in place of the methyl ester of **4.15.** At the same time, the substitutions made in either zone 4 or 5 using the 2,4-dichlorobenzene moiety yield **4.7e** and **4.8e**, that are both as effective as MAL1-271.

4.6 Conclusion and Future Directions

In conclusion, we were able to synthesize several thiadiazine analogues with the emphasis on exchanging the urea moiety in MAL1-271 with a sulfamide group. Modifications were achieved in zones 1, 4, and 5. Some of the analogues were reported here and evaluated against toxic aggregates in the cellular model of Huntington's disease in the lab of Prof. Brodsky. The results identified two lead compounds with similar activity as MAL1-271. Yet, we find that it would be more helpful to validate the use of the ester group in zone 5. As the ester is known to undergo hydrolysis to an acid, it would be necessary and insightful to perform a LC/MS study, for example, to check the stability of this vinylogous carbamate group under the assay conditions.

As seen in Figure 12, we proposed that for the future chemistry development it would be interesting to explore the incorporation of a methylene group, which has not yet been investigated. The second direction is to increase the overall rigidification of the structure, which we proposed to explore with a cyclic linker while maintaining the other moieties that are relevant to the agonist activity. This led to the thesis' final chapter, which showed some pre-liminary results for the construction of a macrocycle-containing analogue.
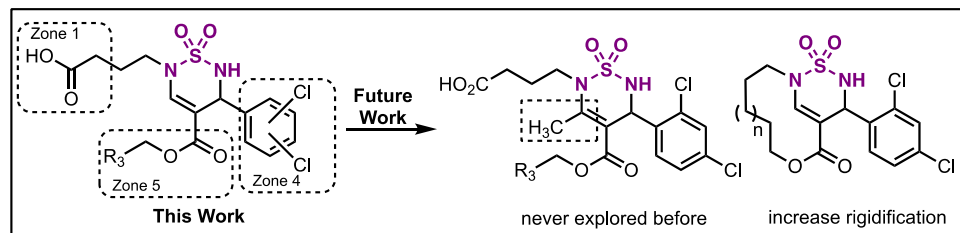
Figure 12. Future directions toward additional analogues

4.7 Experimentals

4.7.1 General

All reactions were carried out under a positive pressure of N_2 in a well-ventilated fume hood unless otherwise noted. Hexanes (ACS grade), ethyl acetate (ACS grade), toluene (ACS grade), and diethyl ether (ACS grade), dichloromethane (ACS grade), acetonitrile (ACS grade), and chloroform (ACS grade) were purchased from Fisher Chemical and used without further purification. Anhydrous tetrahydrofuran was distilled from sodium (10% w/v) under a positive pressure of N_2. Anhydrous dichloromethane and chloroform were distilled from calcium hydride (10% w/v) under a positive pressure of N_2. Commercially available reagents were used without further purification unless otherwise noted. Reactions were monitored by TLC analysis (pre-coated silica gel 60 F254 plates, 250 µm layer thickness) and visualization was accomplished with a 254 nm UV light and by staining with a $KMnO_4$ solution (1.5 g of $KMnO_4$ and 1.5 g of K_2CO_3 in 100 mL of a 0.1% NaOH solution). Flash column chromatography was performed over Silica gel 60 (particle size 0.04-0.063 mm) from EMD chemicals. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker 300, 400, and 500 (equipped with cryoprobe) spectrometers using residual

solvent peaks as internal standard (CDCl$_3$ @ 7.26 ppm ^{1}H NMR, 77.00 ppm ^{13}C NMR; DMSO-d$_6$ @ 2.50 ppm ^{1}H NMR, 39.52 ppm ^{13}C NMR; C$_6$D$_6$ @ 7.16 ppm ^{1}H NMR, 128.06 ppm ^{13}C NMR). Low- resolution mass spectra were recorded on an Agilent Technologies 1260 Infinity II LCMS. High-resolution mass spectra were obtained on a Micromass UK Limited, Q-TOF Ultima API or a Thermo Scientific Exactive Orbitrap LCMS.

4.7.2 Experimentals
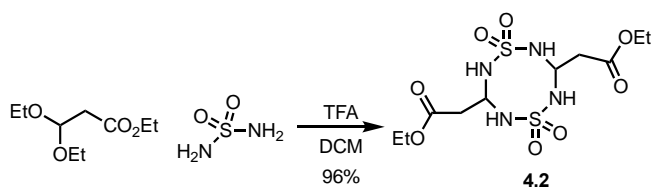
Diethyl 2,2'-((3s,7s)-1,1,5,5-tetraoxido-1,5,2,4,6,8-dithiatetrazocane-3,7-diyl)diacetate (4.2).[84] To a suspension of sulfamide (12.0 g, 118 mmol, 1.0 eq) and TFA (46 mL, 607 mmol, 5.0 eq) in CH$_2$Cl$_2$ (230 mL, [rxn]= 0.5 M) was added ethyl 3,3-diethoxypropanoate (26 mL, 130 mmol, 1.1 eq) over 15 min. The suspension was stirred for 4 h, filtered through a medium glass fritted funnel, washed with CH$_2$Cl$_2$, MeOH, and Et$_2$O to yield **4.2** (22.0 g, 57 mmol, 96%) as an off-white solid: **Mp.** 182-183 °C: **^{1}H NMR (500 MHz, DMSO-d$_6$)** δ 7.53 (4 H, d, J = 9.5 Hz), 5.22-5.15 (2 H, m), 4.06 (4 H, q, J = 7.0 Hz), 2.64 (4 H, d, J = 7.0 Hz), 1.18 (6 H, t, J = 7.5 Hz); **^{13}C NMR (125 MHz, DMSO-d$_6$)** δ 168.5, 62.1, 60.2, 41.1, 14.0; HRMS **ESI$^+$** m/z calcd for C$_{10}$H$_{21}$N$_4$O$_8$S$_2$ [M+H] 389.0801, found 389.0779; **IR** (neat) 3318, 2990, 1717, 1348, 1335, 1048 cm^{-1}.
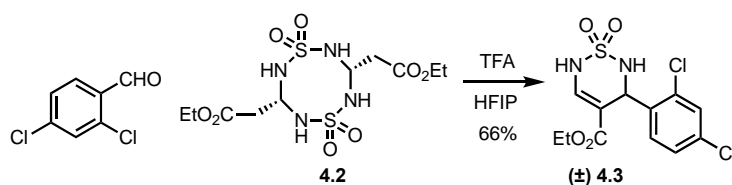
Ethyl 3-phenyl-3,6-dihydro-2*H*-1,2,6-thiadiazine-4-carboxylate 1,1-dioxide (±) 4.3.[85] To a suspension of sulfamide **4.2** (10.0 g, 26 mmol, 1.0 eq) and 2,4-dichlorobenzaldehyde (9.00 g, 51 mmol, 2.0 eq) in HFIP (130 mL, [rxn]= 0.2 M) was treated dropwise TFA (9.65 mL, 128 mmol, 5.0 eq) via a syringe pump. The resulting white mixture was stirred at 35-40 °C for 15 h, quenched with 1/1 EtOAc: sat. NaHCO$_3$, and extracted with EtOAc (3x). The combined organic layers were washed with brine, dried (Na$_2$SO$_4$), and purified by chromatography on SiO$_2$ (15% EtOAc in CH$_2$Cl$_2$) to afford **(±) 4.3** (4.40 g, 12.5 mmol, 66%) as an off-white solid: **Mp.** 192-201 °C: **^{1}H NMR (400 MHz, DMSO-d$_6$)** δ 11.0 (1 H, brs), 8.17 (1 H, d, *J* = 7.2 Hz), 7.62 (1 H, d, *J* = 2.0 Hz), 7.59 (1 H, s), 7.36 (1 H, dd, *J* = 8.4, 2.0 Hz), 7.21 (1 H, d, *J* = 8.4 Hz), 5.59 (1 H, d, *J* = 6.8 Hz), 4.05-3.96 (2 H, m) 1.05 (3 H, t, *J* = 6.8 Hz); **^{13}C NMR (100 MHz, DMSO-d$_6$)** δ 164.7, 139.8, 135.1, 133.9, 133.1, 131.4, 128.7, 126.6, 101.2, 59.8, 54.0, 14.0; HRMS **ESI$^+$** *m/z* calcd for C$_{12}$H$_{11}$N$_2$O$_4$S [M-H] 348.9811, found 348.9892; **IR** (neat) 3269, 3174, 2833, 2185, 1666, 1635, 1586 cm^{-1}.
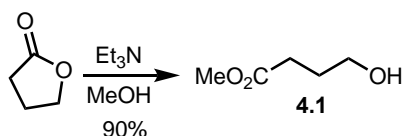
Methyl 4-hydroxybutanoate (4.1).[86] To a solution of γ-butyrolactone (1.0 mL, 13 mmol, 1 eq) in MeOH (65 mL, [rxn]= 0.2 M) was added Et$_3$N (8.8 mL, 63 mmol, 4.9 eq). The solution was stirred at 60 ºC for 16 h and concentrated. The residue was purified by chromatography on SiO$_2$ (5% MeOH in CH$_2$Cl$_2$) and azetroped with hexane (3x) to afford **4.1** (1.37 g, 12 mmol, 90%) as yellow oil: **^{1}H NMR (300 MHz, CDCl$_3$)** δ 3.56-3.50 (5 H, m), 2.32 (2 H, t, *J* = 7.2 Hz), 1.79-1.74 (2 H, m); **^{13}C NMR (75 MHz, CDCl$_3$)** δ 174.4, 61.5, 51.6, 30.5, 27.6.
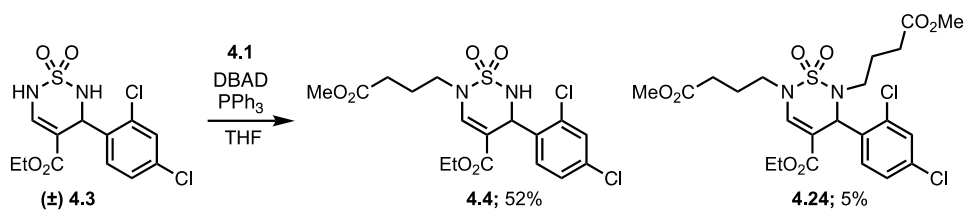
Ethyl 3-(2,4-dichlorophenyl)-6-(4-methoxy-4-oxobutyl)-3,6-dihydro-2*H*-1,2,6-thiadiazine-4-carboxylate 1,1-dioxide (4.4). To a solution of **(±) 4.3** (1.19 g, 3.2 mmol, 1.0 eq), PPh₃ (1.02 g, 3.9 mmol, 1.2 eq), and alcohol **4.1** (0.44 mL, 3.9 mmol, 1.2 eq) in THF (19 mL, [rxn]= 0.17 M) was treated DBAD (0.910 g, 3.9 mmol, 1.2 eq) in one portion at 0 °C. The reaction was warmed to rt, stirred for 15 h, quenched with H_2O, and extracted with CH_2Cl_2 (3x). The combined organic layers were washed with brine, dried ($MgSO_4$), and purified by chromatography on SiO_2 (gradient 20-30% EtOAc in hexane) to yield **4.4** (0.800 g, 95% purity, 1.68 mmol, 52%) as a colorless foam and **4.24** (0.090 g, 0.16 mmol, 5.0%) as s sticky gum. **Ethyl 3-(2,4-ichlorophenyl)-6-(4-methoxy-4-oxobutyl)-3,6-dihydro-2*H*-1,2,6-thiadiazine-4-carboxylate 1,1-dioxide (4.4): ¹H NMR (500 MHz, CDCl₃)** δ ¹H NMR (500 MHz, CDCl₃) δ 7.51 (brs, 1 H), 7.43 (1H, d, $J = 2.0$ Hz), 7.28 (1H, d), 7.20 (1H, dd, $J = 2.0, 9.0$ Hz), 5.90 (1H, d, $J = 8.0$ Hz), 4.91 (1H, d, $J = 8.0$ Hz), 4.07 (2H, dq, $J = 4.0, 7.2$ Hz), 3.70 (3H, brs), 3.68 (2H, dt, $J = 3.0, 6.8$ Hz), 2.46 (2H, t, $J = 7.0$ Hz), 2.09 (2H, dquint, $J = 6.8, 7.0$ Hz), 1.09 (3H, t, $J = 7.2$ Hz); **¹³C NMR (125 MHz, CDCl₃)** δ 173.3, 164.9, 142.4, 134.9,134.6, 133.8, 130.6,129.7, 127.0, 104.6, 60.1, 55.6, 52.0, 49.5, 30.7, 24.8, 14.1; HRMS **ESI+** *m/z* calcd for $C_{17}H_{21}O_6N_2Cl_2S$ [M+H]⁺ 451.0419, found 451.0493; **IR** (neat) 3197, 3064, 2953, 1725, 1664, 1626, 1589, 759 cm⁻¹.

Dimethyl 4,4'-(3-(2,4-dichlorophenyl)-4-(ethoxycarbonyl)-1,1-dioxido-2*H*-1,2,6-thiadiazine-2,6(3*H*)-diyl)dibutyrate (4.24): ¹H NMR (500 MHz, C₆D₆) δ 7.82 (1 H, s), 7.49 (1 H, s), 7.38 (1 H, d, $J = 8.5$ Hz), 6.87 (1 H, dd, $J = 2.0, 8.5$ Hz), 5.85 (1 H, s), 3.94-3.91 (1 H, m), 3.88-4.84 (1

H, m), 3.35-3.31 (7 H, m), 3.19-3.14 (1H, m), 3.06-3.02 (1 H, m), 2.92-2.88 (1 H, m), 2.13-2.11 (3H, m), 2.10-2.02 (1 H, m), 1.96-1.91 (2 H, m), 1.71-1.66 (2 H, m), 0.82 (3 H, t, J = 7.0 Hz) ^{13}**C NMR (125 MHz, C$_6$Cl$_6$)** δ 172.3, 172.0, 141.8, 132.1, 129.3, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.2, 126.4, 102.4, 77.3, 63.2, 60.2, 53.1, 50.9, 50.8, 49.2, 30.3, 29.8, 24.7, 23.2, 13.8; HRMS **ESI**$^+$ m/z calcd for C$_{22}$H$_{29}$O$_8$N$_2$Cl$_2$S [M+H]$^+$ 551.1022, found 551.1011; **IR** (neat) 2956, 1735, 1699, 1627, 1588, 1561 cm^{-1}.
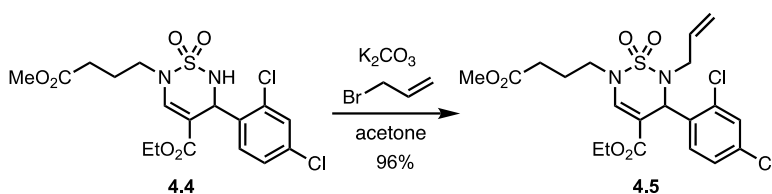
Ethyl 2-allyl-3-(2,4-dichlorophenyl)-6-(4-methoxy-4-oxobutyl)-3,6-dihydro-2H-1,2,6-thiadiazine-4-carboxylate 1,1-dioxide (4.5). To a solution of **4.4** (0.240 g, 0.53 mmol, 1.0 eq) in acetone (1.8 mL, [rxn]= 0.3 M) was added allyl bromide (0.14 mL, 1.6 mmol, 3.0 eq) and K$_2$CO$_3$ (finely ground, 0.367 g, 2.65 mmol, 5.0 eq). The reaction was stirred at rt for 7 h until the conversion was completed by TLC analysis. The reaction mixture was filtered, and the eluent was purified by chromatography on SiO$_2$ (30% EtOAc in hexane; **R$_f$**: 0.25) to yield **4.5** (0.249 g, 0.51 mmol, 96%) as sticky yellow oil: **^{1}H NMR (400 MHz; CDCl$_3$)** δ 7.52 (1 H, s), 7.39 (1 H, d, J = 2.0 Hz), 7.26-7.23 (1 H, m), 7.16 (1 H, dd, J = 8.0, 2.0 Hz), 6.08-5.98 (1 H, m), 5.69 (1 H, s), 5.38-5.27 (2 H, m), 4.14-4.07 (3 H, m), 3.86-3.83 (1 H, m), 3.71 (4 H, m), 3.68-3.57 (1 H, m), 2.44 (2 H, t, J = 7.0 Hz), 2.17 (2 H, s), 2.11- 2.04 (2 H, m), 1.14 (3 H, t, J = 7.0 Hz); **^{13}C NMR (100 MHz; CDCl$_3$)** δ 172., 165.0, 142.1, 134.6, 134.2, 131.6, 131.3, 129.5, 126.6, 121.3, 102.6, 60.8, 60.3, 56.4, 52.0, 49.8, 31.1, 30.5, 25.1, 14.3; HRMS **ESI**$^+$ *m/z* calcd for C$_{20}$H$_{25}$O$_6$N$_2$Cl$_2$S [M+H] 491.0804, found 491.0805; **IR** (neat) 2983, 2952, 1734, 1698, 1626, 1588 cm^{-1}.
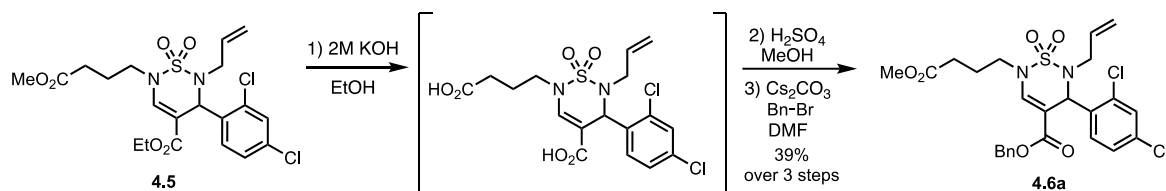
Benzyl 2-allyl-3-(2,4-dichlorophenyl)-6-(4-methoxy-4-oxobutyl)-3,6-dihydro-2*H*-1,2,6-thiadiazine-4-carboxylate 1,1-dioxide (4.6a). To a solution of **4.5** (0.900 g, 1.83 mmol, 1.0 eq) in EtOH (18 mL, [rxn]= 0.1 M) was added 2.0 M KOH (11 mL, 21.9 mmol, 12 eq). The solution was stirred at 80 °C for 15 h, diluted with CH_2Cl_2 (5x), and acidified with 4.0 M HCl until pH=3. The aqueous layer was extracted with EtOAc (3x). The combined organic layers were washed with brine and dried (Na_2SO_4) to afford the crude bis acid as an orange solid, which was used for the next step without further purification.

To a solution of the crude bis acid in MeOH (34 mL, [rxn]= 0.06 M) was added 0.1/25 H_2SO_4/MeOH (0.1 mL). The resulting orange solution was stirred at 50 °C for 5 h until the conversion was completed by LC/MS analysis. The solution was extracted with brine/ CH_2Cl_2 (2x). The organic layers were dried (Na_2SO_4) to afford the crude methyl ester as an orange solid, which was used without further purification.

To a solution of the crude methyl ester (0.90 g, 1.9 mmol, 1.0 eq) in DMF (20 mL, [rxn]= 0.1 M) were treated benzyl bromide (0.4 mL, 2.7 mmol, 1.4 eq) and Cs_2CO_3 (0.820 g, 2.5 mmol, 1.3 eq). The mixture was stirred 22 h, diluted with H_2O (10x), and extracted with CH_2Cl_2 (3x). The combined organic layers were washed with brine, dried (Na_2SO_4), and purified by chromatography on SiO_2 (30% EtOAc in hexane; **R_f:** 0.26) to afford **4.6a** as yellow sticky oil (0.419 g, 0.78 mmol, 39% over 3 steps): **¹H NMR (500 MHz; CDCl₃)** δ 7.59 (1 H, s), 7.38 (1 H, d, *J* = 2.5 Hz), 7.30-7.27 (3 H, m), 7.24 (1 H, d, *J* = 8.5 Hz), 7.12 (1 H, dd, *J* = 8.5, 2.0 Hz), 7.09-

7.08 (2 H, m), 6.04-6.02 (1 H, m), 5.74 (1 H, s), 5.34 (1 H, dd, J = 17, 1.0 Hz), 5.27 (1 H, dd, J =

17, 1.0 Hz), 5.15 (1 H, d, J = 13 Hz), 5.03 (1 H, d, J = 13 Hz), 4.13 (1 H, dd, J = 5.5, 6.5 Hz),

3.85 (1 H, d, J = 5.5, 6.5 Hz), 3.72-3.67 (4 H, m), 3.64-3.59 (1 H, m), 2.42 (2 H, t, J = 7.0 Hz),

2.07 (2 H, q, J = 7.0 Hz); **^{13}C NMR (125 MHz; CDCl$_3$)** δ 127.3, 164.7, 142.6, 135.7, 134.6, 134.5,

134.1, 131.7, 131.1, 129.3, 128.5, 128.2, 127.7, 126.5, 121.2, 101.1, 66.3, 60.3, 56.3, 51.8, 49.8,

30.4, 25.0; HRMS **ESI$^+$** m/z calcd for C$_{25}$H$_{27}$O$_6$N$_2$Cl$_2$S [M+H] 553.0961, found 553.0970; **IR**

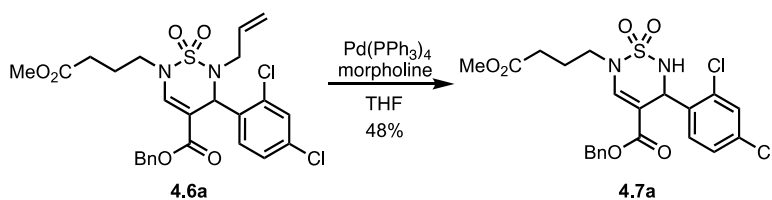(neat) 3220, 2159, 1707, 1624, 1562 cm^{-1}.

Benzyl 3-(2,4-dichlorophenyl)-6-(4-methoxy-4-oxobutyl)-3,6-dihydro-2H-1,2,6-thiadiazine-4-carboxylate 1,1-dioxide (4.7a). To a degassed solution of **4.6a** (0.420 g, 0.76 mmol, 1.0 eq) in

THF (6.0 mL, [rxn]= 0.1 M) were treated Pd(PPh$_3$)$_4$ (0.090 g, 0.08 mmol, 0.1 eq) and dropwise

morpholine (0.90 mL,10 mmol, 14 eq). The mixture was stirred at rt for 5 h until the conversion

was completed by TLC analysis and purified by chromatography on SiO$_2$ (30% EtOAc in hexane,

R$_f$: 0.1) to afford **4.7a** (0.19 g, 0.37 mmol, 48%) as a yellow sticky foam: **^{1}H NMR (500 MHz;**

CDCl$_3$) δ 7.54 (1 H, s), 7.38 (1 H, d, J = 2.0 Hz), 7.38-7.27 (3 H, m), 7.23 (1 H, d, J = 8.5 Hz),

7.13 (1 H, dd, J = 8.0, 2.0 Hz), 7.05 (2 H, dd, J = 8.0, 2.0 Hz), 5.91 (1 H, s), 5.13 (1 H, d, J = 12

Hz), 4.94 (1 H, d, J = 12.5 Hz), 4.90 (1 H, s), 3.69-3.67 (5 H, m), 2.45 (2 H, t, J = 7.0 Hz), 2.10-

2.04 (2 H, m); **^{13}C NMR (125 MHz; CDCl$_3$)** δ 173.3, 164.6, 142.8, 135.6, 135.1, 134.7, 133.7,

130.6, 129.8, 128.6, 128.4, 128.2, 127.2, 104.5, 66.5, 55.8, 52.0, 49.7, 30.7, 24.8.
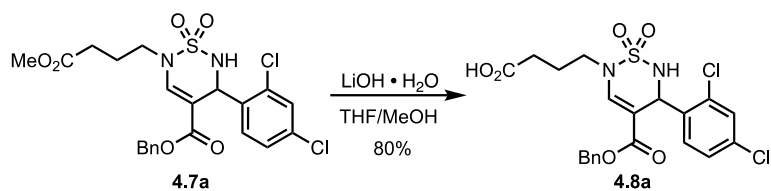
4-(4-((Benzyloxy)carbonyl)-5-(2,4-dichlorophenyl)-1,1-dioxido-5,6-dihydro-2*H*-1,2,6-thiadiazin-2-yl)butanoic acid (4.8a). To a solution of **4.7a** (0.270 g, 0.52 mmol, 1.0 eq) in 1/1 THF/MeOH (5.3 mL, [rxn]= 0.1 M) was added LiOH•H$_2$O (1.0 M solution, 1.1 mL, 1.1 mmol, 2.0 eq). The mixture was stirred at rt for 2 h until the conversion was completed by TLC and LC/MS analysis. The mixture was diluted with CH$_2$Cl$_2$ (5x), acidified with 1.0 M HCl until pH=3 and extracted with 10% MeOH in CH$_2$Cl$_2$ (3x). The combined organic layers were washed with brine, and dried (MgSO$_4$) to afford **4.8a** as a colorless foam (0.260 g, 0.42 mmol, 80%): **^{1}H NMR (500 MHz; CDCl$_3$)** δ 7.56 (1 H, s), 7.36 (1 H, d, *J* = 2.0), 7.29-7.27 (2 H, m, overlapping with CDCl$_3$), 7.19 (1 H, d, *J* = 8.0 Hz), 7.11 (1 H, dd, *J* = 8.0, 2.0 Hz), 7.03-7.01 (2 H, m), 5.89 (1 H, d, *J* = 8.0 Hz), 5.16 (1 H, d, *J* = 8.0 Hz), 5.10 (1 H, d, *J* = 13 Hz), 4.93 (1 H, d, *J* = 13 Hz), 3.73-3.63 (2 H, m), 2.49 (2 H, t, *J* = 7.2 Hz), 2.06 (2 H, q, *J* = 7.2 Hz); **^{13}C NMR (125 MHz; CDCl$_3$)** δ 177.3, 164.8, 142.9, 135.4, 135.1, 134.6, 133.6, 130.6, 129.8, 128.6, 128.4, 128.1, 127.1, 104.4, 66.6, 55.6, 49.3, 30.4, 24.5; HRMS **ESI$^+$** *m/z* calcd for C$_{21}$H$_{21}$O$_6$N$_2$Cl$_2$S [M+H] 499.0499, found 499.0492; **IR** (neat) 3220, 2159, 1707, 1624, 1562 cm^{-1}.

Thiophen-3-ylmethyl 2-allyl-3-(2,4-dichlorophenyl)-6-(4-methoxy-4-oxobutyl)-3,6-dihydro-2*H*-1,2,6-thiadiazine-4-carboxylate 1,1-dioxide (4.6b). To a solution of **4.5** (0.900 g, 1.8 mmol,

1.0 eq) in EtOH (18 mL, [rxn]= 0.1 M) was added 2.0 M KOH (11 mL, 21.9 mmol, 12 eq). The solution was stirred at 80 °C for 15 h, diluted with CH_2Cl_2 (5x), and acidified with 1.0 M HCl until pH=3. The aqueous layer was extracted with EtOAc (3x). The combined organic layers were washed with brine and dried (Na_2SO_4) to afford the crude bis acid as an orange solid, which was used without further purification.

To a solution of the crude bis acid in MeOH (34 mL, [rxn]= 0.06 M) was added 0.1/25 H_2SO_4/MeOH (0.1 mL). The resulting orange solution was stirred at 50 °C for 5 h until the conversion was completed with LC/MS analysis. The solution was extracted with brine/ CH_2Cl_2 (2x). The organic layers were dried (Na_2SO_4) and concentrated to afford the crude methyl ester as an orange solid, which was used without further purification.

To a solution of the crude methyl ester (0.500 g, 0.65 mmol, 1.0 eq) in DMF (5.5 mL, [rxn]= 0.1 M) were added 3-bromo methyl thiophene (0.260 g, 1.4 mmol, 1.3 eq) and Cs_2CO_3 (0.46 g, 1.4 mmol, 1.3 eq). The syringe was transferring the electrophile was rinsed with another 1.0 mL DMF. The mixture was stirred 22 h, diluted with H_2O (10x), and extracted with CH_2Cl_2 (3x). The combined organic layers were washed with brine, dried (Na_2SO_4), and purified by chromatography on SiO_2 (gradient 30-40% EtOAc in hexane; **R_f:** 0.2) to afford **4.6b** as yellow sticky oil (0.501 g, 0.90 mmol, 84% over 3 steps): **^{1}H NMR (500 MHz; CDCl_3)** δ 7.62 (1 H, s), 7.44 (1 H, d, J = 2.0 Hz), 7.32-7.26 (2 H, m), 7.18-7.14 (2 H, m), 6.88 (1 H, d, J = 5.0 Hz), 6.10-6.05 (1 H, m), 5.77 (1 H, s), 5.40-5.33 (2 H, m), 5.18 (1 H, d, J = 13 Hz), 5.10 (1 H, d, J = 13 Hz), 4.16 (1 H, dd, J = 6.5, 15 Hz), 3.89 (1 H, dd, J = 6.5, 15 Hz), 3.78 (4 H, s), 3.76-3.64 (1 H, m), 2.48 (2 H, t, J = 7.5 Hz), 2.11 (2H, q, J = 7.5 Hz); **^{13}C NMR (125 MHz; CDCl_3)** δ 172.9, 164.7, 142.6, 136.6, 134.6, 134.5, 134.1, 131.6, 131.1, 129.3, 127.2, 126.5, 124.0, 121.2, 102.1, 61.5, 60.3, 56.3, 51.9, 49.8, 25.0.
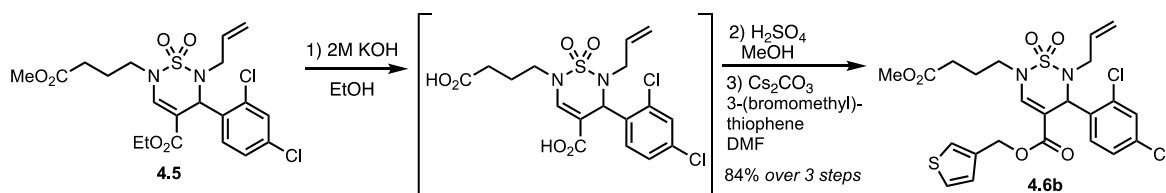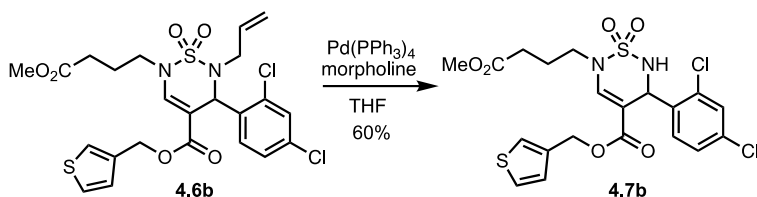
Thiophen-3-ylmethyl 3-(2,4-dichlorophenyl)-6-(4-methoxy-4-oxobutyl)-3,6-dihydro-2*H*-1,2,6-thiadiazine-4-carboxylate 1,1-dioxide (4.7b). To a degassed solution of **4.6b** (0.480 g, 0.86 mmol, 1.0 eq) and morpholine (0.45 mL, 5.14 mmol, 6.0 eq) in THF (4.3 mL, [rxn]= 0.2 M) was added Pd(PPh$_3$)$_4$ (0.020 g, 0.017 mmol, 0.02 eq). The mixture was stirred at rt for 54 h until the conversion was completed by TLC analysis. The mixture was concentrated, and redissolved in EtOAc, washed with water (3x) and brine, dried (MgSO$_4$), and purified by chromatography SiO$_2$ (15% EtOAc in hexane; **R$_f$:** 0.5) to afford **4.7b** (0.269 g, 0.52 mmol, 60%) as a fluffy foam: **^{1}H NMR (300 MHz; CDCl$_3$)** δ 7.54 (1 H, s), 7.37 (1 H, d, *J* = 1.9 Hz), 7.27-7.19 (2 H, m), 7.11 (1H, dd, *J* = 8.4, 1.9 Hz), 7.06 (1 H, d, *J* = 2.6 Hz), 6.78 (1 H, d, *J* = 4.9 Hz), 5.87 (1 H, s), 5.66 (1 H, brs), 5.11 (1 H, d, *J* = 12 Hz), 4.95 (1 H, d, *J* = 12 Hz), 3.65-3.60 (5 H, m), 2.41 (2 H, t, *J* = 7.0 Hz), 2.03 (2 H, q, *J* = 7.0 Hz); **^{13}C NMR (75 MHz; CDCl$_3$)** δ 173.3, 164.7, 142.9, 136.3, 134.8, 134.7, 133.7, 130.5, 129.6, 127.3, 126.8, 126.2, 124.3, 103.9, 61.4, 55.3, 51.9, 49.5, 30.5, 24.7; HRMS **ESI$^+$** *m/z* calcd for C$_{20}$H$_{19}$O$_6$N$_2$Cl$_2$S$_2$ [M+H] 517.0056, found 517.0051.
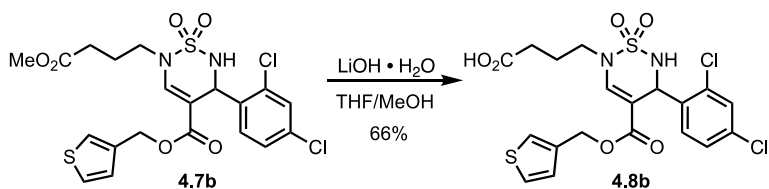
4-(5-(2,4-Dichlorophenyl)-1,1-dioxido-4-((thiophen-3-ylmethoxy)carbonyl)-5,6-dihydro-2*H*-1,2,6-thiadiazin-2-yl)butanoic acid (4.8b). To the solution of **4.7b** (0.100 g, 0.19 mmol, 1.0 eq)

in 1/1 THF/MeOH (1.9 mL, [rxn]= 0.1 M) was treated LiOH•H_2O (1.0 M solution, 0.39 mL, 0.39

mmol, 2.0 eq). The mixture was stirred at rt for 24 h until the conversion was completed with TLC

analysis. The mixture was diluted with CH_2Cl_2 (5x), acidified with 1.0 M HCl until pH=3,

extracted with 10% MeOH in CH_2Cl_2 (3x). The combined organic layers were washed with brine,

and dried (MgSO$_4$) to yield **4.8b** (0.063 g, 0.13 mmol, 66%) as a white solid: **Mp.** 60-64 °C; **^{1}H**

NMR (300 MHz; DMSO-d$_6$) δ 12.2 (1 H, brs), 8.62 (1 H, d, *J* = 7.2 Hz), 7.79 (1 H, brs), 7.62 (1

H, d, *J* = 2.0 Hz), 7.49-7.46 (1 H, m), 7.36-7.31 (2 H, m), 7.22 (1 H, d, *J* = 8.4 Hz), 6.88 (1 H, dd,

J = 3.9, 5.1 Hz), 5.61 (1 H, d, *J* = 7.2 Hz), 5.08, 5.01 (2 H, ABq, *J*AB = 13 Hz), 3.67 (2 H, t, *J* =

7.2 Hz), 2.31 (2 H, t, *J* = 7.5 Hz), 1.83 (2 H, q, *J* = 7.2 Hz); **^{13}C NMR (75 MHz; DMSO-d$_6$)** δ

173.8, 164.3, 143.7, 137.0, 134.6, 133.9, 133.2, 131.4, 128.7, 127.1, 126.7, 126.5, 123.6, 101.6,

60.9, 53.8, 48.7, 30.2, 24.9; HRMS **ESI$^-$** *m/z* calcd for $C_{19}H_{17}O_6N_2Cl_2S_2$ [M-H] 502.98996, found

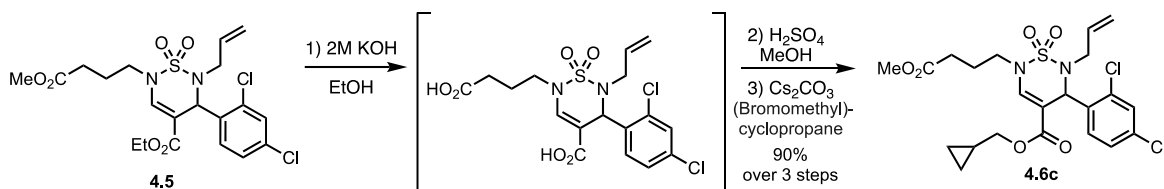502.9909; **IR** (neat) 3676, 2988, 2901, 1393, 1250, 1057, 893 cm^{-1}.

Cyclopropylmethyl   2-allyl-3-(2,4-dichlorophenyl)-6-(4-methoxy-4-oxobutyl)-3,6-dihydro-

2H-1,2,6-thiadiazine-4-carboxylate 1,1-dioxide (4.6c). To a solution of **4.5** (0.900 g, 1.8 mmol,

1.0 eq) in EtOH (18 mL, [rxn]= 0.1 M) was added 2.0 M KOH (11 mL, 22 mmol, 12 eq). The

solution was stirred at 80 °C for 15 h, diluted with CH_2Cl_2 (5x), and acidified with 4.0 M HCl until

pH=3. The aqueous layer was extracted with EtOAc (3x). The combined organic layers were

washed with brine and dried (Na$_2$SO$_4$) to afford the crude bis acid as an orange solid, which was

used without further purification.

To a solution of the crude bis acid in MeOH (34 mL, [rxn]= 0.06 M) was added 0.1/25 H$_2$SO$_4$/MeOH (0.1 mL). The resulting orange solution was stirred at 50 °C for 5 h until the conversion was completed with LC/MS analysis. The solution was extracted with brine/ CH$_2$Cl$_2$ (2x). The combined organic layers were dried (Na$_2$SO$_4$) to afford the crude methyl ester as an orange solid, which was used without further purification.

To a solution of the crude methyl ester (0.320 g, 0.69 mmol, 1.0 eq) in DMF (6.5 mL, [rxn]= 0.1 M) were added Cs$_2$CO$_3$ (0.300 g, 0.91 mmol, 1.3 eq) and (bromomethyl)cyclopropane (0.120 g, 0.91 mmol, 3.0 eq). The mixture was stirred at rt for 15 h, diluted with H$_2$O (10x) and extracted with CH$_2$Cl$_2$ (3x). The combined organic layers were washed with brine, dried (MgSO$_4$), and purified by chromatography on SiO$_2$ (40% EtOAc in hexane, **R$_f$:** 0.4) to afford **4.6c** (0.331 g, 0.63 mmol, 92% over 3 steps) as yellow oil: **^{1}H NMR (400 MHz; CDCl$_3$)** δ 7.54 (1 H, s), 7.39 (1 H, d, *J* = 2.0 Hz), 7.27-7.25 (1 H, m, overlapping with CDCl$_3$), 7.15 (1 H, dd, *J* = 8.0, 2.0 Hz), 6.05-6.00 (1 H, m), 5.71 (1 H, s), 5.39-5.29 (2 H, m), 4.12 (1 H, dd, *J* = 6.4, 14 Hz), 3.90-3.80 (3 H, m), 3.71-3.58 (5 H, m), 3.50-3.40 (2 H, m), 2.44 (2 H, t, *J* = 7.0 Hz), 2.06 (2 H, q, *J* = 7.0 Hz), 0.99-0.94 (1 H, m), 0.47-0.42 (2 H, m), 0.13-0.09 (2 H, m); **^{13}C NMR (100 MHz; CDCl$_3$)** δ 173.0, 165.2, 142.2, 134.7, 134.3, 131.7, 131.3, 129.4, 126.6, 121.3, 102.7, 69.4, 66.0, 60.3, 56.4, 52.0, 49.8, 30.5, 25.1, 15.4, 9.8, 3.2, 3.1.
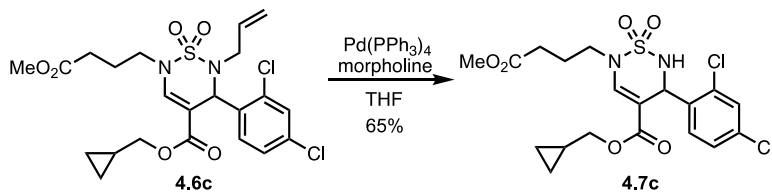
Cyclopropylmethyl 3-(2,4-dichlorophenyl)-6-(4-methoxy-4-oxobutyl)-3,6-dihydro-2*H*-1,2,6-thiadiazine-4-carboxylate 1,1-dioxide (4.7c). To a solution of **4.6c** (0.240 g, 0.46 mmol, 1.0 eq)

and morpholine (0.25 mL, 2.8 mmol, 6.0 eq) in THF (2.3 mL, [rxn]= 0.2 M), which was sparged with N_2 for 30 min, was added Pd(PPh₃)₄ (0.012 g, 0.01 mmol, 0.02 eq). The mixture was stirred for 6 h until the conversion was completed by TLC analysis. The resulting black mixture was concentrated, redissolved in EtOAc, washed with water (3x) and brine, dried (MgSO₄), and purified by chromatography on SiO_2 (15% EtOAc in hexane; **R_f**: 0.4) to afford **4.7c** (0.140 g, 0.29 mmol, 65%) as yellow oil: **¹H NMR (300 MHz; CDCl₃)** δ 7.52 (1 H, s), 7.42 (1 H, d, *J* = 2.1 Hz), 7.27 (1 H, d, *J* = 8.4 Hz), 7.18 (1 H, dd, *J* = 8.4, 2.1 Hz), 5.90 (1 H, s), 5.46 (1 H, brs), 3.84-3.74 (2 H, m), 3.77-3.64 (5 H, m), 2.43 (2 H, t, *J* = 7.2 Hz), 2.08-2.04 (2 H, q, *J* = 7.2 Hz), 0.94-0.85 (1 H, m), 0.45-0.38 (2 H, m), 0.09-0.04 (2 H, m); **¹³C NMR (75 MHz; CDCl₃)** δ 173.3, 165.0, 142.4, 134.8, 134.6, 133.9, 130.6, 129.6, 126.9, 104.6, 69.4, 55.5, 52.0, 49.5, 30.6, 24.8, 9.67, 3.14, 3.07; HRMS **ESI⁻** *m/z* calcd for $C_{19}H_{21}O_6N_2Cl_2S_2$ [M-H] 475.0491 found 475.0488.
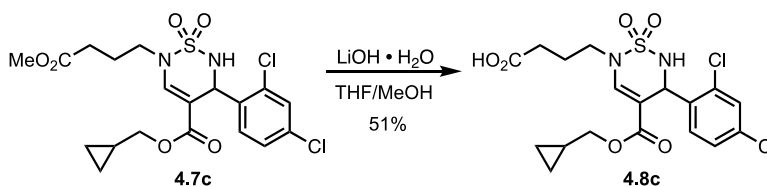
4-(4-(((Cyclopropylmethoxy)carbonyl)-5-(2,4-dichlorophenyl)-1,1-dioxido-5,6-dihydro-2*H*-1,2,6-thiadiazin-2-yl)butanoic acid (4.8c). To a solution of **4.7c** (0.140 g, 0.29 mmol, 1.0 eq) in 1/1 THF/MeOH (3.0 mL, [rxn]= 0.1 M) was added LiOH•H₂O (1.0 M solution, 0.60 mL, 2.0 eq). The mixture was stirred at rt for 4 h until the conversion was completed with TLC analysis, diluted with CH_2Cl_2 (5x), acidified with 1.0 M HCl until pH=3, and extracted with 10 % MeOH in CH_2Cl_2 (3x). The combined organic layers were dried (MgSO₄) and concentrated to afford **4.8c** (0.069 g, 0.15 mmol, 51%) as a white solid: **Mp**. 56-60 °C; **¹H NMR (400 MHz; DMSO-d₆)** δ 12.1 (1 H, brs), 8.63 (1 H, brs), 7.74 (1 H, s), 7.63 (1 H, d, *J* = 2.0 Hz), 7.38 (1 H, dd, *J* = 8.0, 2.0 Hz), 7.24

(1 H, d, J = 8.0 Hz), 5.62 (1 H, s), 3.85-3.76 (2 H, m), 3.66 (2 H, t, J = 7.0 Hz), 2.31 (2 H, t, J = 7.0 Hz), 1.86 (2 H, m), 0.94-0.86 (2 H, m), 0.39-0.35 (2 H, m), 0.09-0.07 (2 H, m); **^{13}C NMR (100 MHz; DMSO-d$_6$)** δ 174.0, 164.6, 143.3, 134.8, 133.9, 133.2, 131.4, 128.7, 126.7, 102.2, 68.0, 53.9, 48.7, 30.3, 24.9, 9.63, 2.79, 2.70; HRMS **ESI[+]** m/z calcd for $C_{18}H_{21}O_6N_2Cl_2S$ [M+H] 463.0491, found 463.0489; **IR** (neat) 3010, 2252, 1701, 1625, 1470 cm^{-1}.
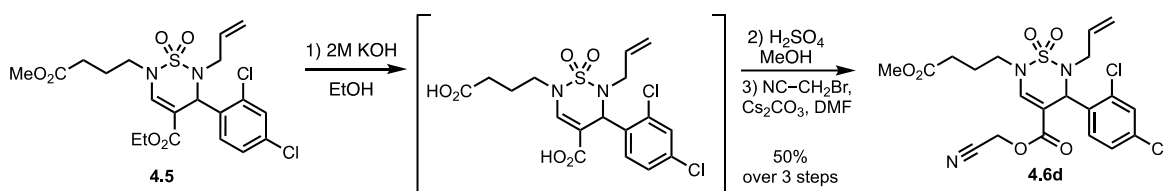

Cyanomethyl 2-allyl-3-(2,4-dichlorophenyl)-6-(4-methoxy-4-oxobutyl)-3,6-dihydro-2*H*-1,2,6-thiadiazine-4-carboxylate 1,1-dioxide (4.6d). To a solution of **4.5** (0.900 g, 1.8 mmol, 1.0 eq) in EtOH (18 mL, [rxn]= 0.1 M) was added 2.0 M KOH (11 mL, 22 mmol, 12 eq). The solution was stirred at 80 °C for 15 h, diluted with CH$_2$Cl$_2$ (5x), and acidified with 4.0 M HCl until pH=3. The aqueous layer was extracted with EtOAc (3x). The combined organic layers were washed with brine, dried (Na$_2$SO$_4$) to afford the crude bis acid as an orange solid crude, which was used without further purification.

To a solution of the crude bis acid in MeOH (34 mL, [rxn]= 0.06 M) was added 0.1/25 H$_2$SO$_4$/MeOH (0.1 mL). The resulting orange solution was stirred at 50 °C for 5 h until the conversion was completed with LC/MS analysis. The solution was extracted with brine/ CH$_2$Cl$_2$ (2x). The organic layers were combined and dried (Na$_2$SO$_4$) to afford the crude methyl ester as an orange crude, which was used without further purification.

To a solution of the crude methyl ester (0.300 g, 0.65 mmol, 1.0 eq) in DMF (6.5 mL, [rxn]= 0.1 M) were added Cs$_2$CO$_3$ (0.270 g, 0.84 mmol, 1.3 eq) and bromo acetonitrile (1.0 M in

DMF, 0.85 mL, 0.85 mmol, 1.3 eq). The reaction mixture was stirred at rt for 15 h, diluted with

H$_2$O (10x) and extracted with CH$_2$Cl$_2$ (3x). The organic layers were washed with brine, dried

(MgSO$_4$), and purified by chromatography on SiO$_2$ (gradient 40-45% EtOAc in hexane; **R$_f$**: 0.2)

to afford **4.6d** (0.329 g, 0.63 mmol, 92% over 3 steps) as yellow oil: **^{1}H NMR (400 MHz; CDCl$_3$)**

δ 7.65 (1 H, s), 7.53 (1 H, d, J = 8.4 Hz), 7.21-7.15 (2 H, m), 6.04-5.96 (1 H, m), 5.67 (1 H, s),

5.40-5.30 (2 H, m), 4.12 (1 H, dd, J = 6.8, 1.6 Hz), 3.86 (1 H, dd, J = 6.8, 1.6 Hz), 3.79-3.72 (4 H,

m), 3.66-3.62 (1 H, m), 2.46 (2 H, t, J = 6.8 Hz), 2.08 (2 H, q, J = 7.2 Hz); **^{13}C NMR (100 MHz;**

CDCl$_3$) δ 172.9, 163.3, 144.6, 135.2, 134.7, 133.5, 131.3, 130.8, 129.8, 126.8, 121.8, 114.4, 99.7,

60.1, 56.5, 52.1, 50.3, 48.6, 30.4, 25.2.

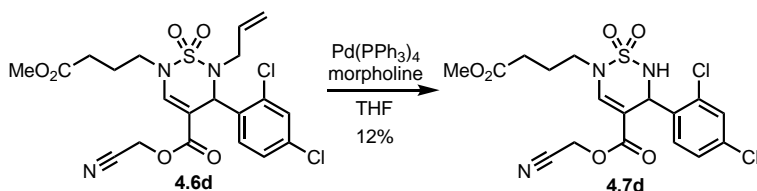**Cyanomethyl   3-(2,4-dichlorophenyl)-6-(4-methoxy-4-oxobutyl)-3,6-dihydro-2*H*-1,2,6-**

thiadiazine-4-carboxylate 1,1-dioxide (4.7d). To a degassed solution of **4.6d** (0.550 g, 1.08

mmol, 1.0 eq) and morpholine (0.570 g, 6.5 mmol, 6.0 eq) in THF (5.5 mL, [rxn]= 0.2 M) was

added Pd(PPh$_3$)$_4$ (0.040 g, 0.03 mmol, 0.03 eq). The mixture was stirred for 72 h until the

conversion was completed by TLC analysis. The resulting black mixture was concentrated,

redissolved in EtOAc, washed with water (3x) and brine, dried (MgSO$_4$), and purified by

chromatography on SiO$_2$ (40% EtOAc in hexane) to afford **4.7d** (0.059 g, 0.13 mmol 12%) as a

white solid: **Mp**. 65-70 °C; **^{1}H NMR (400 MHz, DMSO-d$_6$)** δ 8.78 (1 H, brs), 7.92 (1 H, s), 7.65

(1 H, d, J = 2.0 Hz), 7.38 (1 H, dd, J = 8.4, 2.0 Hz), 7.24 (1 H, d, J = 8.4 Hz); 5.59 (1 H, s), 4.94

(2 H, s); 3.72 (2 H, t, J = 7.2 Hz), 3.61 (3 H, s), 2.40 (2 H, t, J = 7.2 Hz), 1.90 (2 H, q, J = 7.2 Hz);

13C NMR (100 MHz, DMSO-d6) δ 172.7, 163.3, 145.5, 134.2, 133.9, 133.4, 131.3, 128.9, 126.8, 116.0, 99.6, 5=3.6, 51.4, 48.9, 48.8, 29.9, 24.8; **HRMS ESI+** m/z calcd for $C_{17}H_{18}Cl_2N_3O_6S$ [M+H] 462.0287, found 462.0288; **IR** (neat) 3168, 3052, 3019, 2961, 2179, 2023, 1711, 1688 1621, 1591, 1564 cm^{-1}.
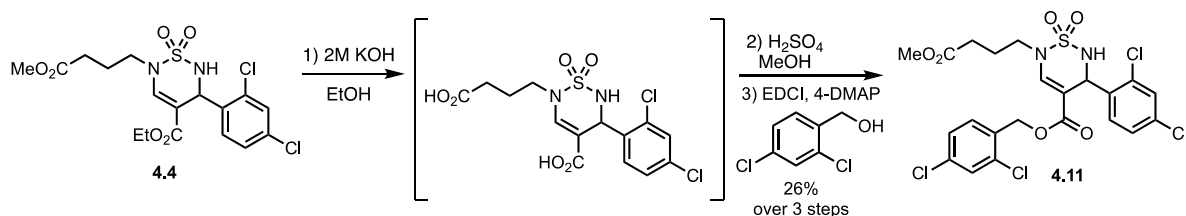
Methyl 4-(4-(2,4-dichlorobenzyl)-5-(2,4-dichlorophenyl)-1,1-dioxido-5,6-dihydro-2*H*-1,2,6-thiadiazin-2-yl)butanoate (4.11). To a solution of **4.4** (0.230 g, 0.51 mmol, 1.0 eq in EtOH (5.2 mL, [rxn]= 0.1 M) was added 2.0 M KOH (3.6 mL, 7.2 mmol, 14 eq). The solution was stirred at 80 °C for 15 h, diluted with CH_2Cl_2 (5x), and acidified with 1.0 M HCl until pH=3. The aqueous layer was extracted with EtOAc (3x). The combined organic layers were washed with brine, and dried (Na_2SO_4) to afford the crude bis acid as an orange solid, which was used without further purification.

To a solution of the crude bis acid in MeOH (18 mL, [rxn]= 0.06 M) was added 0.1/25 H_2SO_4/MeOH (0.1 mL). The resulting orange solution was stirred at 50 °C for 5 h until the conversion was completed with LC/MS analysis. The solution was extracted with brine/ CH_2Cl_2 (2x). The combined organic layers were dried (Na_2SO_4) and concentrated to afford the crude methyl ester as orange solid, which was used without further purification.

To a solution of the crude methyl ester (0.160 g, 0.37 mmol, 1.0 eq) in CH_2Cl_2 (2.4 mL, [rxn]= 0.15 M) were added 2,4-dichlorobenzyl alcohol (0.200 g, 1.1 mmol, 3.0 eq), EDCI (0.29 g, 1.5 mmol, 4.0 eq), 4-DMAP (0.030 g, 0.37 mmol, 1.0 eq), and Et₃N (0.1 mL, 0.70 mmol, 3.0 eq).

The resulting colorless solution was stirred for 23 h and extracted with CH_2Cl_2/ sat. $NaHCO_3$ (3x). The combined organic layers were washed with brine, dried (Na_2SO_4), and purified by chromatography on SiO_2 (30% EtOAc in hexane) to yield **4.11** (0.059 g, 0.10 mmol, 26% over 3 steps) as a clear film: **^{1}H NMR (500 MHz; CDCl$_3$)** δ 7.56 (1 H, s), 7.35 (2 H, dd, J = 8.0, 2.0 Hz), 7.15 (1 H, d, J = 2.0 Hz), 7.14-7.11 (2 H, m), 6.97 (2 H, d, J = 1.0 Hz), 5.89 (1 H, d, J = 14 Hz), 5.19 (1 H, d, J = 14 Hz), 4.99-4.97 (2 H, m), 3.69-3.66 (5 H, m), 2.44 (2 H, t, J = 7.5 Hz), 2.12-2.02 (2 H, m); **^{13}C NMR (125 MHz; CDCl$_3$)** δ 173.3, 164.3, 143,3, 135.2, 135.1, 134.7, 131.9, 131.0, 130.6, 129.6, 127.2, 127.1, 104.0,63.2, 55.7, 52.1, 49.8, 30.7, 24.8; HRMS **ESI$^+$** *m/z* calcd for $C_{22}H_{21}O_6N_2Cl_4S$ [M+H] 580.9868, found 580.9864; **IR** (neat) 3234, 2926, 2855, 1708, 1624, 1591 cm^{-1}.
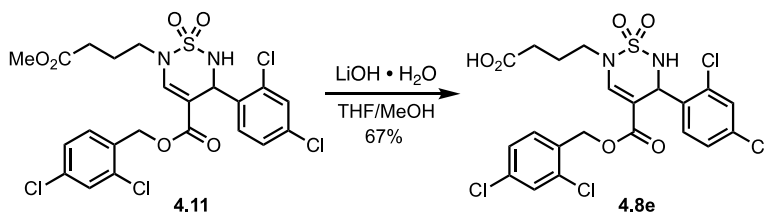

4-(4-(2,4-Dichlorobenzyl)-5-(2,4-dichlorophenyl)-1,1-dioxido-5,6-dihydro-2*H*-1,2,6-thiadiazin-2-yl)butanoic acid (4.8e). To a solution of **4.11** (0.078 g, 0.13 mmol, 1.0 eq) in 4/1 THF/MeOH (1.5 mL, [rxn]= 0.1 M) was added a solution of LiOH•H_2O (0.012 g, 0.27 mmol, 2.0 eq) in H_2O (0.2 mL). The mixture was stirred at rt for 1 h until the conversion was completed by TLC analysis. The mixture was diluted with CH_2Cl_2, acidified until pH=3 with 1.0 M HCl, and extracted with EtOAc (3x). The combined organic layers were washed with brine, dried (Na_2SO_4), and purified by chromatography on SiO_2 (2% MeOH in CH_2Cl_2, spiked with 0.5% AcOH) to afford **4.8e** as a colorless foam (0.051 g, 0.09 mmol, 67%): **^{1}H NMR (500 MHz; DMSO-d$_6$)** δ 12.2 (1 H, brs), 8.66 (1 H, d, J = 7.0 Hz), 7.82 (1 H, s), 7.59-7.58 (2 H, m), 7.37 (1 H, dd, J = 8.0, 2.0 Hz),

7.32 (1 H, dd, J = 8.0, 2.0 Hz), 7.23-7.21 (2 H, m), 5.61 (1 H, d, J = 7.0 Hz), 5.16 (1 H, d, J = 14 Hz), 5.02 (1 H, d, J = 14 Hz), 3.67 (2 H, t, J = 7.5 Hz), 2.31 (2 H, t, J = 7.5 Hz), 1.91-1.83 (2 H, m); **^{13}C NMR (125 MHz; DMSO-d$_6$)** δ 173.8, 164.0, 144.1, 134.5, 133.9, 133.5, 133.3, 132.6, 131.4, 131.1, 128.8, 128.7, 127.2, 126.7, 62.2, 53.7, 48.7, 30.2, 24.9; HRMS **ESI$^+$** m/z calcd for C$_{21}$H$_{19}$O$_6$N$_2$Cl$_4$S [M+H] 566.9712, found 566.9717.
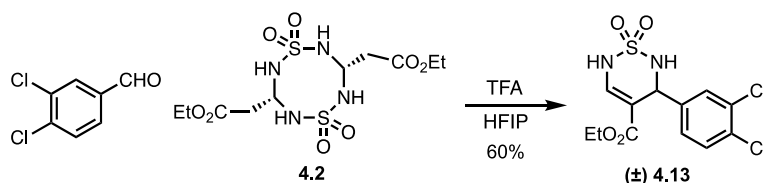
Ethyl 3-(3,4-dichlorophenyl)-3,6-dihydro-2H-1,2,6-thiadiazine-4-carboxylate 1,1-dioxide (± 4.13). To a suspension of sulfamide **4.2** (5.0 g, 1.29 mmol, 1.0 eq) and 3,4-dichlorobenzaldehyde (4.51 g, 25.8 mmol, 2.0 eq) in HFIP (50 mL, [rxn]= 0.2 M) was treated dropwise TFA (4.8 mL, 64.4 mmol, 5.0 eq) via a syringe pump. The resulting white mixture was stirred at 35-40 °C for 18 h, quenched with EtOAc/sat. NaHCO$_3$, and extracted with EtOAc (3x). The combined organic layers were washed with brine, dried (Na$_2$SO$_4$), concentrated to give a yellow residue, which recrystallized with chloroform (3x) to afford **(±) 4.13** (2.69 g, 7.66 mmol, 60%) as a fluffy needle-like crystal: **Mp.** 174-177 °C; **^{1}H NMR (500 MHz; DMSO-d$_6$)** δ 11.0 (1 H, brs), 8.08 (1 H, d, J = 7.0 Hz), 7.56-7.55 (2 H, m), 7.44 (1 H, d, J = 2.0 Hz), 7.23 (1 H, dd, J = 8.0, 2.0 Hz), 5.36 (1 H, d, J = 6.9 Hz), 4.08-3.96 (2 H, m), 1.08 (3 H, t, J = 7.5 Hz); **^{13}C NMR (125 MHz; DMSO-d$_6$)** δ 165.0; 140.4, 139.6, 130.4, 130.0, 129.9, 129.8, 128.3, 101.4, 59.7, 56.0, 14.1; HRMS **ESI$^-$** m/z calcd for C$_{12}$H$_{11}$O$_4$N$_2$Cl$_2$S [M-H] 348.9811, found 348.9809; **IR** (neat) 3281, 2978, 1693, 1620, 1570 cm^{-1}.
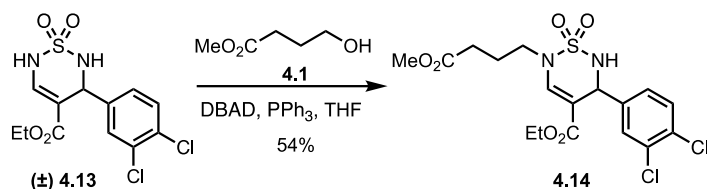
Ethyl 3-(3,4-dichlorophenyl)-6-(4-methoxy-4-oxobutyl)-3,6-dihydro-2*H*-1,2,6-thiadiazine-4-carboxylate 1,1-dioxide (4.14). To a solution of **(±) 4.13** (0.912 g, 2.46 mmol, 1.0 eq) and **4.1** (0.29 g, 2.46 mmol, 1.0 eq) in THF (20 mL, [rxn]= 0.17 M) were added PPh$_3$ (0.653 g, 2.46 mmol, 1.0 eq) and DBAD (0.573 g, 2.46 mmol, 1.0 eq) in one portion at 0 °C. The mixture was stirred at 0 °C for 30 min, stirred at rt for another 15 h, quenched with water, and extracted with CH$_2$Cl$_2$ (3x). The combined organic layers were washed with brine, dried (Na$_2$SO$_4$), and purified by chromatography on SiO$_2$ (30% EtOAc in hexane followed by the second chromatography on SiO$_2$ with 30% acetone in hexane) to yield **4.14** (0.607 g, 1.34 mmol, 54%) as a sticky colorless foam: **^{1}H NMR (500 MHz; CDCl$_3$)** δ 7.48 (1 H, s), 7.45 (1 H, d, *J* = 2.0 Hz), 7.42 (1 H, d, *J* = 8.0 Hz), 7.20 (1 H, dd, *J* = 8.0, 2.0 Hz), 5.53 (1 H, d, *J* = 8.0 Hz), 4.70 (1 H, d, *J* = 8.0 Hz), 4.16-4.04 (2 H, m), 3.73-3.63 (5 H, m), 2.46 (2 H, t, *J* = 7.0 Hz), 2.09-2.05 (2 H, m), 1.13 (3 H, t, *J* = 7.0 Hz); **^{13}C NMR (125 MHz; CDCl$_3$)** δ 173.4, 164,9, 142.3, 138.3, 132.8, 132.6, 130.6, 129.9, 127.2, 105.1, 60.9, 58.2, 52.1, 49.7, 30.8, 24.8, 14.3; HRMS **ESI$^+$** *m/z* calcd for C$_{21}$H$_{21}$O$_6$N$_2$Cl$_2$S [M+H] 499.0491, found 499.0495; **IR** (neat) 3222, 3065, 2893, 2955, 1693, 1625, 1565 cm^{-1}.
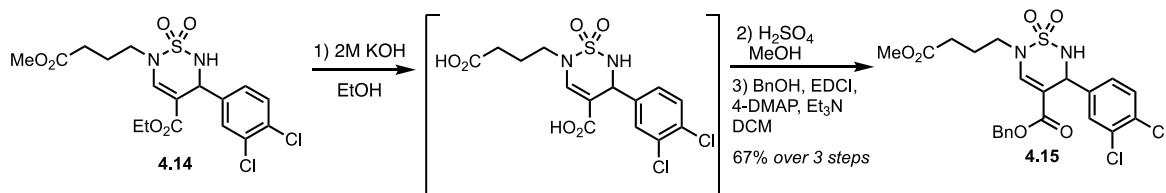

Methyl 4-(4-benzyl-5-(3,4-dichlorophenyl)-1,1-dioxido-5,6-dihydro-2*H*-1,2,6-thiadiazin-2-yl)butanoate (4.15). To a solution of **4.14** (0.580 g, 1.3 mmol, 1.0 eq) in EtOH (13 mL, [rxn]= 0.1

M) was added 2.0 M KOH (6.5 mL, 10 mmol, 9.0 eq). The solution was stirred at 80 °C for 15 h, diluted with CH_2Cl_2 (5x), and acidified with 4.0 M HCl until pH=3. The aqueous layer was extracted with EtOAc (3x). The combined organic layers were washed with brine and dried (Na_2SO_4) to afford the crude bis acid as an orange solid, which was used without further purification.

To a solution of the crude bis acid in MeOH (22 mL, [rxn]= 0.06 M) was added 0.1/25 H_2SO_4/MeOH (0.1 mL). The resulting orange solution was stirred at 50 °C for 5 h until the conversion was completed with LC/MS analysis. The mixture was extracted with brine/ CH_2Cl_2 (2x). The combined organic layers were dried (Na_2SO_4) to afford the crude methyl ester as an orange solid, which was used without further purification.

To a solution of the crude methyl ester (0.200 g, 0.47 mmol, 1.0 eq) in CH_2Cl_2 (2.5 mL, [rxn]= 0.2 M) were added benzyl alcohol (0.33 mL, 1.4 mmol, 3.0 eq), EDCI (0.270 g, 1.4 mmol, 3.0 eq), 4-DMAP (0.030 g, 0.23 mmol, 0.5 eq), and Et_3N (0.21 mL, 1.4 mmol, 3.0 eq). The solution was stirred 15 h, diluted with sat. NH_4Cl, and extracted with CH_2Cl_2 (3x). The combined organic layers were washed with sat. $NaHCO_3$, water, brine, dried (Na_2SO_4), and purified by chromatography on SiO_2 (30% EtOAc in hexane) to afford **4.15** (0.159 g, 0.31 mmol, 67% over 3 steps) as a clear film: **^{1}H NMR (500 MHz; CDCl$_3$)** δ 7.51 (1 H, s), 7.40 (1 H, d, J = 2.0 Hz), 7.35 (1 H, d, J = 8.0 Hz), 7.30-7.28 (3 H, m), 7.15 (1 H, dd, J = 8.0, 2.0 Hz), 7.10-7.08 (2 H, m), 5.51 (1 H, d, J = 8.0 Hz), 5.15 (1 H, d, J = 13 Hz), 4.97 (1 H, d, J = 13 Hz), 4.77 (1 H, d, J = 8.0 Hz), 3.70-3.63 (5 H, m), 2.44 (2 H, t, J = 7.0 Hz), 2.09-2.03 (m, 2 H); **^{13}C NMR (125 MHz; CDCl$_3$)** δ 173.4,164.7, 142.7, 138.2, 135.5, 132.9, 132.7, 130.6, 129.9, 128.7, 128.5, 128.3, 127.2, 104.9, 66.6, 58.3, 52.0, 49.8, 30.8, 24.8; HRMS **ESI$^+$** *m/z* calcd for $C_{22}H_{23}O_6N_2Cl_2S$ [M+H] 513.0648, found 513.0645; **IR** (neat) 3300, 3073, 2964, 2930, 1746, 1707, 1591 cm^{-1}.
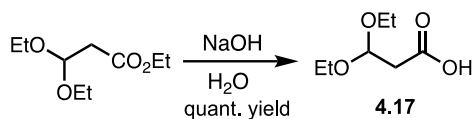
3,3-Diethoxypropanoic acid (4.17).[82] To a solution of ethyl 3,3-diethoxypropanoate (2.00 g, 11

mmol, 1.0 eq) in H_2O (4.2 mL, [rxn]= 2.5 M) was added NaOH (0.68 g, 17 mmol, 1.6 eq). The

mixture was heated in the sealed tube at 100 °C for 1 h, acidified with 12 M HCl until pH= 3 at 0

°C and extracted with EtOAc (2x). After each extraction, the aqueous layer was acidified to pH=3

with 12 M HCl. The combined organic layers were washed with brine and dried (Na_2SO_4) to yield

4.17 (quant.) as light-yellow oil: **^{1}H NMR (400 MHz, DMSO-d$_6$)** δ 12.2 (1 H, brs), 4.82 (1 H, t,

J = 7.2 Hz), 3.62- 3.54 (2 H, m), 3.50-3.42 (2 H, m), 2.50 (2 H, m, overlapping with DMSO-d$_6$),

1.1 (6 H, t, J = 7.2 Hz); **^{13}C NMR (100 MHz, DMSO-d$_6$)** δ 170.9, 99.5, 61.1, 15.2.
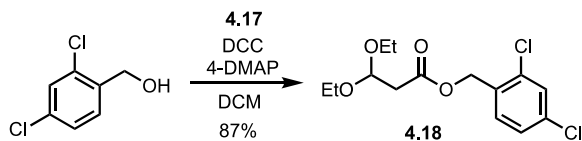
2,4-Dichlorobenzyl 3,3-diethoxypropanoate (4.18). To the solution of **4.17** (1.00 g, 6.20 mmol,

1.0 eq) in CH_2Cl_2 (62 mL, [rxn]= 0.10 M) were added 4-DMAP (0.193 g, 1.60 mmol, 0.25 eq) and

2,4-dichlorobenzyl alcohol (1.96 g, 11.1 mmol, 1.8 eq). The resulting solution was cooled down

to 0 °C, and treated with DCC (1.43 g, 6.7 mmol, 1.1 eq), warmed to rt, and stirred for 18 h, and

filtered over celite. The filtrate was diluted with CH_2Cl_2 (2x), washed with sat. $NaHCO_3$, brine,

dried ($MgSO_4$), filtered, and purified by chromatography on SiO_2 (15% EtOAc in hexane; **R$_f$:** 0.4)

to afford **4.18** (1.66 g, 5.18 mmol, 87%) as colorless oil: **^{1}H NMR (500 MHz; CDCl$_3$)** δ 7.40 (1

H, d, J = 2.0 Hz), 7.36 (1 H, d, J = 8.0 Hz), 7.24 (1 H, dd, J = 8.0, 2.0 Hz), 5.21 (2 H, s), 4.97 (1

H, t, J = 6.0 Hz), 3.70-3.64 (2H, m), 3.57-3.50 (2 H, m), 2.74 (2 H, d, J = 6.0 Hz), 1.18 (6 H, t, J

= 7.2 Hz); **^{13}C NMR (125 MHz; CDCl$_3$)** δ 169.7 134.7, 134.3, 132.3, 130.6, 129.5, 127.3, 99.6, 63.1, 62.1, 40.0, 15.3.
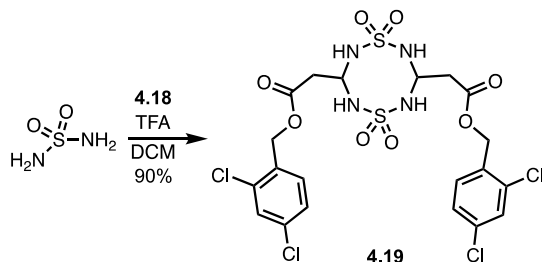
Bis(2,4-dichlorobenzyl)   **2,2'-(1,1,5,5-tetraoxido-1,5,2,4,6,8-dithiatetrazocane-3,7-diyl)diacetate (4.19).** To a suspension of **4.18** (1.70 g, 5.2 mmol, 1.1 eq) and sulfamide (0.450 g, 4.7 mmol, 1.0 eq) in CH$_2$Cl$_2$ (7.5 mL, [rxn]= 0.5 M) was treated dropwise TFA (1.4 mL, 19 mmol, 5.0 eq) via a syringe pump. The mixture was stirred for 4 h and filtered. The precipitate was washed with CH$_2$Cl$_2$ and Et$_2$O to yield **4.19** (1.30 g, 2.1 mmol, 90%) as an off-white solid: **Mp.** 189-193 °C; **^{1}H NMR (500 MHz; DMSO-d$_6$)** δ 7.70-7.67 (6 H, m), 7.57 (2 H, d, *J* = 8.0 Hz), 7.41 (2 H, dd, *J* = 8.2, 2.0 Hz), 5.27-5.20 (4 H, m), 5.17 (2 H, s), 2.78 (4 H, d, *J* = 7.5 Hz); **^{13}C NMR (125 MHz; DMSO-d$_6$)** δ 168.3, 133.4, 133.1, 132.5, 131.1, 128.7, 127.4, 62.5, 61.9, 40.7; **HRMS ESI$^-$** *m/z* calcd for C$_{20}$H$_{19}$O$_8$N$_4$Cl$_4$S$_2$ [M-H] 646.9393, found 646.9411; **IR** (neat) 3325, 2953, 1729, 1590, 1564 cm^{-1}.
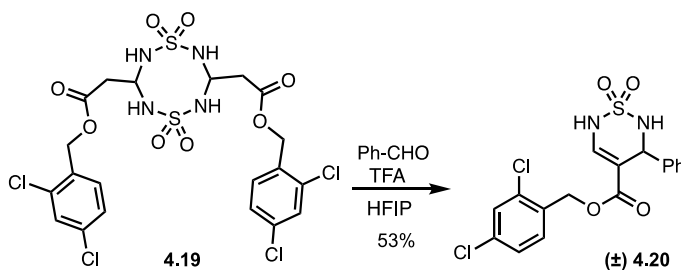
**2,4-Dichlorobenzyl 3-phenyl-3,6-dihydro-2*H*-1,2,6-thiadiazine-4-carboxylate 1,1-dioxide (±
4.20).** To a suspension of **4.19** (0.200 g, 0.30 mmol, 1.0 eq) and distilled benzaldehyde (0.065 g,
0.6 mmol, 2.0 eq) in HFIP (0.6 mL, [rxn]= 0.5 M) was treated dropwise TFA (0.50 mL, 6.0 mmol,
21 eq). The suspension was stirred at 35-40 °C for 68 h and extracted with EtOAc/sat. NaHCO$_3$
(3x). The combined organic layers were washed with brine, dried (Na$_2$SO$_4$), and purified by
chromatography on SiO$_2$ (gradient 15-20% EtOAc in CH$_2$Cl$_2$; **R$_f$**: 0.61) to afford a yellow foam,
which was re-suspended in CH$_2$Cl$_2$/n-pentane and filtered to afford **(±) 4.20** (0.067 g, 0.13 mmol,
53%) as an off-white solid: **^{1}H NMR (500 MHz; DMSO-d$_6$)** δ 10.94 (1 H, brs), 7.99 (1 H, d, *J* =
7.0 Hz), 7.62 (1 H, d, *J* = 2.0 Hz), 7.59 (1 H, s), 7.35 (1 H, dd, *J* = 8.0, 2.0 Hz), 7.29-7.22 (4 H,
m), 7.18 (1 H, d, *J* = 8.0 Hz), 5.36 (1 H, d, *J* = 7.0 Hz), 5.14 (1 H, d, *J* = 13 Hz), 5.05 (1 H, d, *J* =
13 Hz); **^{13}C NMR (125 MHz; DMSO-d$_6$)** δ 164.7, 139.9, 138.8, 133.4, 133.3, 132.9, 131.0, 128.8,
128.0, 127.8, 127.4, 127.3, 101.8, 62.0, 57.4; HRMS **ESI** *m/z* calcd for C$_{17}$H$_{13}$O$_4$N$_2$Cl$_2$S [M-H]
410.9967, found 410.9973.
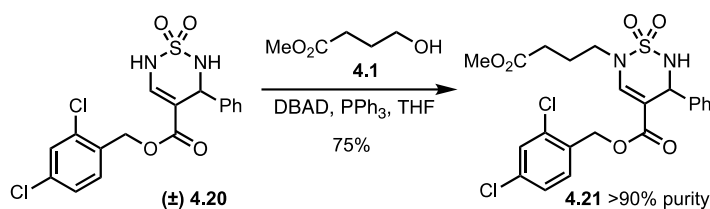
2,4-Dichlorobenzyl 6-(4-methoxy-4-oxobutyl)-3-phenyl-3,6-dihydro-2*H*-1,2,6-thiadiazine-4-
carboxylate 1,1-dioxide (4.21). To a solution of **(±) 4.20** (0.050 g, 0.12 mmol, 1.0 eq) and PPh$_3$
(0.038 g, 0.14 mmol, 1.2 eq) in THF (0.5 mL, [rxn]=0.2 M) were treated alcohol **4.1** (0.024 g, 0.16
mmol, 1.3 eq) and DBAD (0.034 g, 0.14 mmol, 1.2 eq) in one portion. The resulting yellow
solution was stirred for 9 h, quenched with brine, dried (Na$_2$SO$_4$), and purified by chromatography
on SiO$_2$ (30% acetone in hexane; **R$_f$**: 0.21) to afford a semi-clean **4.21** (0.051 g, 0.09 mmol, 75%)

as a light yellow foam: **^{1}H NMR (500 MHz; CHCl$_3$)** δ 7.50 (1 H, s), 7.34-7.30 (6 H, m), 7.06 (1 H, dd, J = 8.0, 2.0 Hz), 6.74 (1 H, d, J = 8.0 Hz), 5.57 (1 H, d, J = 8.0 Hz), 5.10 (1 H, d, J = 13 Hz), 4.98 (1 H, d, J = 13 Hz), 4.72 (1 H, d, J = 8.0 Hz), 3.70-3.61 (5 H, m), 2.49-2.41 (2 H, m), 2.17-2.04 (2 H, m).

4-(4-(((2,4-Dichlorobenzyl)oxy)carbonyl)-1,1-dioxido-5-phenyl-5,6-dihydro-2H-1,2,6-thiadiazin-2-yl)butanoic acid (4.8f). To a solution of **4.21** (0.050 g, 0.10 mmol, 1.0 eq) in 1/1 THF/MeOH (1.1 mL, [rxn]=0.1 M) was added 1.4 M aqueous solution of LiOH•H$_2$O (0.008, 0.2 mmol, 2.0 eq). The mixture was stirred for 5 h until the conversion was completed by TLC analysis. The mixture was diluted with CH$_2$Cl$_2$ (5x), acidified to pH=3 with 1.0 M HCl, and extracted with EtOAc (3x). The combined organic layers were dried (Na$_2$SO$_4$) to afford crude as a light yellow foam, which was triturated in n-pentane to yield **4.8f** (0.025 g, 0.05 mmol, 51%) as an off-white solid: **Mp**.180-185 °C; **^{1}H NMR (500 MHz; DMSO- d$_6$)** δ 7.55 (1 H, d, J = 2.0 Hz), 7.45 (1 H, s), 7.27 (1 H, dd, J = 8.0, 2.0 Hz), 7.21 (2 H, d, J = 7.0 Hz), 7.15-7.11 (2 H, m), 7.06-7.03 (1 H, m), 6.94 (1 H, d, J = 8.0 Hz), 5.09 (1 H, s), 5.03 (1 H, d, J = 14 Hz), 4.86 (1 H, d, J = 14 Hz), 3.26-3.21 (1 H, m), 1.82-1.79 (2 H, m), 1.73-1.72 (2 H, m); **^{13}C NMR (125 MHz; DMSO-d$_6$)** δ 174.8, 166.0, 147.2, 146.1, 134.2, 132.5, 132.3, 129.9, 128.4, 128.0, 127.2, 126.8, 125.0, 99.5, 60.3, 59.8, 49.5, 35.9, 27.3; HRMS **ESI$^-$** *m/z* calcd for C$_{21}$H$_{19}$O$_6$N$_2$Cl$_2$S [M-H] 497.0335, found 497.0342; **IR** (neat) 3833, 3251, 3095, 2934, 2606, 1680, 1651, 1603, 1564 cm^{-1}.

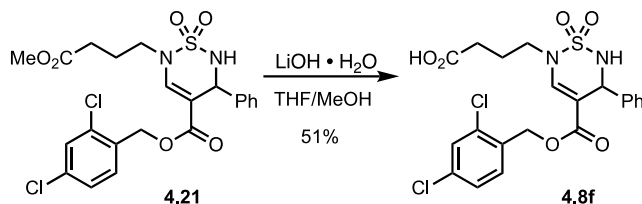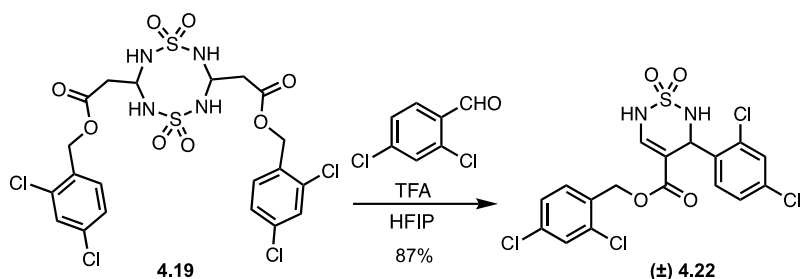**2,4-Dichlorobenzyl 3-(2,4-dichlorophenyl)-3,6-dihydro-2*H*-1,2,6-thiadiazine-4-carboxylate 1,1-dioxide (± 4.22).** To a suspension of **4.19** (0.200 g, 0.3 mmol, 1.0 eq) and 2,4-dichlorobenzaldehyde (0.107 g, 0.6 mmol, 2.0 eq) in HFIP (0.6 mL, [rxn]= 0.5 M) was treated dropwise TFA (0.5 mL, 20 eq). The mixture was stirred at 35-40 °C for 68 h and extracted with EtOAc/sat. NaHCO$_3$ (3x). The combined organic layers were washed with brine, dried (Na$_2$SO$_4$), and purified by chromatography on SiO$_2$ (gradient 15-20% EtOAc in CH$_2$Cl$_2$; **R$_f$**: 0.35) to afford **(±) 4.22** (0.138 g, 0.27 mmol, 87%) as a yellow foam: **^{1}H NMR (500 MHz; CDCl$_3$)** δ 7.64 (1 H, d, *J* = 6.0 Hz), 7.38-7.35 (2 H, m), 7.31 (1 H, brs), 7.21 (1 H, d, *J* = 8.0 Hz), 7.17-7.12 (2 H, m), 7.00 (2 H, d, *J* = 8.0 Hz), 5.93 (1 H, d, *J* = 8.0 Hz), 5.20 (1 H, d, *J* = 13 Hz), 5.01 (1 H, d, *J* = 13 Hz), 4.95-4.87 (1H, brs); **^{13}C NMR (125 MHz; CDCl$_3$)** δ 164.4, 139.4, 135.3, 135.1, 134.6, 133.3, 131.6, 130.9, 130.5, 129.8, 129.5, 127.2, 127.1, 104.4, 63.3, 55.9, 31.1; HRMS **ESI$^-$** *m/z* calcd for C$_{17}$H$_{11}$O$_4$N$_2$Cl$_4$S [M-H] 478.9188, found 478.9192.
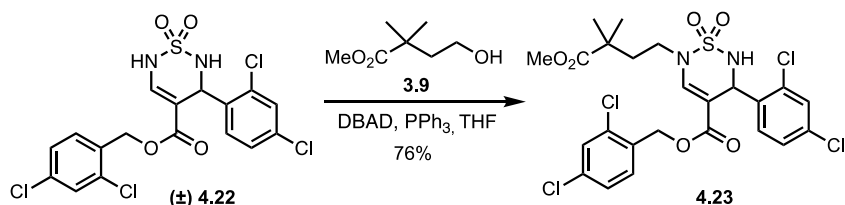
2,4-Dichlorobenzyl 3-(2,4-dichlorophenyl)-6-(4-methoxy-3,3-dimethyl-4-oxobutyl)-3,6-dihydro-2*H*-1,2,6-thiadiazine-4-carboxylate 1,1-dioxide (4.23). To a solution of **(±) 4.22** (0.265 g, 0.55 mmol, 1.0 eq) and PPh$_3$ (0.144 g, 0.55 mmol, 1.0 eq) in THF (1.8 mL, [rxn]= 0.15 M) was

treated **3.9** (0.100 g, 0.57 mmol, 1.1 eq). The syringe containing the alcohol **3.9** was rinsed with

THF (0.1 mL). The mixture was added DBAD (0.126 g, 0.55 mmol, 1.0 eq) in one portion, stirred

for 5 h until the conversion was completed by LC/MS analysis. The mixture was diluted with

CH_2Cl_2 (5x), quenched with brine, dried (Na_2SO_4), and purified by chromatography SiO_2 (gradient

5-10% EtOAc in CH_2Cl_2; **R$_f$:** 0.4) to afford **4.23** (0.257 g, 0.42 mmol, 76%) as a colorless foam:

^{1}H NMR (500 MHz; CDCl$_3$) δ 7.56 (1 H, d, J = 3.5 Hz), 7.35 (1 H, d, J = 2.0 Hz), 7.33 (1 H, d,

J = 2.0 Hz), 7.21 (1 H, d, J = 8.3 Hz), 7.14 (1 H, dd, J = 8.3, 2.0 Hz), 7.11 (1 H, dd, J = 8.3, 2.0

Hz), 6.96 (1 H, d, J = 8.3 Hz), 5.89 (1 H, d, J = 8.0 Hz), 5.23 (1 H, d, J = 8.0 Hz), 5.19 (1 H, d, J

= 13 Hz), 4.97 (1 H, d, J = 13 Hz), 3.67 (3 H, s), 3.65-3.55 (2 H, m), 1.99 (2 H, t, J = 8.0 Hz), 1.23

(6 H, d, J = 2.0 Hz); **^{13}C NMR (125 MHz; CDCl$_3$)** δ 177.5, 164.3, 143.2, 135.0, 134.9, 134.6,

134.5, 134.5, 133.5, 131.8, 130.8, 130.5, 129.6, 129.4, 127.0, 126.9, 103.9, 63.0, 55.5, 52.2, 46.9,

41.1, 39.7, 25.3, 25.2; HRMS **ESI$^-$** m/z calcd for $C_{24}H_{23}O_6N_2Cl_4S$ [M-H] 607, 0025, found

607.0035.
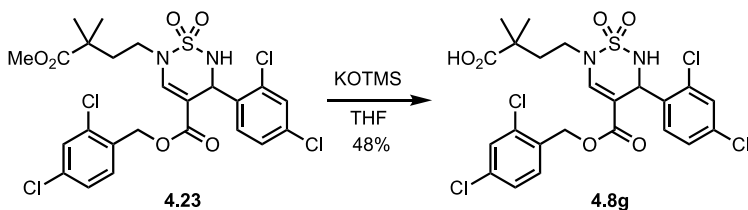
4-(4-((((2,4-Dichlorobenzyl)oxy)carbonyl)-5-(2,4-dichlorophenyl)-1,1-dioxido-5,6-dihydro-

2H-1,2,6-thiadiazin-2-yl)-2,2-dimethylbutanoic acid (4.8g). To a solution of **4.23** (0.120 g, 0.20

mmol, 1.0 eq) in THF (2.5 mL, [rxn]= 0.08 M) was added KOTMS (0.112 g, 0.80 mmol, 4.0 eq).

The mixture was stirred for 5 h until the conversion was completed by TLC analysis. The mixture

was quenched with 0.5 M citric acid (2.0 mL), stirred for 10 min, and extracted with CH_2Cl_2/H_2O

(3x). The combined organic layers were washed with brine and purified by chromatography on

SiO$_2$ (5% MeOH in CH$_2$Cl$_2$, spiked with 0.5% AcOH) to afford a yellow foam, which was triturated in Et$_2$O/n-pentane to yield **4.8g** (0.056 g, 0.095 mmol, 48%) as an off-white solid: **Mp**. 165-168 °C; **^{1}H NMR (500 MHz; DMSO-d$_6$)** δ 12.3 (1H, brs), 8.65 (1 H, d, J = 9.0 Hz), 7.81 (1H, s), 7.59-7.57 (2 H, m), 7.37 (1 H, dd, J = 8.0, 2.0 Hz), 7.32 (1 H, dd, J = 8.0, 2.0 Hz), 7.22-7.18 (2 H, m), 5.60 (1 H, d, J = 7.2 Hz), 5.15 (1 H, d, J = 13 Hz), 5.02 (1 H, d, J = 13 Hz), 3.67-3.62 (2 H, m), 1.91-1.85 (2 H, m), 1.14 (6 H, s); **^{13}C NMR (125 MHz; DMSO-d$_6$)** δ 178.1, 163.9, 144.0, 134.5, 133.8, 133.5, 133.5, 133.3, 132.6, 131.4, 131.0, 128.8, 128.7, 127.2, 126.7, 101.6, 62.1, 53.7, 46.4, 24.9, 24.6; HRMS **ESI$^-$** m/z calcd for C$_{23}$H$_{23}$O$_6$N$_2$Cl$_4$S [M-H] 595.0025, found 595.0018; **IR** (neat) 3288, 3072, 2944, 2979, 2571, 1707, 1694, 1628 cm^{-1}.

5.0 Thiadiazine-Containing Macrocycles as the Potential Hsp70 Agonists

"Maybe I made a mistake yesterday, but yesterday's me is still me. I am who I am today, with all my faults. Tomorrow I might be a tiny bit wiser, and that's me, too. These faults and mistakes are what I am, making up the brightest stars in the constellation of my life. I have come to love myself for who I was, who I am, and who I hope to become... Tell me your story. I want to hear your voice, and I want to hear your conviction. No matter who you are, where you're from, your skin color, gender identity: speak yourself. Find your name, find your voice by speaking yourself."-RM (2018)

5.1 Importance of Macrocycles in Medicinal Chemistry

Functionalized macrocycles and medium-sized rings are prevalent across multiple areas of chemistry, from drug discovery to nanomaterials.[87-91] Interest in macrocycles partially stems from the fact that many of these structures are present in natural-product secondary metabolites. Among 100,000 different secondary metabolized products, 3% of them are macrocycles. These macrocyclic metabolites are routinely excreted as signaling molecules between cells of the same species or as chemical warfare between microbes competing for scarce resources. Thus, it is not surprising that many of these macrocyclic scaffolds have relevant therapeutic effects as antiviral,[92] immunosuppressive,[93, 94] anti-fungal,[95] antibacterial,[96] and anticancer[97] (Figure 13).
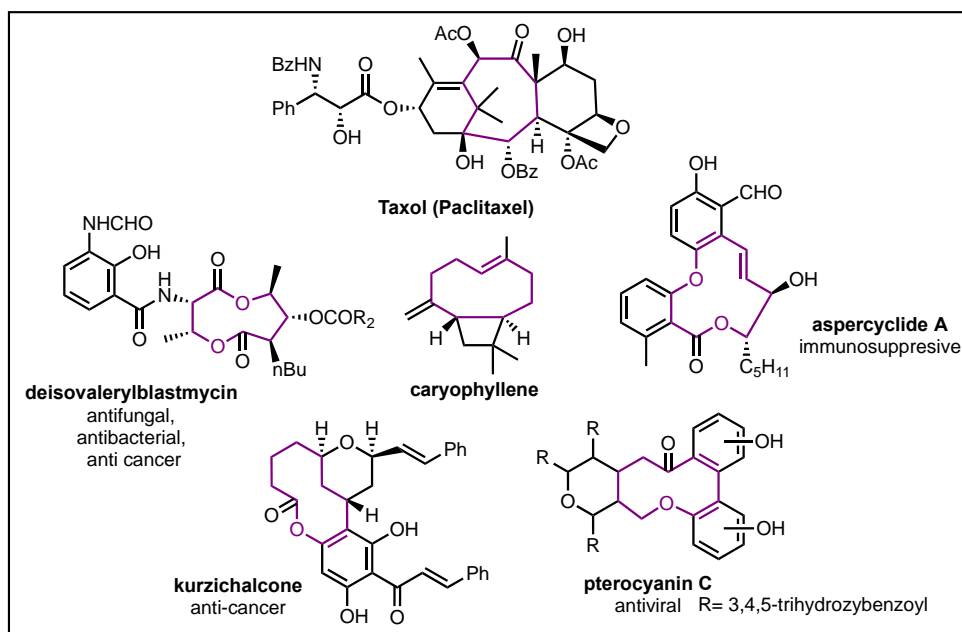
Figure 13. Select examples of drugs and natural products carried the medium-sized rings

Regardless of their biological potential, macrocyclic scaffolds remain under-represented in pharmaceuticals. They are rarely found in the top 200 brand names and the top 200 generic drugs.[87, 88] Couple drugs with the large macrocyclic scaffolds are the 12-membered ring antibiotic vancomycin,[98-101] and the 19-membered ring Hsp90 inhibitor, geldanamycin.[98-100] Other well-known compounds with the medium-sized rings (8-11 atoms) are the anticancer drug Taxol® and a common sesquiterpene caryophyllene, that are found in many types of essential oils (Figure 13)

5.2 Advantages in the Incorporation of Macrocycles

The incorporation of macrocycles or medium-sized rings have proven to provide both benefits and challenges.[91, 92] Given their natural-product origins, macrocycles often show high-affinity for the targeted proteins. Due to their structural pre-organization and restricted rotational

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bonds, the entropic costs are considered to be lower when these macrocycles are bound to the targeted proteins.

Macrocycles also offer several advantages over their acyclic counter partners. With multiple functional groups scattered in a compact structure, macrocycles can cover a larger chemical space without requiring multiple linker chains, which is a common problem found in acyclic compounds. Several beautiful examples from literature have showed that combining acyclic counterparts into a cyclized complex resulted in higher affinity,[102, 103] activity,[103] potency,[104] and selectivity.[105-107]

5.3 Challenges in the Construction of Macrocycles

It is a monumental effort to construct macrocycles let alone to modify specific regions of the structures, which leads to the practical hurdles in creating library of macrocycles for a purpose of the high-throughput screen in drug discovery. The first and major limiting factor is the complexity of the macrocycles.[108] While the cyclization of chains with >10 atoms suffers from the entropic cost to get two reactive termini into close proximation, the cyclization of chains of 8-11 atoms is hampered by the enthalpic cost, which comes from the ring strain in the transition state.[109]

9-, 10-, and 11-membered rings are the most synthetically challenging since they are affected from both enthalpic and entropic costs. A competitive intermolecular process like dimerization could arise and lead to the reactions running at the high dilution, which can be impractical at a process or manufacturing level. In certain types of the cyclization, choosing the right catalyst and the additives could require a lot of efforts, making macrocycle a less compelling target. Nonetheless, macrocyclic scaffolds pose intriguing yet unique intellectual problem to

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organic chemists. Numerous efforts were spent in creating new chemistry and designing novel strategy to form the macrocyclic library for the high throughput screen purposes, however, the majority of the useful results were achieved in the area of peptide macrocycles.[110]
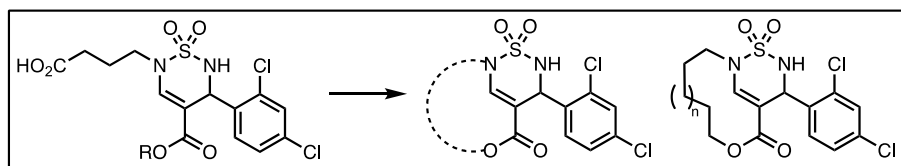
Figure 14. New avenue for additional analogues, thiadizine-containing macrocycles

As seen Figure 14, we were interested in incorporating a medium-sized ring into the thiadiazine skeleton for an exploratory purpose. We also aimed for several purposes when making the incorporation: (1) to create new interactions between the scaffold and the Hsp70-Hsp40 complex (2) to explore the chemistry of a unique motif that has not been studied previously (3) to contribute an intellectual effort to the field of macrocycles by answering a critical synthetic question, how to incorporate a macrocycle in an efficient way. Therefore, we envisioned forming a ring from the N atoms of the sulfamide to the vinylogous ester while maintaining the critical 2,4-dichlorobenzyl substituent.

5.4 Progress toward the Thiadiazine-Containing Macrocycles

5.4.1 Rationale for the Key Disconnection, RCM

We decided to first prepare a 10-membered ring with a key disconnection to be the ring closing metathesis (RCM) between the allylic ester and the homoallylic side chain (Figure 15). The reasons for this disconnection stemmed from the fact that we have a robust route to the precursors of the RCM and the field of RCM has considerably matured over the past two decades.
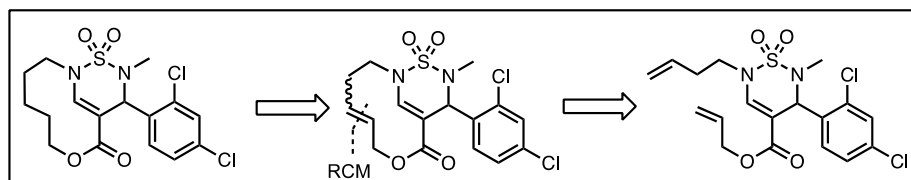
Figure 15. The key disconnection, RCM, for the construction of a 10-membered ring

5.4.2 RCM in the Construction of Medium-Sized Macrocycles

RCM is now an established strategy that is utilized widely in the construction of macrocycles. Given the development of more stable and robust catalysts over the past two decades, RCM is a well-defined and powerful synthetic tool for organic chemists.[111-117] However, due to the high constrain of the challenging medium-sized rings (8-11 atoms), the effectiveness of RCM for their formations is not well-precedented. Typically, these formations required a high dilution and temperature, and in certain cases, a high catalyst loading. In some cases, the slow addition of the catalyst and the use of appropriate additives are also required to facilitate the difficult

cyclization. Select examples where 10-membered rings were generated by the catalytic RCM reactions are drawn in Figure 16.[118-123]
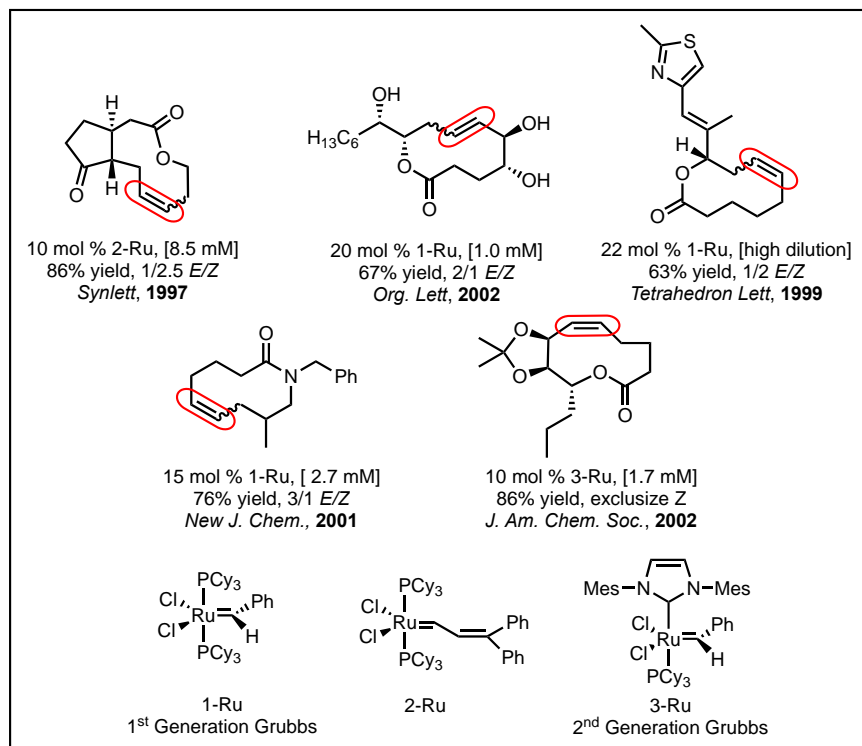
Figure 16. Select examples of 10-membered rings generated with RCM

5.4.3 Preliminary Study toward the Construction of a Thiadiazine-Containing Macrocycle
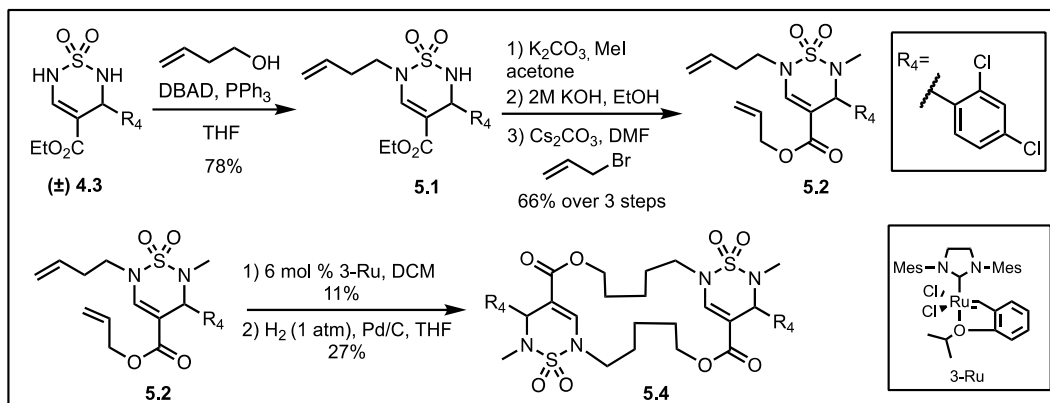
Scheme 13. The first RCM attempt for a construction of the 10-membered macrocycles

As illustrated in Scheme 13, the heterocycle (±) **4.3** (for preparation, see Section 4.4) was subjected to the Mitsunobu alkylation with homoallylic alcohol to form **5.1** in 78%. As we were concerned about the chelation of the remaining N atom of the sulfone to the Ru catalyst, we decided to methylate this position. The methyl-substituted product was then hydrolyzed and alkylated with allylic bromide to furnish diene **5.2** in 66% over 3 steps. The diene **5.2** was then subjected to the RCM in CH_2Cl_2 at reflux in the presence of 6 mol% of the 2nd generation Hoveyda-Grubbs catalyst (labelled as 3-Ru) to generate only the dimerized product **5.3** (*E*-alkene) in 11% yield.[124] This dimer **5.3** was reduced with H_2 and Pd/C to give **5.4** in 27% yield, and the structure was confirmed with X-ray crystallography (Figure 17). The crystal structure of **5.4** also clarified that the dimer **5.3** was indeed a head-to-tail dimer.
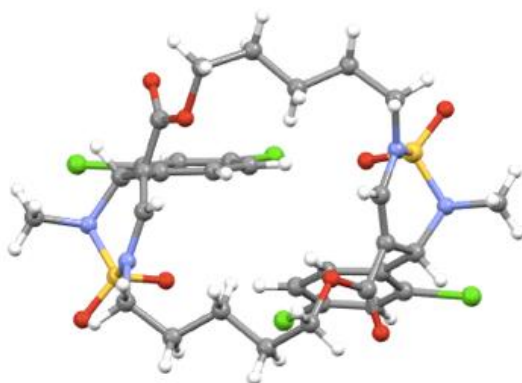
Figure 17. X-ray analysis of the dimer 5.4 (CCDC 2083322)

The formation of the dimer **5.3** could be explained due to the relatively high concentration (15 mM) being used while typically related RCM performed in concentrations ranging from 1-5 mM. The second reason, which was proposed by the Fürstner group, was the formation of a six-membered chelate catalytic species, which potentially sequestered the catalyst (Figure 18).[125] Several protocols from the same group have also demonstrated that the use of a Lewis acid-type additive such as titanium isopropoxide, Ti(OiPr)4 in presences of the early developed Ru-based catalysts, could prevent the catalyst poisoning. In light of this, we are considering the use of the early developed Ru-based catalyst such as the 2nd generation Grubbs catalyst with the addition of Ti(OiPr)4. We are also considering substrate modification such as additional substituents like the gem-dimethyl groups on the homoallylic linker to facilitate the cyclization.
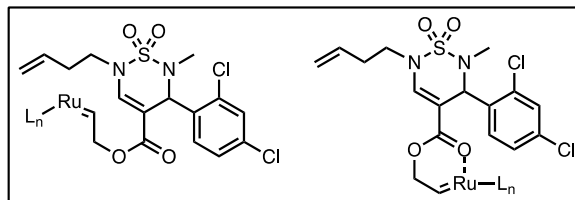
Figure 18. Potential chelate formation in the RCM reaction

Macrocycles are valuable yet challenging scaffolds. We have started the development of the thiadiazine-containing macrocycles and considered RCM as the first promising approach. Besides the substrate and condition modifications for the RCM, a different key disconnection such as lactonization, as illustrated in Figure 19, is also being considered.

Lactonization
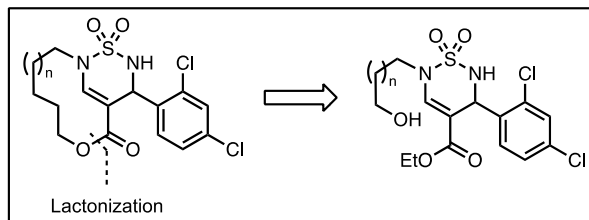
Figure 19. Future disconnections to be considered for a construction of the macrocycle

5.5 Experimentals

5.5.1 General

All reactions were carried out under a positive pressure of N_2 in a well-ventilated fume hood unless otherwise noted. Hexanes (ACS grade), ethyl acetate (ACS grade), toluene (ACS grade), and diethyl ether (ACS grade), dichloromethane (ACS grade), acetonitrile (ACS grade), and chloroform (ACS grade) were purchased from Fisher Chemical and used without further purification. Anhydrous tetrahydrofuran was distilled from sodium (10% w/v) under a positive pressure of N_2. Anhydrous dichloromethane was distilled from calcium hydride (10% w/v) under a positive pressure of N_2. Commercially available reagents were used without further purification unless otherwise noted. Reactions were monitored by TLC analysis (pre-coated silica gel 60 F254

plates, 250 µm layer thickness) and visualization was accomplished with a 254 nm UV light and by staining with a KMnO$_4$ solution (1.5 g of KMnO$_4$ and 1.5 g of K$_2$CO$_3$ in 100 mL of a 0.1% NaOH solution). Flash column chromatography was performed over Silica gel 60 (particle size 0.04-0.063 mm) from EMD chemicals. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker 300, 400, and 500 (equipped with cryoprobe) spectrometers using residual solvent peaks as internal standard (CDCl$_3$ @ 7.26 ppm ^{1}H NMR, 77.00 ppm ^{13}C NMR; DMSO-d$_6$ @ 2.50 ppm ^{1}H NMR, 39.52 ppm ^{13}C NMR). Low-resolution mass spectra were recorded on an Agilent Technologies 1260 Infinity II LCMS. High- resolution mass spectra were obtained on a Micromass UK Limited, Q-TOF Ultima API or a Thermo Scientific Exactive Orbitrap LCMS.

5.5.2 Experimentals
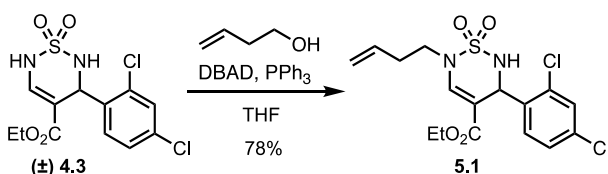
Ethyl   6-(but-3-en-1-yl)-3-(2,4-dichlorophenyl)-3,6-dihydro-2*H*-1,2,6-thiadiazine-4-carboxylate 1,1-dioxide (5.1). To a solution of (±) **4.3** (0.200 g, 0.56 mmol, 1.0 eq) and but-3-en-1-ol (0.048 g, 0.68 mmol, 1.2 eq), and PPh$_3$ (0.179 g, 0.68 mmol, 1.2 eq) in THF (3.8 mL, [rxn]= 0.15 M) was added DBAD (0.147 g, 0.68 mmol, 1.2 eq) in 2-3 portions. The resulting yellow solution was stirred for 16 h, diluted with CH$_2$Cl$_2$ (5x), washed with brine, dried (MgSO$_4$), and purified by chromatography on SiO$_2$ (100% CH$_2$Cl$_2$, **R$_f$:** 0.29) to afford **5.1** (0.180 g, 0.44 mmol, 78%) as colorless oil: **^{1}H NMR (400 MHz; CDCl$_3$)** δ 7.48 (1 H, brs), 7.42 (1 H, d, *J* = 1.8 Hz), 7.24 (1 H, d, *J* = 8.4 Hz, overlapping with CDCl$_3$), 7.19 (1 H, dd, *J* = 8.4 Hz), 5.89 (1 H, d, *J* = 6.0 Hz), 5.85-5.75 (1 H, m), 5.21-5.16 (2 H, m), 4.89 (1 H, brs), 4.11-3.99 (2 H, m), 3.76-3.68 (1 H,

m), 3.66-3.58 (1 H, m), 2.52 (2 H, q, $J = 7.1$ Hz), 1.08 (3 H, t, $J = 7.1$ Hz); **^{13}C NMR (100 MHz; CDCl$_3$)** δ 164.8, 142.7, 135.1, 134.7, 133.8, 133.6, 130.6, 129.8, 127.1, 118.9, 104.2, 60.8, 55.8, 49.9, 34.2, 14.2; HRMS **ESI$^+$** *m/z* calcd for $C_{16}H_{19}O_4N_2Cl_2S$ [M+H] 405.0437, found 405.0438.
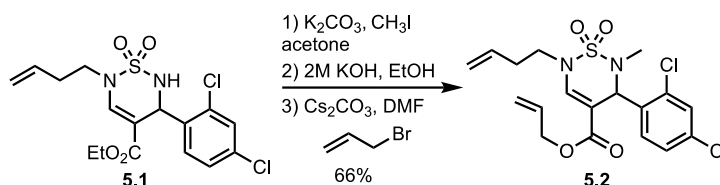
Allyl 6-(but-3-en-1-yl)-3-(2,4-dichlorophenyl)-2-methyl-3,6-dihydro-2*H*-1,2,6-thiadiazine-4-carboxylate 1,1-dioxide (5.2). To a suspension of **5.1** (1.30 g, 3.2 mmol, 1.0 eq) and K$_2$CO$_3$ (2.66 g, 19 mmol, 6.0 eq) in ACN (50 mL, [rxn]=0.06 M) was treated MeI (1.2 mL, 19 mmol, 6.0 eq). The mixture was stirred for 15 h, filtered over a pad of celite to afford a yellow crude, which was used without further purification.

To a solution of the yellow residue in EtOH (30 mL, [rxn]= 0.1 M) was added 2.0 M KOH solution (19 mL, 39 mmol, 12 eq) in one portion. The solution was stirred at 80 °C for 5 h, diluted with EtOAc, acidified with 4.0 M HCl until pH= 3, extracted with EtOAc (3x). The combined organic layers were washed with brine and dried (MgSO$_4$) to afford the crude acid, which was used without further purification.

To a solution of the crude acid in DMF (32 mL, [rxn]= 0.06 M) were added Cs$_2$CO$_3$ (1.40 g, 4.2 mmol, 1.3 eq) and allyl bromide (0.33 mL, 3.9 mmol, 1.2 eq). The mixture was stirred for 15 h and extracted with EtOAc/H$_2$O (3x). The combined organic layers were washed with brine, dried (MgSO$_4$), and purified by chromatography on SiO$_2$ (20% EtOAc in PE; **R$_f$**: 0.28) to afford **5.2** (0.920 g, 2.1 mmol, 66% over 3 steps) as thick yellow oil: **^{1}H NMR (400 MHz; CDCl$_3$)** δ 7.58 (1 H, s), 7.41 (1 H, d, $J = 2.0$ Hz), 7.20 (1 H, d, $J = 8.4$ Hz), 7.16 (1 H, dd, $J = 8.4, 2.0$ Hz), 5.83-

5.73 (1 H, m), 5.53 (1 H, s), 5.22-5.09 (4 H, m), 4.58-4.53 (2 H, m), 3.68-3.66 (2 H, m), 3.00 (3 H, s), 2.54-2.48 (2 H, m); ^{13}**C NMR (100 MHz; CDCl$_3$)** δ 165.1, 142.5, 135.0, 134.7, 134.0, 133.4, 131.2, 131.1, 129.5, 126.6, 119.0, 118.1, 99.9, 65.3, 63.4, 50.1, 40.0, 34.4; HRMS **ESI$^+$** *m/z* calcd for C$_{18}$H$_{21}$O$_4$N$_2$Cl$_2$S [M+H] 431.593 found 431.0588; **IR** (neat) 3081, 2933, 2118, 1698, 1622, 1588, 1561 cm^{-1}.
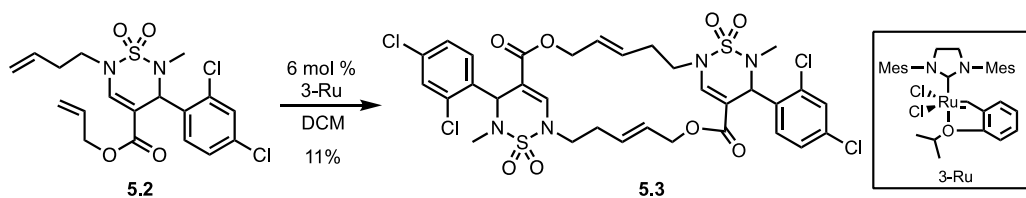
(4E,9E,16E,21E)-10,22-Bis(2,4-dichlorophenyl)-11,23-dimethyl-7,19-dioxa-12,24-dithia-1,11,13,23-tetraazatricyclo[19.3.1.1^{9,13}]hexacosa-4,9(26),16,21(25)-tetraene-8,20-dione 12,12,24,24-tetraoxide (5.3). To a solution of **5.2** (0.800 g, 1.9 mmol, 1.0 eq) in CH$_2$Cl$_2$ (125 mL, [rxn]= 0.015 M) was added the 2nd generation Hoveyda-Grubbs (0.074 g, 0.11 mmol, 0.06 eq) at rt. The resulting green solution was degassed by freeze-pump-thaw (3x) and heated at reflux for 11 h until the conversion was completed by TLC analysis. The dark yellow mixture was exposed to air for 1 hour and purified by chromatography on SiO$_2$ (gradient 15-60% EtOAc in hexane; **R$_f$:** 0.10 in 50%) to afford **5.3** (0.078 g, 0.10 mmol, 11%) as a light brown solid: ^{1}**H NMR (500 MHz; CDCl$_3$)** δ 7.44-7.42 (1 H, s), 7.42 (1 H, d, *J* = 2.0 Hz), 7.18 (1 H, d, *J* = 8.0 Hz), 7.15 (1 H, d, *J* = 2.0 Hz), 5.74 (1 H, td, *J* = 15, 5.0 Hz), 5.54 (1 H, s), 5.51-5.46 (1 H, m), 4.79 (1 H, dd, *J* = 14, 5.0 Hz), 4.49 (1 H, dd, *J* = 14, 3.5 Hz), 3.79-3.74 (1 H, m), 3.65-3.60 (1 H, m), 3.61 (3 H, s), 2.46 (2 H, m); ^{13}**C NMR (125 MHz; CDCl$_3$)** δ 165.0, 143.2, 135.2, 134.9, 134.0, 131.1, 129.7, 129.4, 128.6, 126.7, 98.8, 64.5, 63.4, 50.5, 40.1, 32.9; HRMS **ESI$^+$** *m/z* calcd for C$_{32}$H$_{33}$O$_8$N$_4$Cl$_4$S$_2$ [M+H] 805.0488, found 805.0480; **IR** (neat) 2931, 1692, 1623, 1588, 1563 cm^{-1}.
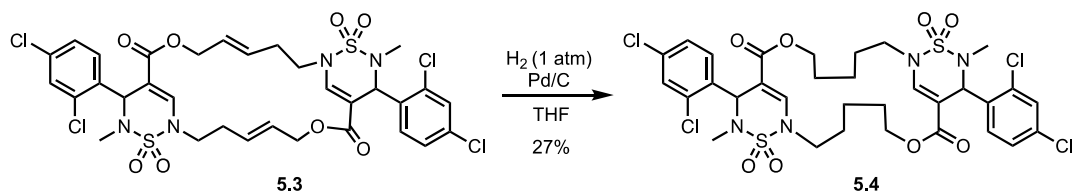
(9*E*,21*E*)-10,22-Bis(2,4-dichlorophenyl)-11,23-dimethyl-7,19-dioxa-12,24-dithia-1,11,13,23-tetraazatricyclo[19.3.1.1^{9,13}]hexacosa-9(26),21(25)-diene-8,20-dione 12,12,24,24-tetraoxide (5.4). To a solution of **5.3** (0.048 g, 0.06 mmol, 1.0 eq) in THF (4 mL, [rxn]= 0.03 M) was added 10% Pd on carbon (0.024 g, 0.006 mmol, 0.1 eq) at rt under N_2. The N_2 was then replaced with H_2, and the reaction mixture was stirred at rt for 15 h until the conversion was completed by TLC analysis. The mixture was filtered over a thin pad of celite. The eluent was concentrated to yield white solid, which was recrystallized with CH_2Cl_2 to yield **5.7** (0.013 g, 0.016 mmol, 27%) as a needle-like crystal: **^{1}H NMR (500 MHz; CDCl$_3$)** δ 7.82 (1 H, s), 7.63 (1 H, d, *J* = 2.0 Hz), 7.10-7.05 (2 H, m), 5.47 (1 H, s), 4.22-4.19 (1 H, m), 4.06-4.02 (1 H, m), 3.78-3.75 (2 H, m), 2.92 (3 H, s), 1.69-1.60 (1 H, m), 1.51-1.46 (1 H, m); **^{13}C NMR (125 MHz; CDCl$_3$)** δ 164.7, 143.1, 134.6, 134.3, 133.3, 131.4, 128.7, 126.6, 98.3, 64.0, 62.8, 54.9, 50.9, 29.5, 28.1, 23.0; HRMS **ESI$^-$** *m/z* calcd for $C_{32}H_{37}O_8N_4Cl_4S_2$ [M-H] 809.0801, found 809.0808.

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