TECHNISCHE UNIVERSITÄT DARMSTADT

Synthesis and investigation of 2-substituted hetero-/arylacetic acids as bottom groups for selective inhibitors of the FK506-binding protein 51 (FKBP51)

Synthese und Untersuchung von 2-substituierten hetero-/arylessigsäuren als bottom groups für selektive Inhibitoren des FK506-bindenden Proteins 51 (FKBP51)

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von Fabian Henrik Knaup

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Fabian Henrik Knaup

2 Acknowledgments

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I thank Dr. Margherita Springer from the Max Planck institute for psychiatry in Munich for performing the snapshot pharmacokinetic study of compound **68b** and SAFit2 and Saskia Wedel for measuring the activity of **68a** and **68b** on TRPV1 receptor desensitization.

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3 Abstract

In recent years the FK506-binding protein 51 (FKBP51) has emerged as a potential target for treating depression, chronic pain, or diabetes. The first selective FKBP51 inhibitors, SAFit1 and SAFit2, contain a crucial cyclohexyl group in the bottom group section, enabling selectivity over the closest homolog FKBP52. After discovering these lead compounds, all structure-based optimizations were conducted in the context of the cyclohexyl group for inducing selectivity for FKBP51. Therefore, first, different approaches for synthesizing 2,2-disubstituted acetic acids were investigated, and the respective SAFit1 analogs were analyzed by structure-affinity relationships.

During the structure-based optimization of the SAFit bottom group to enhance the overall physicochemical properties and metabolic stability, a thiophene scaffold was identified that could enable selectivity for FKBP51. For the first time since the discovery of the SAFit ligands, a suitable replacement for the highly efficient cyclohexyl motif was identified.

Additionally, thiophene-containing SAFit analogs have a high binding affinity for FKBP51 and can discriminate between FKBP51 and its closest homolog, FKBP52. The co-crystal structures of those analogs also revealed that the novel scaffold enables selectivity by binding to the previously identified transient binding pocket, which is characterized by the displacement of the phenylalanine residue in position 67 towards the solvent phase. Moreover, besides high binding affinity and selectivity for FKBP51, the most promising SAFit2 analog **68b** of this series also possesses an acceptable pharmacokinetic profile *in-vivo*. As a result, the thiophene-containing SAFit2 analogs are promising tool compounds for investigating FKBP51 in animal models.

4 Zusammenfassung

In den letzten Jahren hat sich das FK506-bindende Protein 51 (FKBP51) als Zielprotein für die Behandlung von stressbedingten Krankheiten z.B. chronischen Schmerzen, Diabetes oder Depressionen erwiesen. Die ersten selektiven FKBP51-Inhibitoren, SAFit1 und SAFit2, enthalten eine Cyclohexylgruppe im Bereich der *bottom group*, die die Selektivität gegenüber dem nächsten Homologen FKBP52 ermöglicht. Im Kontext dieser Leitverbindungen wurden alle strukturbasierten Optimierungen mit der Cyclohexylgruppe durchgeführt, um eine Selektivität für FKBP51 zu erreichen. Daher wurden zuerst verschiedene Ansätze zur Synthese von 2,2-disubstituierten Essigsäuren untersucht und die entsprechenden SAFit1-Analoga durch Struktur-Affinitätsbeziehungen analysiert.

Während der strukturbasierten Optimierung der SAFit *bottom group* zur Verbesserung der physikalisch-chemischen Eigenschaften und der metabolischen Stabilität wurde ein Thiophen-Baustein identifiziert, der Selektivität für FKBP51 ermöglichen konnte. Zum ersten Mal seit der Entdeckung der SAFit-Liganden wurde ein geeigneter Ersatz für das hocheffiziente Cyclohexyl-Motiv gefunden.

Darüber hinaus weisen thiophenhaltige SAFit-Analoga eine hohe Bindungsaffinität für FKBP51 auf und können zwischen FKBP51 und seinem engsten Homologen, FKBP52, unterscheiden. Die Co-Kristallstrukturen dieser Analoga zeigten zudem, dass der neuartige Baustein Selektivität ermöglicht, indem es an die zuvor identifizierte transiente Bindungstasche bindet, die sich nach der Verdrängung von Phe^{67} in FKBP51 bildet. Darüber hinaus besitzt das vielversprechendste SAFit2-Analog **68b** dieser Reihe neben einer hohen Bindungsaffinität und Selektivität für FKBP51 auch ein akzeptables pharmakokinetisches Profil *in vivo*. Somit sind die thiophenhaltigen SAFit2-Analoga vielversprechende Verbindungen für die Untersuchung von FKBP51 in Tiermodellen.

5 Declaration of contributions

The determination of the binding affinities for FKBP51 and FKBP52 by fluorescent polarization assay of the synthesized compounds was performed by Wisley Oki Sugiarto, Dr. Stephanie Merz, Dr. Patrick Purder, and Dr. Tim Heymann.

Thomas Geiger performed the characterization of the synthesized compounds by a NanoBRET assay.

The co-crystallization of the ligands in complex with the FK1 domain of FKBP51 was performed, measured, and analyzed by Dr. Christian Meyners. We also thank HZB for allocating synchrotron radiation beamtime, and we would like to acknowledge the help and support of Manfred Weiss and the whole MX team during the experiment.

Dr. Margherita Springer from the Max Planck Institute for Psychiatry in Munich performed the snapshot pharmacokinetic study of compound 68b and SAFit2 in BL6 mice.

6 Table of Contents

7 Introduction

7.1 Biology of FKBP51 and its closest homolog FKBP52

The FK506-binding protein 51 (FKBP51) is an immunophilin and co-chaperon first discovered during the studies of progesterone receptor (PR) complexes in 1990.[1–3] FKBP51 is a 51 kDa large protein that binds to the natural product FK506 and thereby is a member of the FKBP protein family.[4] In contrast to the first discovered and smallest member of the FKBP family, FKBP12, inhibition of the peptidyl-prolyl cis/trans isomerase activity of FKBP51 by FK506 does not result in the formation of a tertiary complex with calcineurin, thereby not resulting in immunosuppressant effects.[4,5]

In general, FKBP51 is a multi-domain peptidyl-prolyl cis/trans isomerase (PPIase) protein comprising a FK1, which contains the PPIase activity, a FK2 domain and a tetratricopeptide repeat protein (TPR) domain (**Figure 1**).[6–8] Unlike the FK1 domain, the structurally similar FK2 domain lacks measurable PPIase activity.[9] Nevertheless, it was suggested that the FK2 domain could enhance the cooperativity of protein-protein interactions of FKBP51.[8] The TPR domain of FKBP51 consists of 7 anti-parallel alpha helices and induces binding of FKBP51 to the MEEDV motif of Hsp90.[10,11] Upon binding of FKBP51 to Hsp90 through its TPR domain, the formed FKBP51-Hsp90 complex can be recruited by various steroid hormone receptors, especially the glucocorticoid receptor (GR).[9,12] During the investigation of glucocorticoid resistance in squirrel monkeys and human cell lines, the FKPB51-Hsp90 complex was identified as a potent inhibitor of GR sensitivity.[13,14] As a result, the binding affinity of the glucocorticoid dexamethasone to the GR was reduced significantly.[14]

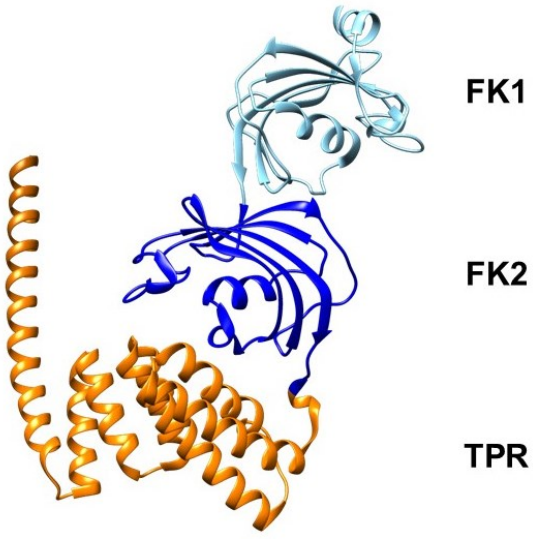


Figure 1: Protein structure of FKBP51 (PDB: 5OMP). The protein consists of a peptidyl-prolyl cis/trans isomerase (PPIase) containing a FK1 domain (light blue), a FK2 domain (blue), and a tetratricopeptide repeat protein (TRP) domain (orange).

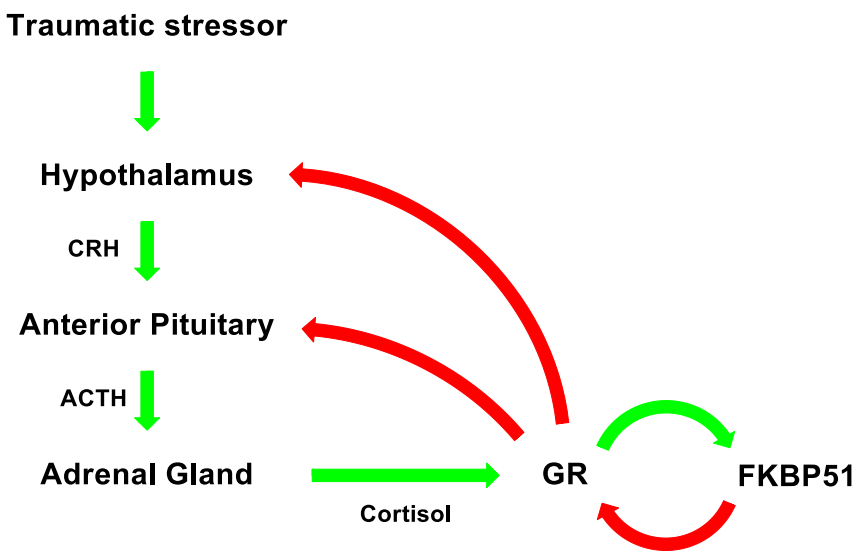


Figure 2: Illustration of the function of the HPA axis upon traumatic stress. First, the stress is registered by the hypothalamus, resulting in the secretion of CRH. This leads to the release of ACTH in the anterior pituitary, leading to cortisol secretion in the adrenal gland. Once the GR in the brain is activated by cortisol, the secretion of CRH and ACTH is shut down, resulting in the termination of the stress response.[15,16]

Therefore, FKBP51 is a crucial modulator of the GR, which plays a critical role in the termination of HPA axis-induced stress responses by a negative feedback loop (**Figure 2**).[15–17] Additionally, the transcription of FKBP51, encoded by the *FKBP5* gene, can be induced by the activated GR or glucocorticoids, progestin, and androgenic hormones.[8,18–20] Therefore, dysregulation of FKBP51 is often associated with higher overall cortisol levels, resulting in higher transcription levels of *FKBP5* and thereby reducing the negative feedback of the GR to the hypothalamus and anterior pituitary upon stimuli.[21–23] As a result, overexpression of FKBP51 is a risk factor in developing stress-related diseases, such as depression, obesity, and chronic pain.[24–32]

Besides FKBP51, there is a second Hsp90 binding co-chaperone in the FKBP family, FKBP52. This 52 kDa large FKBP is, as well as FKBP51, a PPIase-containing multi-domain protein comprising a FK1, a FK2, and C-terminal TPR domain.[33,34] FKBP52 has a sequence identity of around 70% compared to FKBP51, and their tertiary conformations are similar (**Figure 3**).[33] Since FKBP52 contains a TPR domain that can induce binding to Hsp90, FKBP52 is a known modulator of various steroid hormone receptors, such as the glucocorticoid, progesterone, and androgen receptor.[35–37] In contrast to FKBP51, binding of the FKBP52-Hsp90 complex to the GR results in a potentiation of GR signaling, and therefore FKBP52 is a positive modulator of the GR.[37] While FKBP51 and FKBP52 have similar binding affinities for Hsp90, the FKBP51-Hsp90 complex is preferentially incorporated in the apo PR and apo GR complex compared to the FKBP52-Hsp90 complex.[9,34,38] Therefore, FKBP51 acts as an antagonist to the highly homologous FKBP52 in GR signaling, and the balance between both co-chaperones is critical for the homeostasis of the HPA axis.[39] As a result, the inhibition of FKBP51 is a promising drug target for the treatment of stress-related diseases.[40]

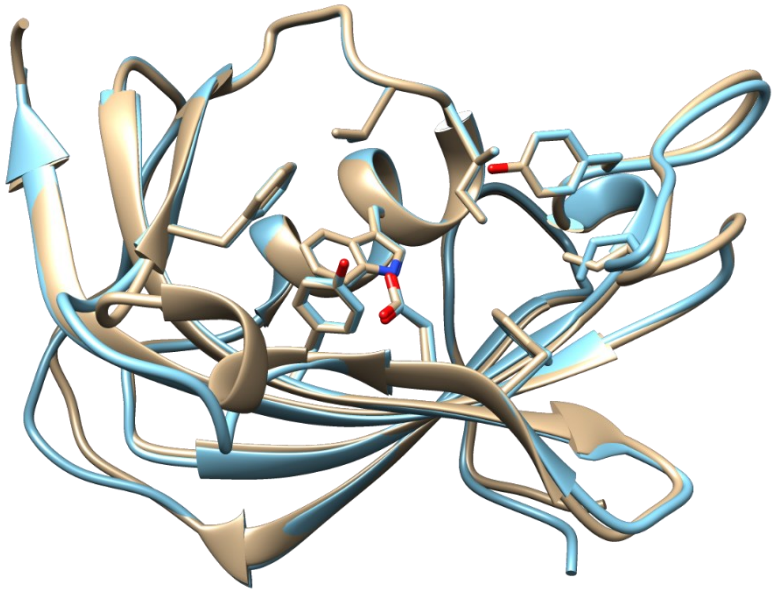


Figure 3: Overlay of the co-crystal structures of the FK1 domain of FKBP51 (beige, PDB: 4DRI) and FKBP52 (cyan, PDB: 4DRJ) bound to Rapamycin. For visualizing the highly similar tertiary structure (ribbon) of both FK1 domains, the ligand was removed, and the residues, which interact with Rapamycin by hydrogen bonding (Asp^{68}, Ile^{87}, and Tyr^{113}) and hydrophobic interactions (Tyr^{57}, Phe^{67}, Phe^{77}, Val^{86}, Trp^{90}, and Ile^{122}), are shown as sticks.

To further understand the function of FKBP51 *in-vivo*, FKBP51 knockout mice were generated and studied in the context of different environmental stressors.[25,26,41] These initial studies showed that mice lacking FKBP51 had significantly enhanced stress-coping and antidepressant-like behavior while not showing any adverse effects.[25,26,41] In contrast, the knockout of FKBP52 in male mice led to the development of several defects in the reproductive organs, highlighting the importance of FKBP52 in androgen receptor complexes.[35,42] Based on the results, it is necessary to inhibit FKBP51 selectively to reduce the potential adverse effects of the inhibition of FKBP52.

7.2 Development of the first selective FKBP51 inhibitors

The first discovered inhibitors for FKBP12 were Rapamycin and FK506 (**Figure 4**).[43,44] These natural products can inhibit the peptidyl-prolyl cis/trans isomerase activity of most FKBPs upon binding to the pipecolic core motif to the PPIase domain.[45] Additionally, FK506 and Rapamycin can form ternary complexes upon binding to FKBP12 with calcineurin (for FK506) and mTOR (for Rapamycin), resulting in the immunosuppressant effects of those drugs.[43,44] Because the formation of the ternary complexes is highly dependent on the structure of the macrocyclic linker, initial FKBP ligand development focused on altering the structure in the macrocyclic linker to prevent the immunosuppressant effect.[46,47] Nevertheless, these first non-immunosuppressive inhibitors were still heavily relying on the complex structures of FK506 and Rapamycin.[46,47]

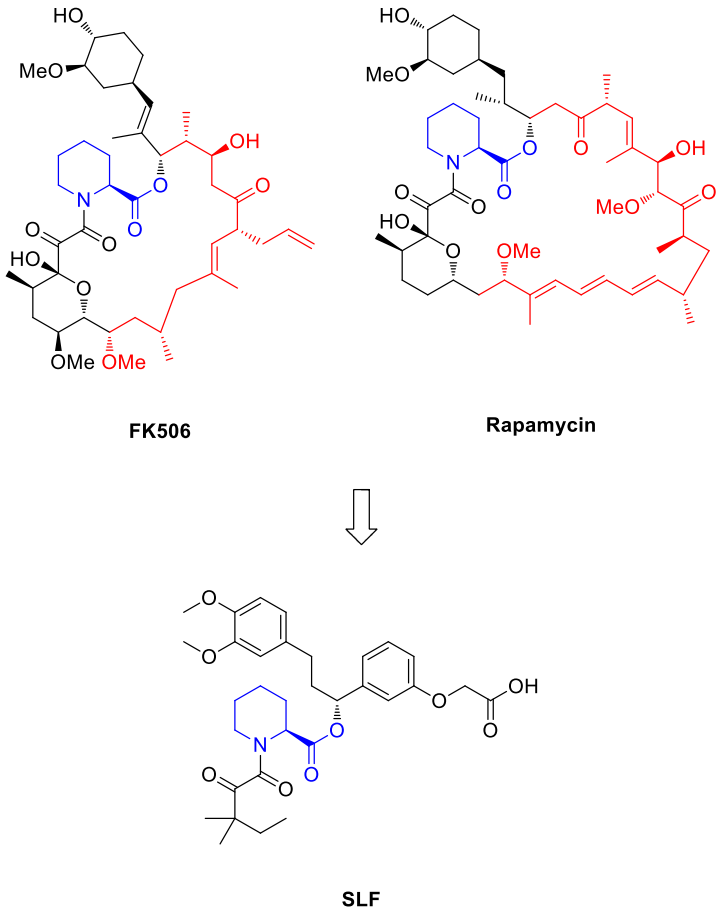


Figure 4: Chemical structures of FK506, Rapamycin, and SLF. The pipecolic core is highlighted in blue, and the macrocyclic linkers that induce the immunosuppressant effects of the natural products FK506 and Rapamycin are highlighted in red.

The first highly potent and non-immunosuppressant synthetic ligand for FKBP12 was SLF, which only contains the pipecolic core and the ketoamide functionality of FK506.[48,49] Since the natural products Rapamycin and FK506 bind to most FKBPs with high binding affinity, the binding affinities for six different FKBPs of SLF was determined by a fluorescent polarization assay.[45] While SLF shows a high binding affinity for FKBP12 (12 nM), it's affinity is significantly reduced for FKBP51 (3.10 µM) and

FKBP52 (2.60 µM). Co-crystallization of FK506 and SLF in complex with FKBP51 (**Figure 5**) and subsequent structure-affinity relationship studies led to the development of a variety of SLF analogs with improved binding affinities for FKBP51.[50–52] Further development of those inhibitors, resulted in the discovery of a rigid [4.3.1] bicyclic scaffold that mimics the pipecolic core, leading to low nanomolar binding affinities for FKBP12, FKBP51, and FKBP52.[53–57] Nevertheless, the bicyclic ligands only showed pan-selectivity for FKBP12 versus FKBP51 and no selectivity over FKBP52.

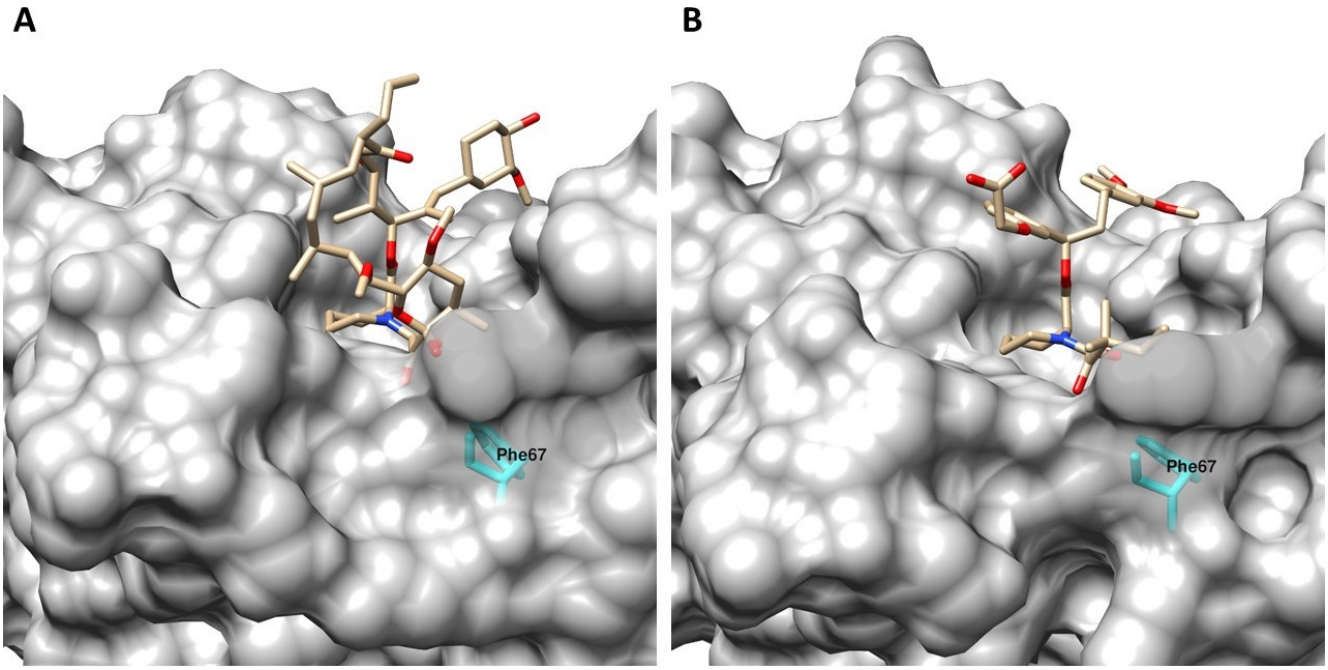


Figure 5: Co-crystal structures of FK506 and SLF (in sticks) in complex with the FK1 domain of FKBP51. The Phe^{67} is highlighted in cyan. A) Co-crystal structure of FK506 (PDB: 3O5R). B) Co-crystal structure of SLF (PDB: 4DRK).

During the investigation of ligands that induce FKBP12 dimerization, Clackson *et al.* remodeled the FKBP12-ligand interface by introducing a sub-pocket.[58] This was achieved by a chemical genetic approach, leading to optimized ligands that bind specifically to mutated proteins, resulting in highly potent and selective ligands.[58] Gaali et al. transferred this approach to FKBP51 and generated the mutants FKBP51^{F67V} and FKBP52^{F67V}. In addition, a series of mutant selective FKBP inhibitors were synthesized. Interestingly, when the binding affinity of those ligands was determined for the wild-type proteins, the ligand iFit1 still showed weak binding in the µM range for FKBP51 and selectivity over FKBP52 (**Figure 6**).[59] The high-resolution co-crystal structure of iFit1 in complex with FKBP51 revealed that the allyl group in the Cα-position of the amide bond is displacing the previously mutated Phe^{67}, resulting in the formation of a transient binding pocket (**Figure 7A**). Based on these results, Gaali *et al.* replaced the identified allyl group of iFit1 with various alkyl groups, resulting in the discovery of SAFit1.[59] The high-resolution co-crystal structure of SAFit1 confirmed that the newly introduced cyclohexyl group is inducing the same conformational rearrangement of Phe^{67} as previously observed. Additionally, the

cyclohexyl group enhances the hydrophobic interaction of SAFit1 in the transient binding pocket, resulting in a single-digit nanomolar binding affinity for FKBP51 while achieving high selectivity. Due to the impaired flexibility of Phe[67] in FKBP52 by the bulk of the residues Thr[58], Trp[60], and Val[129], this structural rearrangement is disfavored for FKBP52, resulting in the observed selectivity of SAFit1 for FKBP51. The discovery of SAFit1 led to one of the first selective FKBP51 inhibitors, but it still lacks cell permeability due to a carboxylic acid functionality.[59] Therefore, the carboxylic acid of SAFit1 was replaced by a morpholine group (SAFit2), resulting in increased cell permeability and better overall pharmacokinetic parameters. Furthermore, SAFit2 can pass the blood-brain barrier.[59]

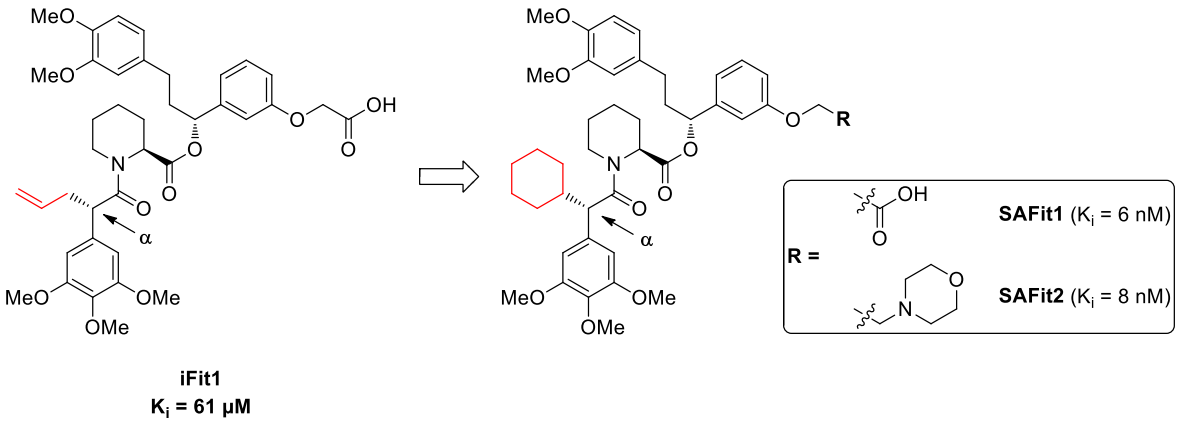


Figure 6: Chemical structures of iFit1, SAFit1, and SAFit2 and their respective K_i values for FKBP51. The selectivity-inducing residue in the Cα -position is highlighted in red.

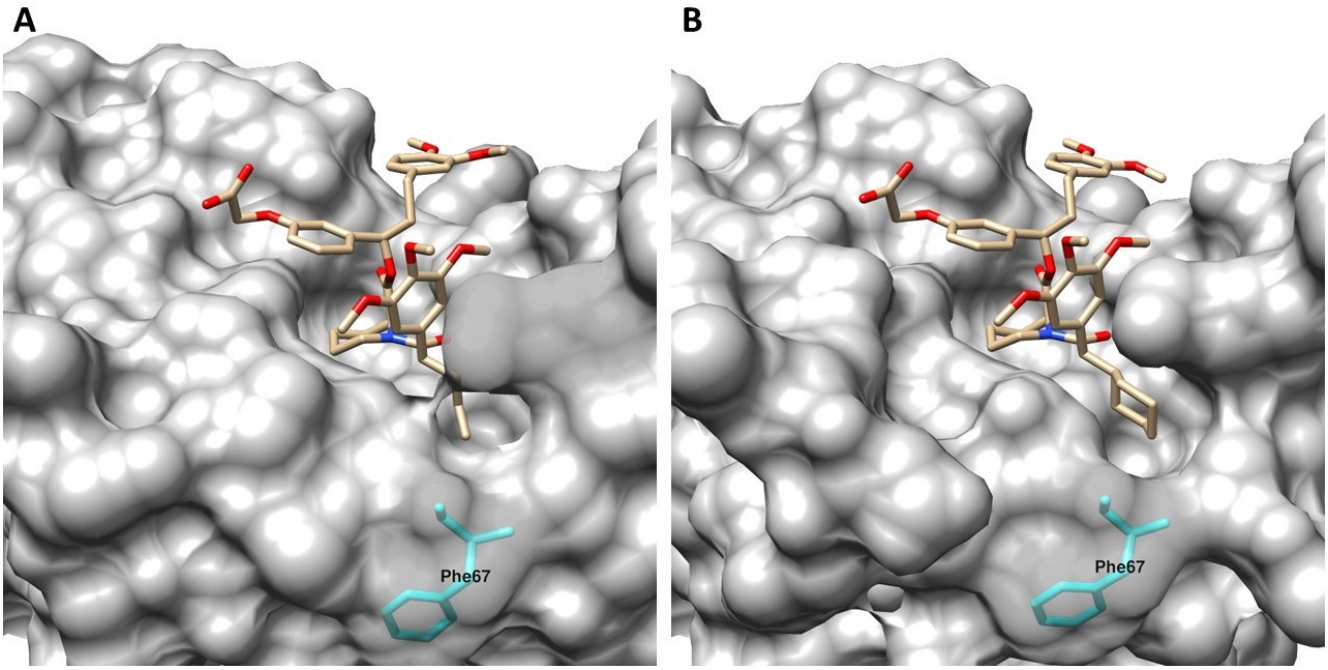


Figure 7: Co-crystal structures of iFit1 and SAFit1 (in sticks) in complex with the FK1 domain of FKBP51. The Phe[67] is highlighted in cyan. A) Co-crystal structure of iFit1 (PDB: 4TW6). B) Co-crystal structure of SAFit1 (PDB: 8CCA).

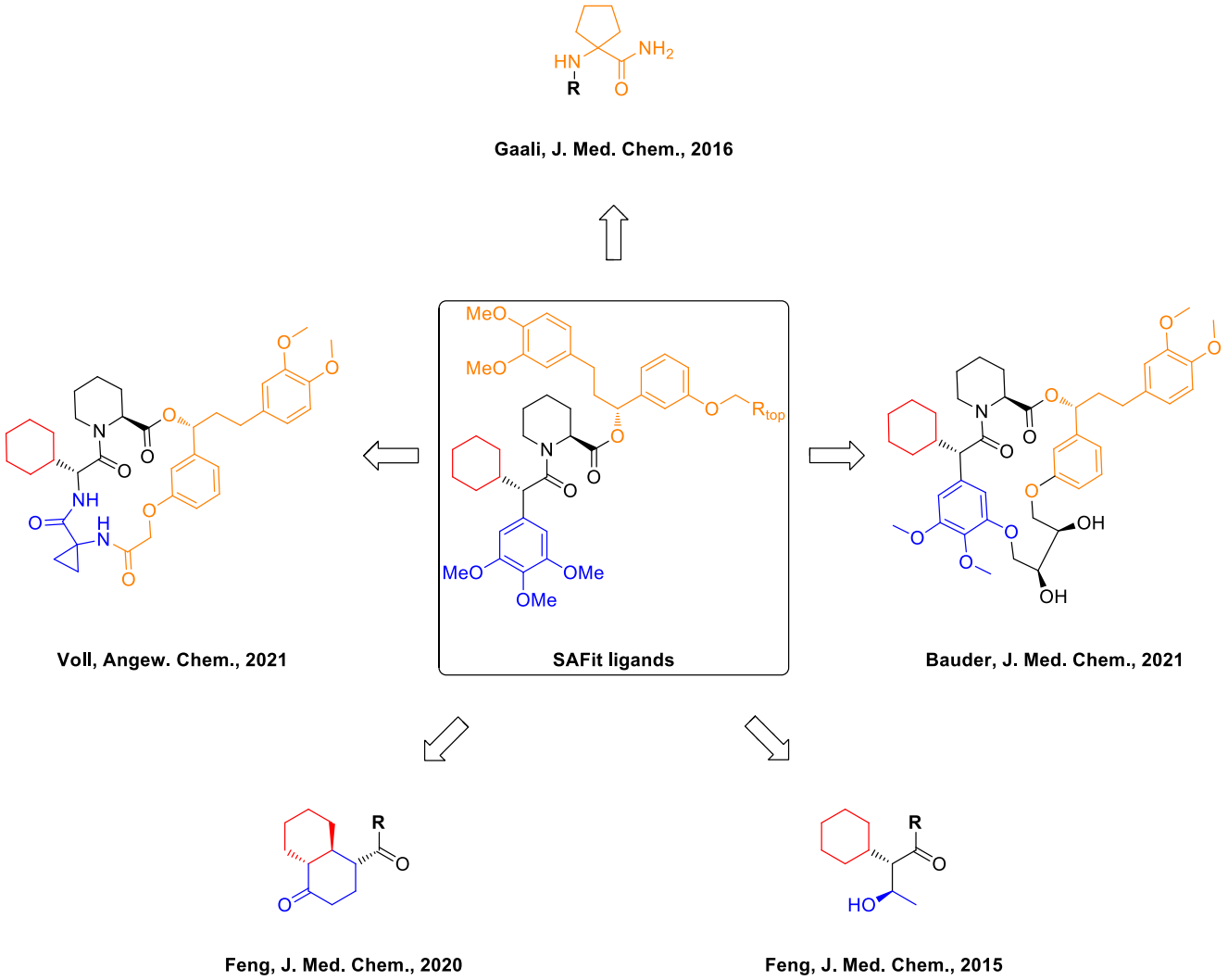


Figure 8: Overview of the previous lead optimization studies of the SAFit inhibitors. The top group is shown in orange, and the bottom group comprises the selectivity-inducing cyclohexyl moiety (R_2) in red and the R_1 group in blue.

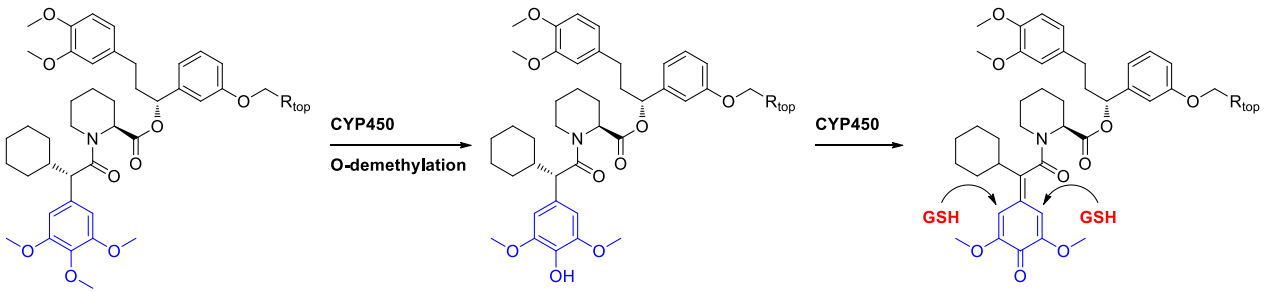


Figure 9: Proposed reaction for the cytochrome P450 mediated O-demethylation and oxidation of the 3,4,5-trimethoxyphenyl group of SAFit ligands. Additionally, potential sites for forming glutathione (GSH) adducts are indicated.

Since the discovery of the SAFit2, multiple structure-affinity relationships of this class have been conducted (**Figure 8**).[60–64] One major objective of these lead optimization studies was to improve the pharmacokinetic parameters of the SAFit ligands by lowering the molecular weight and increasing metabolic stability.[60–64] Especially, the di- and trimethoxy phenyl groups in the bottom and top group section of SAFit ligands are electron-rich aromatic systems and, therefore, susceptible to cytochrome-

mediated O-demethylation followed by oxidation resulting in the formation of reactive metabolites (**Figure 9**).[65,66] To reduce the potential risk of forming reactive metabolites and lower the overall molecular weight, various scaffolds have systematically replaced the standard top and bottom groups.[61,62,67]

However, none of the novel SAFit-based analogs showed better overall properties regarding binding affinity, selectivity, and pharmacokinetic properties than SAFit2.[59,68] Therefore, SAFit2 is still the current gold-standard for the functional investigation of FBP51 *in-vivo*.[29–31,69] Nevertheless, SAFit2 still lacks drug-like properties and is beyond Lipinski's rule of five, so further optimization of the SAFit inhibitors is necessary.[70,71]

7.3 Synthesis of 2-substituted arylacetic acids as bottom groups for SAFit ligands

In general, the SAFit ligand scaffold comprises a top group (orange), a pipecolate as the core group (black), and a bottom group containing the selectivity-inducing cyclohexyl group (R_2, red) and the 3,4,5-trimethoxyphenyl group (R_1, blue) (**Figure 10**).[59] In the standard SAFit synthesis, the enantiopure bottom group is usually coupled to the previously synthesized SAFit core-/top group in the final step of the synthesis.[59,62,67]

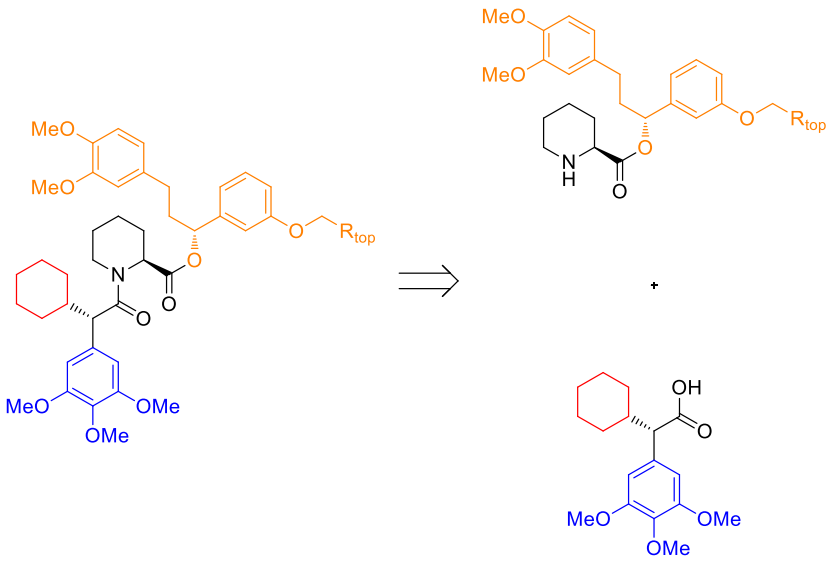


Figure 10: Retrosynthetic analysis of the synthesis of SAFit ligands.

Since the absolute (*S*)-configuration at the Cα-position of the bottom group is crucial for the selective inhibition of FKBP51, the standard bottom group is synthesized enantiopure by asymmetric alkylation.[61,63,67] In this reaction sequence (**Figure 11A**), the commercially available arylacetic acid is first activated by forming the pentafluorophenol ester. Next, the Evans auxiliary is introduced by nucleophilic substitution of the pentafluorophenol ester. Afterward, the asymmetric alkylation with 3-bromcyclohexene takes place, and obtained diastereomers are separated, following the reduction of the alkene and cleavage of the auxiliary. The enantiopure standard SAFit bottom group can be synthesized over 5 steps. However, this optimized synthetic approach has a limited application for upscaling to an industrial scale since it involves a multi-step process and diastereomeric separation. Therefore, in an industrial setup, diastereomeric recrystallization is preferred during upscaling (**Figure 11B**). This approach allows the racemic alkylation of the arylacetic acid in one step, following the separation of the enantiomers by diastereomeric recrystallization.[72–74] Additionally, recovering the 'wrong' enantiomer, followed by racemization and diastereomeric recrystallization, can increase the overall yield of this process.[74]

As an alternative to the auxiliary mediated asymmetric alkylation of arylacetic acids or the diastereomeric recrystallization, Stivala *et al.* reported a direct approach for the asymmetric alkylation

of arylacetic acids (**Figure 11C**).[75] This method achieves the stereoselective alkylation of arylacetic acids using a chiral lithium amide base, which directs the electrophile attack.[75–77] Additionally, Stivala et al. showed that the stereoselectivity of this reaction increases with more sterically demanding and chiral alkylating agents.[75] Even though Stivala *et al.* only reported the alkylation of phenylacetic acid with cyclopentyl iodide, this method might apply to synthesizing 2-cyclohexyl-containing bottom groups as well. However, the authors of this study mentioned that the observed stereoselectivity is highly dependent on the quality of the used n-butyllithium, making it difficult to troubleshoot this reaction.[75]

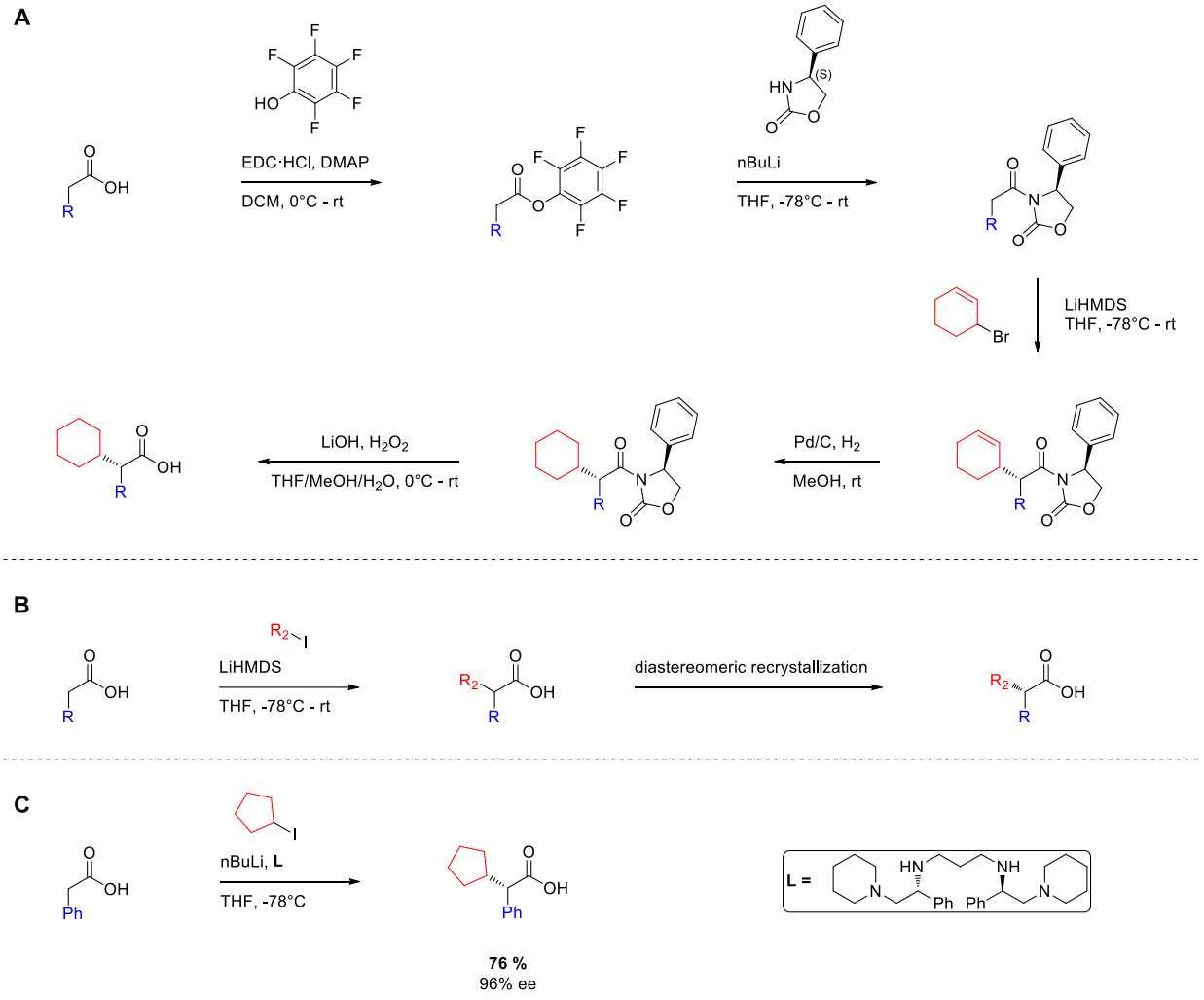


Figure 11: Synthesis of enantiopure 2-substituted arylacetic acids starting from an arylacetic acid (R in blue represents various aryl groups). A) Enantioselective alkylation of arylacetic acids mediated by the Evans auxiliary. B) Asymmetric alkylation of arylacetic acids and diastereomeric recrystallization. C) Direct stereoselective alkylation of arylacetic acids using a chiral lithium amide base.

The currently available methods for synthesizing enantiopure 2,2-disubstituted arylacetic acids tolerate many different alkylating agents and functional groups. Nevertheless, all approaches assume that the used arylacetic acids are readily availability.[74,75,78,79] Since the scope and commercial availability of poly-substituted aryl acetic acids is limited, thereby exploring the R_1 position of bottom groups in the context of selective FKBP51 inhibitors in systematic structure-affinity relationships is compromised. As a result

of the need for arylacetic acids as building blocks for drug discovery and natural product synthesis, different methods have been developed to make arylacetic acids more accessible (**Figure 12**).[80]

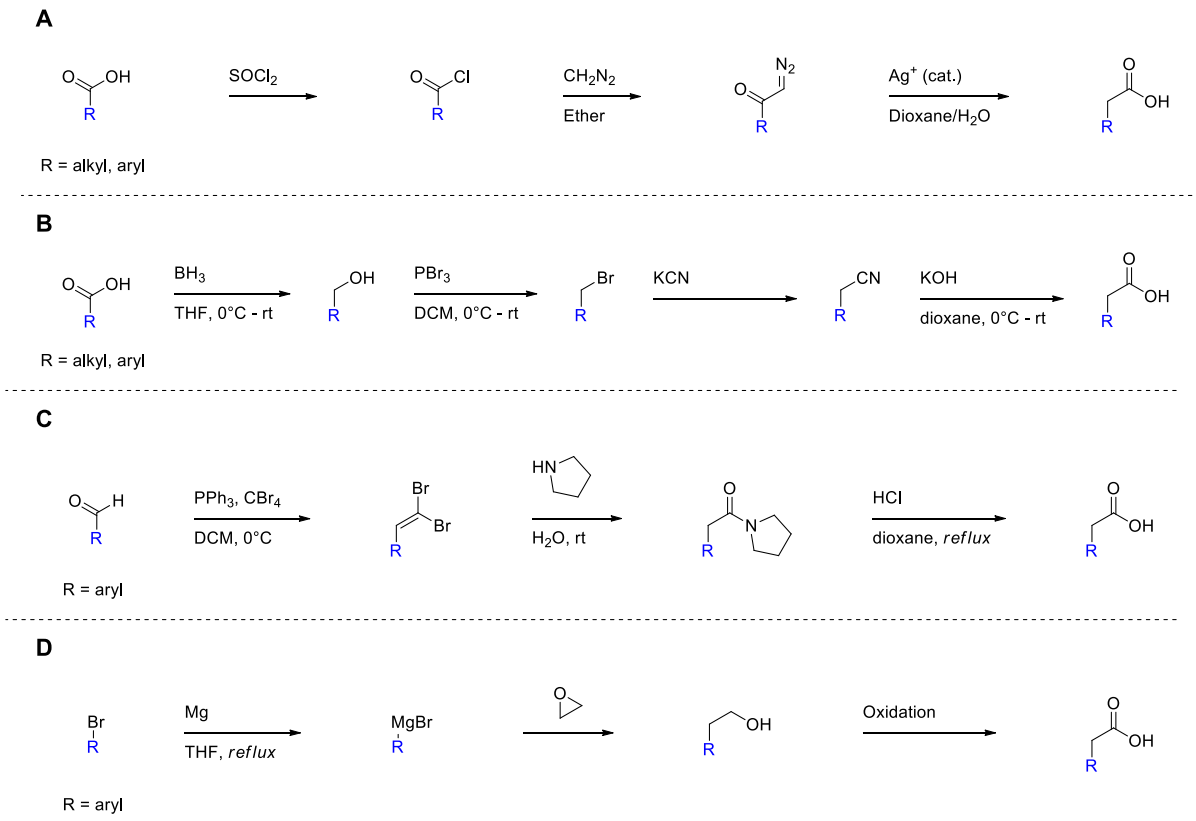


Figure 12: Overview of different synthetic methods for synthesizing 2-substituted acetic acids. A) Arndt-Eistert homologation reaction. B) Multistep one-carbon elongation of carboxylic acids. C) One-carbon elongation of aryl aldehydes. D) Synthesis of 2-substituted acetic acids from the corresponding aryl bromide.

Most developed methods for synthesizing arylacetic acids utilize the one-carbon elongation of more accessible precursors, such as benzoic acids and aryl aldehydes. The most prominent homologation reaction of carboxylic acids is the Arndt-Eistert reaction (**Figure 12A**).[81,82] The carboxylic acid is first converted to a diazo ketone by reacting the respective acid chloride with diazomethane. Subsequently, Wolff rearrangement of the diazo ketone and hydration of the formed ketene leads to the formation of the corresponding elongated acid in high yields. This one-carbon homologation reaction is widely used to elongate α-amino acids to the corresponding β-amino acids.[81] It tolerates a broad scope of substrates as well as functional groups. The major disadvantage of this method is the potential safety hazards involved in generating and handling diazomethane.[83] Therefore, safer alternatives for the Arndt-Eistert homologation reaction have been developed. For example, route B utilizes the availability of various carboxylic acids analogously to the Arndt-Eistert reaction.[84]

In contrast to the Arndt-Eistert reaction, this route involves a multistep sequence starting with the reduction of the carboxylic acid to an alcohol, following the transformation of the alcohol to a good leaving group (for example: Br, OTs, OMs). Subsequently, nucleophilic substitution with a cyanide

source and hydrolysis of the formed nitrile under acidic or alkaline conditions results in the formation of the one-carbon elongated carboxylic acid.[84] Because the hydrolyzation of nitriles usually involves strong acidic or alkaline conditions, the application of this method is limited to functional groups and protection groups that can tolerate these harsh conditions. As a result, Huh *et al.* developed a method for the one-carbon elongation of aryl aldehydes (**Figure 12C**).[85] This method converts aryl aldehydes to the corresponding dibromo alkene by a Wittig-type dibromo olefination. The dibromo alkene is then reacted with pyrrolidine in the presence of water, resulting in amide formation, which is subsequently cleaved to the corresponding arylacetic acid. This synthetic approach allows the synthesis of para-nitrile and para-nitro-containing arylacetic acids. Besides the synthetic routes A-C, route D utilizes commercially available aryl bromides (**Figure 12D**).[86,87] By reacting the corresponding Grignard reagent with ethylene oxide and oxidation of the formed primary alcohol, various arylacetic acids can be obtained from the respective aryl bromides.[86,87]

Overall, the synthesis of enantiopure 2,2-substituted acetic acids generally involves a multistep synthesis, making this approach impractical for rapid structure-based drug discovery. Furthermore, the synthesis gets more complex and time-consuming when the corresponding acetic acid analogs are not commercially available. As a result, different synthetic approaches for the enantioselective α-arylation of 2-substituted acetic acids have been developed (**Figure 13**).[88] The methods usually consist of the stereoselective α-arylation of silyl ketene acetals (A), silyl ketene hemiaminals (B), sterically demanding aryl esters by Hiyama coupling (C), and reductive cross-coupling of 2-substituted α-chloroesters (D).[89–92] The subsequent cleavage of the obtained 2,2-disubstituted esters or amides results in the formation of the enantiopure 2-substituted arylacetic acid.

In general, all the presented methods achieve stereoselective α-arylation by utilizing novel chiral transition metal complexes as catalysts and various arylating agents, such as aryl triflates, aryl iodonium salts, aryl silanes, and aryl iodides.[89–92] Furthermore, the stereoselective α-arylation tolerates a broad scope of functional groups. However, the α-arylation is only studied in the context of various substituted aryl groups and linear or branched alkyl groups in R_2, but only one example includes the cyclohexyl group (route D).[92]

In conclusion, various methods have been developed to synthesize 2,2-disubstituted acetic acids. However, most methods that allow the enantioselective introduction of various aryl-/heteroaryl groups only tolerate simple alkyl groups in the R_2 position. As a result, these methods have limited application for synthesizing 2-cyclohexyl-containing arylacetic acids as bottom groups for selective FKBP51 inhibitors.

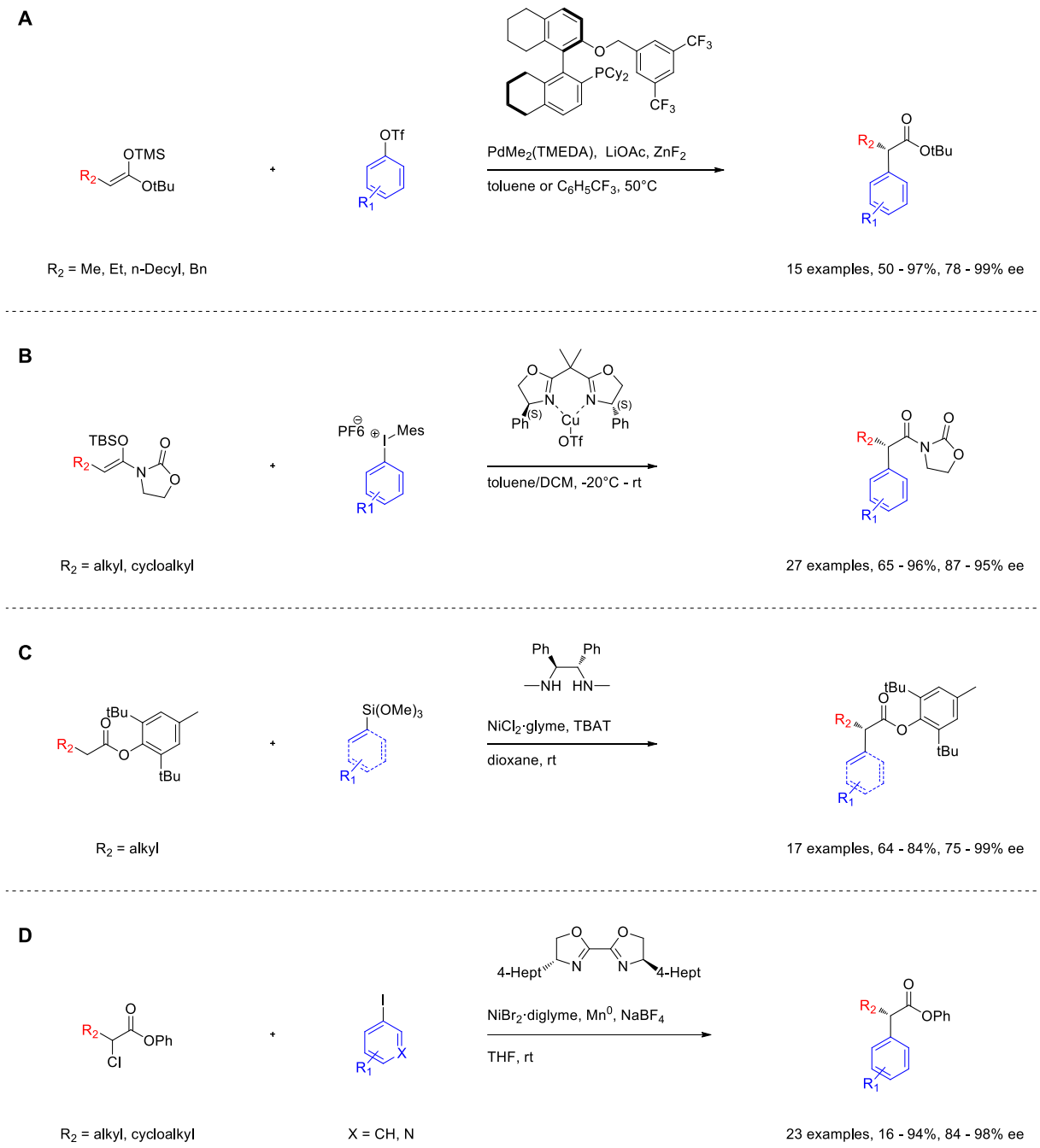


Figure 13: Overview of various methods for the α-arylation of silyl ketene acetals, silyl ketene hemiaminals, acetic acid esters, and α-chloroacetic acid esters. A) Palladium-catalyzed α-arylation of silyl ketene acetals. B) Copper(I)-catalyzed α-arylation of silyl ketene aminals. C) Enantioselective α-arylation by Hiyama coupling. D) Enantioselective α-arylation by reductive cross-coupling.

8 Aim

The discovery of the first selective FKBP51 inhibitors, SAFit1 and SAFit2, resulted in the highly potent and selective FKBP51 ligands. Nevertheless, the discovered inhibitors lack drug-like properties due to their high molecular weight, low metabolic stability, and poor physicochemical properties.[59] Therefore, SAFit1 and SAFit2 are considered chemical tool compounds to elucidate the biological function of FKBP51, and further lead optimization is needed. As a result of the relatively large and complex structures of SAFit1 and SAFit2, this thesis only focused on optimizing the bottom group. Generally, the standard SAFit bottom group consists of a 2,2-disubstituted acetic acid with a 3,4,5-trimethoxyphenyl group in R_1 and a cyclohexyl group in R_2. Especially the stereo configuration and substituent in the R_2-position are crucial for inducing selectivity over FKBP52 and achieving high potency, whereas the 3,4,5-trimethoxyphenyl group contributes to the overall binding affinity.[59,60]

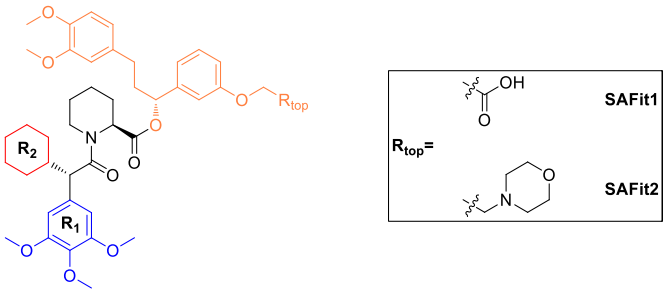


Figure 14: A) Chemical structures of SAFit1 and SAFit2. FKBP51 selective inhibitors contain a top group (orange), a pipecolic core (black), and a bottom group. The bottom group comprises a selectivity-inducing cyclohexyl residue (R_2, red) and a trimethoxyaryl moiety (R_1, blue).

Because di- and trimethoxy phenyl groups are electron-rich aromatic systems, they are susceptible to cytochrome-mediated O-demethylation followed by oxidation resulting in the formation of reactive metabolites.[65,66] To reduce the potential risk of forming reactive metabolites, it was decided to identify a suitable replacement for the 3,4,5-trimethoxy phenyl group in the R_1 while retaining high potency and selectivity for FKBP51. Therefore, the primary objective of this thesis was to synthesize different 2-cyclohexyl-containing aryl-/heteroaryl acetic acids as bottom groups for the selective inhibition of FKBP51. Additionally, the influence of these novel bottom groups in terms of binding affinity, selectivity, and metabolic stability was investigated by rational lead optimization.

9 Results and discussion

9.1 Synthesis and immobilization of the SAFit1 core-/top group

The first selective inhibitors of FKBP51, SAFit1 and SAFit2, differ only in the top group's functionality. SAFit1 contains a carboxylic acid that results in high solubility in aqueous media and high binding affinity but reduces cell permeability and limits its usage to non-cell-based *in-vitro* assays.[59] SAFit2, on the other hand, has a morpholine instead of a carboxylic acid in the top group section, which increases cell permeation while maintaining similar binding affinities.[93] Therefore, SAFit1 is primarily used as a reference compound for ligand development, while SAFit2 is considered the best option for cell-based assays and *in-vivo* experiments.

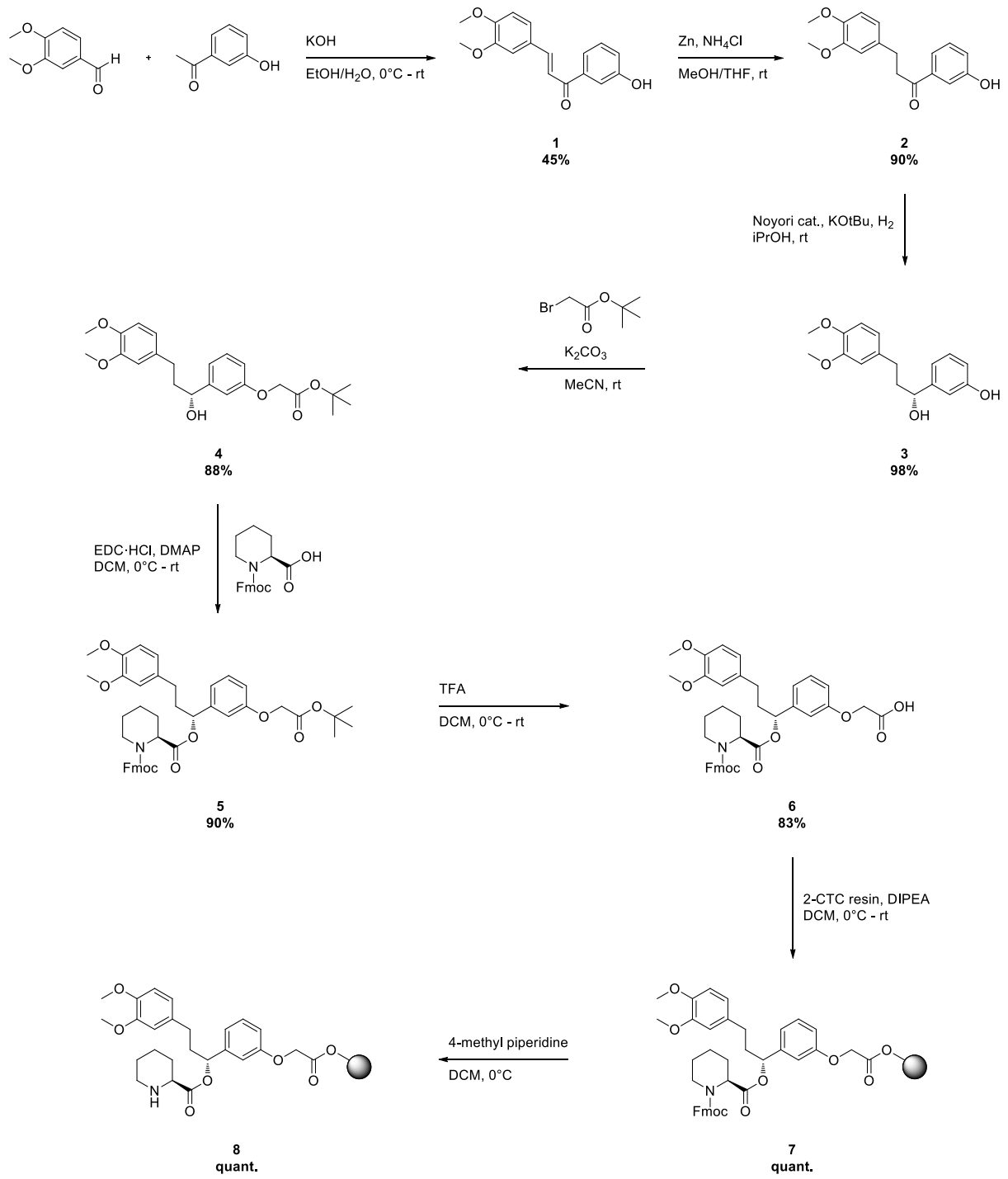
Because this thesis aimed to investigate the impact of various bottom groups on binding affinity and selectivity for FKBP51, the effect of different bottom groups was first examined in the context of the SAFit1 scaffold. The most promising novel bottom groups were then coupled to the SAFit2 core-/top group further to evaluate the effect on pharmacokinetic parameters and cellular activity. To accomplish this, the SAFit top group alcohol **3** was first synthesized using a 3-step sequence developed by Dr. Andreas Voll, which allows the introduction of the different top group residues by alkylation of the phenol, resulting in the formation of the SAFit1 or SAFit2 top group in high yields.

The first step of the reaction sequence involves the synthesis of an alpha-beta unsaturated ketone by Claisen-Schmidt condensation of the non-enolizable aldehyde and acetophenone (**Scheme 1**). However, due to incomplete conversion in this reaction, only moderate yields of **1** were obtained. Afterward, the aldol product **1** was reduced using zinc[94], followed by precipitation of **2** by adding water and recrystallizing from methanol. Finally, ketone **2** was reduced by Noyori asymmetric hydrogenation[95], resulting in the exclusive formation of the chiral alcohol **3** with an overall yield of 42%.

The SAFit1 core/top group was then obtained by alkylating the phenol group of **3** with tert-butyl bromoacetate and subsequent esterification with Fmoc-protected (*S*)-pipecolic acid.[96] Next, the tert-butyl ester was deprotected, and compound **6** was immobilized on 2-chlorotrityl resin, yielding the loaded solid support **7**. Subsequent Fmoc deprotection results in the formation of **8**.

In general, the immobilization of the SAFit1 core-/top group allows the fast generation of small libraries and simple purification. Additionally, the 2-chlortrityl resin has the advantage that the product can be cleaved off the solid support under mildly acidic conditions, unlike usually used conditions for the tert-butyl ester deprotection, which can lead to side product formation in complex molecules such as SAFit1 analogs.

Scheme 1: Synthesis of the SAFit1 core-/top group and immobilization on 2-chlorotrityl resin.



9.2 Replacement of the para methoxy group of the SAFit1 bottom group

Feng et al. previously reported that the systematic removal of the methoxy groups of the 3,4,5-trimethoxyphenyl residue of the bottom group led to a drastic decrease in binding affinity.[60] Additionally, removing the para methoxy group of SAFit1 resulted in a 30-fold decrease in binding affinity. In contrast, removing one of the meta methoxy groups led to a reduction of only 10-fold.

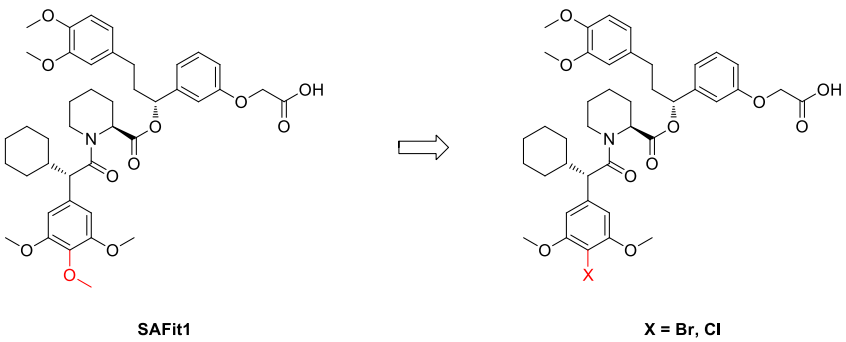
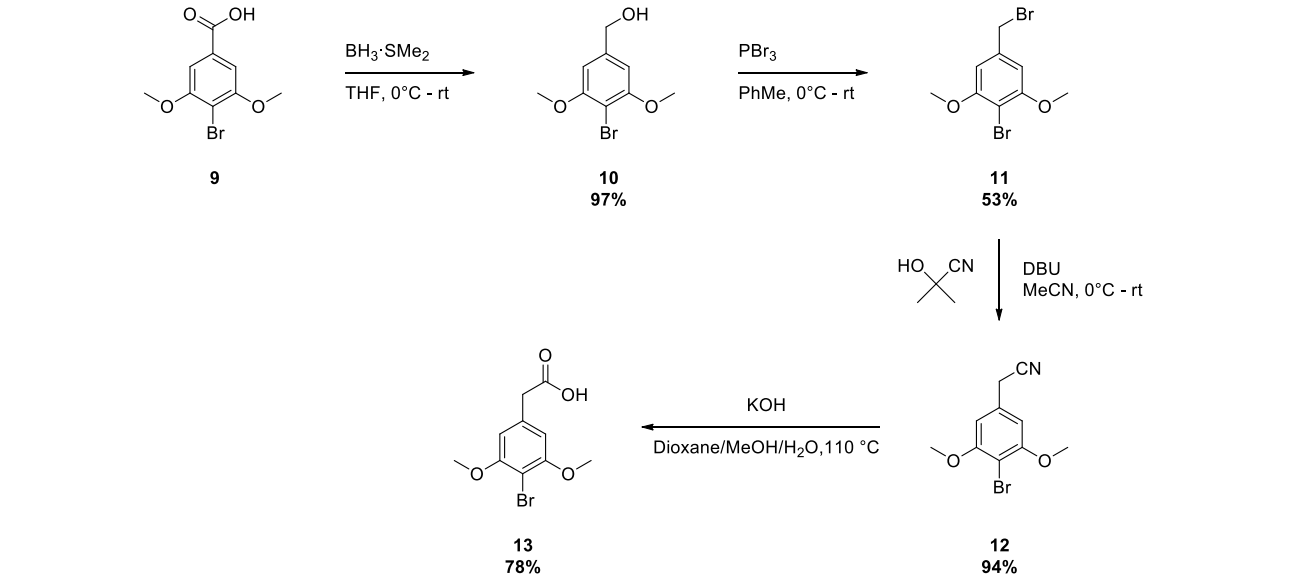


Figure 15: Rationale for substituting the para methoxy group (red) of SAFit1 by a bromine and chlorine atom (red).

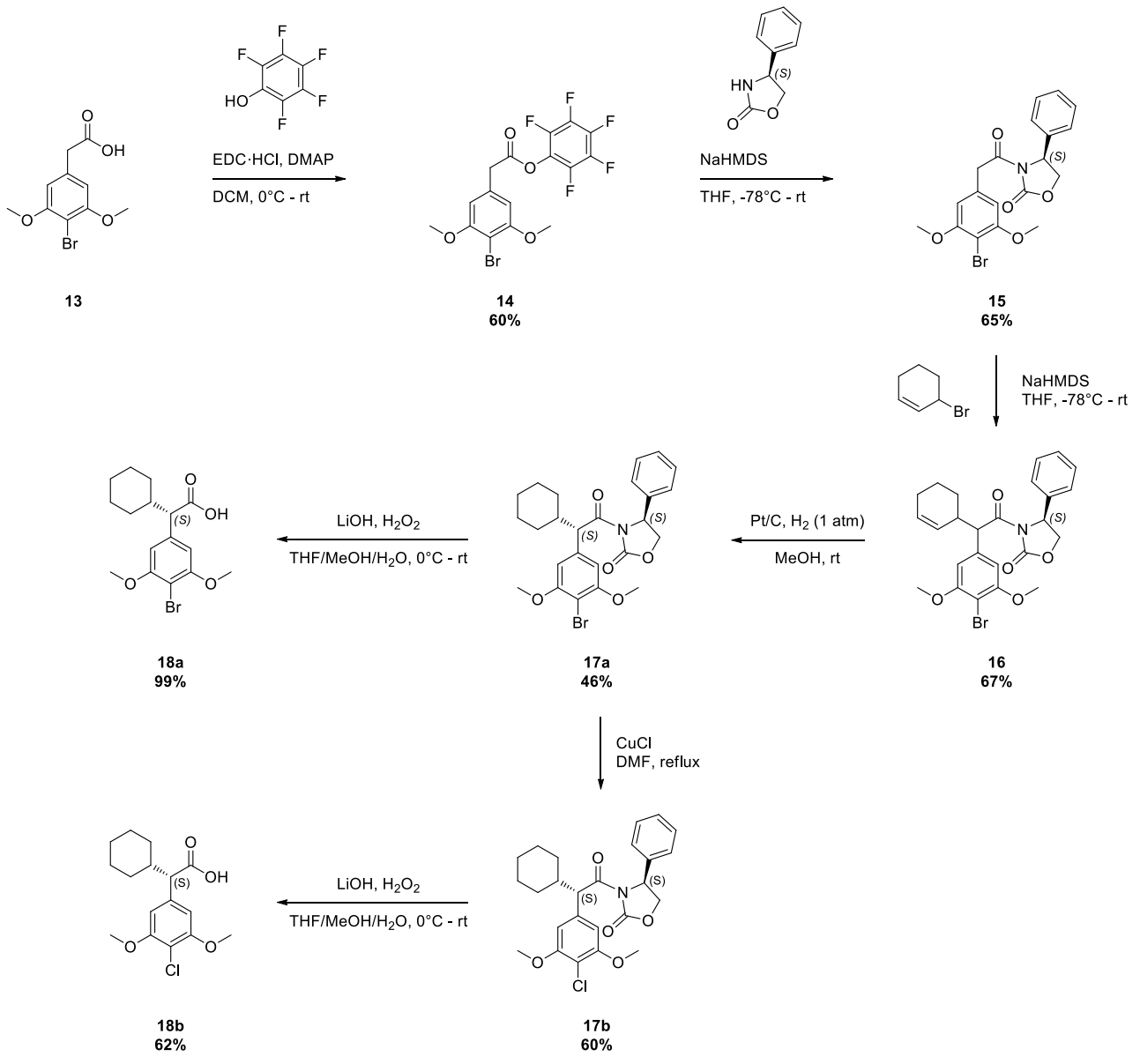
Based on this observation, the para methoxy group of the bottom groups seems to affect the overall binding affinity more than the ones in the meta positions. Because the para methoxy group is orientated towards the solvent in the co-crystal structure of SAFit1 and doesn't show any beneficial interactions with the protein, it appears that this specific methoxy group is stabilizing the solvation of the ligand-protein complex. Furthermore, the para methoxy group is forced out of the ring plane by steric and electronic effects of the neighboring methoxy groups, which remain in a planar conformation to the ring system.[97] Because the para methoxy group somewhat rigidifies the position of the meta methoxy groups, the importance of the para methoxy group was further investigated by substituting it with a bromine and chlorine atom (**Figure 15**).

Since arylacetic acid **13** was not commercially available, it was synthesized from the corresponding commercially available benzoic acid **9** by homologation (**Scheme 2**).[98] First, carboxylic acid **9** was reduced with borane to the corresponding alcohol. Then **10** was reacted with PBr_3 via SN_2 reaction to form **11**.[99] Subsequently, compound **12** was derived by SN_2 reaction of **11** with in-situ generated cyanide from cyanoacetohydrine.[100] Finally, nitrile **12** was hydrolyzed under alkaline conditions.[101] Overall, the arylacetic acid **13** was obtained with a yield of 36% over 4 steps. The enantiopure bottom groups **18a** and **18b** were synthesized from compound **13** by Evans auxiliary mediated asymmetric alkylation.[79,102] Therefore, **13** was first converted into the activated ester **14** by Steglich esterification with pentafluorophenol and reacted with the Evans auxiliary.[103] Then **15** was alkylated using 3-bromocyclohexene. Because aryl bromides are susceptible to lithiation reaction, NaHMDS was used instead of LiHMDS. In the next step, the double bond of alkene **16** was reduced, and the diastereomers were separated by column chromatography, yielding compound **17a**.

Scheme 2: Synthesis of the enantiopure bottom groups **18a** and **18b** and coupling to the SAFit1 core-/top group.



9 | **10** 97% | **11** 53% | **12** 94% | **13** 78%



13 | **14** 60% | **15** 65% | **16** 67% | **17a** 46% | **18a** 99% | **17b** 60% | **18b** 62%

When Palladium was used instead of Platinum, the reaction was completed within 5 min, and the debrominated product was observed as the major.[104] In the end, the Evans auxiliary was cleaved by the in-situ generated lithium hydroperoxide resulting in the formation of the enantiopure bottom group **18a**.[105]

The chlorinated enantiopure carboxylic acid **18b** was synthesized by treating compound **17a** first with CuCl, resulting in compound **17b** by SN_{Ar} reaction (**Scheme 3**).[106] Subsequently, the Evans auxiliary was cleaved off, which resulted in the formation of enantiopure **18b**. The obtained bottom groups **18a** and **18b** were then coupled to the SAFit1 core-/top group, and the SAFit1 analogs **19a** and **19b** were tested by FP assay for their binding affinities towards FKBP51 and FKBP52. The binding affinities were then compared to SAFit1 and compound **19c** (**Table 1**).

Scheme 3: Synthesis of the SAFit1 analogs **19a** and **19b**.

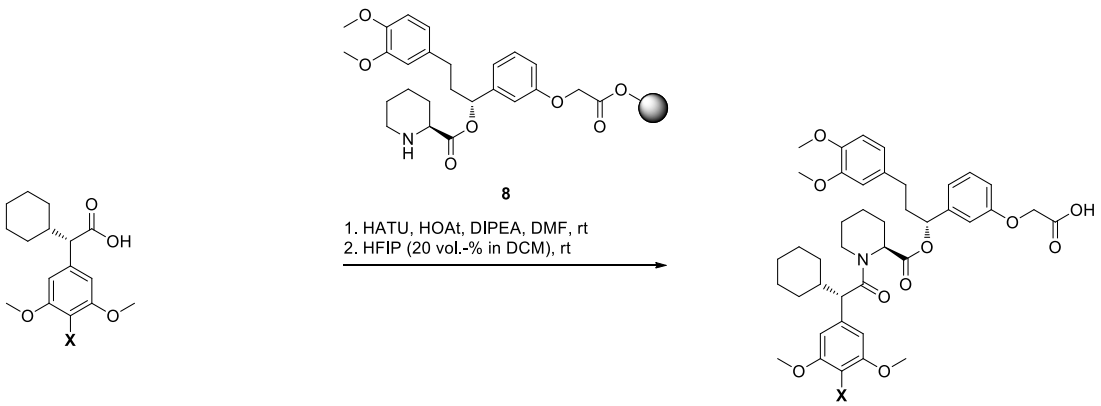


Table 1: Overview of the determined binding affinities of SAFit1, **19a**, **19b,** and **19c**.* reported values by Feng et al.

Entry	X	FKBP51 K_i [nM]	FKBP52 K_i [nM]
SAFit1	-OMe	6 ± 0.4	3859 ± 2395
19a	-Br	53 ± 7	>10000
19b	-Cl	50 ± 6	3339 ± 1434
19c	-H	110*	>50000*

Compounds **19a** and **19b** show an approximately 12-fold reduction in binding affinity for FKBP51 compared to SAFit1. Moreover, **19a** and **19b** possess almost identical binding affinities for FKBP51, but a slight loss of selectivity over FKBP52 is observed for compound **19b**. Compared to the previously

published compound **19c**, the binding affinities of **19a** and **19b** are reduced by factor two. Based on this observation, the 2-fold increased binding affinity of **19a** and **19b** is most likely associated with the steric effects of para substitution on the orientation of the meta methoxy group.

Because the immense difference in binding affinity of SAFit1, **19a**, and **19b** can't be explained by any rational interaction of the para substituent with the protein, we had a closer look at the thermodynamics of the ligand-protein binding process. Shimokhina et al. demonstrated that the binding process of ligand L to the protein P could be illustrated as a Born-Haber cycle (**Figure 16**).[107]

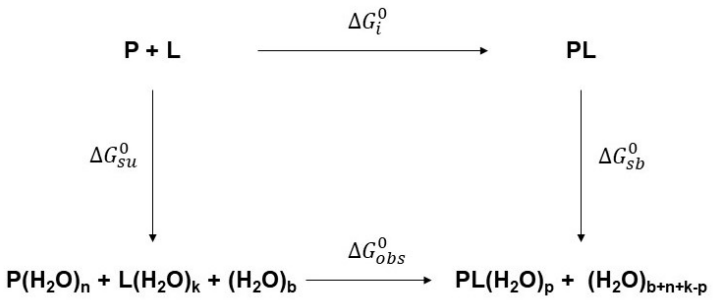


Figure 16: Born–Haber cycle for ligand L binding to protein P, showing the relationship between the observed free energy of binding ΔG_{obs}^0, the intrinsic energy ΔG_i^0 , and the solvation free energies of unbound P+L (ΔG_{su}^0) and bound PL (ΔG_{sb}^0) species.

Because the Gibbs free energy is a state function, the observed standard free energy ΔG_{obs}^0 can be derived by Eq. 1 from the intrinsic free energy of the binding process of P and L and the difference in free energy of solvation of the bound protein-ligand complex (ΔG_{sb}^0) and the unbound species (ΔG_{su}^0).[107]

$$\Delta G_{obs}^0 = \Delta G_i^0 + \left[\Delta G_{sb}^0 - \Delta G_{su}^0\right] \qquad \text{(Eq. 1)}$$

Since ΔG_{sb}^0 represents the free solvation energy of the protein-ligand complex and ΔG_{su}^0 is the sum of the free solvation energy of the ligand and the protein, Eq. 1 can be expressed as Eq. 2.[107]

$$\Delta G_{obs}^0 = \Delta G_i^0 + \left[\Delta G_{solPL}^0 - \left(\Delta G_{solP}^0 + \Delta G_{solL}^0\right)\right] \qquad \text{(Eq. 2)}$$

Based on the assumption that the substitution of the para methoxy by a bromine and chlorine atom doesn't disrupt any significant ligand-protein interaction, ΔG_i^0 for SAFit1, compounds **19a** and **19b** should be almost constant. Furthermore, the free energy of the solvation of the protein (ΔG_{solP}^0) is constant and ΔG_{solL}^0 should be in a similar range for SAFit1, **19a**, and **19b** since only the para position of the bottom group was altered. In consideration of these assumptions and Eq. 2, the difference in binding affinities results from different energies for ΔG_{solPL}^0.

Based on this analysis, it was assumed that the para methoxy group of SAFit1 mainly contributes to the solvation of the protein-ligand complex rather than to a beneficial protein-ligand interaction resulting

from the orientation of the meta methoxy groups. Additionally, the high-resolution co-crystal structure of SAFit1 in complex with the FK1 domain of FKBP51 suggests that 2 water molecules are participating in a hydrogen bonding network with the three methoxy groups of SAFit1 (**Figure 17**).

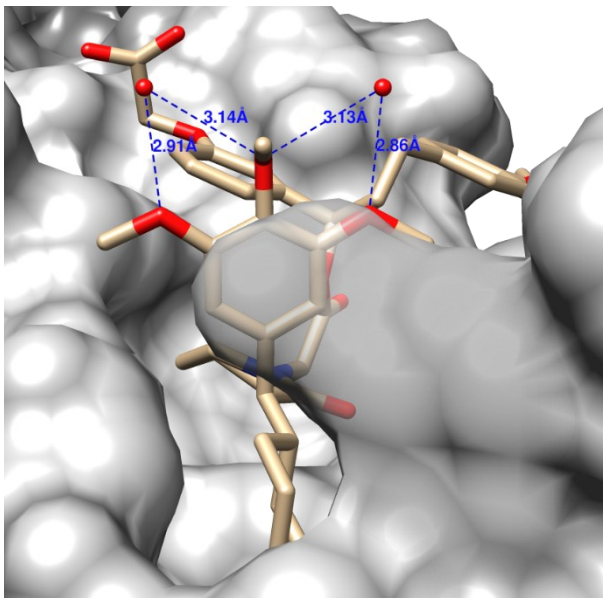


Figure 17: Co-crystal structure of SAFit1 (beige sticks) in complex with the FK1 domain of FKBP51 (grey surface, PDB: 8CCA). The two water molecules that participate in the formation of a hydrogen bonding network with the bottom group are shown as red dots and the distance to the oxygen atoms of the methoxy groups was measured.

9.3 Synthesis of different bottom groups by Suzuki coupling

In previous studies, different bottom groups have been screened by either Evans auxiliary-mediated enantioselective or racemic alkylation of various acetic acid analogs. Because the enantioselective alkylation of acetic acid analogs involved a multistep synthesis in resolving the stereocenter in the C-α position of the carboxylic acid, this approach was not efficient enough for the fast screening of different bottom groups. Therefore, it was decided to synthesize different bottom groups racemically and couple them to the SAFit1 core-/top group. The obtained diastereomeric mixtures should be tested for their binding affinity for FKBP51, and potential hits could then be synthesized diastereomeric pure and tested again.

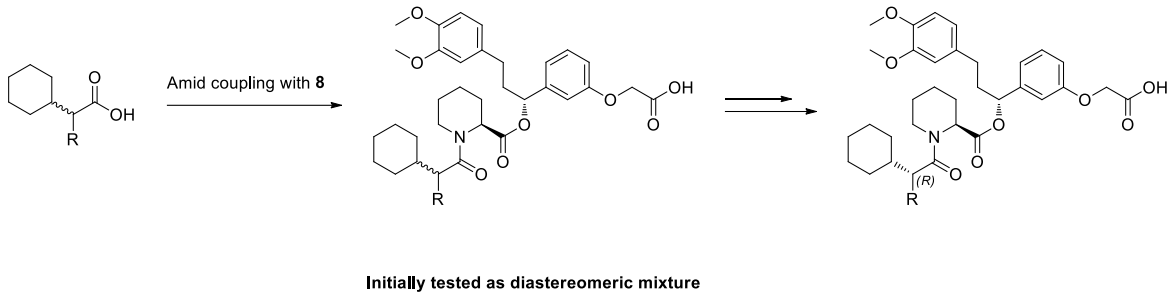
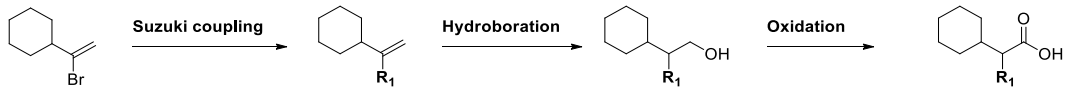


Figure 18: Synthesis of racemic bottom groups and testing them as diastereomeric mixtures.

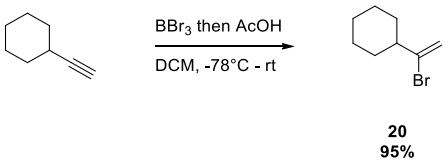
Since the commercial availability and scope of different aryl-/heteroarylacetic acids are limited, a novel synthesis route for the bottom group synthesis was developed. Using a Suzuki coupling, this novel approach introduces different substituted aryl/-heteroaryl residues in the R_1-position while already containing the cyclohexyl residue (**Scheme 4**).[108,109] Subsequent hydroboration and oxidation of the primary alcohol results in the formation of racemic bottom groups.

Scheme 4: General reaction sequence of the synthesis of bottom groups for selective FKBP51 inhibitors by Suzuki coupling.



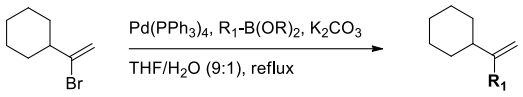
First, the starting material **20** was synthesized using boron tribromide mediated addition of HBr to the commercially available alkyne. In this reaction, only the formation of the Markovnikov product was observed in high yields.[110]

Scheme 5: Synthesis of the precursor **20**.

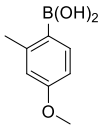
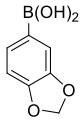
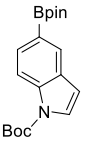


The obtained (1-bromovinyl)cyclohexane **20** was then converted with various aryl-/heteroaryl boronic acids or boronic pinacol esters via palladium-catalyzed Suzuki coupling to the compounds **21a-g** in moderate to good yields (**Table 2**).

Table 2: Overview of the scope and yields of the Suzuki coupling reaction of **21a-g**.



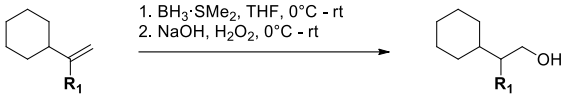
20 **21a-g**

Entry	R_1-B(OR)$_2$	Yield [%]	Entry	R_1-B(OR)$_2$	Yield [%]
21a		90%	**21d**		90%
21b		45%	**21e**		53%
21c		74%	**21f**		79%
			21g		53%

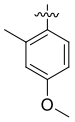
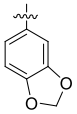
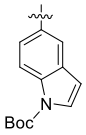
In general, the Suzuki coupling under the standard conditions of (1-bromovinyl)cyclohexane **20** using $Pd(PPh_3)_4$ and K_2CO_3 in a mixture of THF/H_2O (9:1) tolerated a broad scope of heterocyclic- and aryl boronic acids or their corresponding boronic pinacol esters, as well as functional groups such as nitriles, methoxy groups, and Boc, protected indoles. In the next step, alkenes **21a-g** were hydroborated with $BH_3{\cdot}SMe_2$ complex in THF (**Figure 4**). The selectivity of this reaction was predictable since the boron reagent preferentially adds to the least sterically hindered carbon atom of the alkene in an anti-Markovnikov fashion. Moreover, the disubstituted alkenes **21a-g** are highly sterically hindered in the 1-position of the alkene, resulting in higher regioselectivity.[111,112] It is noteworthy that higher sterically demanding boranes, such as 9-BBN, didn't show any formation of the hydroboration product even under elevated temperatures. This observation is mainly caused due to the steric hindrance of the used alkenes. Subsequent oxidation of the in-situ generated boranes led to the formation of the primary alcohols **22a-f** in moderated yields. Only alkene **21e** didn't show any conversion during the hydroboration under the standard conditions since nitrogen-containing heterocycles such as pyridines

and pyrimidines are known to form highly stable complexes with boranes.[113] Therefore, the formed pyridine and pyrimidine borane complexes need to be activated by iodine to increase the reactivity of the formed borane complexes and allow intramolecular hydroboration.[114] In the case of the pyridine-containing alkene **21d**, the hydroboration and subsequent oxidation under these optimized conditions allowed the formation of the primary alcohol **22d**. Still, in the case of the pyrimidine analog **21e**, no reaction was observed. Overall, the selective synthesis of the primary alcohols via Brown hydroboration from the corresponding alkenes was possible for a broad scope of substrates. Nevertheless, some optimization was needed for N-heterocyclic compounds.

Table 3: Overview of the hydroboration reaction of compounds **22a-f**.



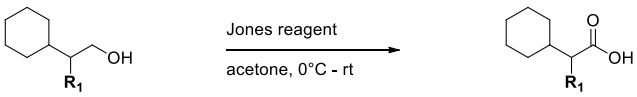
22a-g **22a-f**

Entry	R_1	Yield [%]	Entry	R_1	Yield [%]
22a		57%	**22d**[a]		55%[a]
22b		52%	**22e**		61%
22c		44%	**22f**		54%

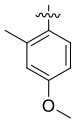
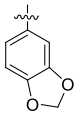
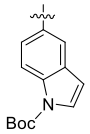
[a] 1. $BH_3 \cdot SMe_2$, then I_2, DCM, 0°C – rt; 2. NaOH, H_2O_2, THF, 0°C – rt.

The alcohols **22a-f** were oxidized via Jones oxidation to the corresponding bottom groups (**Table 4**).[115] In this reaction, only compounds **23a** and **23b** were obtained in moderate yields, and only low yields were obtained during the oxidation of compound **23d**. This is mainly caused by the ability of pyridines to form stable complexes with chromium(VI)oxide.[116] The formation of the metal-pyridine complex generally reduces the reactivity, resulting in the oxidation of primary alcohols to the corresponding aldehydes.[117] For compounds **23c**, **23e**, and **23f**, only decomposition was observed during the oxidation of the primary alcohols under these conditions. Moreover, milder oxidization strategies, such as forming the corresponding aldehyde and subsequent Pinnick oxidation, were not successful for these substrates.

Table 4: Overview of the oxidation of the alcohols **22a-f** to the final bottom groups **23a-f**.



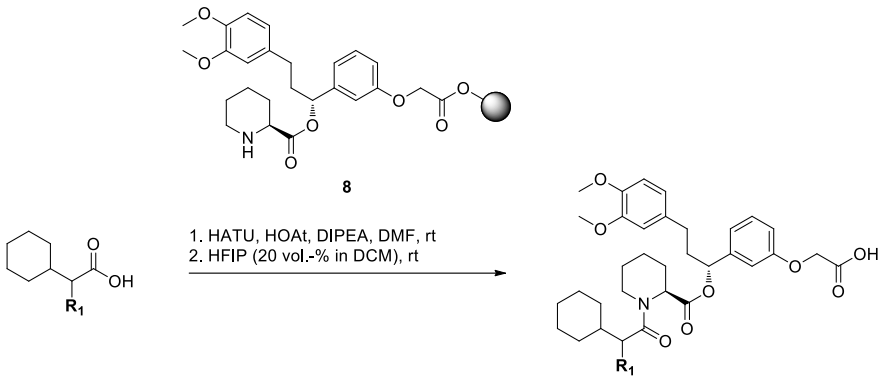
22a-f **23a-f**

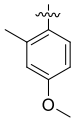
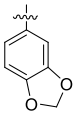
Entry	R_1	Yield [%]	Entry	R_1	Yield [%]
23a		72%	**23d**		39%
23b		76%	**23e**		decomposition
23c		decomposition	**23f**		decomposition

In the final step, the obtained bottom groups **23a-b** and **23d** were coupled to the SAFit1 core-/top group via HATU coupling, and the resulting selective FKBP51 ligands were cleaved off the solid support by the treatment of the resin with a 20 vol.-% solution of HFIP in DCM. After the purification of compounds **24a-c**, the binding affinities for FKBP51 and FKBP52 were determined by FP assay.[45] (**Table 5**) In general, compounds **24a-c** were obtained in moderate yields, but the yield of compound **24a** was reduced due to the higher steric hindrance by the ortho methyl group of **23a**. Moreover, it seems that ortho substitution in the R_1 position reduces binding affinity for FKBP51 compared to the unsubstituted ligand **19c**. Therefore, ortho substitution in the R_1 position is not beneficial for the binding affinity of FKBP51 ligands.

In this series, the best ligand was compound **24b**. Nevertheless, the linkage of the methoxy groups in the 3 and 4 positions with a methylene linker doesn't result in an increased binding affinity. This observation indicates that the methyl group of the meta methoxy group might contribute to a hydrophobic interaction of the ligand with the protein. Since this interaction is not possible in the dioxolane scaffold, the reduced binding affinity can be explained. Moreover, the introduction of 3-pyridinyl moiety in the R_1 position led to a decreased binding affinity for FKBP51 compared to a phenyl residue (K_i = 847 nM). Based on this observation, the nitrogen atom in the 3-pyridinyl residue of the compound doesn't contribute to any new interaction with the protein but is somewhat decreasing binding affinity.

Table 5: Overview of the binding affinities of the SAFit1 analogs **24a-c**.



Entry	R_1	Yield [%]	FKBP51 K_i [nM]	FKBP52 K_i [nM]
24a		44%	4035 ±718	>10000
24b		67%	116 ± 15	7823 ± 4013
24c		63%	1365 ± 198	>10000

Overall, the new synthetic route allows the fast synthesis of bottom groups with residues in the R_1 position compatible with the conditions used during the hydroboration and the subsequent oxidation. Especially more complex substrates such as N and S-containing heterocycles are not compatible with the hydroboration conditions since boranes are known to form stable complexes with nitrogen and sulfur atoms, resulting in low conversions even under optimized reaction conditions. Moreover, the conditions in this reaction sequence only allow substrates with functional groups resistant to strong reductive and oxidative conditions. Further, this synthesis does not tolerate more complex heterocyclic substrates such as furans, pyrimidines, and indoles. In the end, this synthetic methodology offers the fast synthesis of bottom groups for selective FKBP51 inhibitors but only allows the introduction of relatively simple residues. Therefore, this synthesis is not feasible for the broad screening of various residues in the bottom group's R1 position, and other methodologies are more feasible.

9.4 Investigation of 1,2,3-triazoles as bottom group scaffolds

Previous research has shown that changing the substitution of the 3,4,5-trimethoxyphenyl group doesn't benefit the binding affinity for FKBP51. Based on these findings, this study focuses on other possible interactions that can be targeted for developing more effective and selective ligands for FKBP51.
In a previous SAR study, the interaction of the hydrogen bond between Asp^{68} of FKBP51 and the natural products rapamycin and FK506 was investigated.[50] Therefore, different smaller groups, such as chiral alcohols, ketones, and ethers, were introduced in place of the 3,4,5-trimethoxyphenyl group. However, no hydrogen bonding was observed in the high-resolution co-crystal structures of those ligands in the complex with FKBP51.[60] This may be since another amino acid, Tyr^{57}, is a better hydrogen bond donor. As a result, this study focuses on altering the chemical structure of the bottom group by using a 5-membered heterocycle instead of a 6-membered ring system. This structural change might allow the formation of new hydrophobic and hydrophilic interactions of the bottom group with FKBP51. (**Figure 19**)

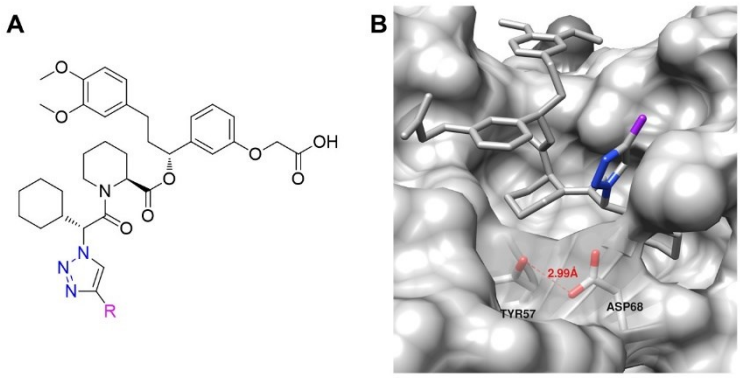
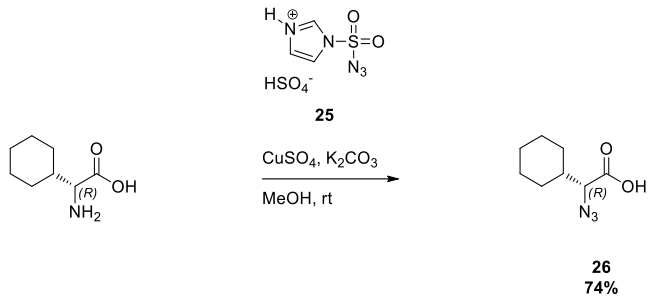


Figure 19: A) General chemical structure of the SAFit1 triazole analogs. B) Modeled structure of the triazole containing SAFit1 analog in complex with FKBP51. The nitrogen atoms of the triazole are highlighted in blue, and the exit vector for the residues R is highlighted in purple.

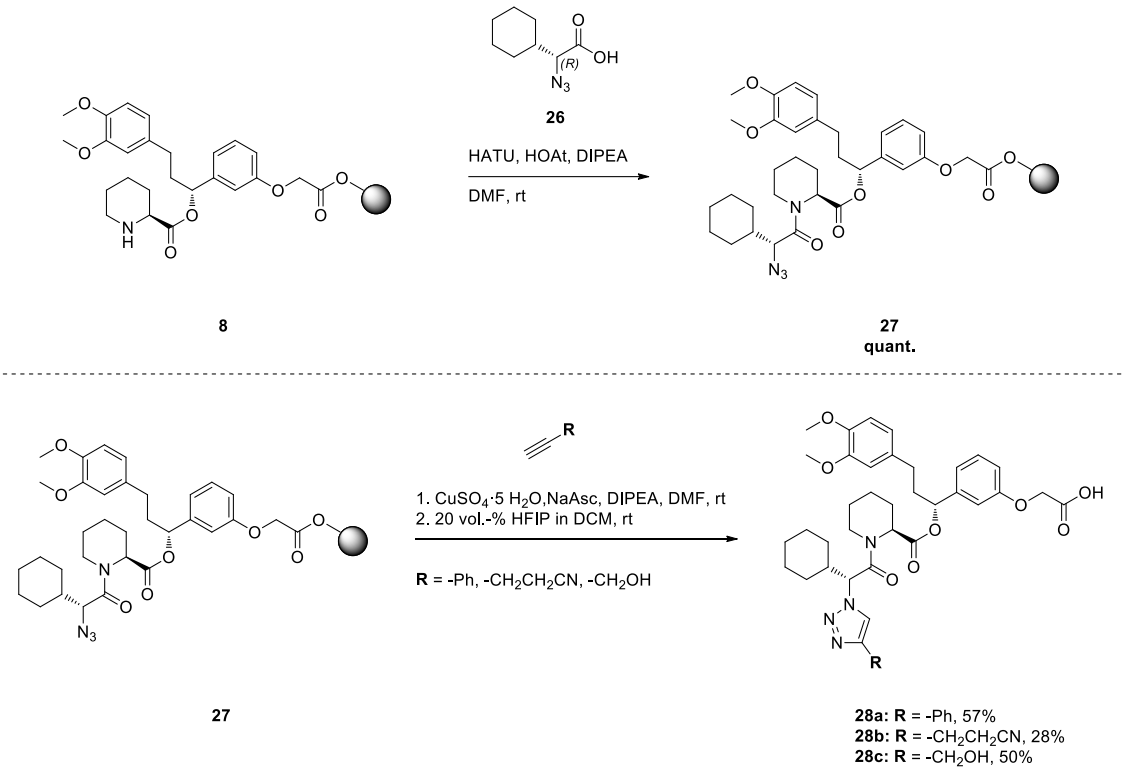
Due to the limited commercial availability of substituted heteroarylacetic acids, this study initially focused on 4-substituted triazole as a starting point for introducing 5-membered heterocycles. This scaffold can be selectively synthesized and derivatized by copper-catalyzed click reaction. This approach allowed us the generation of a small library with a high diversity of residues in the 4-substituted triazoles, which would allow the creation of a diverse library of 4-substituted triazoles.
For the click reaction, the commercially available amino acid was first converted into the corresponding 2-azido-2-acetic acid **26** by copper-catalyzed azidation reaction under retention of the stereo configuration (
Scheme 6).[118] It is noteworthy that the free base and hydrochloride salt of the azide transfer reagent **25** can spontaneously decompose during preparation and upon prolonged storage.[119–121] Therefore, the

more stable and safe hydrogen sulfate salt was prepared for the azidation reaction according to the procedure of Potter et al.[122]

Scheme 6: Synthesis of the azido acid **26**.



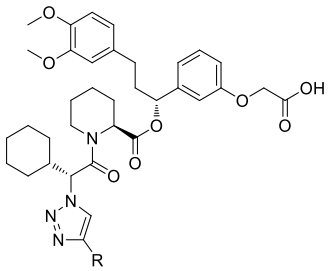
Scheme 7: Synthesis of the SAFit1 analogs **28a-c**.



The obtained 2-azido-2-acetic acid was then coupled to the immobilized SAFit1 core/top group by standard HATU coupling (**Scheme 7**), and the click reaction was performed on the solid phase. Subsequent cleavage of the products **28a-c** from the solid support, with HFIP and purification via preparative HPLC, resulted in moderate yields of compounds **28a-c**. The triazole-containing SAFit1 analogs **28a-c** were tested via FP assay for their binding affinities for FKBP51 and FKBP52.[45] (**Table 6**). Overall, the determined binding affinities showed that the triazole moiety was not tolerated since the binding affinities for compounds **28a-c** are in the µM range. One reason for this observation might be that the nitrogen atoms of the triazole are disrupting vital ligand-protein interactions, such as the hydrogen bond between the carbonyl oxygen of the amide bond and Tyr[113]. Interestingly, the analog containing phenyl ring in the 4-position (**28a**) has a higher binding affinity for FKBP51 than compounds

28b and **28c**. This observation might be explained by enhanced hydrophobic interactions of the phenyl ring with the protein surface, leading to an increased binding affinity for compound **28a**.

Table 6: Overview of the determined binding affinities of compound **28a-c**.



Entry	R	FKBP51 K_i [µM]	FKBP52 K_i [µM]
28a		1.89 ± 0.23	>100
28b		9.06 ± 1.32	>100
28c		5.97 ± 2.04	>100

Overall, the SAFit1 analogs containing triazoles at the bottom show selectivity over FKBP52. Nevertheless, the binding affinity of compounds **28a-c** decreased by 500 – 1000-fold for FKBP51 compared to SAFit1 (

Table 6).

In conclusion, the structure-affinity relationship of compounds **28a-c** showed that a 1,2,3-triazoles are not tolerated by FKBP51 as bottom group scaffolds.

9.5 Identification of a potential novel scaffold for the selective inhibition of FKBP51

A bioisosteric replacement strategy is a rational approach for optimizing lead compounds in modern drug discovery. Replacing certain functional groups or scaffolds with isosteric elements ideally leads to a better pharmacological profile with similar or improved biological affinity.[123–126] In recent years, various lead optimization studies have shown that replacing a phenyl group with a thiophene scaffold can retain similar biological activity.[127–130] In addition, 2,5-disubstituted thiophene-containing scaffolds have superior metabolic properties compared to unsubstituted thiophenes.[131] By blocking the 2,5-position of thiophenes, the CYP450-mediated epoxidation and S-oxidation generally can be reduced, avoiding the formation of reactive metabolites and, therefore, the toxicity of the ligand.[131–133]

In previous SAR studies, attempts to remove or replace the 3,4,5-trimethoxyphenyl group of SAFit1 led to no significant improvement. It was therefore decided to apply a bioisosteric replacement approach to enhance the overall properties. As a result, the phenyl group of **29** was isosterically substituted with a 2-thienyl group, respectively. Furthermore, the potential of halogenating the 5-position of thiophenes was investigated (**Figure 20**).

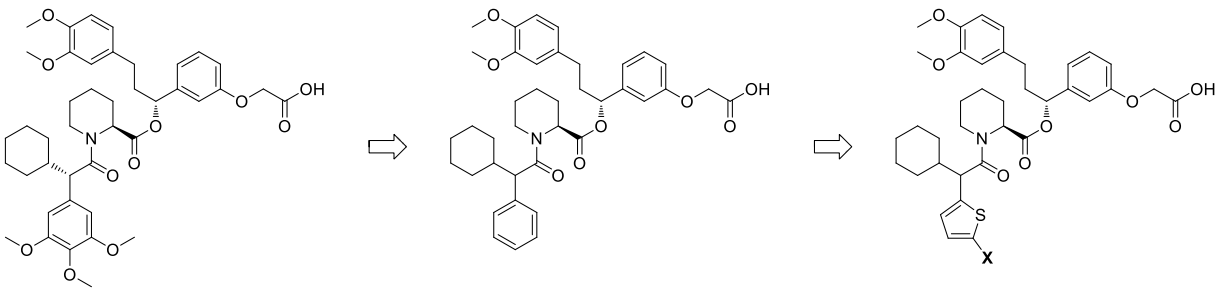
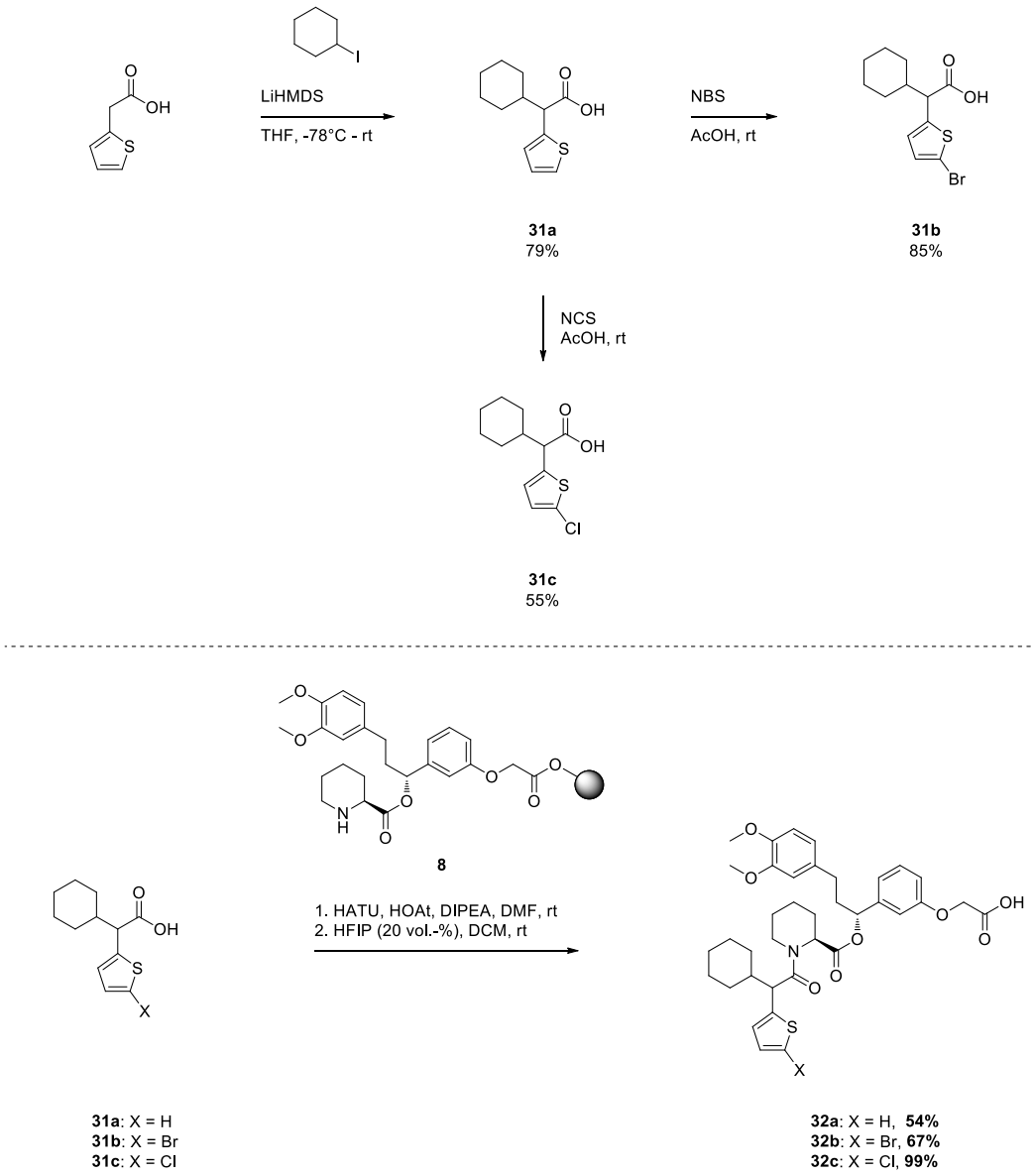


Figure 20: The removal of the three methoxy groups of SAFit1 results in a significant loss of binding affinity for FKBP51 (**29**). Therefore, the phenyl group should be replaced by a 2-thienyl group.

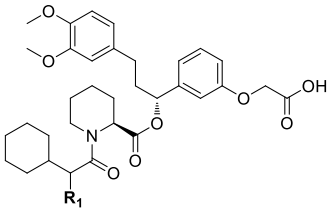
The 2-thienyl-containing bottom group **31a** was synthesized in one step by alkylating 2-thiopheneacetic acid with iodocyclohexane, resulting in a yield of 79% (**Scheme 8**). Subsequent halogenation in the 5-position of the thiophene group with NBS or NCS in acetic acid at room temperature yielded **31b** and **31c**. Overall, the bromination of **31a** resulted in a higher yield than the chlorination, which is likely due to the greater reactivity of NBS. Furthermore, the chlorination of **31a** resulted in the formation of a significant amount of dichlorinated side-product, which lowered the yield of **31c**. The second chlorination of **31c** is unselective, resulting in an inseparable mixture of the 3,5-dichloro and 4,5-dichloro-regioisomer. Therefore, dichloro-substituted thiophenes were not considered in the SAR study.

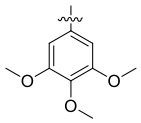
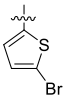
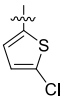
Scheme 8: Synthesis of the 2-thienyl-containing bottom groups **31a-c** and the SAFit1 analogs **32a-c**.



In the final step of the synthesis, the racemic bottom groups **31a-c** were coupled to the immobilized SAFit1 core-/top group via HATU coupling. The resulting SAFit1 analogs **32a-c** were cleaved off the solid support using a 20 vol.-% solution of HFIP in DCM, and the diastereomeric mixtures of SAFit1 analogs **32a-c** were then purified. The binding affinities of the new SAFit1 analogs for FKBP51 and FKBP52 were then determined by FP assay. To further understand the effect of replacing the aryl residue with a 2-thienyl group, the determined binding affinities of the diastereomeric mixtures **32a-c** were compared to the respective diastereomeric mixtures of SAFit1 (SAFit1*) and compound **29** (**Table 7**).

Table 7: Binding affinities of diastereomeric mixtures of SAFit1, compounds **32a-c** and **29**. *All compounds were tested as diastereomeric mixtures with (R/S) at the Cα position of the amide bond.



Entry	R_1	FKBP51 K_i [nM]	FKBP52 K_i [nM]
SAFit1*		15 ± 4	3959 ± 2395
29*		847 ± 91	>10000
32a*		442 ± 39	>10000
32b*		223 ± 31	>10000
32c*		159 ± 26	>10000

The comparison of the binding affinities for FKBP51 of SAFit1* and **29** showed that removing the three methoxy groups led to a significant decrease in binding affinity, as previously reported by Feng *et al.*[67] Replacing the phenyl group of **29** in R_1 with a 2-thienyl group (**32a**) resulted in a 2-fold increase in binding affinity from 847 nM to 442 nM. In addition, halogenation in the 5-position of the 2-thienyl moiety further increases the binding affinity (**32b**), while compound **32c** is the best compound in this SAR series, with a binding affinity of 159 nM. Notably, adding a single chlorine atom in the 5-positon of the 2-thienyl group resulted in an approximately 3-fold increase in binding affinity compared to the unsubstituted compound **29**. Furthermore, compound **32c** binds only 10-fold weaker than SAFit*, showing that the bioisosteric replacement is a practical approach for optimizing selective FKBP51 inhibitors.

Based on these encouraging findings, Dr. Christian Meyners obtained high-resolution co-crystal structures of the diastereomeric mixtures of compounds **32a** and **32c** in complex with the FK1 domain of FKBP51 to identify potential new interactions of this novel thiophene-containing scaffolds (**Figure 21**). It was assumed that only one of the diastereomers would be presented in the co-crystal structures of **32a** and **32c**. It was hypothesized that the diastereomer with the correct stereo configuration at the Cα position of the amide bond and the higher binding affinity for FKBP51 would be observed in the

obtained co-crystal structures. In the previously obtained co-crystal structure of SAFit1 in complex with the FK1 domain of FKBP51, the selectivity-inducing cyclohexyl moiety is buried in a transient binding pocket, resulting from the displacement of Phe^{67} (**Figure 21A**). To our great surprise, in the high-resolution co-crystal structures of **32a** and **32c**, only the (*S*)-diastereomer is observed in complex with FKBP51, where the 2-thienyl and 5-chloro-2-thienyl group is located in the transient binding pocket (**Figure 21B & C**). The displacement of the cyclohexyl moiety caused by thiophene-containing scaffolds leads to a change in conformation where the cyclohexyl group is now in the former position of the 3,4,5-trimethoxyphenyl residue of SAFit1.

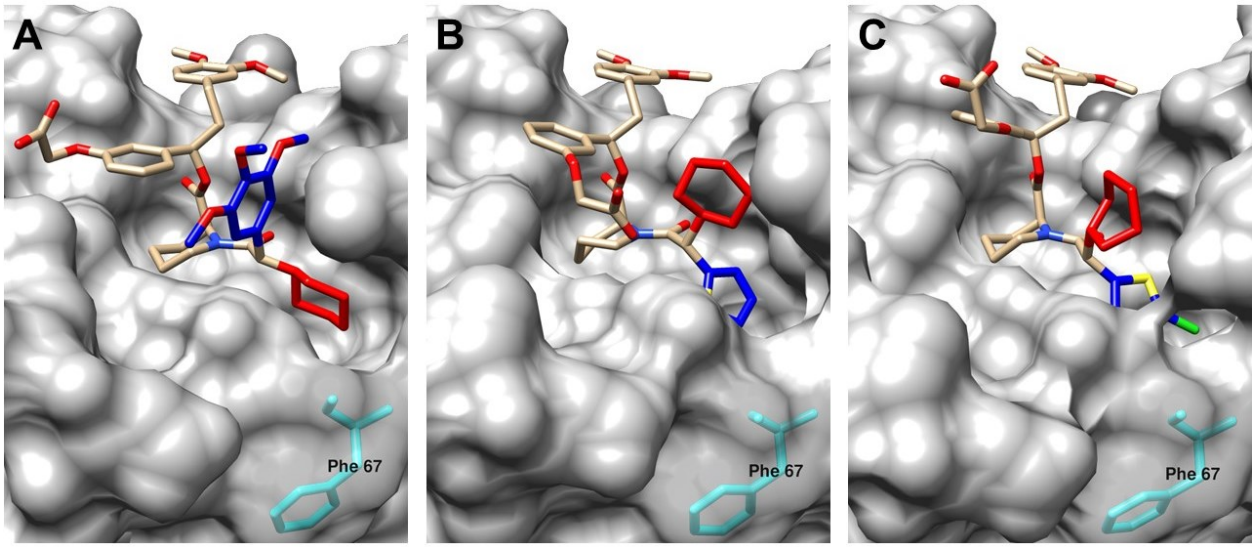


Figure 21: Co-crystal structure of SAFit1 analogs (sticks) in complex with the FK1 domain of FKBP51 (grey surface, the displaced Phe^{67} highlighted in cyan). The cyclohexyl moiety is shown in red, the moieties in the R_1 position in blue, and the rest of the ligands in pale orange. (A) The co-crystal structure of SAFit1 (PDB: 8CCA). (B) The co-crystal structure of compound **32a** (PDB: 8CCH). (C) The co-crystal structure of compound **32c** (PDB: 8CCB).

In addition, the orientation of the Phe^{67} is not impacted by the novel 2-thienyl and 5-chloro-2-thienyl scaffolds, which results in the formation of the transient binding pocket that is crucial for the selective inhibition of FKBP51. Based on the observation that only the diastereomers of **32a** and **32c**, with (*S*) configuration at the Cα-position, were observed, it was inferred that these diastereomers should possess a higher binding affinity (**Figure 22**). Additionally, the absence of the (*R*) diastereomer suggests that the novel thiophene-containing scaffolds might be better residues for inducing selectivity for FKBP51 than the cyclohexyl group.

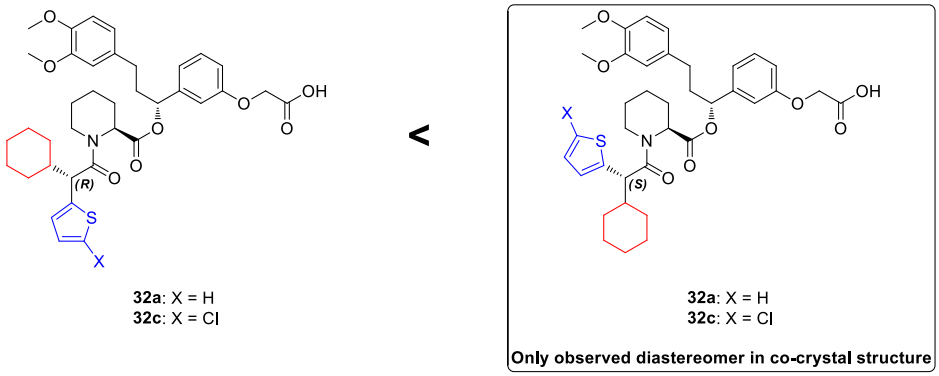


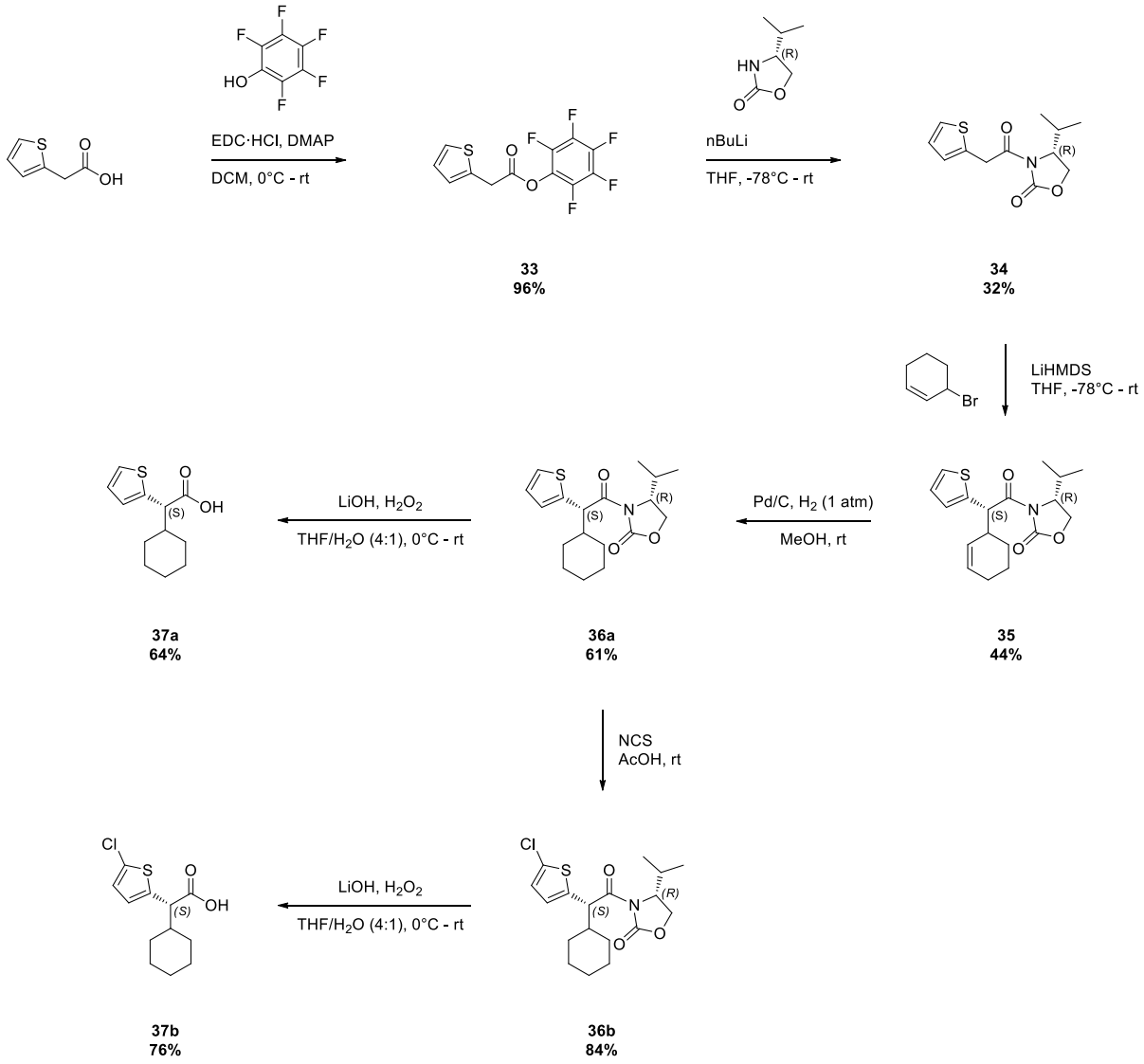
Figure 22: Chemical structures of the (R) and (S) diastereomers of compounds **32a** and **32c**.

To demonstrate that the thiophene scaffold is a suitable for inducing selectivity over FKBP52 and that the (*S*)-diastereomer of compounds **32a** and **32c** is the better binding one, the SAFit analogs **32a** and **32c** were synthesized diastereomerically pure. To achieve this, the bottom groups of compounds **37a** and **37b** were synthesized using Evans auxiliary mediated asymmetric alkylation (**Scheme 9**).[78,79] In the first step, 2-thiopheneacetic acid was converted with pentafluorophenol by Steglich esterification to obtain the activated ester **33**. Next, the Evans auxiliary was introduced by nucleophilic substitution of the deprotonated oxazolidinone with compound **33**, resulting in a yield of only 32%. This was due to the formation of various side products, one of them being the acylated product of compound **34**, which forms from the reaction of the enolate from **34** with the activated ester **33**. Despite the low yield during this reaction, various conditions for introducing the Evans auxiliary were screened, but none resulted in higher yields than the initial conditions. Therefore, the reaction sequence was continued without further optimization. In the next step, **34** was alkylated first with 3-bromochyclohexene, and the obtained diastereomers (*d.r.* = 4:1) were then separated. Next, the alkene was reduced by palladium-catalyzed hydrogenation, followed by cleavage of the Evans auxiliary, yielding the enantiopure bottom group **37a**. In addition to synthesizing the enantiopure bottom group **37a**, the 5-chlorinated analog was also synthesized. The chlorination of the 2-thienyl group in the 5-position was achieved by treating compound **36a** with NCS. After removing the Evans auxiliary, the enantiopure bottom group **37b** was obtained.

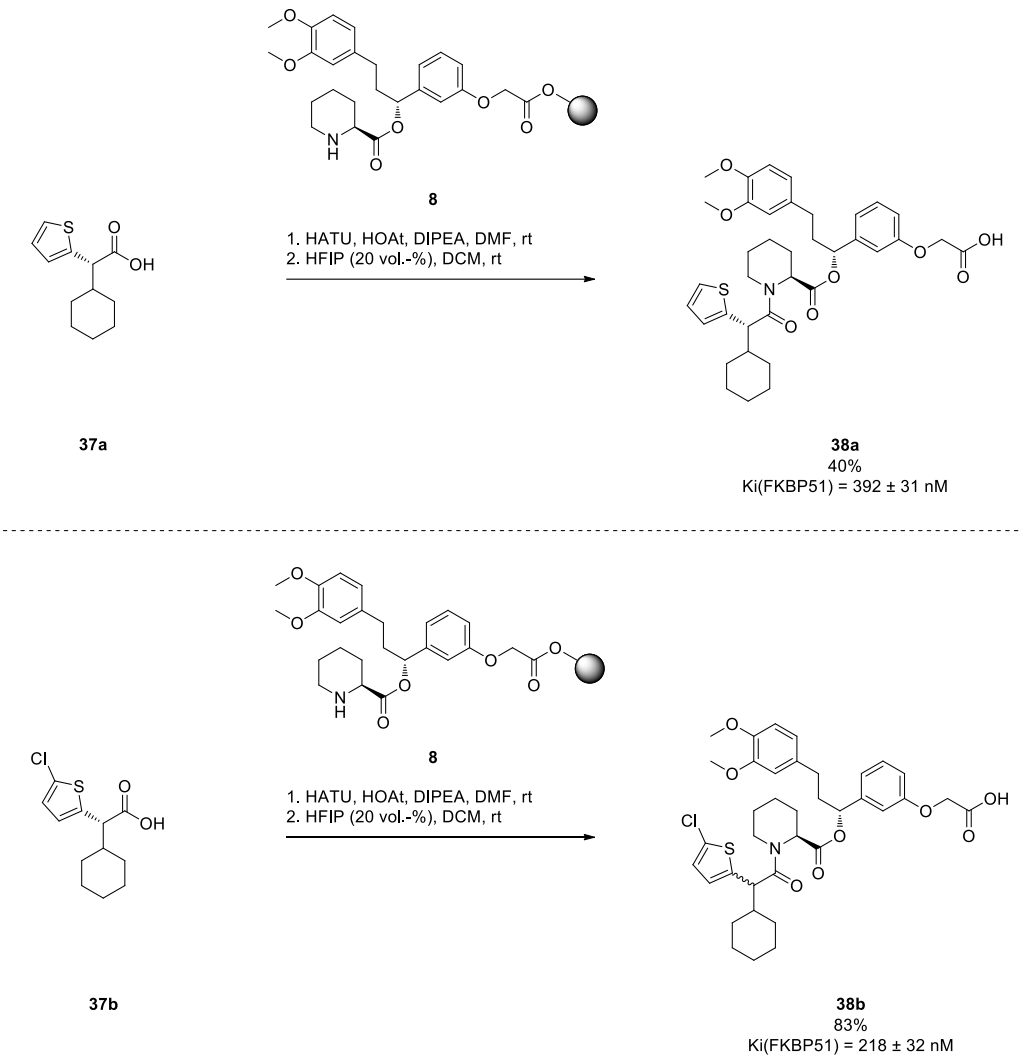
In the final step of this reaction sequence, the enantiopure (S) bottom groups **36a-b** were coupled to the SAFit1 core-/ top group, and the binding affinity for FKBP51 and FKBP52 of **37a-b** were determined (**Scheme 10**). The binding affinity for compound **37a** is 392 nM, slightly better than the respective diastereomeric mixture **32a**. These results suggest that thiophenes are at least as effective as the cyclohexyl group in binding affinity and, more importantly, inducing selectivity for FKBP51. During the coupling of **37b** was coupled to the immobilized SAFit1 core-/ top group. Analysis of the NMR data from compound **38b** revealed that epimerization occurred at the Cα-position during amide coupling.
Moreover, the determined binding affinities for compound **38b** were similar to the ones of the diastereomeric mixture **32c**. Because the epimerization during the amide coupling reaction was only

observable in the NMR and not in HPLC, optimizing the coupling conditions was not feasible. As a result, compound **38b** could not be synthesized diastereomerically pure.

Scheme 9: Synthesis of the enantiopure bottom groups **37a-b** by Evans auxiliary mediated asymmetric alkylation.



Scheme 10: Synthesis of the diastereomeric pure SAFit1 analogs **38a-b**.



In conclusion, a potential novel scaffold for the selective inhibition of FKBP51 has been discovered through bioisosteric replacement and testing diastereomeric mixtures of SAFit1 analogs. Notably, this method led to the identification of thiophene-containing scaffolds that can bind to the transient binding pocket upon the displacement of Phe[67]. Additionally, thiophene-containing bottom groups may serve as novel scaffolds for the selective inhibition of FKBP51, leading to the development of improved ligands.

9.6 Investigation of smaller thiophene-containing bottom groups for the selective inhibition of FKBP51

The potential of using this novel thiophene-containing scaffold for the selective inhibition of FKBP51 raised interest, as replacing the cyclohexyl moiety of SAFit1 and SAFit2 usually led to a decrease in binding affinity and selectivity.[59] To understand how the thiophene group in the R2 position induces selectivity for FKBP51, the effects of removing and reducing the substituent in R_1 were investigated.

To this end, 2-thiopheneacetic acid was coupled to the immobilized SAFit1 core-/top group by HATU coupling, yielding compound **39** (**Scheme 11**). Because alpha-keto amides were commonly used as bottom group scaffolds in the early development of unselective FKBP ligands, it was also decided to synthesize compound **42**, which contains a 2-thienyl-a-ketoamide as the bottom group.[51,52] Compound **42** was synthesized by cleaving the commercially available alpha-keto ester **40** with NaOH, followed by amide coupling to the SAFit1 core-/top group. In the end, the binding affinities of **39** and **41** for FKBP51 and FKBP52 were determined by FP assay (**Table 8**).

Scheme 11: Synthesis of the SAFit1 analogs **39** and **41**.

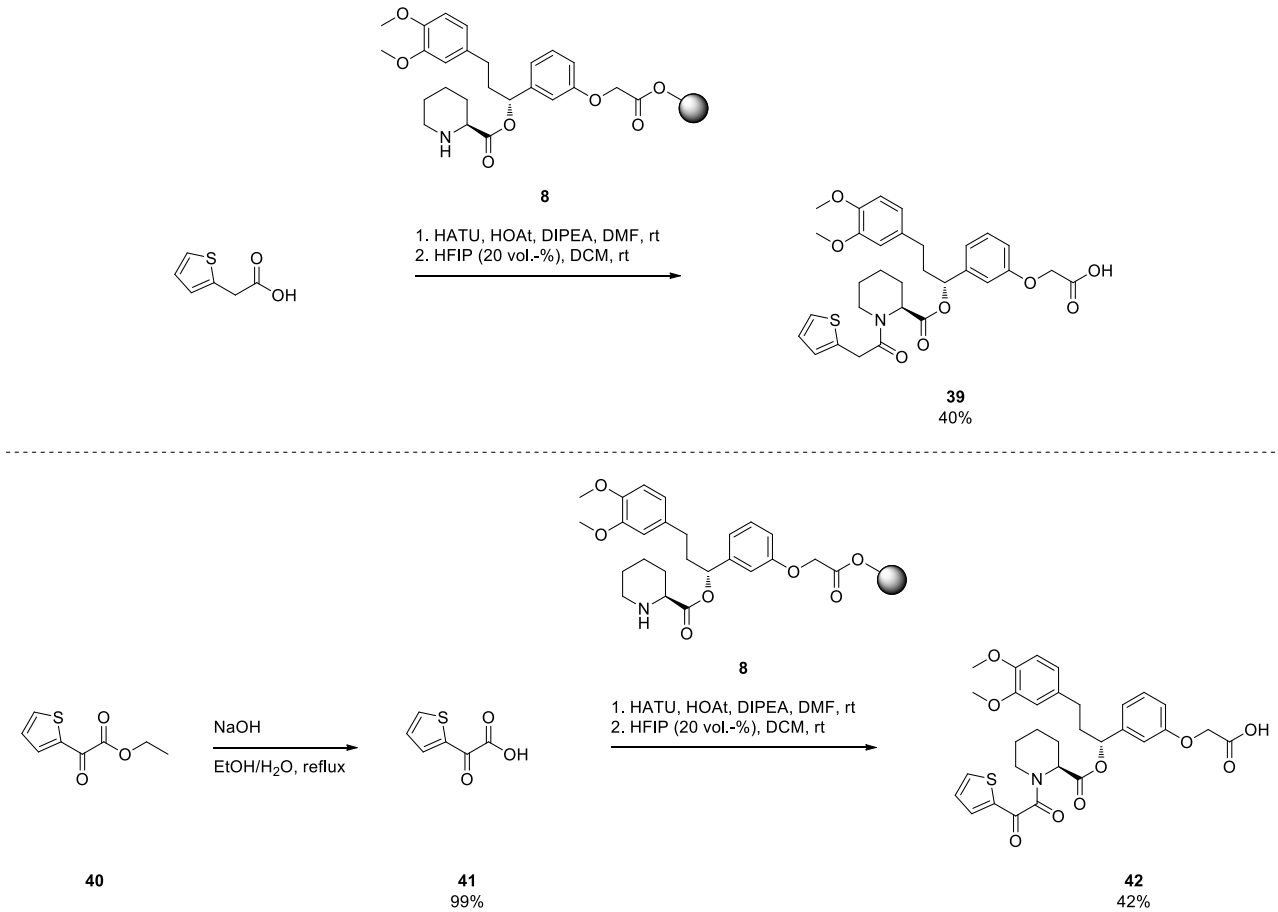
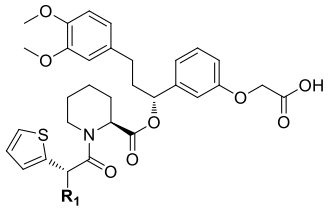


Table 8: Overview of the determined binding affinities of SAFit1 analogs **32a**, **39,** and **42**.



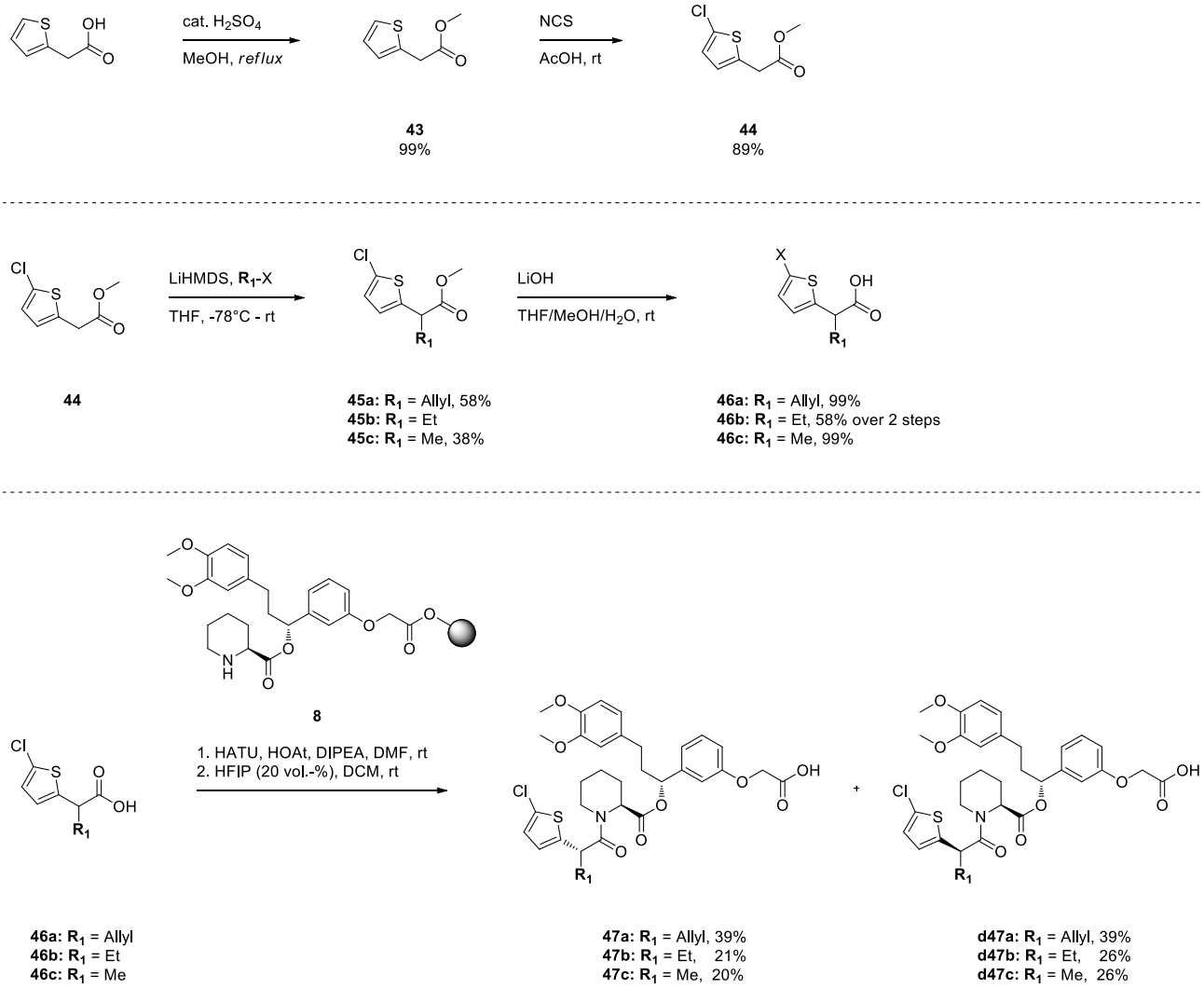
Entry	R_1	FKBP51 K_i [nM]	FKBP52 K_i [nM]
32a		392 ± 31	> 10000
39		>10000	> 8000
42		1885 ± 238	7311 ± 1460

The results of the FP assay revealed that compound **39**, containing an unsubstituted 2-thiopheneacetic acid, has a poor binding affinity for FKBP51 and FKBP52. In addition, **39** is unselective for FKBP51. However, when a carbonyl group is introduced in the Cα-position (**42**), an increased binding affinity and selectivity for FKBP51 is observed. Nevertheless, compound **32a**, containing a cyclohexyl group in R_1, still shows the best binding affinity and selectivity for FKBP51. These findings highlight that the substituent in R_1 plays a crucial role in inducing selectivity in thiophene-containing SAFit1 analogs. Therefore, it was decided to systematically reduce the cyclohexyl group to allyl, ethyl, and methyl groups. Because the 5-chlorothiophene-containing scaffold (**32c**) showed the best binding so far, the SAR study was conducted within the context of 5-chloro-2-thienyl groups.

First, ester **43** was synthesized by Fischer esterification of 2-thiopheneacetic acid with methanol, followed by selective chlorination of **43** in the 5-position of the thiophene (**Scheme 12**). Different alkyl groups were introduced by the alkylation of **43** with allyl bromide, ethyl iodide, and methyl iodide. Finally, the methyl esters were cleaved using LiOH, yielding the racemic bottom groups **46a-c**.

The final step of the synthesis involved coupling **46a-c** to the SAFit1 core-/top group and separating the pair of diastereomers using preparative HPLC. It was impossible to determine the absolute configuration of the bottom group by NOESY experiments because the inter-atomic distance between the stereogenic hydrogens of the bottom group and pipecolate was too large. Therefore, both diastereomers **47a-c** and **d47a-c** were tested for their binding affinities towards FKBP51 and FKBP52 by FP assay. For all pairs of diastereomers, only one diastereomer showed high binding affinities and selectivity for FKBP51 (**Figure 23**). As a result, the stereocenter of the better binding diastereomers **47a-c** was assigned to the (*S*)-configuration, which allows the binding of the thiophene to the transient binding pocket of FKBP51 (**Table 9**).

Scheme 12: Synthesis of the SAFit1 analogs **47a-c** and their diastereomers **d47a-c**.



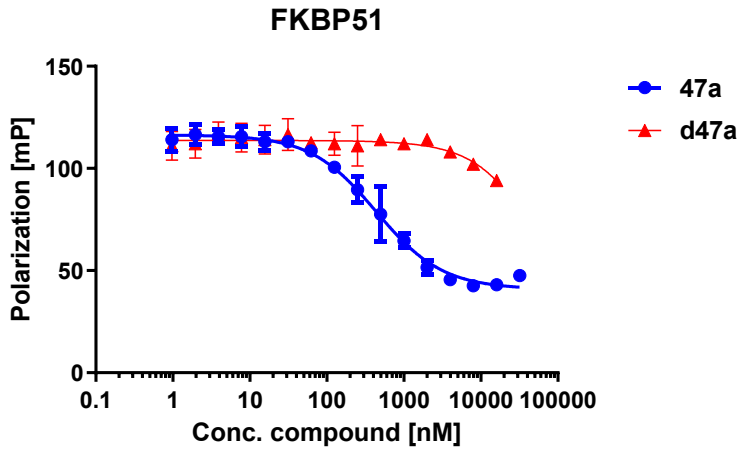
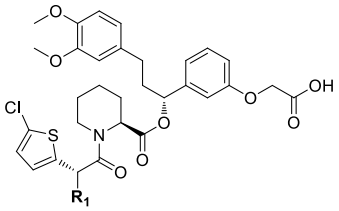


Figure 23: Representative FP assay of compound **47a** and **d47a** for FKBP51 measured by Wisely Oki Sugiarto.

Table 9: Overview of the binding affinities of compounds **47a-c**.



Entry	R_1	FKBP51 K_i [nM]	FKBP52 K_i [nM]
32c*		154 ± 26*	> 10000*
47a		223 ± 19	> 10000
47b		210 ± 11	> 10000
47c		300 ± 25	> 50000

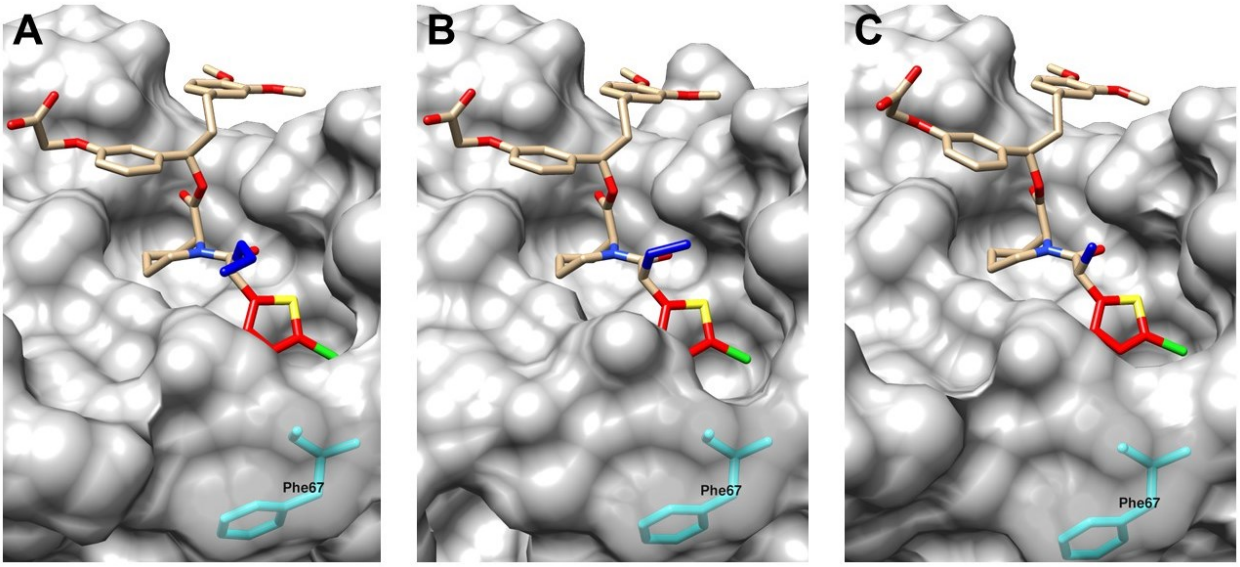


Figure 24: Comparison of the co-crystal structures of compounds **47a-c**, which were measured by Dr. Christian Meyners. (A) The co-crystal structure of **47a** in complex with the FK1 domain of FKBP51 (PDB: 8CCF). (B) The co-crystal structure of **47b** in complex with the FK1 domain of FKBP51 (PDB: 8CCE). (C) The co-crystal structure of **47c** in complex with the FK1 domain of FKBP51 (PDB: 8CCC).

In general, the systematic reduction in the R_1 position of compound **32a** led to ligands with moderate binding affinities and good selectivity for FKBP51. Interestingly, the methyl group alone in R_1 (**47c**) was sufficient to induce selectivity while maintaining moderate binding affinity (300 nM). This observation is consistent with the finding of Feng *et al.*, who also reduced the R_1 position systematically in the context of the cyclohexyl group and showed that a methyl group is the smallest residue to induce selectivity.[67] To gain further insight into the interaction of the different alkyl groups with FKBP51, high-resolution co-

crystal structures of **47a-c** in complex with the FK1 domain of FKBP51 were obtained (**Figure 24**). The co-crystal structures of **47a-c** show that the stereo configuration at the Cα position of the bottom group was assigned correctly, and the 5-chlorothiophene is binding to the transient binding pocket in FKBP51, causing the displacement of Phe^{67}. Moreover, the alkyl groups in R_1 of **47a-c** are partially solvent exposed and engage in a hydrophobic interaction with Ile^{122}.

Overall, the systematic reduction of the R_1 position of compound **32c** resulted in FKBP51 selective ligands with moderate binding affinity. Furthermore, the newly identified 5-chlorothiophene group can induce selectivity over FKBP52, even when only a methyl group is present. To further understand the influence of the 5-chlorothiophene group in terms of binding affinity for FKBP51, the 5-chlorothiophene moiety of compound **47c** was replaced by a phenyl and para-chlorophenyl group. This systematic replacement should clarify whether or not the thiophene scaffold is a contributing factor to the observed binding affinity of compound **47c**.

To obtain the methylated bottom groups **51** and **53**, esters **49** and **52** were alkylated with methyl iodide (**Scheme 13**). The methyl esters were then cleaved using LiOH, and the resulting bottom groups, **51** and **53**, were coupled to the SAFit1 core-/top group. The pair of diastereomers were separated using preparative HPLC, and the binding affinities of **54a-b** were determined by FP assay (**Table 10**). Replacing the cyclohexyl moiety of compound **55** with a 5-chlorothiophene group (**47c**) significantly improves the binding affinity for FKBP51 by 4-fold. However, introducing a phenyl group in R_2 decreases the binding affinity of **54a** compared to **47c**. Furthermore, introducing a chlorine atom in the para position of the phenyl ring (**54b**) does not affect the overall binding affinity. Nevertheless, compounds **54a-b** show a slightly higher selectivity for FKBP51, but are by far not as selective as **47c**.

Scheme 13: Synthesis of the SAFit1 analogs **54a-b** and their diastereomers **d54a-b**.

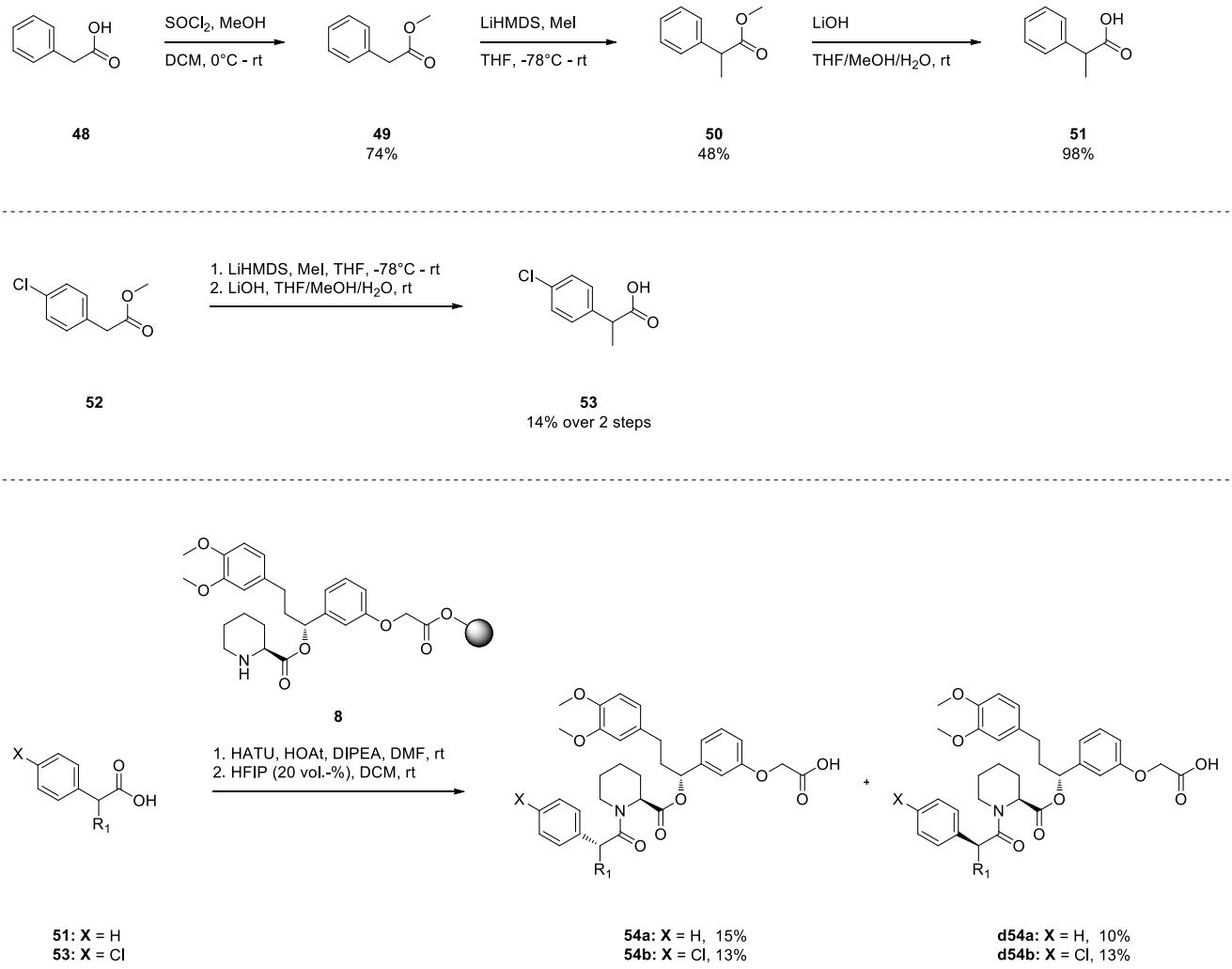
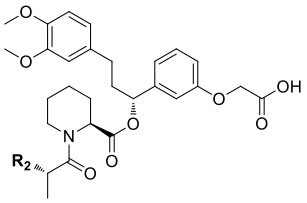
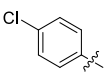


Table 10: Overview of the determined binding affinities of **47c**, **54a-c**, **55**. *reported by Feng *et al.*[67]



Entry	R_2	FKBP51 K_i [nM]	FKBP52 K_i [nM]
47c		300 ± 25	>10000
54a		4951 ± 1422	>10000
54b		4953 ± 755	>10000
55*		1200*	>1000*

In conclusion, this SAR study of the R_2 position suggests that the 5-chlorothiophene group enhances the hydrophobic interaction with the protein but cannot be replicated by a bioisosteric replacement such as a para-chlorophenyl group. Additionally, this SAR analysis provided further evidence that the 5-chlorothiophene can be used as a replacement for the cyclohexyl moiety in the context of selective FKBP51 inhibitors and that even smaller alkyl groups in R_1 can induce selectivity.

9.7 Exploration of different thiophene-containing moieties in R_2 for the selective inhibition of FKBP51

Identifying thiophene-containing bottom groups for selective inhibition of FKBP51 even in the context of smaller bottom groups (**47c**) raised the question if this group is also compatible with the highly preferred 3,4,5-trimethoxyphenyl residue in R_1. Toward this end, it was decided to explore different substituted thiophene groups. Therefore, a new synthetic route was established to introduce different substituents in R_2 (**Figure 25**).

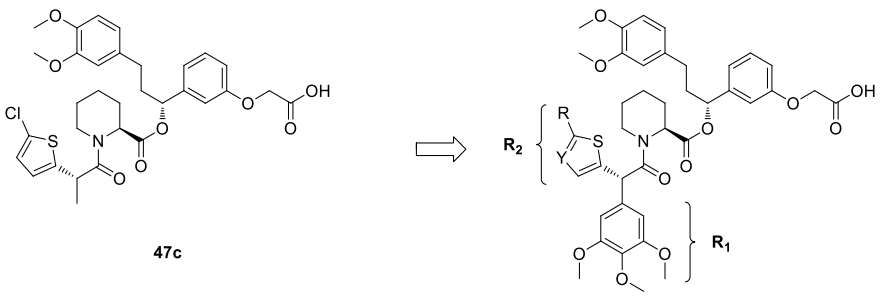
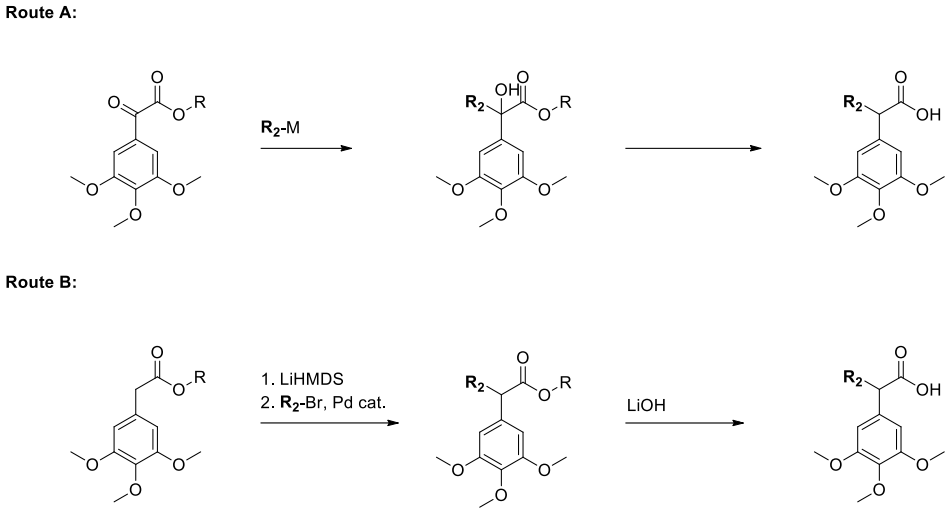


Figure 25: General chemical structure and rationale to explore different thiophene-containing groups in the context of the SAFit1 trimethoxyphenyl subgroup.

Generally, two approaches can be used to introduce different aryl groups in the R_2 position (**Scheme 14**). One approach would be the addition of an organometal reagent (for example, Grignard, organolithium, or Reformatski reagent) to an alpha-keto acid/ester resulting in the formation of the corresponding alpha-hydroxy acid/ester. In the next step, the hydroxy group would be then removed by a deoxygenation reaction, followed by cleavage of the ester (**Scheme 14**, Route A). The other option would be to introduce different aryl groups by palladium-catalyzed alpha arylation of ester enolates and subsequent cleavage of the ester (**Scheme 14**, Route B).[134–137]

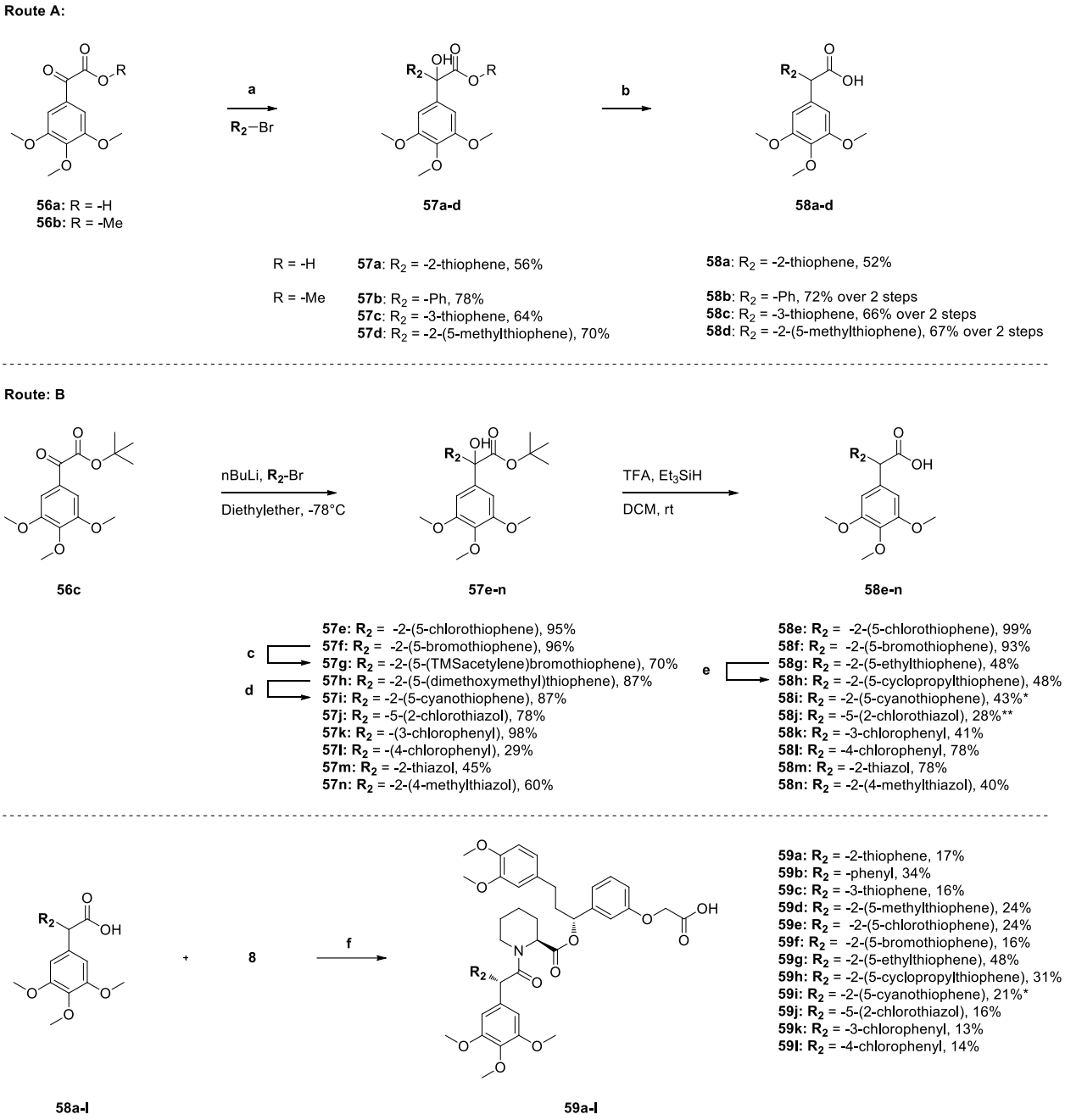
Overall, both synthetic routes would allow the fast generation of a library with different aryl-/heteroaryl groups in the R_2 position. Because the alpha arylation of esters is highly dependent on the electronic properties of the used aryl-/heteroaryl bromides and palladium-catalyst, it was decided to use Route A to introduce various groups in R_2 in the context of the SAFit bottom group.

Scheme 14: Synthesis of 2-aryl-/heteroaryl-containing SAFit bottom groups.



The synthesis of **58a-d** started from the reaction of α-keto acid **56a** or methyl- α-keto ester **56b**. Hereby, different aryl-/heteroaryl organolithium and Grignard reagents were used, yielding **57a-d** in moderate yields (**Scheme 15**, Route A). Next, the α-hydroxy groups of **57a-d** were selectively removed by deoxygenation using triethyl silane and catalytical amounts of $InCl_3$, followed by ester hydrolysis for compounds **57b-d**. Using this reaction sequence, the 2-substituted 3,4,5-trimethoxyphenylacetic acids **58a-d** were obtained in moderate yields over 2 or 3 steps, respectively. Since not all *in-situ* generated organolithium compounds were stable at room temperature, it was necessary to add the ester to the formed organolithium reagent at low temperatures. To suppress further side reactions, the methyl ester was replaced by the more sterically demanding tert-butyl ester (**56c**). This did not only result in increased yields for the formation of the α-hydroxy esters **57e-n** but also allowed the deoxygenation and tertbutyl ester cleavage in one step by using a mixture of triethyl silane in TFA (**Scheme 15**, Route B). This optimized procedure provided the 2-substituted 3,4,5-trimethoxyphenylacetic acids **58e-n** with good overall yields. Afterward, the obtained racemic carboxylic acids **58a-n** were coupled to the immobilized SAFit1 core-/top group **8**. Preparative HPLC separated the diastereomeric mixtures, and the binding affinity for FKBP51 and FKBP52 was determined by FP assay for all diastereomers. For the carboxylic acids **58m** and **58n**, coupling to the SAFit1 core-/top group were unsuccessful, even after screening various coupling conditions. Therefore, these compounds were not considered in the SAR analysis. As observed before, only one diastereomer showed good binding to FKBP51, resulting in the assignment of the absolute configuration, as shown in **Table 11**.

Scheme 15: Synthesis of the SAFit1 analogs **59a-l**.



Reagents and conditions: a) for **57a**, **57c**: R_2-Br, iPrMgCl LiCl, THF 0°C – rt; for **57b**: PhLi, THF, -78°C; for **57d**: R_2-Br, tertBuLi, THF, -78°C; b) for **58a**: $InCl_3$, Et_3SiH, DCM, rt; for **58b–d**: 1. $InCl_3$, Et_3SiH, DCM, rt; 2. LiOH, THF/MeOH/H_2O (3:1:1), rt; c) Trimethylsilylacetylene, Pd(dppf)Cl_2, CuI, Et_3N, 75°C; d) I_2, 28 wt.-% $NH_{3(aq.)}$, THF, rt; e) cyclopropylboronic acid, Pd(OAc)$_2$, SPhos, K_3PO_4, toluene/H_2O (20:1), 100°C. f) 1. for **59a**, **59d, 59e**: trichloracetonitrile, PPh_3, DIPEA, DCM, rt; for **59b**: COMU, pyridine, DMF, rt; for **59c**: T3P, pyridine, DMF, rt; for **59f-h, 59j-l**: oxalyl chloride, DIPEA, DCM, 0°C – rt; for **59i**: TCFH, NMI, DCM, rt. 3. 20 vol.-% HFIP in DCM, rt. *for **58i**: Ph_2SiHCl, $InCl_3$, DCM, rt; ** for **58j**: 1. TFA, DCM, rt; 2. NaI, H_3PO_3, MsOH (40 wt.-% in water), 40°C.

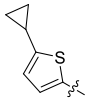
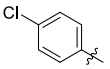
The unsubstituted 2- and 3-thiophene analogs (**59a** and **59c**) showed similar binding affinities compared to a simple phenyl group in R_2 (**59b**) and a 30-fold reduction compared to SAFit1. Interestingly, upon substitution of the 5-position of the 2-thienyl group by a methyl group (**59d**), chlorine (**59e**), and bromine atom (**59f**), the binding affinity for FKBP51 increased drastically by 25-fold, resulting in a single-digit nanomolar affinity. This observation suggests that the 5-substituted 2-thiophenes are suitable bioisosteric replacements for the cyclohexyl group in the SAFit1 scaffold. Moreover, larger substituents, such as ethyl (**59g**), cyclopropyl (**59h**), and nitrile group(**59i**), were tolerated in the 5-position but resulted in a slight decrease in binding affinity. When a 5-chloro-2-thiazol group (**59j**) was used instead of the 5-chloro-thien-2-yl group, the binding affinity decreased by around 3-fold. This indicates that the introduction of a nitrogen atoms into the heterocycle results in enhanced interactions within the transient binding pocket of FKBP51. Additionally, the replacement with a meta- and para-substituted phenyl group (**59k** and **59l**) results in a decrease of binding affinity by 5-fold compared to **59e**. Overall, all obtained analogs **59a-l** showed good to excellent binding affinities for FKBP51 (<150 nM) while retaining high selectivity over FKBP52.

To confirm the correct assignment of the absolute configuration of the bottom groups and binding mode of this series, representative co-crystal structures of **59e** and **59g** in complex with the FK1 domain of FKBP51 were obtained (**Figure 26**). The co-crystal structures of **59e** and **59g** clearly show that the configurations were assigned correctly. Also, the 5-substituted thiophene groups are positioned in the transient binding pocket, as predicted (**Figure 26A&C**). Interestingly, the residue of Ile^{121} undergoes a conformational change in the co-crystal structure of **59g**, which leads to reduced hydrophobic interaction with the 3,4,5-trimethoxyphenyl group, possibly leading to a reduction of the observed binding affinity for FKBP51 compared to **59e**.

The overlay of the structure of **59e** and **59g** with SAFit1 also shows that the 5-substituted thiophenes and cyclohexyl group have the same orientation, resulting in the engagement in hydrophobic interactions in the transient binding pocket (**Figure 26B&D**). In both ligands, R_1 and R_2 are in an orthogonal orientation to each other, resulting in an almost identical binding mode as SAFit1.

In conclusion, 5-substituted thiophenes also proved to be an excellent bioisosteric replacement for the cyclohexyl group in 3,4,5-trimethoxyphenyl-containing FKBP51 selective ligands. Furthermore, upon replacement of the cyclohexyl moiety, the binding mode is not altered, resulting in almost identical binding affinities for compound **59e** compared to SAFit1. Furthermore, slightly larger substituents in the 5-position of thiophenes are tolerated by FKBP51, resulting in lower binding affinities.

Table 11: Overview of the determined binding affinities for SAFit1 analogs **59a-l**.

Entry	R_2	FKBP51 K_i [nM]	FKBP52 K_i [nM]	Entry	R_2	FKBP51 K_i [nM]	FKBP52 K_i [nM]
SAFit1		6 ± 0.4	3859 ± 2395	**59g**		13 ± 1	>1,000
59a		103 ± 12	10171 ± 923	**59h**		41 ± 5	>1,000
59b		118 ± 9	43792 ± 9206				
59c		140 ± 19	17951 ± 2463	**59i**		23 ± 3	2728 ± 332
59d		7 ± 1	1030 ± 286	**59j**		12 ± 1	1516 ± 304
59e		4 ± 1	950 ± 215	**59k**		22 ± 2	12910 ± 2748
59f		7 ± 1	436 ± 56	**59l**		26 ± 2	1725 ± 425

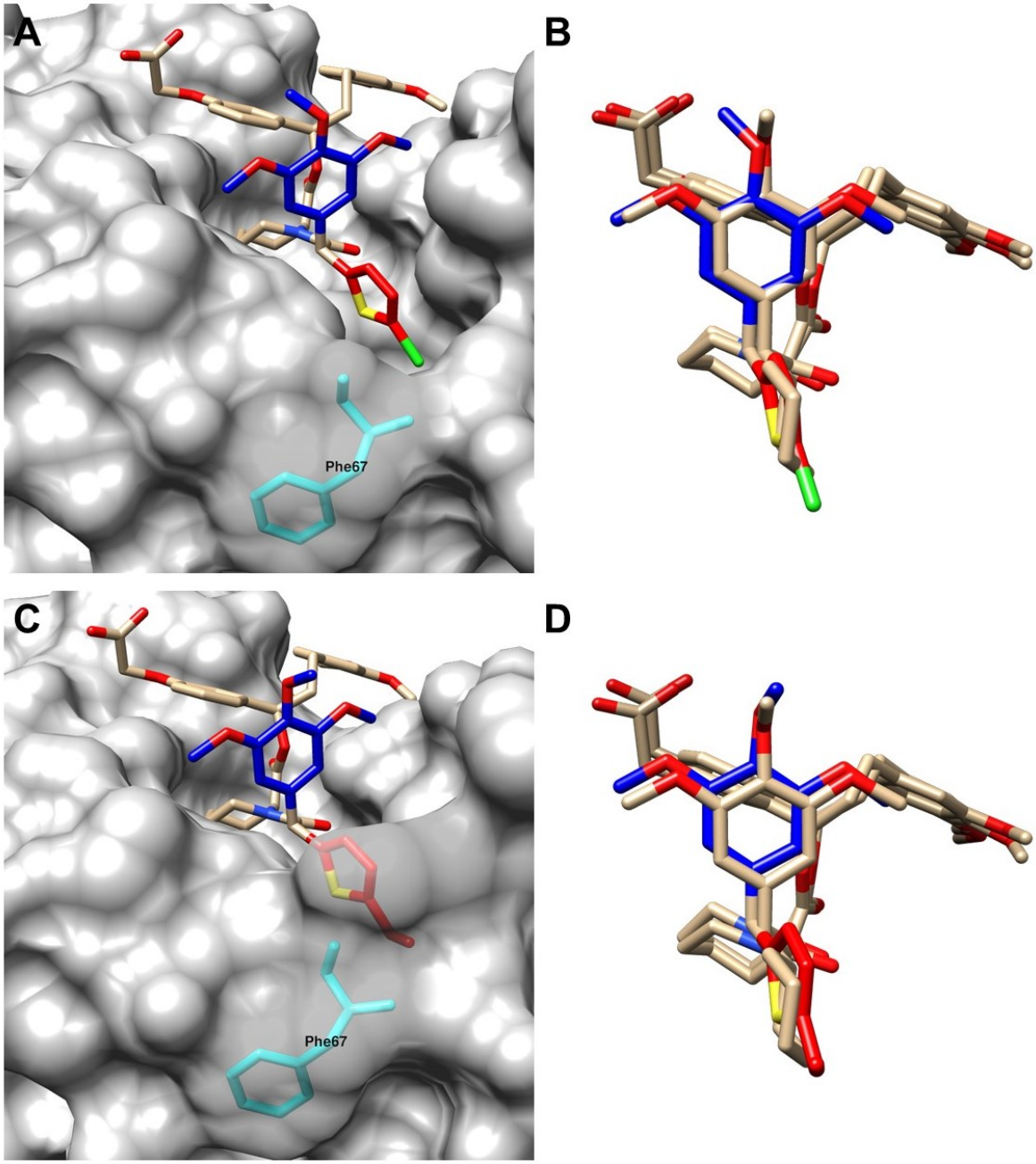


Figure 26: Co-crystal structures of compounds **59e** and **59g** (sticks) in complex with the FK1 domain of FKBP51 (Phe^{67} highlighted in cyan), solved by Dr. Christian Meyners. The (5-chloro)thien-2-yl moiety is shown in red, the aryl moiety is in blue, and the rest of the ligand is in pale orange. (A) The co-crystal structure of compound **59e** in complex with the FK1 domain of FKBP51 (PDB: 8CCD). (B) Superposition of compound **59e** with SAFit1 (pale orange). (C) The co-crystal structure of compound **59g** in complex with the FK1 domain of FKBP51. (D) Superposition of compound **59g** with SAFit1 (pale orange).

9.8 Synthesis and *in-vivo* evaluation of thiophene-containing SAFit2 analogs

The development of the first selective inhibitors of FKBP51 SAFit1 and SAFit2 resulted in ligands with binding affinities of 6 nM and 8 nM. Both ligands showed similar binding affinities for the FK1 domain of FKBP51 (**Figure 27**). Still, when these ligands were tested for their binding affinities in a cellular NanoBRET assay, SAFit2 had an approximately 4-fold higher binding affinity for FKBP51.[93]

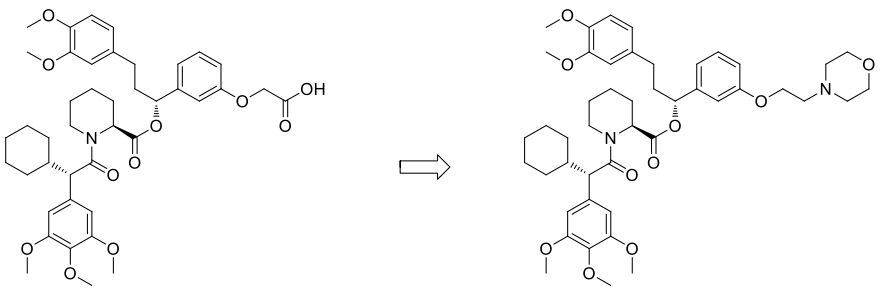


Figure 27: Chemical structures and binding affinities for FKBP51 of SAFit1 and SAFit2.

The reason for this observation is probably the difference in cell permeability. SAFit1 shows lower binding affinities for FKBP51 in the nano BRET assay mainly due to the polar carboxylic acid residue in the top group, leading to reduced cell permeability. Therefore, using a morpholine group instead results in a better permeability and, ultimately, a 4-fold increase in binding affinity in the nano BRET assay. Moreover, the morpholine residue even allows SAFit2 to pass the blood-brain barrier. These properties make SAFit2 the current gold standard for investigating FKBP51 in various cell-based assays and *in-vivo* experiments.[25,29–31,59,69,93,138,139]

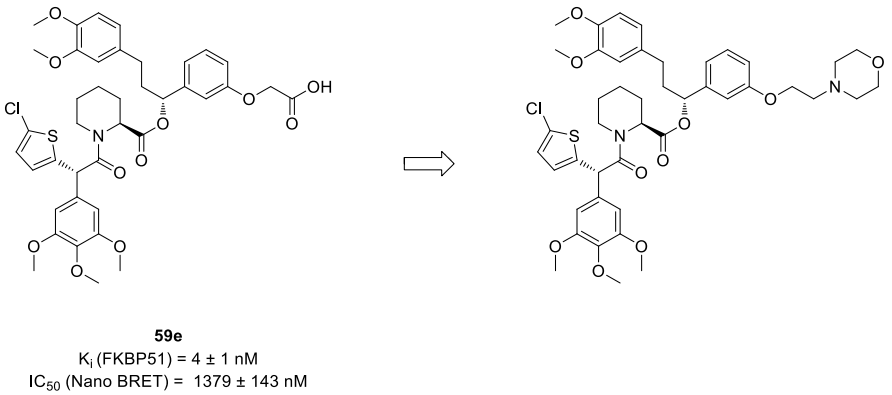


Figure 28: Transformation of compound **59e** into its SAFit2 analog.

During the investigation of novel scaffolds for the selective inhibition of FKBP51, only SAFit1 analogs were synthesized mainly due to the access to solid-supported synthesis and the resulting solubility in

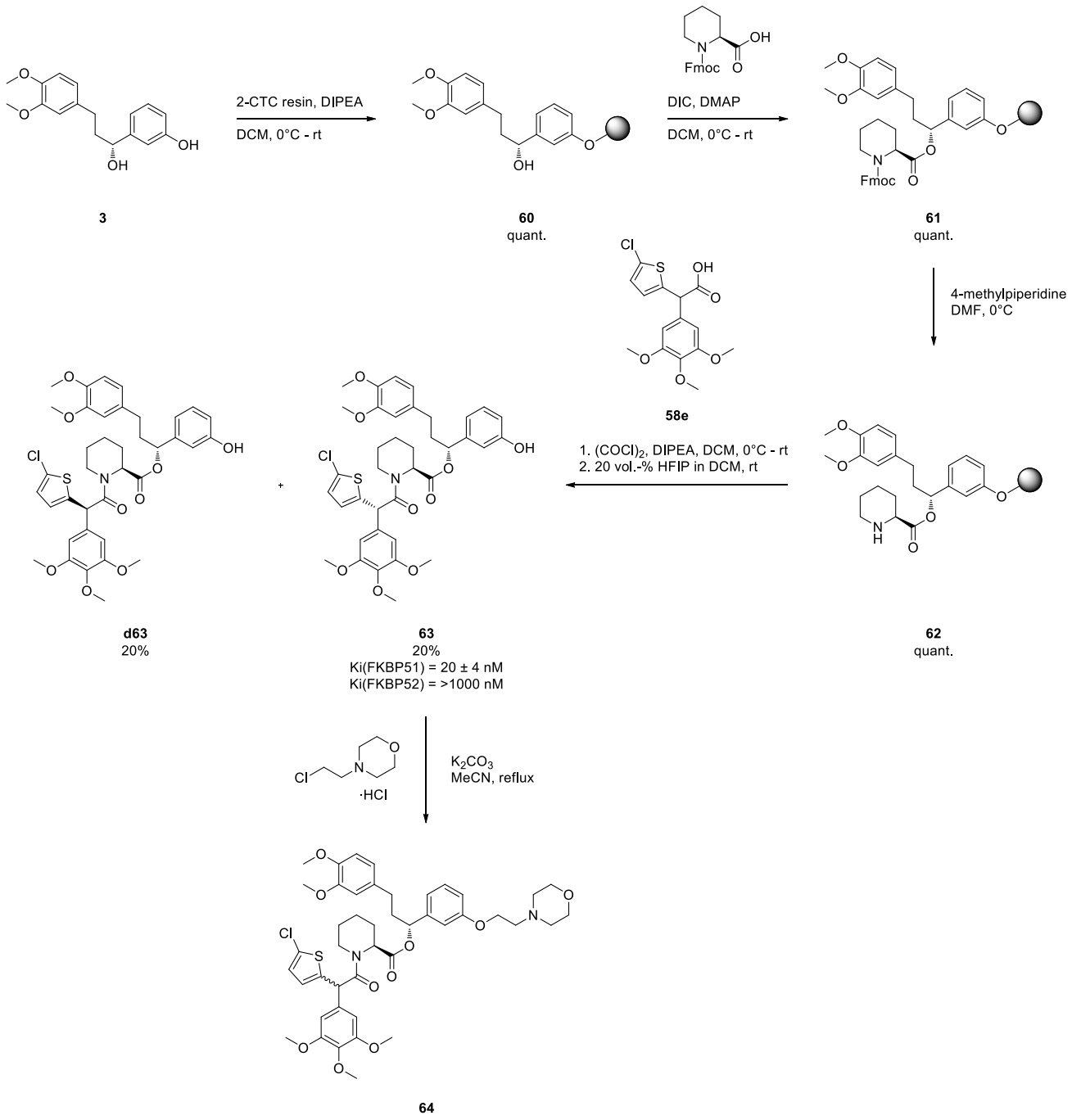
aqueous media. For a better comparison between the current gold standard SAFit2 and the best ligand of this series (**59e**), the latter was converted into the corresponding SAFit2 analog (**Figure 28**).

First, compound **63** was synthesized and then modified to a SAFit2 analog in a subsequent step by introducing the morpholine functionality via alkylation of the phenolic hydroxy group (**Scheme 16**). This synthesis route also allows the further derivatization of the residues in the top group. Therefore, the synthesis of the SAFit2 analog **64** was accomplished by solid phase synthesis. First, the top group alcohol **3** was immobilized with the phenolic hydroxy group on 2-chlorotrityl resin. Then the chiral alcohol was esterified with the Fmoc-protected pipecolic acid via Steglich esterification. Next, the Fmoc protection group was removed by treatment with 4-methylpiperidine, and the resulting amino group was coupled to the bottom group **58e** by amidation. Finally, the obtained diastereomeric mixture was cleaved off the solid support by treatment with a 20 vol.-% solution of HFIP in DCM, and the diastereomers were separated by preparative HPLC. Compound **63** was tested via FP assay for the binding affinity for FKBP51. Surprisingly, compound **63** still has a binding affinity of 20 nM for FKBP51, even though lacking the carboxylic acid functionality in the top group. This suggests that in this position, the absence of top group functionality has only a minor influence on the binding affinity for FKBP51 but can also influence solubility or cell permeability.

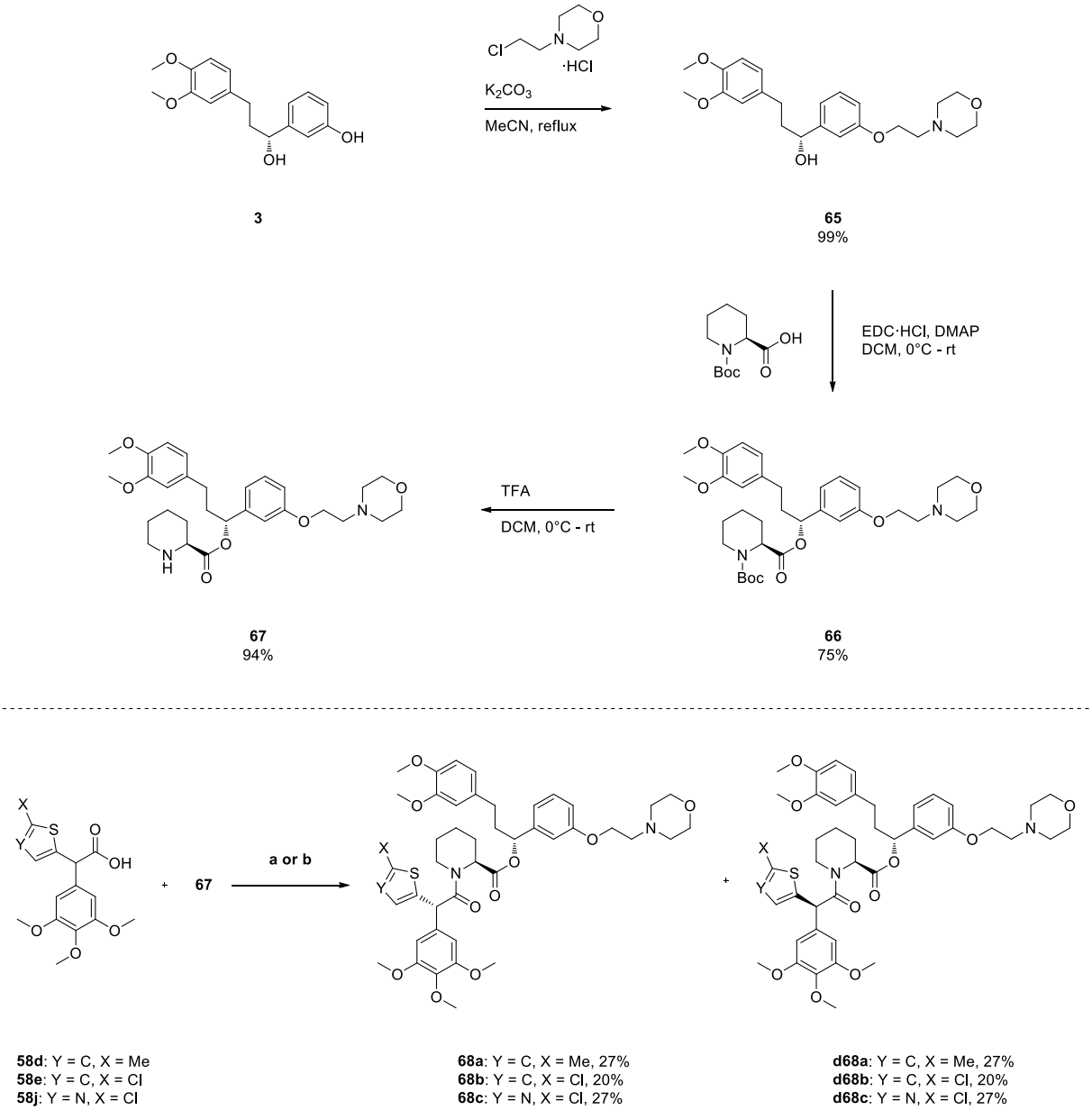
The obtained diastereomeric pure compound **63** was then alkylated with 4-(2-chloroethyl)morpholine in the presence of potassium carbonate. During this reaction, full epimerization of the bottom group has been observed, resulting in a diastereomeric mixture. This observation is mainly caused due to the acidity of the hydrogen atom in the Cα-position of the amide bond.

Based on this observation, it was decided to synthesize compound **67** by the classic SAFit2 synthesis (**Scheme 17**). In this synthesis, the top group alcohol **3** is first alkylated with 4-(2-chloroethyl)morpholine in the presence of potassium carbonate, followed by Steglich esterification of the chiral hydroxy group with the Boc-protected pipecolic acid. In the next step, the Boc protection group of compound **66** is removed by treatment with TFA in DCM to obtain the SAFit2 core-/top group in an overall yield of 70%.

Scheme 16: Synthesis of compound **64** on solid phase and alkylation to **63**.



Scheme 17: Classic synthesis of SAFit2 analogs **68a-c**.



Reagents and conditions: a) for **68a**: **58d**, **67**, TCFH, NMI, MeCN, rt; for **68c**: **58j**, **67**, TCFH, NMI, MeCN, rt; b) for **68b**: **58e**, **67**, oxalyl chloride, DIPEA, DCM, 0°C – rt.

Finally, the bottom groups **58d**, **58e,** and **58j** are coupled to the SAFit2 core-/top group by either forming the acid chloride and subsequent reaction with **67** in the presence of DIPEA (a) or by N-acyl imidazolium mediated amid coupling (b). Afterward, the obtained diastereomers of **68a-c** were separated by preparative HPLC, and the binding affinities for **68a-c** were determined in an FP and a NanoBRET assay (**Table 12**).

Table 12: Overview of the binding affinities, NanoBRET data, and the determined *in-vivo* pharmacokinetic parameters of SAFit2 analogs **68a-c**, by Dr. Margherita Springer and Dr. Mathias Schmidt.

Entry	R_2	FKBP51 K_i [nM]	FKBP52 K_i [nM]	Nano BRET FKBP51 IC_{50} [nM]	In-vivo pharmacokinetic parameters in BL6 mice			
					c_{max} [ng/mL]	t_{max} [h]	$t_{1/2}$ [h]	V_D [L]
SAFit2		8 ± 1	2159 ± 175	196 ± 14	3061.0	1	3.7	0.1
68a		17 ± 3	775 ± 273	613 ± 58	-	-	-	-
68b		19 ± 3	774 ± 245	584 ± 41	989.8	0.5	4.3	0.3
68c		26 ± 2	1725 ± 425	-	-	-	-	-

The compounds **68a** and **68b** have an approximately 3-fold decreased binding affinity for FKBP51 compared to SAFit2 with binding affinities of 17 nM and 19 nM. Interestingly when the thienyl moiety in the 5-position is substituted with a chlorine atom instead of a methyl group, the binding affinity for FKBP52 is the same. When the 5-chlorothienyl moiety of compound **68b** is substituted with a 2-chlorothiazole moiety (**68c**), the binding affinity decreases to 26 nM, and the selectivity for FKBP51 increases from 40-fold to 66-fold. Moreover, the calculated clogP of compound **68c** is slightly lower than compound **68b** and SAFit2. The compounds **68a-b** were also tested in a nano BRET assay for their binding affinity for FKBP51. Compounds **68a-b** have 3-fold decreased IC_{50} values for FKBP51 compared to SAFit2. Since the IC_{50} depends on the binding affinity for FKBP51 and the cell permeability, the 3-fold decrease in IC_{50} values probably corresponds to the 3-fold decreased binding affinity of **68a-b** compared to SAFit2 measured in the FP assay. Based on this observation, it is assumed that compounds **68a-b** can permeate cells to the same extent as SAFit2, even though the clogP is slightly higher. Overall, the novel SAFit2 analogs **68a-b** possess binding affinities in the lower nanomolar range and are selective for FKBP51. Furthermore, compounds **68a-b** seem to have cell permeability in the same range of SAFit2 if the 3-fold reduction in binding affinity for FKBP51 is considered.

Based on the results in **Table 12**, a snapshot PK study of compound **68b** was performed by Dr. Margherita Springer in the research group of Dr. Mathias V. Schmidt at the Max-Planck-institute for Psychiatry in Munich. The PK study of **68b** was performed on BL6 mice intraperitoneally injected with 10 mg/kg body weight of compound **68b** or SAFit2. The blood plasma concentration of **68b** was then determined throughout 6 h and compared to the data of SAFit2.

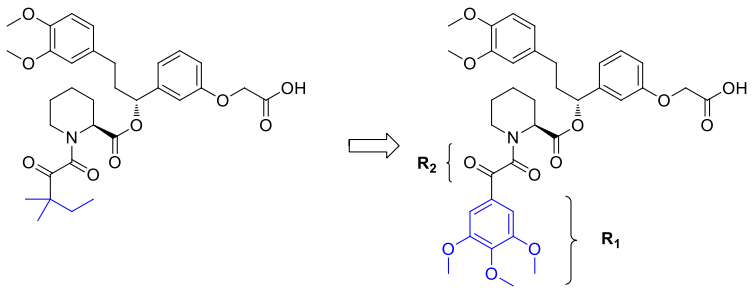
The results of the PK study of compound **68b** and SAFit2 show that the maximum plasma concentration of compound **68b** is reached after 30 min with c_{max} = 990 ng/mL and is approximately 3-fold lower than for SAFit2 with 3061 ng/mL after 1 h of treatment. This suggests that compound **68b** has an acceptable

PK profile, with a longer half-life time, higher volume of distribution, and shorter t_{max} compared to SAFit2. Compound **68b** was also detected in brain tissue samples, indicating that **68b** can pass the blood-brain barrier. Furthermore, **68b** showed accumulation in gonadal white adipose tissue due to its high lipophilicity.

In conclusion, the introduction of the novel scaffold for the selective inhibition of FKBP51 results in an acceptable *in-vivo* PK profile of **68b**, allowing it to be used as a chemical tool compound for *in-vivo* experiments to elucidate the function of FKBP51 in animal models further.

9.9 Synthesis of selective SLF analogs by Claisen Ireland rearrangement

In early ligand development of synthetic FKBP ligands, Holt et al. discovered the first fully synthetic FKBP12 ligand SLF.[48,49] This inhibitor showed good binding affinities for FKBP12 but low binding affinities for FKBP51 and FKBP52.[51] Furthermore, this ligand was unselective for both proteins. Therefore, Gopalakrishnan et al. evaluated different bottom groups in the R_1 position of SLF by replacing the tert-pentyl group with different residues. In this study, Gopalakrishnan found that upon replacement of the tert-pentyl group of SLF by a 3,4,5-trimethoxyphenyl group (**69**), the binding affinities for FKBP12 and FKBP52 were the same, but the binding affinity for FKBP51 decreased (**Figure 29**).[51]



SLF
IC_{50}(FKBP12) = 0.17 µM
IC_{50}(FKBP51) = 8.4 µM
IC_{50}(FKBP52) = 10.5 µM

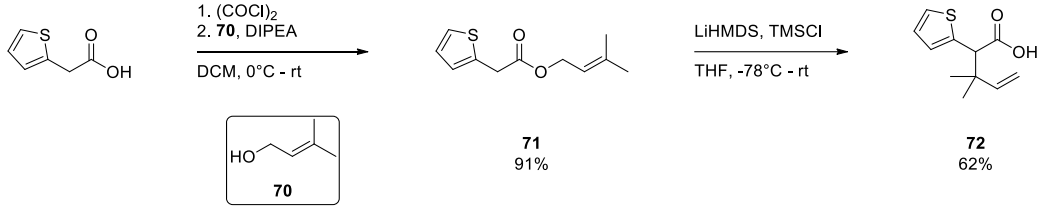
69
IC_{50}(FKBP12) = 0.15 µM
IC_{50}(FKBP51) = 19.3 µM
IC_{50}(FKBP52) = 11.6 µM

Figure 29: Chemical structures and binding affinities of SLF and compound **69**.

Based on these results and the commercial availability of the 3,4,5-trimethoxyphenylacetic acid, the identified 3,4,5-trimethoxyphenyl group in the R_1 position of **69** was used as the bottom group for the development of the first selective FKBP51 inhibitors SAFit1 and SAFit2.[59] Moreover, the unpolar nature of the tert-pentyl group of SLF makes this group not appealing for drug-like compounds. Because the introduction of the selectivity-inducing cyclohexyl moiety in the R_2 of SAFit ligands was never combined with the tert-pentyl group in the R_1 position, but the binding affinity for FKBP51 of SLF is 2-fold higher than of compound **69**, it was decided to investigate the potential of this scaffold.[51] It was hypothesized that the newly identified selectivity-inducing 2-thienyl moiety would result in even better binding affinities for FKBP51. Moreover, this study was meant to show how highly sterically hindered residue in the R_1 position would influence the binding affinity, selectivity, and spatial orientation of the 2-thienyl moiety in the co-crystal structure of FKBP51.

In general, alkyl groups in the R_2 position of the bottom groups were introduced by alkylation of the enolate of the corresponding ester with primary and secondary alkyl bromide or iodides. Because tertiary alkyl bromides undergo an elimination reaction, via an E1 mechanism, under strongly basic conditions, this synthetic approach is unsuitable for synthesizing tert-pentyl-containing bottom groups.[140] Therefore, it was decided to introduce the tert-pentyl moiety by Claisen-Ireland rearrangement, followed by a reduction of the obtained alkene (**Scheme 18**).[141–143]

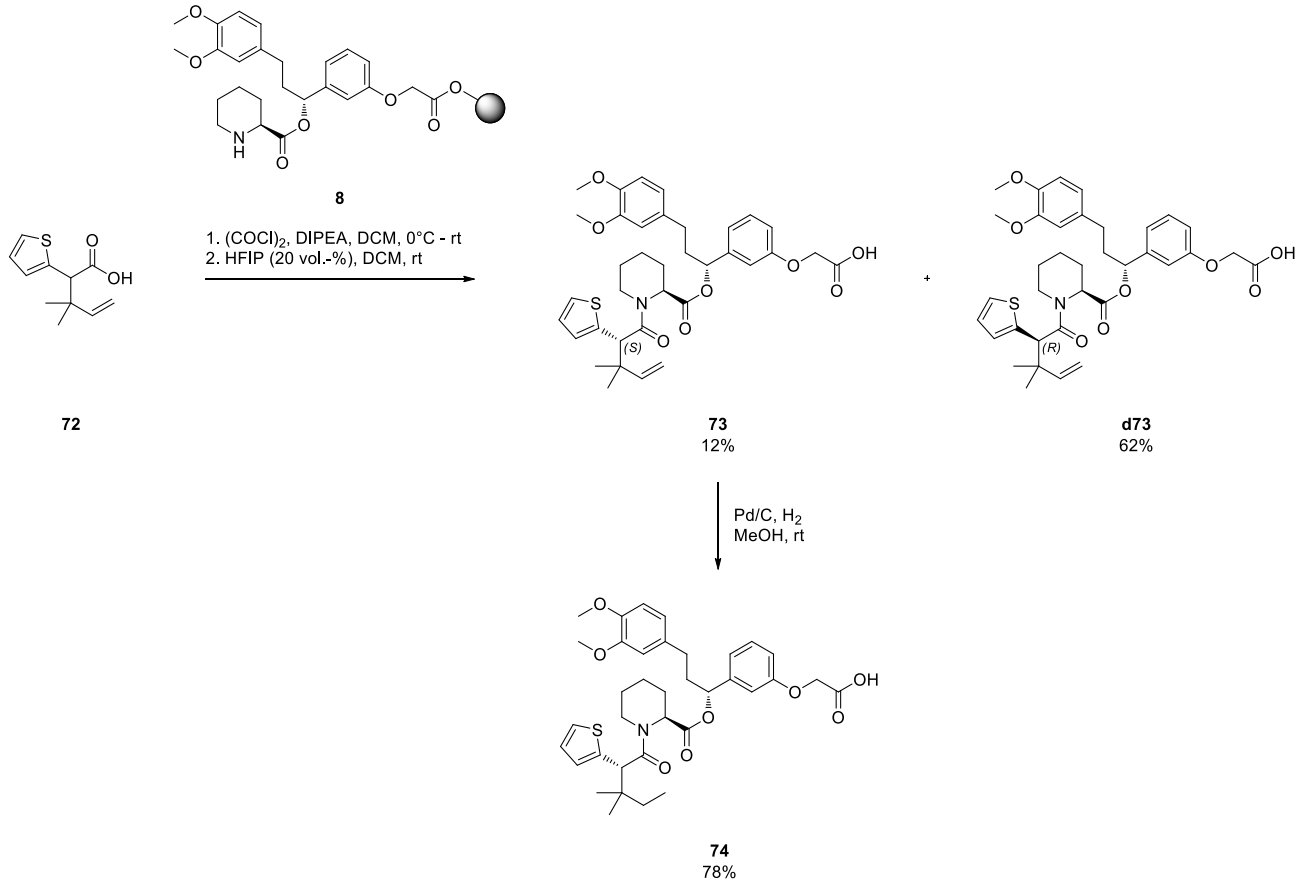
Scheme 18: Synthesis of bottom group **72** by Claisen Ireland rearrangement.



First, the acid chloride of the commercially available 2-thienylacetic acid was formed by treatment with oxalyl chloride, followed by esterification with alcohol **70** in the presence of DIPEA. Next, **71** was converted by Claisen-Ireland rearrangement to bottom group **72**. In this reaction, the enolate of **71** was formed by the treatment with LiHMDS at -78°C, followed by the addition of TMS chloride, which resulted in the formation of the corresponding silyl enol ether. Upon warming to room temperature, the formed silyl enol ether spontaneously undergoes a [3,3]-sigmatropic rearrangement resulting in the formation of **72** after acidic hydrolysis.

Next, compound **72** was coupled to the immobilized SAFit1 core-/top group **8** via acid chloride-mediated amide coupling in the presence of DIPEA. Interestingly, during a test cleavage via LC/MS, a diastereomeric ratio of 2:1 compound **73** and **d73** was observed. After the formed product was cleaved off, the solid support and the diastereomers were separated via preparative HPLC. Because of the product distribution of 2:1, compound **73** was only obtained in a low yield of 12%, and **d73** was the major diastereomer (62%) formed during this reaction. In the last step of the reaction sequence, the tert-pentenyl residue of compound **73** was reduced by palladium-catalyzed hydrogenation. This reaction led to the SLF analog **74** in high yields.

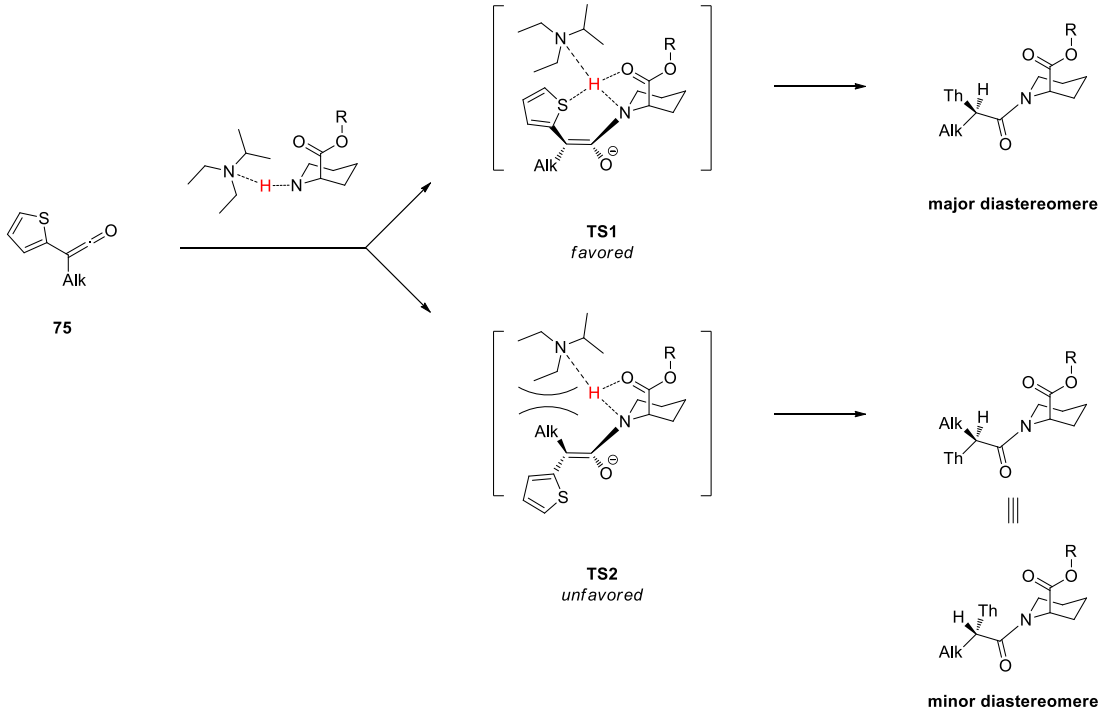
Scheme 19: Synthesis of the SLF analogs **73** and **74**.



The surprising diastereoselectivity observed during the synthesis of compound **73**/**d73** can be explained by the partial formation of the ketene **72** from the reaction of the formed acyl chloride with DIPEA, possibly because the prepared acid chloride was treated with DIPEA at 0°C before it was added to the immobilized core-/top group of SAFit1. In previous studies, Yamagami *et al.* showed that the in-situ generated ketene formation could result in highly diastereoselective esterification with (R)-pantolactone.[144] During their mechanistic studies, they also found that the diastereoselectivity observed in this reaction was highly dependent on converting the acyl chlorides into the corresponding ketenes. Moreover, they suggested that a stable hydrogen-bonding interaction between the used amine base and the formed ketene acetals results in a high rotational barrier of the conformational equilibrium resulting in a highly diastereoselective reaction. Based on these findings, the proposed mechanism of Yamagami *et al.* was applied to the amide coupling of compound **73** with the SAFi1 core-/top group (**Scheme 20**). The two possible transition states of the formed ketene hemiaminals TS1 and TS2 indicate that DIPEA and the nitrogen atom of the pipecolic core of SAFit1 can form a hydrogen-bonding interaction with the abstracted proton of the amine group. In addition, the orientation of the thienyl moiety in TS1 allows the participation of the sulfur atom of the thiophene in the proposed hydrogen-bonding interaction of the DIPEA-ketene hemiaminal complex, resulting in a higher rotational barrier for the transition of TS1 in TS2.

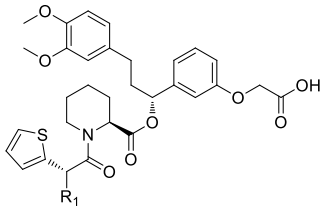
Furthermore, the bulkier tert-pentenyl group (Alk) in TS2 can reduce the rate of deprotonation of DIPEA due to sterically hindrance, and the alkenyl moiety can't participate in the hydrogen-bonding interaction resulting in a lower rotational barrier. Because TS1 is the more stable of both transition states with a higher rotational barrier, the interconversion of TS2 in TS1 is favored, resulting in the formation of larger quantities of diastereomer **d73** than **73**. Overall, the diastereomeric ratio of 2:1 during the formation of compounds **d73** and **73** can be explained by the analysis of the proposed transition states and by the partial formation of ketene **75** during this reaction. Moreover, the observed stereoselective addition of thiophene containing ketene **75** could allow a potential route for synthesizing enantiomeric pure thiophene-containing bottom groups by diastereoselective esterification of the racemic bottom groups with chiral alcohols similar to the work of Yamagami *et al.*, which is used in an industrial scale.[145]

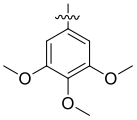
Scheme 20: Proposed mechanism for the diastereoselective amid coupling of **72** to the SAFit1 core-/top group **8**.



The binding affinities for FKBP51 and FKBP52 ligands **73** and **74** were determined by FP assay. The results were then compared to compound **59a** to evaluate the influence of the replacement of the 3,4,5-trimethoxyphenyl residue in terms of binding affinities compared to the tert-pentyl group of compound **74** (**Table 13**).

Table 13: Overview of the determined binding affinities of SLF analogs **59a**, **73**, and **74**.



Entry	X	FKBP51 K_i [nM]	FKBP52 K_i [nM]
59a		103 ± 12	10171 ± 923
73		329 ± 29	>10000
74		270 ± 33	>10000

Comparing the binding affinities of compounds **59a**, **73**, and **74** reveals that the 3,4,5-trimethoxyphenyl residue in the R_1 position of compound **59a** has the best binding affinity for FKBP51 with 103 nM when a 2-thienyl moiety is present in the R_2 position. In contrast, introducing a tert-pentenyl group (**73**) or tert-pentyl group (**74**) results in a 3-fold decrease in binding affinity for FKBP51, but both compounds show selectivity towards FKBP51. Unfortunately, the replacement of the 3,4,5-trimethoxyphenyl group by a tert-pentyl didn't show the hypothesized trend analogous to their unselective counterparts.

To further understand how the replacement of the 3,4,5-trimehoxyphenyl group with a tert-pentyl group influences the binding mode, a high-resolution co-crystal structure of **73** in complex with the FK1 domain of FKBP51 was obtained (**Figure 30A**). As expected, the thiophene group again binds to the transient binding pocket, which is crucial for selectivity over FKBP52. Additionally, the tert-pentenyl group is located in a similar position as the 3,4,5-trimethoxyphenyl group. The superposition of the bound ligands **73** and SAFit1 reveals that the top group ring bearing the carboxylic acid is forced out of its previous position, reducing the ability of the ligand to interact with the protein (**Figure 30B**). One reason for this observation is a possible clash of the top group section with the tert-pentenyl group probably causes the conformational change of the top group of **73**. This would align well with the observed 3-fold reduction in binding affinity for FKBP51. In conclusion, introducing a tert-pentyl group in R_1 is tolerated in the context of selective FKBP51 inhibitors. However, further reduction and optimization of the SAFit 1 top group is required.

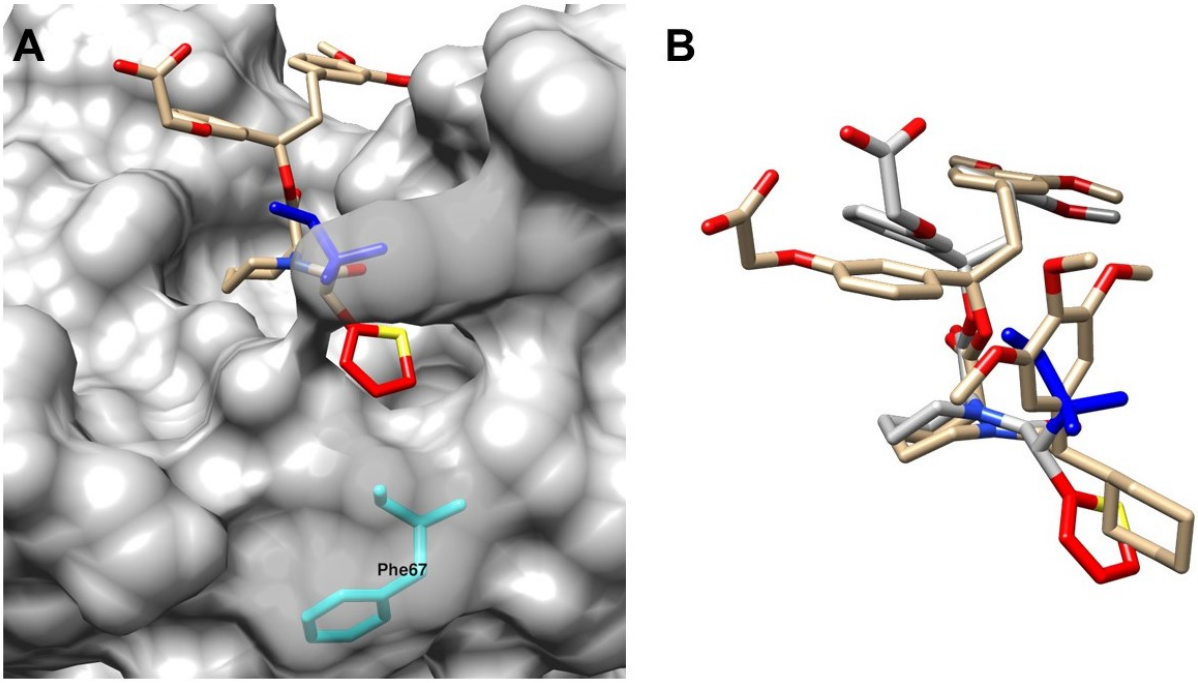
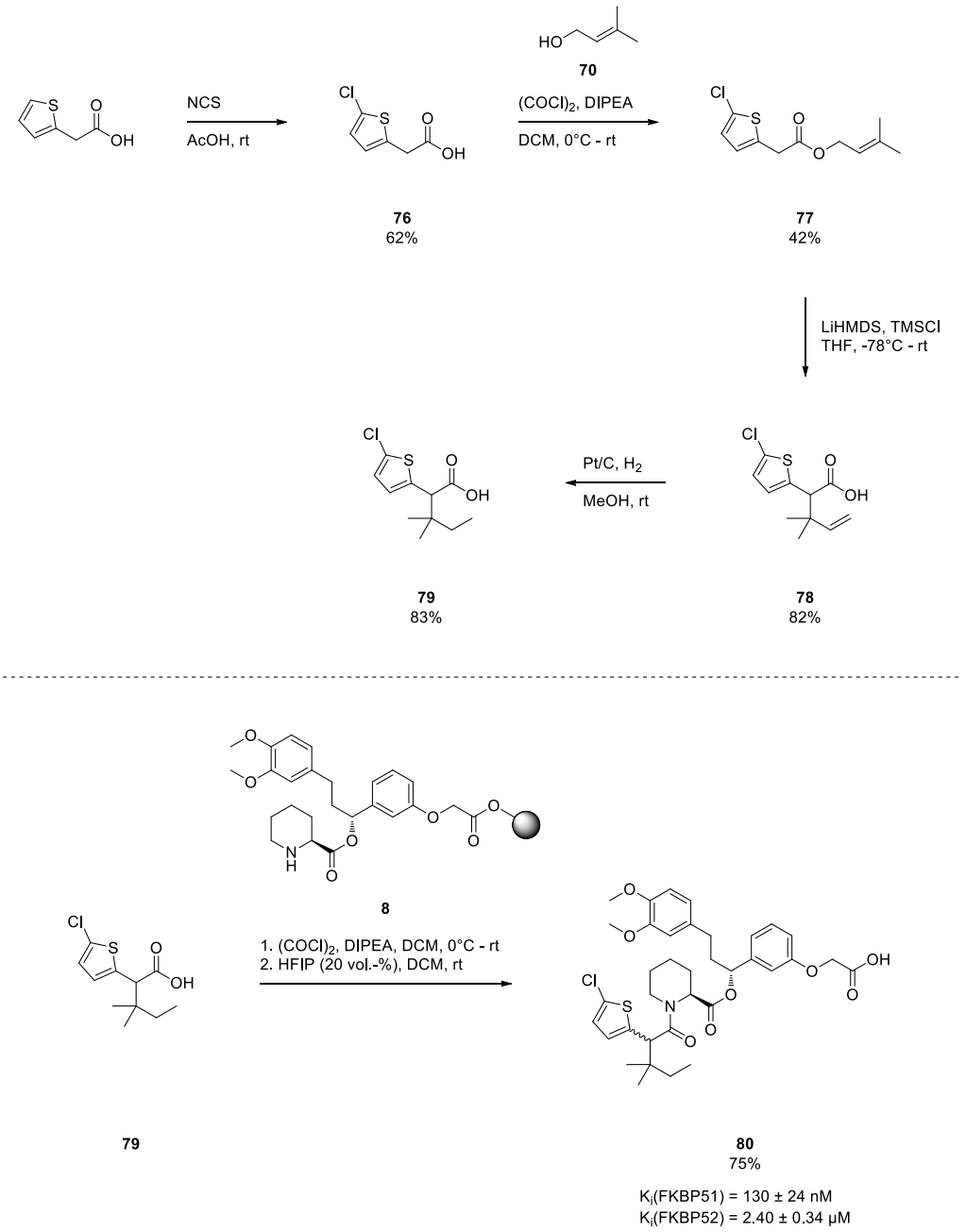


Figure 30: Co-crystal structure of compound **73** (sticks) in complex with the FK1 domain of FKBP51 (Phe^{67} highlighted in cyan). The thien-2-yl moiety is shown in red, the tertpentyl moiety is in blue, and the rest of the ligand is in pale orange. (A) The co-crystal structure of compound **73** in complex with the FK1 domain of FKBP51. (B) Superposition of compound **73** (grey sticks) with SAFit1 (pale orange).

Because the substitution of the 2-thienyl group in the 5-position increased the binding affinity significantly in previous SAR studies, the influence of the chlorination in the 5-position of the 2-thienyl moiety was also investigated here (**Scheme 21**). Unfortunately, selective late-stage chlorination of ligand **74** is not possible because the chlorination would take place at the dimethoxyphenyl ring of the top group. Therefore, the 2-thienylacetic acid was first chlorinated using NCS and was then esterified with alcohol **70** to form compound **76**. After the Claisen-Ireland rearrangement of **76** and subsequent reduction of the alkene, the tert-pentyl containing bottom group **79** was obtained in an overall yield of 18% over 4 steps.

Finally, the bottom group **79** was coupled to the immobilized SAFit1 core-/top group **8**, and compound **80** was cleaved off the solid support. After coupling **79** to the SAFit1 core-/top group, only a mixture of diastereomers of compound **80** was obtained, which was inseparable via preparative HPLC even after extensive optimization. The analysis of the ^{1}H NMR of **80** showed that the ratio between diastereomers is 1:1, and no stereoselectivity was observed in contrast to the amide coupling reaction of compound **72**. This indicates that no ketene formation occurred during the amide coupling of **79**, which results in a 1:1 ratio of **80**.

Scheme 21: Synthesis and determined binding affinities of SLF analog **80**.



Nevertheless, the diastereomeric mixture of compound **80** was tested via FP assay for the binding affinity for FKBP51 and FKBP51. The determined Ki value for FKBP51 increases by 2-fold upon chlorination of the thienyl moiety in the 5-position but is still significantly lower than the best selective FKBP51 ligands.

In conclusion, this study showed that the Claisen-Ireland rearrangement is a suitable synthetic approach for synthesizing tert-pentenyl and tert-pentyl-containing bottom groups. In general, these bottom groups are tolerated by FKBP51 but disrupt the hydrophobic interaction of the top group, resulting in a lower binding affinity for FKBP51.

10 Conclusion

In this thesis, various approaches for synthesizing bottom group analogs have been explored, as well as the structure affinity relationship of the replacement of the 3,4,5-trimethoxyphenyl group of SAFit1 and SAFit2. In addition, a novel scaffold for inducing selectivity over FKBP52 has been identified by testing diastereomeric mixtures of SAFit1 analogs. Further exploration of the structure-affinity relationship of these novel thiophene-containing bottom groups revealed that substitution of the 5-position enhances the binding affinity for FKBP51. All obtained thiophene-containing SAFit analogs showed high selectivity and moderate to excellent binding affinities for FKBP51.

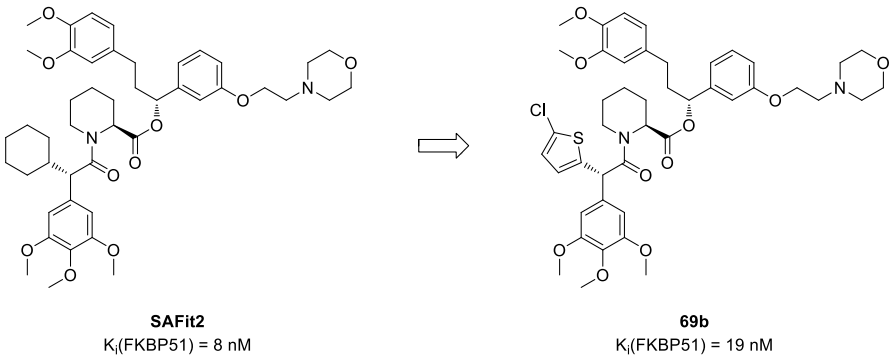


Figure 31: Chemical structures of SAFit2 and the most promising SAFit2 analog **69b**.

Additionally, the most promising tool, compound **69b,** was synthesized, and a Snapshot PK study in mice determined the pharmacokinetic properties. The results of this study indicate that the novel SAFit2 analogs **69b** has an acceptable PK profile, with slightly better properties than SAFit2. This led to the assumption that the 5-chlorothiophenes are suitable replacements for the cyclohexyl moiety, which is crucial for the selectivity over FKBP52 in SAFit ligands. In conclusion, over 30 novel SAFit bottom groups have been synthesized and characterized by FP assay. Furthermore, for some promising compounds, high-resolution co-crystal structures were obtained. In the future, further lead optimization of the tool compound **69b** should focus on reducing the molecular weight of the ligand.

11 Experimental section

11.1 General information

Solvents and reagents

All reagents were purchased from ABCR, Sigma Aldrich, Novabiochem, Carl Roth, Fluorochem, BLD Pharm, TCI and were used without further purification. Dry solvents have been purchased from Acros Organics and Sigma Aldrich and were used without further purification.

Reactions

All reactions have been conducted under an argon atmosphere and with dry solvents unless water was present in the reaction mixture.

Thin-layer chromatography (TLC)

Aluminum plates coated with silica 60 F_{254nm} were used for analytical chromatography (Merck). The compound spots were visualized by UV light and/or by staining the TLC plate with Hanessian (5 g $Ce(SO_4)_2$, 25 g $NH_4Mo_7O_{24} \cdot 4\ H_2O$ in 40 mL H_2O and 50 mL H_2SO_4), Ninhydrin (0.5 g Ninhydrin in 100 mL EtOH and 5 mL AcOH), or Permanganate stain (1.5 g $KMnO_4$, 10 g K_2CO_3, 1.25 mL 10% NaOH in 200 mL H_2O). The calculated R_f values and used eluents are described in every procedure.

Column chromatography

Normal phase column chromatography was performed either by manual flash chromatography on silica (SiO2) from Macherey−Nagel (particle size 0.04−0.063 mm) or by automated flash chromatography on a Biotage Isolera.

Preparative HPLC

Reverse-phase purifications were performed with an Interchim puriFlash 5.250 system fitted with a Luna® 5 µm C18(2) 100 Å, LC column (250 x 21.2 mm). Eluents were 0.1% TFA in water (solvent A) and 0.1% TFA in acetonitrile (solvent B). The method used is described in the procedure of the specific compound. The detailed measurement parameters are outlined in the analytical section of every compound.

Nuclear magnetic resonance spectroscopy (NMR)

All ^{1}H and ^{13}C-NMR spectra have been measured at the NMR facility at the department of chemistry at Technische Universität Darmstadt (TUD). on a Bruker AC 300, AR300 or DRX500. Chemical shifts for 1H and 13C are given in ppm (δ). Deuterated chloroform ($CDCl_3$), dimethyl sulfoxide (DMSO-d6), and methanol (MeOD)were used as solvents, and the spectra were calibrated according to their corresponding peak. The multiplicities are abbreviated as follows: singlet (s), doublet (d), triplet (t), quartet (q), doublet of doublets (dd), doublet of doublets (ddd), doublet of triplets (dt), multiplet (m). Only the major peaks were reported in the case of rotamers and diastereomers for SAFit-like analogs.

Analytical HPLC/MS

Analytical LC−MS (liquid chromatography−mass spectrometry) measurements were performed on an Agilent 1260 Infinity II System consisting of a 1260 Infinity II flexible pump, a vial sampler, a multicolumn thermostat fitted with a Poroshell 120 3 mm × 150 mm, 2.7 µm EC-C18 column or a Poroshell 120 50 mm × 2.1 mm, 1.9 µm EC-C18, and a diode array detector connected to a 6125B MSD single quadrupole detector. Eluents were 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B). The detailed measurement parameters are outlined in the analytical section of every compound.

Chiral HPLC

Chiral HPLC measurements were performed on a Beckman System Gold System consisting of a Beckman System Gold 125S Solvent Module, a vial sampler, a Daicel chemical industry Ltd, Chiralcel OD-H, Normal Phase analytical column (250 × 4.6 mm, 5 µm), and a Beckman System Gold Diode Array Detector Module 168. The eluents were n-hexane (solvent A) and isopropanol (solvent B). The detailed measurement parameters are outlined in the analytical section of every compound.

High-resolution mass spectra (HRMS)

HRMS measurements were obtained by the Mass Spectrometry Department of the Technical University of Darmstadt using a Bruker Daltonics Impact II mass spectrometer (quadrupole time-of-flight).

Fluorescence polarization assay (FP assay)

The competitive fluorescence polarization assay was performed by Wisely Oki Sugiarto according to Kozany *et al.*[45] All tested compounds had a purity of >95% at 220 nm. The data was analyzed by Prism 6.0 (GraphPad Software), and the Ki values were calculated by fitting to the equation provided by Kozany *et al.*[45]

NanoBRET assay

The NanoBRET assay was performed by Thomas Geiger, following the procedure developed by Gnatzy *et al.*[93] All tested compounds had a purity of >95% at 220 nm. The data was analyzed by Prism 6.0 (GraphPad Software), and the IC_{50} values were calculated by fitting to the equation provided by Gnatzy et al.[93]

Crystallization

The co-crystallization was performed by Dr. Christian Meyners and the complexes were prepared by mixing FKBP51FK1 A19T, C103A, and C107I (14-140) at 10-20 mg/ml with a slight molar excess of ligand previously dissolved at 20 mM in DMSO. Crystallization was performed at room temperature using the hanging drop vapor-diffusion method, equilibrating mixtures of 1 µl protein complex and 1 µl reservoir against 500 µl reservoir solution. Crystals were obtained from reservoir solutions containing 16-32% PEG-3350, 0.2 M NH_4-acetate, 0.1 M HEPES-NaOH pH 7.5, and for **32a**, additionally 10% ethylene glycol. Crystals were fished, cryoprotected with 30% PEG-3350, 0.2 M NH_4-acetate, 0.1 M HEPES-NaOH pH 7.5, and 10% ethylene glycol, and flash frozen in liquid nitrogen.

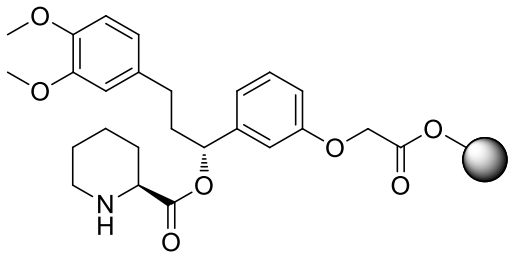
Structure solution and refinement

The crystallographic experiments were performed by Dr. Christian Meyners on the BL14.1 beamline at the Helmholtz-Zentrum BESSY II synchrotron in Berlin, Germany.[146] Diffraction data were integrated with DIALS or XDS and further processed with the implemented programs of the CCP4i and CCP4i2 interface.[147–151] The data reduction was conducted with Aimless.[150,152,153] Crystal structures were solved by molecular replacement using Phaser.[154] Iterative model improvement and refinement were performed with Coot and Refmac5.[155–160] The dictionaries for the compounds were generated wit PRODRG implemented in CCP4i.[161] Residues facing solvent channels without detectable side chain density were truncated.

11.2 General procedures

General procedure for the Fmoc-deprotection of the immobilized SAFit1 core-/top group (7)

(*R*)-3-(3,4-Dimethoxyphenyl)-1-(3-(2-methoxy-2-oxoethoxy)phenyl)propyl (*S*)-piperidine-2-carboxylate (8)



The resin **7** was weight into a syringe equipped with a filter and was swelled for 10 min with DCM. The resin was washed twice with DMF and then treated with a cold (0℃) solution of 4-methyl piperidine (20 vol.-% in DMF) for 5 min. The resin was washed twice with DMF, and the Fmoc deprotection was repeated three times. In the end, the resin was washed twice with DCM and was ready for the amide coupling.

HPLC (5 – 100 % solvent B, 3 min) R_t = 1.559 min, purity (220 nm): 99%
Mass (ESI^+): m/z: calculated 458.21 $[M+H]^+$, found 458.20$[M+H]^+$

General procedure A: HATU coupling of the bottom groups with the immobilized SAFit1 core-/top group

A 10 mL filter syringe was charged with resin **7** and was first Fmoc-deprotected following the general procedure. Then the deprotected resin **8** was treated with a preactivated solution containing the respective carboxylic acid, HATU, HOAt, and DIPEA in DMF. The resulting suspension was shaken at rt overnight. Then the resin was filtered and washed twice with DMF, followed by DCM. Finally, the product was cleaved off the resin by treating it with a solution of 20 vol.-% HFIP in DCM for 1 h. After removal of the solvent under reduced pressure and purification via preparative HPLC, the respective compounds were obtained as colorless solids.

General procedure B: Amide coupling of bottom groups with the immobilized SAFit1 core-/top group by *in-situ* generated acyl chlorides

A 10 mL filter syringe was charged with resin **7** and was first Fmoc-deprotected following the general procedure. In a flask, the respective carboxylic acid was dissolved in DCM. Then PPh_3 was added, followed by trichloroacetonitrile. The resulting solution was stirred for 1h at rt. After the complete formation of the acid chloride, the Fmoc-deprotected resin **8** was treated first with a solution of DIPEA in DCM, followed by the acid chloride solution. The resulting suspension was shaken for 1h at rt. Then the resin was filtered and washed twice with DCM. Finally, the product was cleaved off the resin by treating it with a solution of 20 vol.-% HFIP in DCM for 1 h. After removal of the solvent under reduced pressure and purification via preparative HPLC, the respective compounds were obtained as colorless solids.

General procedure C: Amide coupling of bottom groups with the immobilized SAFit1 core-/top group with acyl chlorides

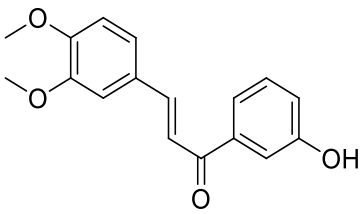
A 10 mL filter syringe was charged with resin **7** and was first Fmoc-deprotected following the general procedure. In a flask, the respective carboxylic acid was dissolved in DCM and cooled to 0°C. Then oxalyl chloride was added, and the resulting solution was stirred at rt for 1h. After the complete formation of the acid chloride, the solvent was removed under reduced pressure, and the obtained acid chloride was redissolved in DCM and cooled to 0°C. Then DIPEA was added, and the Fmoc-deprotected resin **8** was treated with the acid chloride solution. The resulting suspension was shaken for 1h at rt. Then the resin was filtered and washed twice with DCM. Finally, the product was cleaved off the resin by treating it with a solution of 20 vol.-% HFIP in DCM for 1 h. After removal of the solvent under reduced pressure and purification via preparative HPLC, the respective compounds were obtained as colorless solids.

General procedure D: Amide coupling of bottom groups with the immobilized SAFit2 core-/top group by N-methylimidazolium amides

In a 5 mL flask, the respective carboxylic acid and SAFit2 core-/top group **67** (1.10 eq.) was dissolved in MeCN (0.1 M). Then N-methylimidazole (3.50 eq.) was added, followed by TCFH (1.20 eq.). The resulting solution was then stirred at rt until completion. After the complete conversion of the carboxylic acid, the solvent was removed under reduced pressure, and the obtained crude was purified via preparative HPLC. The respective compounds were obtained as colorless solids.

11.3 Synthesis of the immobilized SAFit1 core-/top group

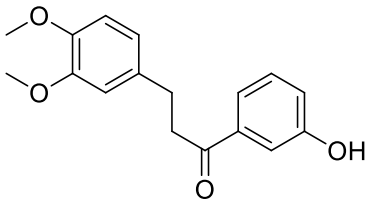
3-(3,4-Dimethoxyphenyl)-1-(3-hydroxyphenyl)prop-2-en-1-one (1)



A 1 L flask was charged with 3,4-dimethoxybenzaldehyde (22.00 g, 132 mmol, 1.00 eq.) and 3-hydroxy acetophenone (18.03 g, 132 mmol, 1.00 eq.). Then 177 mL ethanol was added, and the solution was cooled to 0°C. Afterward, KOH (34.96 g, 530 mmol, 4.00 eq.) in 110 mL water was added dropwise at this temperature. The reaction mixture was then allowed to warm to room temperature overnight. After completion of the reaction, 220 g ice was added, followed by the dropwise addition of 136 mL conc. HCl. The remaining ethanol was then removed under reduced pressure, and the aq. phase was extracted with 300 mL EtOAc three times. The combined org. layers were then washed with brine, dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The crude was then purified via column chromatography (CH/EtOAc, 0 – 50%). After the removal of the solvent, the title compound was obtained as a yellow solid.

Yield: 17.05 g (59.97 mmol, 45%)
TLC (CH/EtOAc, 1:1): R_f = 0.49
HPLC (5 – 100 % solvent B, 3 min) R_t = 1.794 min, purity (220 nm): 99%
Mass (ESI^+): m/z: calculated 228.99 $[M\text{-}OH]^+$, found 229.34 $[M\text{-}OH]^+$
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 (dd, *J* = 15.7, 1.4 Hz, 1H), 7.67 (dt, *J* = 2.9, 1.5 Hz, 1H), 7.56 (ddd, *J* = 7.7, 1.6, 1.0 Hz, 1H), 7.39 – 7.34 (m, 2H), 7.21 (dd, *J* = 8.4, 2.0 Hz, 1H), 7.14 – 7.11 (m, 1H), 7.01 (s, 1H), 6.87 (dd, *J* = 8.4, 1.3 Hz, 1H), 3.93 (s, 3H), 3.92 (s, 3H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 191.10, 156.74, 151.73, 149.35, 145.87, 139.83, 129.96, 127.85, 123.52, 120.90, 120.46, 119.96, 115.41, 111.28, 110.38, 56.13, 56.12.

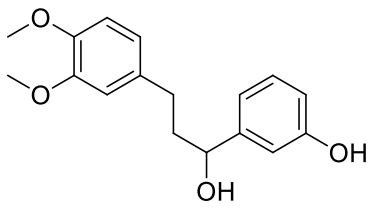
3-(3,4-Dimethoxyphenyl)-1-(3-hydroxyphenyl)propan-1-one (2)



A 1 L flask was charged with zinc dust (39.20 g, 600 mmol, 10.00 eq.), ammonium chloride (32.07 g, 600 mmol, 10.00 eq.), and 500 mL MeOH. Then a solution of the α,β-unsaturated ketone **1** (17.05 g, 60 mmol, 1.00 eq.) was added dropwise as a solution in 83 mL MeOH and 60 mL THF. After complete addition, the reaction mixture was stirred for one hour at room temperature and filtered. Finally, the title compound **2** was precipitated by the slow addition of water, filtered, and washed with water. The title compound was dried under reduced pressure and was obtained as a colorless solid.

Yield: 15.39 g (53.75 mmol, 90%)
TLC (CH/EtOAc, 1:1): R_f = 0.53
HPLC (5 – 100 % solvent B, 3 min) R_t = 1.796 min, purity (220 nm): 95%
Mass (ESI$^+$): m/z: calculated 287.12 $[M+H]^+$, found 287.20 $[M+H]^+$
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.54 (t, *J* = 2.0 Hz, 1H), 7.50 – 7.47 (m, 1H), 7.29 (t, *J* = 7.9 Hz, 1H), 7.08 (dd, *J* = 8.1, 2.5 Hz, 1H), 6.80 – 6.73 (m, 3H), 3.83 (s, 3H), 3.83 (s, 3H), 3.25 (t, *J* = 7.6 Hz, 2H), 2.99 (t, *J* = 7.6 Hz, 2H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 200.55, 156.68, 148.88, 147.40, 138.18, 133.81, 129.98, 120.83, 120.53, 120.32, 114.72, 111.99, 111.53, 56.02, 55.91, 40.87, 29.94.

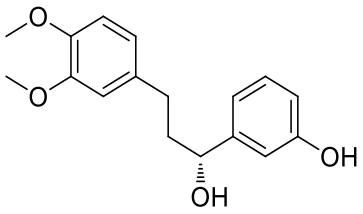
3-(3-(3,4-Dimethoxyphenyl)-1-hydroxypropyl)phenol (rac-3)



A 10 mL dried flask was charged with **2** (151 mg, 0.53 mmol, 1.00 eq.), and 5 mL dry THF was added. Then $NaBH_4$ (20 mg, 0.55 mmol, 1.05 eq.) was added, and the solution was stirred at rt overnight. The reaction mixture was quenched by adding 5 mL 1 M HCl and was then extracted with 10 mL EtOAc three times. The combined org. layers were then washed with brine, dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The crude product was then purified by column chromatography (CH/EtOAc, 0 – 50%). After the removal of the solvent, the title compound was obtained as a colorless solid.

Yield: 132 mg (0.46 mmol, 87%)
TLC (CH/EtOAc, 1:1): R_f = 0.42
HPLC (5 – 100 % solvent B, 3 min) R_t = 1.710 min, purity (220 nm): 98%
Chiral HPLC (n-heptane/iPrOH 20 % B, 20 min) $R_{t,1}$ = 13.83 min, $R_{t,2}$ = 15.05 min, e.r. = 1:1
Mass (ESI^+): m/z: calculated 311.14 $[M+Na]^+$, found 311.00 $[M+Na]^+$
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.17 (t, J = 7.8 Hz, 1H), 6.86 (t, J = 2.0 Hz, 1H), 6.83 (dt, J = 7.6, 1.3 Hz, 1H), 6.77 – 6.72 (m, 2H), 6.71 – 6.67 (m, 2H), 4.61 (dd, J = 7.8, 5.3 Hz, 1H), 3.83 (s, 3H), 3.82 (s, 3H), 2.70 – 2.54 (m, 2H), 2.12 – 1.92 (m, 2H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 156.39, 148.95, 147.32, 146.34, 134.48, 129.81, 120.39, 118.17, 114.87, 112.97, 112.06, 111.52, 73.94, 55.97, 40.47, 31.70.

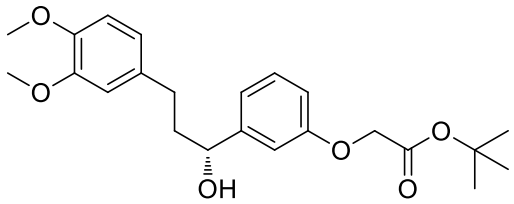
(R)-3-(3-(3,4-Dimethoxyphenyl)-1-hydroxypropyl)phenol (3)



A mixture of **2** (5.01 g, 17.50 mmol, 1.00 eq.), potassium tert-butoxide (2.34 g, 21.01 mmol, 1.20 eq.), and 175 mL of isopropanol was degassed for 15 min with argon. Then the catalyst was added, and the resulting solution was degassed for an additional 15 min. Finally, 1 atm of hydrogen was applied, and the reaction mixture was stirred overnight. After the conversion of ketone **2**, the reaction mixture was filtered through a pad of silica, and the solvent was removed under reduced pressure. The title compound was obtained as a brownish oil and was used without further purification.

Yield: 4.97 g (17.23 mmol, 98%)
TLC (CH/EtOAc, 1:1): R_f = 0.41
HPLC (5 – 100 % solvent B, 3 min) R_t = 1.683 min, purity (220 nm): 99%
Chiral HPLC (n-heptane/iPrOH 20 % B, 20 min) $R_{t,1}$ = 13.73 min, $R_{t,2}$ = 15.08 min, e.r. = 99:1
Mass (ESI^+): m/z: calculated 311.13 $[M+Na]^+$, found 311.20 $[M+Na]^+$
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.17 (t, J = 7.8 Hz, 1H), 6.86 (t, J = 2.0 Hz, 1H), 6.83 (dt, J = 7.6, 1.3 Hz, 1H), 6.77 – 6.72 (m, 2H), 6.71 – 6.67 (m, 2H), 4.61 (dd, J = 7.8, 5.3 Hz, 1H), 3.83 (s, 3H), 3.82 (s, 3H), 2.70 – 2.54 (m, 2H), 2.12 – 1.92 (m, 2H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 156.39, 148.95, 147.32, 146.34, 134.48, 129.81, 120.39, 118.17, 114.87, 112.97, 112.06, 111.52, 73.94, 55.97, 40.47, 31.70.

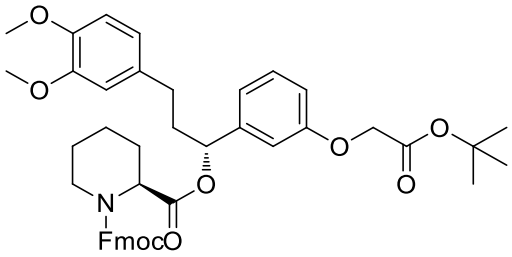
Tert-butyl (R)-2-(3-(3-(3,4-dimethoxyphenyl)-1-hydroxypropyl)phenoxy)-acetate (4)



A 250 mL flask was charged with **3** (4.08 g, 14.16 mmol, 1.00 eq.), potassium carbonate (2.94 g, 21.24 mmol, 1.50 eq.), and 60 mL dry acetonitrile. The resulting suspension was stirred for 10 min at rt before adding tert-butyl bromoacetate (2.30 mL, 15.58 mmol, 1.10 eq.). The resulting mixture was then stirred overnight. After the complete conversion of the starting material, the reaction was diluted with acetonitrile, filtered, and the solvent was removed under reduced pressure. The crude product was then purified by column chromatography (CH/EtOAc, 0 – 30 %). The title compound was obtained as a yellowish oil.

Yield: 5.01 g (12.45 mmol, 88%)
TLC (CH/EtOAc, 3:1): R_f = 0.17
HPLC (30 – 100 % solvent B, 3 min) R_t = 1.765 min, purity (220 nm): 96%
Mass (ESI$^+$): m/z: calculated 425.20 $[M+Na]^+$, found 425.00 $[M+Na]^+$
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.25 (t, *J* = 8.2 Hz, 1H), 6.99 – 6.88 (m, 2H), 6.83 – 6.76 (m, 2H), 6.75 – 6.70 (m, 2H), 4.65 (dd, *J* = 7.7, 5.3 Hz, 1H), 4.50 (s, 2H), 3.85 (s, 3H), 3.85 (s, 3H), 2.64 (tdd, *J* = 14.2, 11.3, 7.4 Hz, 2H), 2.31 (d, *J* = 16.0 Hz, 1H), 2.13 – 1.95 (m, 2H), 1.49 (s, 9H).
^{13}C NMR (75 MHz, $CDCl_3$) δ 168.06, 158.12, 148.87, 147.21, 146.60, 134.45, 129.54, 120.24, 119.13, 113.59, 112.23, 111.84, 111.35, 82.39, 73.62, 65.67, 55.96, 55.85, 40.67, 31.63, 28.08.

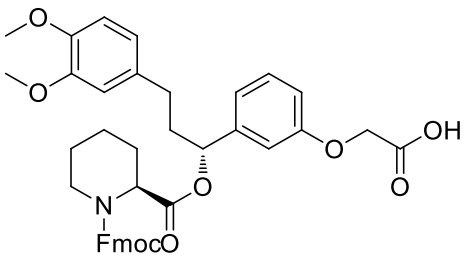
1-((9*H*-fluoren-9-yl)methyl) 2-((*R*)-1-(3-(2-(*tert*-butoxy)-2-oxoethoxy)phenyl)-3-(3,4-dimethoxyphenyl)propyl) (*S*)-piperidine-1,2-dicarboxylate (5)



A mixture of **4** (4.97 g, 12.35 mmol, 1.00 eq.), (S)-*N*-Fmoc-piperidine-2-carboxylic acid (4.78 g, 13.59 mmol, 1.10 eq.), DMAP (0.15 g, 1.24 mmol, 0.10 eq.) and 62 mL dry DCM was cooled to 0°C. Then small portions of EDC·HCl (2.84 g, 14.82 mmol, 1.20 eq.) were added over 10 min. The reaction mixture was then stirred for an additional 15 min at this temperature, and then the ice bath was removed. After 90 min, the reaction mixture was diluted with 150 mL DCM, washed with 1 M HCl (200 mL), and the aq. phase was extracted three times with 50 mL DCM. The combined org. layers were then dried over $MgSO_4$, and the solvent was removed under reduced pressure. The crude was then purified by column chromatography (CH/EtOAc, 0 – 25%), and the solvent was removed to obtain compound **5** as a colorless solid. (8.16 g, 11.09 mmol, 90 %).

Yield: 8.16 g (11.09 mmol, 90%)
TLC (CH/EtOAc, 3:1): R_f = 0.27
HPLC (50 – 100 % solvent B, 3 min) R_t = 2.273 min, purity (220 nm): 97%
Mass (ESI$^+$): m/z: calculated 758.34 $[M+Na]^+$, found 758.00 $[M+Na]^+$
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.81 – 7.71 (m, 2H), 7.61 (t, *J* = 8.9 Hz, 1H), 7.48 (dd, *J* = 21.4, 7.6 Hz, 1H), 7.42 – 7.15 (m, 5H), 7.00 – 6.88 (m, 2H), 6.79 (dd, *J* = 36.1, 8.4 Hz, 2H), 6.70 – 6.56 (m, 2H), 5.86 – 5.73 (m, 1H), 4.97 (dd, *J* = 74.2, 5.5 Hz, 1H), 4.55 – 4.28 (m, 4H), 4.21 – 4.07 (m, 2H), 3.85 (s, 6H), 3.21 – 2.95 (m, 1H), 2.62 – 2.42 (m, 2H), 2.34 (q, *J* = 9.8, 7.5 Hz, 1H), 2.24 – 2.18 (m, 1H), 2.08 – 1.99 (m, 1H), 1.77 – 1.66 (m, 3H), 1.48 (s, 9H), 1.33 – 1.24 (m, 2H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 171.01, 167.96, 158.12, 156.46, 148.91, 147.34, 144.18, 143.95, 141.78, 141.35, 133.54, 129.74, 127.75, 127.12, 125.16, 120.16, 120.03, 119.99, 114.01, 113.30, 111.74, 82.41, 76.24, 67.85, 65.81, 55.97, 55.86, 47.29, 38.13, 31.20, 28.09, 26.90, 24.86, 20.90, 14.28.

2-(3-((*R*)-1-(((*S*)-1-(((9*H*-fluoren-9-yl)methoxy)carbonyl)piperidine-2-carbonyl)oxy)-3-(3,4-dimethoxyphenyl)propyl)phenoxy)acetic acid (6)



A 100 mL flask was charged with **5** (8.06 g, 10.95 mmol, 1.00 eq.) and 38.5 mL dry DCM. The solution was cooled to 0℃, and 16.5 mL TFA was added dropwise. After complete addition, the ice bath was removed, and the reaction mixture was stirred for 3 h at rt. Finally, the solvent was removed, and the crude product was purified by column chromatography (CH/EtOAc + 1% FA, 0 – 50%). After the removal of the solvent, the title compound **6** was obtained as a colorless solid.

Yield: 6.15 g (9.05 mmol, 83%)

TLC (CH/EtOAc + 1% FA, 1:1): R_f = 0.29

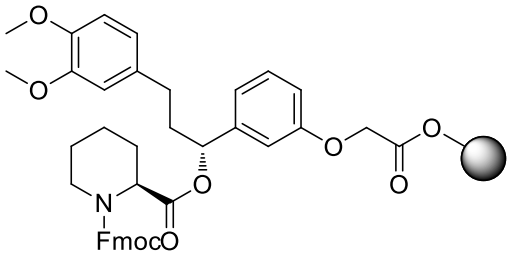
HPLC (50 – 100 % solvent B, 3 min) R_t = 1.838 min, purity (220 nm): 99%

Mass (ESI$^+$): m/z: calculated 702.28 $[M+Na]^+$, found 702.00 $[M+Na]^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.79 – 7.69 (m, 2H), 7.59 (d, *J* = 8.3 Hz, 1H), 7.51 – 7.43 (m, 1H), 7.44 – 7.18 (m, 5H), 7.01 – 6.88 (m, 2H), 6.87 (d, *J* = 10.5 Hz, 1H), 6.77 (t, *J* = 9.5 Hz, 1H), 6.69 (d, *J* = 8.6 Hz, 1H), 6.64 – 6.56 (m, 1H), 5.81 – 5.68 (m, 1H), 5.13 – 4.90 (m, 1H), 4.72 – 4.58 (m, 2H), 4.54 (s, 1H), 4.49 – 4.36 (m, 2H), 4.27 (t, *J* = 7.2 Hz, 1H), 4.19 – 4.03 (m, 2H), 3.85 (s, 6H), 3.27 – 3.13 (m, 1H), 2.69 – 2.42 (m, 2H), 2.34 (dd, *J* = 24.6, 13.4 Hz, 1H), 2.24 – 2.13 (m, 1H), 2.11 – 1.97 (m, 2H), 1.85 – 1.62 (m, 3H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 172.27, 170.81, 164.10, 157.91, 147.40, 143.99, 143.76, 142.13, 141.34, 141.32, 133.46, 129.85, 127.77, 127.13, 125.10, 120.24, 120.03, 114.91, 111.76, 111.53, 111.42, 76.42, 68.16, 65.18, 60.60, 55.98, 55.90, 47.18, 42.02, 38.15, 31.37, 27.01, 24.78, 20.83.

Immobilization of 2-(3-((*R*)-1-(((*S*)-1-(((9*H*-fluoren-9-yl)methoxy)carbonyl)piperidine-2-carbonyl)-oxy)-3-(3,4-dimethoxyphenyl)propyl)phenoxy)acetic acid (7)



A 250 mL dried flask was charged with 2-chlorotrityl resin (9.91 g, 11.71 mmol, 2.00 eq., loading = 1.181 mmol/g) and 60 mL dry DCM. The resin was allowed to swell for 10 min before compound **6** (3.98 g, 5.86 mmol, 1.00 eq.) was added dropwise in 20 mL dry DCM. Finally, 5.10 mL DIPEA (29.28 mmol, 5.00 eq.) was added, and the resulting slurry was stirred at rt overnight. After complete immobilization of the starting material, 2 mL of dry methanol was added, and the solution was stirred for 1 h to cap the resin. Then the resin was filtered through a silica frit and was washed three times with 50 mL DCM. The obtained resin was dried under vacuum, and the loading of compound **7** was calculated using the following formula:

$$\frac{(m_{total} - m_{resin}) \cdot 10^3}{\left(MW_{compound\ 6} - MW_{HCl}\right) \cdot m_{total}} = loading\ \left[\frac{mmol}{g}\right]$$

Because different batches of this compound were used during the synthesis of SAFit1 analogs, the loading of compound **7** is shown in every procedure of the amide coupling.

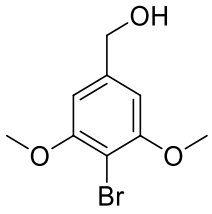
Analytics for the micro cleavage of an aliquot:

HPLC (50 – 100 % solvent B, 3 min) R_t = 1.838 min, purity (220 nm): 99%

Mass (ESI^+): m/z: calculated 702.28 $[M+Na]^+$, found 702.00 $[M+Na]^+$

11.4 Synthesis of the para bromo and para chloro containing SAFit bottom group

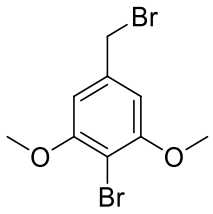
(4-Bromo-3,5-dimethoxyphenyl)methanol (10)



In a dried 250 mL flask, 4-brom-3,5-dimethoxybenzoic acid (**9**, 20 g, 76.61 mmol, 1.00 eq.) was dissolved in 153 mL dry THF. Then 76.61 mL of a 2 M $BH_3 \cdot SM_2$ solution in THF was added dropwise. After complete addition, the reaction was stirred at room temperature overnight. The reaction mixture was quenched by carefully adding 1 M HCl and was extracted three times with ethyl acetate. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. Compound **10** was obtained as a colorless solid.

Yield: 18.38 g (74.39 mmol, 97%)
TLC (CH/EtOAc, 3:1): R_f = 0.41
HPLC (5 – 100 % solvent B, 20 min) R_t = 10.683 min, purity (220 nm): 99%
Mass (ESI^+): m/z: calculated 228.99 $[M\text{-}OH]^+$, found 229.34 $[M\text{-}OH]^+$
^{1}H NMR (500 MHz, $CDCl_3$) δ 6.58 (s, 2H), 4.66 (s, 2H), 3.89 (s, 6H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 157.31, 141.85, 103.18, 99.88, 77.41, 77.16, 76.91, 65.30, 56.61.

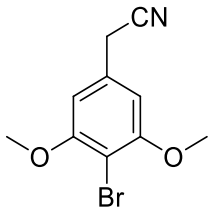
2-Bromo-5-(bromomethyl)-1,3-dimethoxybenzene (11)



In a 250 mL flask, **10** (18.38 g, 74.39 mmol, 1.00 eq.) was dissolved in 150 mL toluene under an argon atmosphere. Then 7.77 mL phosphorous tribromide (22.14 g, 74.39 mmol, 1.00 eq.) was added dropwise to the solution. The reaction was stirred for 7 h at room temperature and quenched by adding 100 mL of ice water. The mixture was transferred to a separatory funnel, and the organic layer was washed two times with water. The organic phase was dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The crude was then filtered over a silica plug using CH as the eluent, and the solvent was removed. The title compound **11** was obtained as a colorless oil.

Yield: 12.19 g (39.33 mmol, 53%)
TLC (CH): R_f = 0.44
HPLC (5 – 100 % solvent B, 20 min) R_t = 13.850 min, purity (220 nm): 92%
Mass (ESI^+): m/z: calculated 228.99 $[M-Br]^+$, found 229.30 $[M-Br]^+$
^{1}H NMR (300 MHz, $CDCl_3$) δ 6.60 (s, 2H), 4.45 (s, 2H), 3.91 (s, 6H).
^{13}C NMR (76 MHz, $CDCl_3$) δ 157.35, 138.37, 107.13, 105.57, 101.35, 77.58, 77.16, 76.74, 56.67, 33.48.

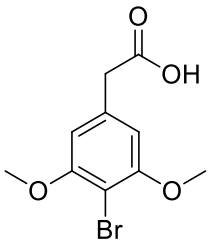
2-(4-Bromo-3,5-dimethoxyphenyl)acetonitrile (12)



A 250 mL flask was charged with **11** (12.19 g, 39.33 mmol, 1.00 eq.), acetone cyanohydrin (4.69 g, 55.11 mmol, 1.40 eq.), and 100 mL acetonitrile. Then a solution of DBU (6.45 mL, 43.22 mmol, 1.10 eq.) in 55 mL acetonitrile is added dropwise at 0°C over the course of 1h. After complete addition, the ice bath was removed, and the reaction mixture was stirred at room temperature overnight. The solvent was removed under reduced pressure, and the residue was taken up in 200 mL diethyl ether and washed with 100 mL water. The aq. phase was then extracted with 100 mL diethyl ether three times. The combined org. layers were then washed with brine, dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The title compound was obtained as a colorless solid.

Yield: 9.42 g (36.78 mmol, 94%)
TLC (CH/EtOAc, 3:1): R_f = 0.29
HPLC (5 – 100 % solvent B, 20 min) R_t = 14.267 min, purity (220 nm): 90%
^{1}H NMR (300 MHz, $CDCl_3$) δ 6.52 (s, 1H), 3.91 (s, 4H), 3.73 (s, 1H).
^{13}C NMR (76 MHz, $CDCl_3$) δ 157.58, 130.41, 117.29, 104.40, 100.71, 56.59, 23.96.

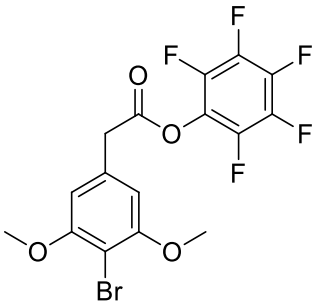
2-(4-Bromo-3,5-dimethoxyphenyl)acetic acid (13)



A 250 mL flask was charged with **12** (9.42 g, 36.78 mmol, 1.00 eq.), KOH (24.15 g, 43.04 mmol, 11.70 eq.), and 117 mL methanol/H_2O/dioxane (3:2.5:3). The resulting mixture was then heated to 110°C overnight. After cooling to rt, the reaction mixture was diluted with ice-cold water and acidified using conc. HCl. The aq. phase was then extracted three times with 200 mL EtOAc and the combined org. phases were washed with brine, dried over $MgSO_4$, and the solvent was removed under reduced pressure. Compound **13** was obtained as an orange solid after recrystallization from ethanol.

Yield: 7.94 g (28.86 mmol, 78%)
TLC (CH/EtOAc + 1% FA, 1:1): R_f = 0.48
HPLC (5 – 100 % solvent B, 20 min) R_t = 13.483 min, purity (220 nm): 95%
Mass (ESI$^+$): m/z: calculated 274.98 $[M+H]^+$, found 275.19 $[M+H]^+$
^{1}H NMR (300 MHz, $CDCl_3$) δ 6.50 (s, 2H), 3.89 (s, 6H), 3.61 (s, 2H).
^{13}C NMR (76 MHz, $CDCl_3$) δ 176.77, 157.29, 133.88, 106.15, 100.28, 56.64, 41.47.

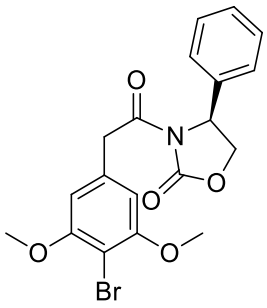
Perfluorophenyl 2-(4-bromo-3,5-dimethoxyphenyl)acetate (14)



A 100 mL flask was charged with **13** (3.97 g, 14.45 mmol, 1.00 eq.), pentafluorophenol (3.05 g, 15.89 mmol, 1.10 eq.), DMAP (0.53 g, 4.33 mmol, 0.30 eq.) and 30 mL DCM. The solution was cooled to 0°C, and EDC·HCl (2.93 g, 15.89 mmol, 1.10 eq.) was added in small portions. After complete addition, the ice bath was removed, and the reaction mixture was stirred overnight at rt. The reaction mixture was quenched by adding brine and extracted three times with DCM. The combined org. layers were then washed with 1 M HCl, dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The crude was then purified by column chromatography (CH/EtOAc, 5:1). The title compound was obtained as a colorless oil.

Yield: 3.84 g (8.70 mmol, 60%)
TLC (CH/EtOAc, 5:1): R_f = 0.38
HPLC (50 – 100 % solvent B, 20 min) R_t = 9.144 min, purity (220 nm): 99%
^{1}H NMR (500 MHz, $CDCl_3$) δ 6.56 (s, 2H), 3.93 (s, 2H), 3.91 (s, 6H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 167.04, 157.51, 142.27, 140.30, 140.17, 139.05, 137.01, 132.64, 105.84, 100.69, 56.65, 40.59.

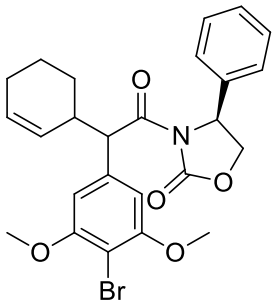
(S)-3-(2-(4-Bromo-3,5-dimethoxyphenyl)acetyl)-4-phenyloxazolidin-2-one (15)



A dried 250 mL flask was charged with (S)-phenyl-oxazolidone (1.70 g, 10.44 mmol, 1.20 eq.) and 44 mL dry THF. The resulting solution was then cooled to -78°C, and 10.44 mL NaHMDS (1 M in THF, 10.44 mmol, 1.20 eq.) was added dropwise. The resulting slurry was stirred for 1 h at this temperature before **14** (3.84 g, 8.69 mmol, 1.00 eq.) was added dropwise in 40 mL THF. After complete addition, the reaction mixture was allowed to warm to rt overnight. The reaction was quenched by the addition of sat. NH_4Cl solution and was extracted three times with DCM. The combined org. layers were then washed with brine, dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The crude was then purified by column chromatography (CH/EtOAc, 3:1). The title compound was obtained as a colorless solid.

Yield: 2.39 g (5.69 mmol, 65%)
TLC (CH/EtOAc, 2:1): R_f = 0.22
HPLC (50 – 100 % solvent B, 20 min) R_t = 7.000 min, purity (220 nm): 95%
Mass (ESI^+): m/z: calculated 420.04 $[M+H]^+$, found 420.08 $[M+H]^+$
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34 – 7.31 (m, 3H), 7.19 – 7.15 (m, 2H), 6.46 (s, 2H), 5.43 (dd, J = 8.8, 4.0 Hz, 1H), 4.72 – 4.66 (m, 1H), 4.32 (d, J = 14.6 Hz, 1H), 4.26 (ddd, J = 9.0, 4.1, 0.7 Hz, 1H), 4.16 (d, J = 14.6 Hz, 1H), 3.80 (s, 6H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 170.05, 157.11, 153.65, 138.72, 133.90, 129.26, 128.93, 126.14, 106.37, 99.95, 70.02, 57.90, 56.56, 41.91.

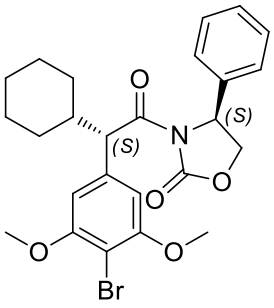
(4S)-3-(2-(4-Bromo-3,5-dimethoxyphenyl)-2-(cyclohex-2-en-1-yl)acetyl)-4-phenyl-oxazolidin-2-one (16)



In a 100 mL dried flask, **15** (2.25 g, 5.35 mmol, 1.00 eq.) was dissolved in 27 mL THF, and the solution was cooled to -78°C. Then 6.44 mL NaHMDS (1 M in THF, 6.44 mmol, 1.20 eq.) was added dropwise, and the resulting solution was stirred for 1h at -78°C. Then 1.25 mL 3-bromcyclohexene (10.70 mmol, 2.00 eq.) was added, and the solution was stirred for 1h at -78°C before it was allowed to warm to rt overnight. The reaction mixture was quenched by the addition of sat. NH_4Cl solution and was extracted with diethyl ether. The combined org. layers were then washed with brine, dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The crude was then purified by column chromatography (CH/EtOAc, 5:1). The title compound was obtained as a colorless solid.

Yield: 1.80 g (3.61 mmol, 67%)
TLC (CH/EtOAc, 5:1): R_f = 0.28
HPLC (60 – 80 % solvent B, 20 min) $R_{t,d1}$ = 9.418 min, $R_{t,d2}$ = 10.441 min, purity (220 nm): 99%
Mass (ESI^+): m/z: calculated 500.10 $[M+H]^+$, found 500.23 $[M+H]^+$
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35 – 7.24 (m, 5H), 6.61 (s, 2H), 5.29 (dd, J = 8.8, 3.8 Hz, 1H), 4.80 (td, J = 26.9, 11.2 Hz, 1H), 4.58 – 4.47 (m, 1H), 4.13 (dd, J = 9.0, 3.8 Hz, 1H), 3.80 (s, 6H), 1.58 (dddq, J = 10.7, 8.2, 5.5, 2.7 Hz, 1H), 1.40 – 0.97 (m, 7H), 0.84 – 0.73 (m, 1H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 173.02, 171.80, 156.95, 153.33, 139.18, 137.81, 129.10, 128.78, 126.03, 105.68, 99.94, 69.48, 58.11, 56.52, 53.78, 39.44, 26.08, 25.21, 20.38.

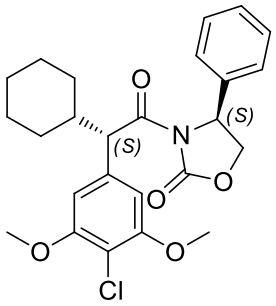
(S)-3-((S)-2-(4-Bromo-3,5-dimethoxyphenyl)-2-cyclohexylacetyl)-4-phenyloxazolidin-2-one (17a)



In a 100 mL flask, **16** (1050 mg, 2.09 mmol, 1.00 eq.) was dissolved in 42 mL methanol. The solution was then degassed for 10 min with argon and platinum on carbon (41 mg, 0.02 mmol, 1 mol-%, 10 wt.-%) was added. Afterward, the suspension was flushed with hydrogen for 10 min before 1 atm of hydrogen was applied. The suspension was then stirred for 3 h before it was filtered through a short plug of silica. After the solvent was removed, the crude was purified by column chromatography (DCM/CH, 3:1). The title compound was obtained as a colorless solid.

Yield: 480 mg (0.96 mmol, 46%)
TLC (DCM/CH, 4:1): R_f = 0.53
HPLC (60 – 80 % solvent B, 20 min) R_t = 12.179 min, purity (220 nm): 99%
Mass (ESI$^+$): m/z: calculated 502.12 $[M+H]^+$, found 502.54 $[M+H]^+$
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.46 – 7.31 (m, 5H), 6.66 (s, 2H), 5.35 (dd, J = 8.8, 3.7 Hz, 1H), 4.84 (d, J = 10.8 Hz, 1H), 4.59 (t, J = 8.9 Hz, 1H), 4.22 (dd, J = 8.9, 3.7 Hz, 1H), 3.88 (s, 6H), 2.07 – 1.95 (m, 1H), 1.62 – 1.49 (m, 3H), 1.37 – 1.20 (m, 2H), 1.15 – 1.03 (m, 3H), 0.98 – 0.71 (m, 2H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 173.58, 157.02, 153.64, 139.37, 138.33, 129.30, 128.93, 126.04, 105.87, 99.87, 69.57, 58.29, 56.66, 54.85, 42.14, 31.56, 30.31, 26.33, 25.93, 25.86.

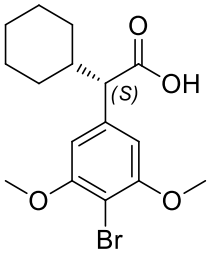
(S)-3-((S)-2-(4-Chloro-3,5-dimethoxyphenyl)-2-cyclohexylacetyl)-4-phenyloxazolidin-2-one (17b)



In a 10 mL flask, x (44 mg, 0.08 mmol, 1.00 eq.) and CuCl (10 mg, 0.09, 1.10 eq.) were dissolved in 5 mL dry DMF. The resulting solution was then refluxed for 2 days. After cooling to rt, the reaction mixture was filtered through a plug of silica, and the solvent was removed under reduced pressure. The crude was then purified by column chromatography (CH/EtOAc, 3:1). The title compound was obtained as a colorless solid.

Yield: 24 mg (0.05 mmol, 60%)
TLC (CH/EtOAc, 3:1): R_f = 0.32
HPLC (60 – 80 % solvent B, 20 min) R_t = 11.145 min, purity (220 nm): 95%
Mass (ESI^+): m/z: calculated 458.17 $[M+H]^+$, found 458.28 $[M+H]^+$
^{1}H NMR (500 MHz, Chloroform-d) δ 7.43 – 7.31 (m, 5H), 6.69 (s, 2H), 5.36 (dd, J = 8.7, 3.7 Hz, 1H), 4.84 (d, J = 10.7 Hz, 1H), 4.59 (t, J = 8.9 Hz, 1H), 4.22 (dd, J = 8.9, 3.7 Hz, 1H), 3.89 (s, 6H), 2.04 – 1.95 (m, 1H), 1.62 – 1.49 (m, 3H), 1.36 – 1.24 (m, 3H), 1.14 – 1.04 (m, 2H), 0.98 – 0.74 (m, 2H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 173.64, 156.06, 153.63, 139.39, 137.19, 129.30, 126.06, 109.77, 105.88, 69.58, 58.31, 56.59, 54.85, 42.19, 31.57, 30.33, 26.36, 25.88.

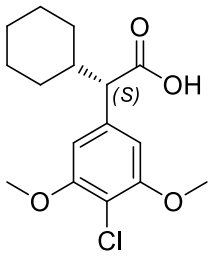
(S)-2-(4-Bromo-3,5-dimethoxyphenyl)-2-cyclohexylacetic acid (18a)



A 25 mL flask was charged with **17a** (295 mg, 0.59 mmol, 1.00 eq.), and 10 mL of THF/H_2O (8:5) was added. Then LiOH (28 mg, 1.17 mmol, 2.00 eq.) was added, followed by 0.30 mL aq. H_2O_2 (2.94 mmol, 5.00 eq., 30 wt.-% in H_2O). The resulting solution was then stirred at rt overnight. The reaction mixture was diluted by adding water and then acidified with 1 M HCl (pH < 2). The cloudy aq. layer was then extracted three times with diethyl ether and the combined org. layers were dried over $MgSO_4$. After removal of the solvent under reduced pressure, the crude was purified by column chromatography (CH/EtOAc + 1% FA, 3:1). The title compound was obtained as a colorless solid.

Yield: 209 mg (0.59 mmol, 99%)
TLC (CH/EtOAc + 1% FA, 3:1): R_f = 0.28
HPLC (5 – 100 % solvent B, 20 min) R_t = 15.745 min, purity (220 nm): 99%
Mass (ESI$^+$): m/z: calculated 357.06 $[M+H]^+$, found 357.30 $[M+H]^+$
^{1}H NMR (300 MHz, $CDCl_3$) δ 6.55 (s, 2H), 3.88 (s, 6H), 3.20 (s, 1H), 2.08 – 1.84 (m, 2H), 1.83 – 1.59 (m, 3H), 1.43 – 1.22 (m, 2H), 1.19 – 1.00 (m, 3H), 0.95 – 0.72 (m, 1H).
^{13}C NMR (75 MHz, $CDCl_3$) δ 179.29, 157.17, 138.09, 105.29, 100.19, 59.18, 56.65, 41.07, 32.04, 30.40, 26.33, 26.03.

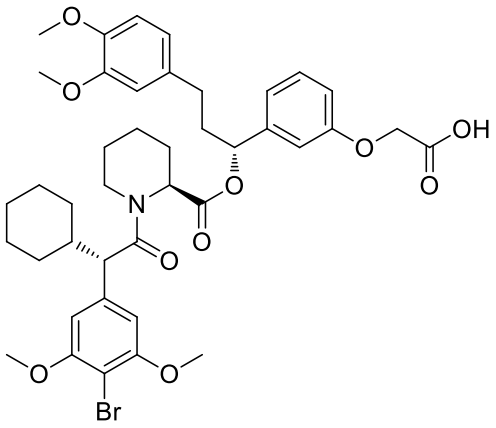
(S)-2-(4-Chloro-3,5-dimethoxyphenyl)-2-cyclohexylacetic acid (18b)



A 10 mL flask was charged with **17b** (23 mg, 0.05 mmol, 1.00 eq.), and 2 mL of THF/H_2O (8:5) was added. Then LiOH (3 mg, 0.10 mmol, 2.00 eq.) was added, followed by 0.03 mL aq. H_2O_2 (0.25 mmol, 5.00 eq., 30 wt.-% in H_2O). The resulting solution was then stirred at rt overnight. The reaction mixture was diluted with water and was then acidified with 1 M HCl (pH < 2). The cloudy aq. layer was then extracted three times with diethyl ether and the combined org. layers were dried over $MgSO_4$. After removal of the solvent under reduced pressure, the crude was purified by column chromatography (CH/EtOAc + 1% FA, 3:1). The title compound was obtained as a colorless solid.

Yield: 10 mg (0.03 mmol, 62%)
TLC (CH/EtOAc + 1% FA, 3:1): R_f = 0.36
HPLC (50 – 100 % solvent B, 20 min) R_t = 6.950 min, purity (220 nm): 99%
Mass (ESI$^+$): m/z: calculated 313.12 $[M+H]^+$, found 313.40 $[M+H]^+$
^{1}H NMR (500 MHz, $CDCl_3$) δ 6.58 (s, 2H), 3.89 (s, 6H), 3.18 (d, J = 10.6 Hz, 1H), 2.01 – 1.87 (m, 2H), 1.78 – 1.73 (m, 1H), 1.69 – 1.62 (m, 2H), 1.41 – 1.28 (m, 2H), 1.19 – 1.05 (m, 3H), 0.82 – 0.71 (m, 1H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 178.76, 156.21, 136.97, 110.10, 105.30, 59.07, 56.59, 41.12, 32.06, 30.42, 26.35, 26.04.

2-(3-((R)-1-(((S)-1-((S)-2-(4-Bromo-3,5-dimethoxyphenyl)-2-cyclohexylacetyl)-piperidine-2-carbonyl)oxy)-3-(3,4-dimethoxyphenyl)propyl)phenoxy)acetic acid (19a)



Resin **8** (191 mg, 0.14 mmol, 2.00 eq., loading: 0.73 mmol/g) was swollen in a syringe with 4 mL DCM for 10 min and was washed twice with 2 mL dry DMF. Then a solution of the acid **18a** (25 mg, 0.07 mmol, 1.00 eq.), HATU (53 mg, 0.14 mmol, 2.00 eq.), HOAt (19 mg, 0.14 mmol, 2.00 eq.), and DIPEA (50 µL, 0.28 mmol, 4.00 eq.) in 2 mL dry DMF was added. The resulting suspension was shaken overnight, and the resin was washed with 2 mL DMF followed by three times 2 mL DCM. Finally, the product was cleaved by treating the resin with a solution of 20 vol.-% HFIP in DCM for 1h. After removal of the solvent under reduced pressure, the crude was purified by column chromatography (CH/EtOAc + 1% FA, 1:1). The title compound was obtained as a colorless foam.

Yield: 26 mg (32.69 µmol, 46%)

TLC (CH/EtOAc + 1% FA, 1:1): R_f = 0.44

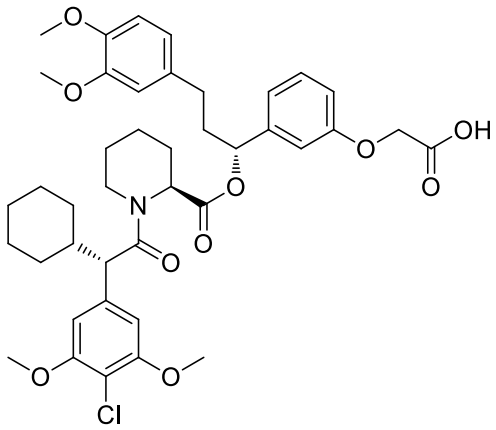
HPLC (30 – 100 % solvent B, 3 min) R_t = 2.200 min, purity (220 nm): 95%

HRMS (ESI$^+$): m/z: calculated 796.26909 $[M+H]^+$, found 796.26755 $[M+H]^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.11 (t, J = 7.8 Hz, 1H), 6.78 (dd, J = 8.3, 2.5 Hz, 1H), 6.71 (d, J = 7.8 Hz, 1H), 6.67 – 6.62 (m, 2H), 6.59 (s, 1H), 6.20 (s, 2H), 5.63 – 5.34 (m, 2H), 4.74 – 4.54 (m, 2H), 3.82 (d, J = 0.8 Hz, 2H), 3.79 (d, J = 0.9 Hz, 3H), 3.78 (s, 3H), 3.53 (s, 6H), 3.32 (d, J = 9.1 Hz, 2H), 2.82 – 2.71 (m, 1H), 2.63 – 2.55 (m, 1H), 2.49 – 2.43 (m, 1H), 2.28 (d, J = 13.6 Hz, 1H), 2.09 – 2.00 (m, 1H), 1.97 – 1.90 (m, 1H), 1.83 (d, J = 12.2 Hz, 1H), 1.68 (dd, J = 11.0, 4.7 Hz, 2H), 1.59 – 1.52 (m, 3H), 1.47 – 1.35 (m, 1H), 1.28 – 1.15 (m, 3H), 1.10 – 0.96 (m, 3H), 0.89 – 0.82 (m, 1H), 0.73 – 0.63 (m, 1H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 174.50, 173.13, 169.91, 158.15, 156.96, 149.13, 147.61, 142.69, 137.64, 133.47, 129.73, 120.37, 119.55, 115.94, 111.85, 111.55, 109.04, 105.56, 76.74, 65.74, 56.48, 56.11, 56.07, 55.75, 52.67, 43.68, 40.98, 38.53, 33.16, 31.67, 30.77, 27.36, 26.63, 26.38, 26.33, 25.42, 21.02.

2-(3-((R)-1-(((S)-1-((S)-2-(4-Chloro-3,5-dimethoxyphenyl)-2-cyclohexylacetyl)-piperidine-2-carbonyl)oxy)-3-(3,4-dimethoxyphenyl)propyl)phenoxy)acetic acid (19b)



Resin **8** (80 mg, 0.06 mmol, 2.00 eq., loading: 0.73 mmol/g) was swollen in a syringe with 2 mL DCM for 10 min and was washed twice with 2 mL dry DMF. Then a solution of the acid **18b** (9 mg, 0.03 mmol, 1.00 eq.), HATU (22 mg, 0.06 mmol, 2.00 eq.), HOAt (8 mg, 0.06 mmol, 2.00 eq.), and DIPEA (20 µL, 0.14 mmol, 4.00 eq.) in 1 mL dry DMF was added. The resulting suspension was shaken overnight, and the resin was washed with 2 mL DMF followed by three times 2 mL DCM. Finally, the product was cleaved by treating the resin with a solution of 20 vol.-% HFIP in DCM for 1h. After removal of the solvent under reduced pressure, the crude was purified by column chromatography (CH/EtOAc + 1% FA, 3:1). The title compound was obtained as a colorless foam.

Yield: 15 mg (19.94 µmol, 71%)

TLC (CH/EtOAc + 1% FA, 3:1): R_f = 0.32

HPLC (30 – 100 % solvent B, 3 min) R_t = 2.177 min, purity (220 nm): 99%

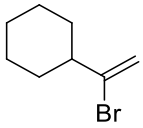
HRMS (ESI$^+$): m/z: calculated 752.31960 $[M+H]^+$, found 752.31791 $[M+H]^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.18 (t, J = 7.9 Hz, 1H), 6.84 (dd, J = 8.3, 2.6 Hz, 1H), 6.77 (d, J = 7.8 Hz, 1H), 6.74 – 6.72 (m, 1H), 6.70 (d, J = 7.9 Hz, 1H), 6.68 – 6.64 (m, 2H), 6.31 (s, 2H), 5.49 (dd, J = 8.9, 4.8 Hz, 1H), 5.44 (d, J = 5.3 Hz, 1H), 4.67 (q, J = 16.3 Hz, 2H), 3.85 (s, 3H), 3.85 (s, 3H), 3.61 (s, 6H), 3.38 (d, J = 9.0 Hz, 1H), 2.85 (td, J = 13.4, 3.1 Hz, 1H), 2.64 – 2.56 (m, 1H), 2.54 – 2.45 (m, 1H), 2.34 (d, J = 13.7 Hz, 1H), 2.14 – 2.05 (m, 1H), 2.04 – 1.93 (m, 2H), 1.91 – 1.83 (m, 1H), 1.75 – 1.69 (m, 2H), 1.65 – 1.58 (m, 2H), 1.51 – 1.42 (m, 2H), 1.31 – 1.21 (m, 4H), 1.18 – 1.07 (m, 2H), 0.96 – 0.84 (m, 2H), 0.79 – 0.68 (m, 1H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 173.11, 171.28, 169.95, 158.13, 155.98, 149.12, 147.59, 142.63, 136.57, 133.46, 129.72, 120.36, 119.53, 115.78, 111.85, 111.55, 109.33, 105.51, 76.67, 65.68, 56.40, 56.10, 56.05, 55.71, 52.63, 43.66, 41.04, 38.51, 33.13, 31.62, 30.76, 27.32, 26.62, 26.37, 26.32, 25.42, 21.01.

11.5 Synthesis of SAFit bottom groups by Suzuki coupling

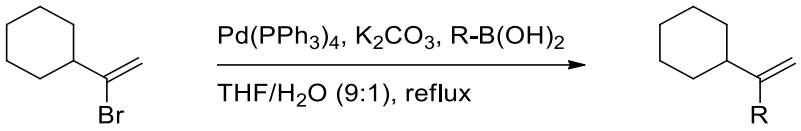
(1-Bromovinyl)cyclohexane (20)



In a dried 250 mL flask, cyclohexylacetylene (5.00 g, 46.22 mmol, 1.00 eq.) was dissolved in 125 mL DCM and the obtained solution was cooled to -78°C. Then 23.11 mL boron tribromide (1 M in DCM, 23.11 mmol, 0.50 eq.) was added dropwise, and the solution was allowed to warm to rt overnight. The reaction mixture was quenched by adding 55 mL acetic acid, and the resulting solution was stirred for an additional hour at rt. Then water was added, and the aq. phase was extracted with DCM three times. The combined org. layers were then washed with brine, dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure (>350 mbar). After purification of the crude via column chromatography (n-pentane), the title compound was obtained as a colorless liquid.

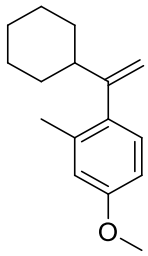
Yield: 8.26 g (43.68 mmol, 95%)
TLC (n-pentane): R_f = 0.81
^{1}H NMR (300 MHz, $CDCl_3$) δ 5.55 (s, 1H), 5.37 (d, J = 1.8 Hz, 1H), 2.22 – 2.11 (m, 1H), 1.98 – 1.87 (m, 2H), 1.84 – 1.74 (m, 2H), 1.77 – 1.64 (m, 1H), 1.34 – 1.23 (m, 4H), 0.98 – 0.70 (m, 1H).
^{13}C NMR (75 MHz, $CDCl_3$) δ 141.43, 114.24, 48.67, 32.30, 26.14.

General procedure for the Suzuki coupling of 20 to 21a-g



In a dried flask equipped with a reflux condenser on top, bromoalkene **20** (1.00 eq.), $Pd(PPh_3)_4$ (0.05 eq.), K_2CO_3 (3.00 eq.), and the boronic acid (1.10 eq.) is dissolved in degassed THF/H_2O (9:1). The resulting mixture is then refluxed overnight. After the reaction mixture is cooled to rt, water is added, and the resulting mixture is extracted three times with diethyl ether. The combined org. layers are washed with brine, dried over $MgSO_4$, filtered, and the solvent is removed under reduced pressure. Finally, the obtained crude is purified by column chromatography.

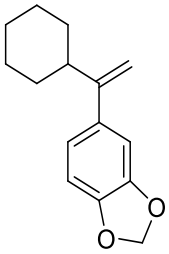
1-(1-Cyclohexylvinyl)-4-methoxy-2-methylbenzene (21a)



Following the general procedure, compound **21a** was synthesized using: **20** (168 mg, 0.89 mmol, 1.00 eq), $Pd(PPh_3)_4$ (51 mg, 0.04 mmol, 0.05 eq.), K_2CO_3 (368 mg, 2.67 mmol, 3.00 eq.) and 4-methoxy-2-methylphenylboronic acid (162 mg, 0.98 mmol, 1.10 eq.) in 8.90 mL degassed THF/H_2O (9:1). After purification via column chromatography (CH/EtOAc, 9:1) compound **21a** was obtained as a colorless oil.

Yield: 184 mg (0.64 mmol, 90%)
TLC (CH): R_f = 0.26
HPLC (50 – 100 % solvent B, 20 min) R_t = 13.300 min, purity (254 nm): 93%
Mass (ESI^+): m/z: calculated 231.17 $[M+H]^+$, found 231.36 $[M+H]^+$
^{1}H NMR (300 MHz, $CDCl_3$) δ 6.88 (d, J = 8.3 Hz, 1H), 6.68 – 6.56 (m, 2H), 5.03 (t, J = 1.6 Hz, 1H), 4.71 (dd, J = 1.9, 0.7 Hz, 1H), 3.71 (s, 3H), 2.17 (s, 3H), 1.78 – 1.65 (m, 4H), 1.62 – 1.56 (m, 1H), 1.21 – 1.04 (m, 6H).
^{13}C NMR (75 MHz, $CDCl_3$) δ 158.25, 155.04, 136.70, 136.49, 129.71, 115.45, 111.60, 110.51, 55.29, 45.15, 32.23, 26.91, 26.56, 20.41.

5-(1-cyclohexylvinyl)benzo[d][1,3]dioxole (21b)



Following the general procedure, compound **21b** was synthesized using: **20** (201 mg, 1.62 mmol, 1.00 eq), $Pd(PPh_3)_4$ (94 mg, 0.08 mmol, 0.05 eq.), K_2CO_3 (673 mg, 4.87 mmol, 3.00 eq.) and 3,4-(methylenedioxo)phenylboronic acid (404 mg, 2.43 mmol, 1.50 eq.) in 8.11 mL degassed THF/H_2O (9:1). After purification via column chromatography (CH) compound **21b** was obtained as a colorless oil.

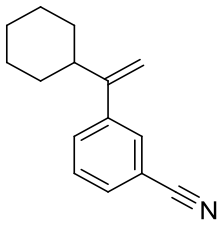
Yield: 137 mg (0.59 mmol, 45%)

TLC (CH/EtOAc, 20:1): R_f = 0.61

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.85 – 6.74 (m, 3H), 5.95 (s, 2H), 5.07 (s, 1H), 4.93 (s, 1H), 2.38 – 2.28 (m, 1H), 1.86 – 1.78 (m, 3H), 1.78 – 1.69 (m, 2H), 1.35 – 1.28 (m, 2H), 1.21 – 1.15 (m, 2H), 0.91 – 0.85 (m, 1H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 154.65, 147.60, 146.74, 137.41, 120.02, 109.78, 108.05, 107.44, 101.06, 42.95, 32.89, 27.00, 26.60.

3-(1-Cyclohexylvinyl)benzonitrile (21c)



Following the general procedure, compound **21c** was synthesized using: **20** (203 mg, 1.07 mmol, 1.00 eq), $Pd(PPh_3)_4$ (62 mg, 0.05 mmol, 0.05 eq.), K_2CO_3 (445 mg, 3.22 mmol, 3.00 eq.) and 3-cyanophenylboronic acid (196 mg, 1.18 mmol, 1.10 eq.) in 10.74 mL degassed THF/H_2O (9:1). After purification via column chromatography (CH/EtOAc, 19:1) compound **21c** was obtained as a colorless oil.

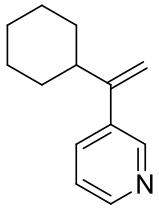
Yield: 167 mg (0.79 mmol, 74%)

TLC (CH/EtOAc, 19:1): R_f = 0.55

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.60 (t, J = 1.7 Hz, 1H), 7.56 – 7.53 (m, 2H), 7.41 (t, J = 7.8 Hz, 1H), 5.16 (s, 1H), 5.10 (t, J = 1.2 Hz, 1H), 2.40 – 2.32 (m, 1H), 1.82 – 1.77 (m, 3H), 1.74 – 1.70 (m, 1H), 1.34 – 1.13 (m, 6H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 153.20, 144.30, 131.25, 130.65, 130.46, 129.11, 119.14, 112.62, 112.47, 42.59, 32.64, 26.80, 26.41.

3-(1-Cyclohexylvinyl)pyridine (21d)



Following the general procedure, compound **21d** was synthesized using: **20** (204 mg, 1.08 mmol, 1.00 eq), $Pd(PPh_3)_4$ (62 mg, 0.05 mmol, 0.05 eq.), K_2CO_3 (447 mg, 3.24 mmol, 3.00 eq.) and 3-pyridinylboronic acid (159 mg, 1.29 mmol, 1.10 eq.) in 10.80 mL degassed THF/H_2O (9:1). After purification via column chromatography (CH/EtOAc, 5:1) compound **21d** was obtained as a colorless oil.

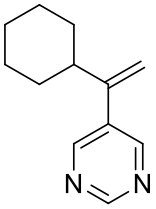
Yield: 182 mg (0.97 mmol, 90%)

TLC (CH/EtOAc, 5:1): R_f = 0.21

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.60 (dd, J = 2.4, 0.9 Hz, 1H), 8.51 (dd, J = 4.8, 1.6 Hz, 1H), 7.63 (dt, J = 7.9, 2.0 Hz, 1H), 7.28 – 7.23 (m, 1H), 5.19 (s, 1H), 5.12 (s, 1H), 2.44 – 2.37 (m, 1H), 1.87 – 1.77 (m, 4H), 1.76 – 1.71 (m, 1H), 1.36 – 1.29 (m, 2H), 1.23 – 1.17 (m, 3H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 151.95, 148.39, 148.18, 138.36, 134.01, 123.12, 112.32, 42.63, 32.61, 26.81, 26.43.

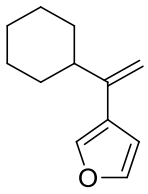
5-(1-Cyclohexylvinyl)pyrimidine (21e)



Following the general procedure, compound **21e** was synthesized using: **20** (179 mg, 0.94 mmol, 1.00 eq), $Pd(PPh_3)_4$ (55 mg, 0.05 mmol, 0.05 eq.), K_2CO_3 (392 mg, 2.83 mmol, 3.00 eq.) and pyrimidine-5-boronic acid (117 mg, 0.94 mmol, 1.10 eq.) in 9.40 mL degassed THF/H_2O (9:1). After purification via column chromatography (CH/EtOAc, 5:1) compound **21e** was obtained as a colorless oil.

Yield: 94 mg (0.49 mmol, 53%)
TLC (CH/EtOAc, 3:1): R_f = 0.25
^{1}H NMR (500 MHz, $CDCl_3$) δ 9.10 (s, 1H), 8.68 (s, 2H), 5.23 (s, 1H), 5.20 – 5.20 (m, 1H), 2.40 – 2.34 (m, 1H), 1.83 – 1.78 (m, 4H), 1.38 – 1.28 (m, 3H), 1.22 – 1.15 (m, 3H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 157.55, 154.76, 148.77, 135.85, 114.13, 42.41, 32.45, 26.66, 26.29.

3-(1-Cyclohexylvinyl)furan (21f)



Following the general procedure, compound **21f** was synthesized using: **20** (152 mg, 0.81 mmol, 1.00 eq), $Pd(PPh_3)_4$ (47 mg, 0.04 mmol, 0.05 eq.), K_2CO_3 (335 mg, 2.42 mmol, 3.00 eq.) and 3-furanylboronic acid (99 mg, 0.89 mmol, 1.10 eq.) in 8.10 mL degassed THF/H_2O (9:1). After purification via column chromatography (CH) compound **21f** was obtained as a colorless oil.

Yield: 112 mg (0.64 mmol, 79%)

TLC (CH): R_f = 0.52

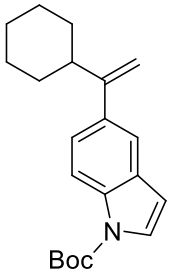
HPLC (50 – 100 % solvent B, 20 min) R_t = 10.750 min, purity (254 nm): 97%

Mass (ESI^+): m/z: calculated 177.12 $[M+H]^+$, found 177.10 $[M+H]^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.45 – 7.44 (m, 1H), 7.36 (t, J = 1.7 Hz, 1H), 6.52 – 6.49 (m, 1H), 5.18 (d, J = 1.2 Hz, 1H), 4.93 (t, J = 1.2 Hz, 1H), 2.24 – 2.16 (m, 1H), 1.92 – 1.87 (m, 1H), 1.84 – 1.78 (m, 1H), 1.76 – 1.71 (m, 1H), 1.37 – 1.32 (m, 2H), 1.31 – 1.28 (m, 1H), 1.27 – 1.23 (m, 3H), 0.93 – 0.80 (m, 1H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 145.72, 143.13, 138.63, 127.24, 108.86, 108.07, 42.85, 33.14, 27.07, 26.63.

Tert-butyl 5-(1-cyclohexylvinyl)-1H-indole-1-carboxylate (21g)



Following the general procedure, compound **21g** was synthesized using: **20** (150 mg, 1.21 mmol, 1.00 eq), $Pd(PPh_3)_4$ (44 mg, 0.04 mmol, 0.05 eq.), K_2CO_3 (771 mg, 3.63 mmol, 3.00 eq.) and 1-boc-5-indoleboronic acid pinacol ester (449 mg, 1.45 mmol, 1.20 eq.) in 6.05 mL degassed THF/H_2O (9:1). After purification via column chromatography (CH/EtOAc, 20:1) compound **21g** was obtained as a colorless oil.

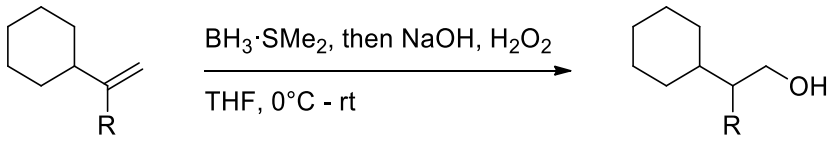
Yield: 207 mg (0.64 mmol, 53%)

TLC (CH/EtOAc, 20:1): R_f = 0.61

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.98 (d, J = 8.7 Hz, 1H), 7.50 (d, J = 3.7 Hz, 1H), 7.43 (d, J = 1.8 Hz, 1H), 7.22 (dd, J = 8.6, 1.8 Hz, 1H), 6.47 (d, J = 3.7 Hz, 1H), 5.07 (d, J = 1.4 Hz, 1H), 4.93 (d, J = 1.5 Hz, 1H), 2.45 – 2.34 (m, 1H), 1.81 – 1.76 (m, 2H), 1.70 (dt, J = 12.8, 3.3 Hz, 2H), 1.66 – 1.62 (m, 1H), 1.59 (s, 9H), 1.29 – 1.21 (m, 2H), 1.15 – 1.06 (m, 3H).

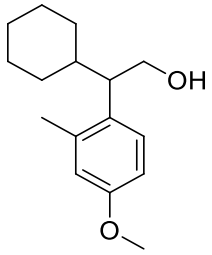
^{13}C NMR (126 MHz, $CDCl_3$) δ 155.47, 149.92, 137.93, 134.51, 130.68, 126.32, 123.50, 118.99, 114.77, 110.07, 107.59, 83.74, 43.19, 32.91, 28.36, 27.02, 26.63.

General procedure for the synthesis of primary alcohol via Brown hydroboration



In a dried flask, the 1,1-disubstituted alkene **21a-g** (1.00 eq.) is dissolved in THF, and the resulting solution is cooled to 0°C. Then borane dimethyl sulfide complex (2 M in THF, 1.05 eq.) is added dropwise, and the resulting solution is stirred at rt for 2 h. After complete conversion of the alkene, the solution is again cooled to 0°C. Then a 3 M solution of NaOH in water (1.50 eq.) and aq. H_2O_2 (30 wt.-%, 3.00 eq.) is added simultaneously. The reaction is allowed to warm to rt overnight. The mixture is then quenched by adding water and extracted three times with EtOAc. The combined org. layers are washed with brine, dried over $MgSO_4$, filtered, and the solvent is removed under reduced pressure. Finally, the primary alcohol is purified by column chromatography.

2-Cyclohexyl-2-(4-methoxy-2-methylphenyl)ethanol (22a)



Following the general procedure, compound **22a** was synthesized using: Alkene **21a** (184 mg, 0.80 mmol, 1.00 eq), 0.43 mL borane dimethyl sulfide complex (2 M in THF, 0.84 mmol, 1.05 eq.), 0.40 mL NaOH (3 M in water, 1.20 mmol, 1.50 eq.) and 0.25 mL aq. H_2O_2 (30 wt.-% 2.40 mmol, 3.00 eq.) in 1.60 mL THF. After purification via column chromatography (CH/EtOAc, 7:1), compound **22a** was obtained as a colorless oil.

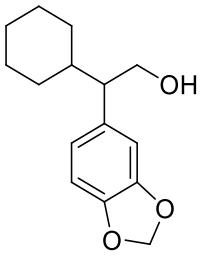
Yield: 89 mg (0.36 mmol, 57%)

TLC (CH/EtOAc, 5:1): R_f = 0.23

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.10 (dd, J = 8.5, 1.3 Hz, 1H), 6.77 – 6.71 (m, 2H), 3.91 (ddd, J = 10.9, 4.9, 1.4 Hz, 1H), 3.78 (s, 3H), 3.76 – 3.72 (m, 1H), 2.87 (td, J = 9.1, 4.8 Hz, 1H), 2.30 (s, 3H), 1.95 – 1.89 (m, 1H), 1.78 – 1.73 (m, 1H), 1.65 – 1.57 (m, 2H), 1.56 – 1.50 (m, 1H), 1.43 – 1.38 (m, 1H), 1.28 – 1.23 (m, 1H), 1.15 – 1.00 (m, 3H), 0.88 – 0.79 (m, 1H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 157.68, 139.39, 132.26, 127.35, 116.00, 111.78, 65.01, 55.21, 48.15, 40.78, 31.40, 31.36, 26.66, 26.61, 26.53, 20.61.

2-(Benzo[d][1,3]dioxol-5-yl)-2-cyclohexylethanol (22b)



Following the general procedure, compound **22b** was synthesized using: Alkene **21b** (119 mg, 0.52 mmol, 1.00 eq), 0.27 mL borane dimethyl sulfide complex (2 M in THF, 0.55 mmol, 1.05 eq.), 0.26 mL NaOH (3 M in water, 0.78 mmol, 1.50 eq.) and 0.18 mL aq. H_2O_2 (30 wt.-% 1.56 mmol, 3.00 eq.) in 2.00 mL THF. After purification via column chromatography (CH/EtOAc, 5:1), compound **22b** was obtained as a colorless oil.

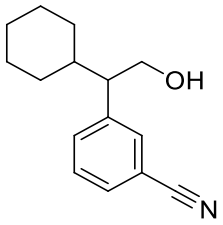
Yield: 67 mg (0.27 mmol, 52%)

TLC (CH/EtOAc, 5:1): R_f = 0.27

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.78 – 6.60 (m, 3H), 5.93 (s, 2H), 3.94 – 3.69 (m, 2H), 2.52 – 2.44 (m, 1H), 1.89 – 1.69 (m, 2H), 1.65 – 1.57 (m, 2H), 1.50 – 1.40 (m, 2H), 1.28 – 1.22 (m, 1H), 1.16 – 1.04 (m, 2H), 1.04 – 0.92 (m, 1H), 0.85 – 0.75 (m, 1H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 147.99, 146.34, 135.57, 122.12, 108.63, 108.32, 101.00, 64.91, 54.59, 40.04, 31.49, 31.30, 26.58, 26.46.

3-(1-Cyclohexyl-2-hydroxyethyl)benzonitrile (22c)



Following the general procedure, compound **22c** was synthesized using: Alkene **21c** (135 mg, 0.64 mmol, 1.00 eq), 0.34 mL borane dimethyl sulfide complex (2 M in THF, 0.67 mmol, 1.05 eq.), 0.32 mL NaOH (3 M in water, 0.96 mmol, 1.50 eq.) and 0.22 mL aq. H_2O_2 (30 wt.-% 1.92 mmol, 3.00 eq.) in 2.00 mL THF. After purification via column chromatography (CH/EtOAc, 5:1), compound **22c** was obtained as a colorless oil.

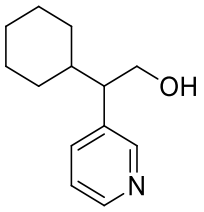
Yield: 65 mg (0.28 mmol, 44%)

TLC (CH/EtOAc, 3:1): R_f = 0.24

^{1}H NMR (500 MHz, DMSO-d_6) δ 7.93 (s, 1H), 7.72 – 7.66 (m, 2H), 7.34 – 7.30 (m, 2H), 4.44 (t, *J* = 5.0 Hz, 1H), 3.79 – 3.66 (m, 2H), 1.90 – 1.83 (m, 1H), 1.71 – 1.63 (m, 2H), 1.61 – 1.55 (m, 2H), 1.40 – 1.35 (m, 1H), 1.26 – 1.20 (m, 1H), 1.13 – 1.01 (m, 2H), 0.96 – 0.88 (m, 1H), 0.76 – 0.67 (m, 1H).

^{13}C NMR (126 MHz, DMSO-d_6) δ 168.15, 143.17, 133.69, 131.65, 127.77, 127.51, 124.90, 62.40, 53.68, 38.85, 30.99, 30.17, 26.09, 26.00, 25.87.

2-Cyclohexyl-2-(pyridin-3-yl)ethanol (22d)



In a 10 mL flask, **21d** (118 mg, 0.63 mmol, 1.00 eq.) was dissolved in 2 mL THF, and the solution was cooled to 0°C. Then 0.33 mL borane dimethyl sulfide complex (2 M in THF, 0.66 mmol, 1.05 eq.) was added dropwise, and the solution was stirred for 1 h at rt. After the complete formation of the pyridine-borane complex, the solvent was removed under reduced pressure, and the residue was dissolved in 2 mL DCM. Then iodine (80 mg, 0.32 mmol, 0.50 eq.) was added, and the solution was stirred overnight at rt. The reaction mixture was cooled to 0°C, and methanol was added. Then 0.63 mL NaOH (3 M in water, 1.89 mmol, 1.50 eq.) and 0.36 mL aq. H_2O_2 (30 wt.-% 3.15 mmol, 3.00 eq.) was added simultaneously, and the resulting mixture was stirred at rt for 5 h. Finally, the reaction mixture was diluted with water and extracted with EtOAc three times. The combined org. layers were washed with brine, dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The obtained crude was purified via column chromatography (EtOAc), and the title compound was obtained as a colorless solid.

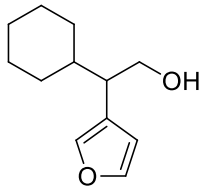
Yield: 71 mg (0.35 mmol, 55%)

TLC (EtOAc): R_f = 0.18

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.31 (dd, J = 5.3, 1.8 Hz, 2H), 7.51 (dt, J = 7.8, 2.1 Hz, 1H), 7.18 (dd, J = 7.9, 4.8 Hz, 1H), 3.88 (dd, J = 6.4, 4.7 Hz, 2H), 2.61 – 2.47 (m, 1H), 1.90 – 1.82 (m, 1H), 1.74 – 1.55 (m, 4H), 1.40 – 1.32 (m, 1H), 1.25 – 1.21 (m, 1H), 1.12 – 1.01 (m, 2H), 1.01 – 0.91 (m, 1H), 0.81 – 0.71 (m, 1H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 150.09, 147.33, 138.21, 136.52, 123.41, 63.79, 52.24, 39.31, 31.39, 31.00, 26.42, 26.37, 26.32.

2-Cyclohexyl-2-(furan-3-yl)ethanol (22e)



Following the general procedure, compound **22e** was synthesized using: Alkene **21f** (96 mg, 0.54 mmol, 1.00 eq), 0.29 mL borane dimethyl sulfide complex (2 M in THF, 0.57 mmol, 1.05 eq.), 0.27 mL NaOH (3 M in water, 0.82 mmol, 1.50 eq.) and 0.19 mL aq. H_2O_2 (30 wt.-% 1.63 mmol, 3.00 eq.) in 1.10 mL THF. After purification via column chromatography (CH/EtOAc, 5:1), compound **22e** was obtained as a colorless oil.

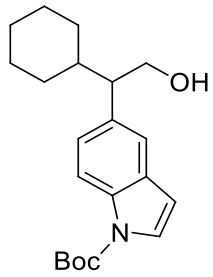
Yield: 67 mg (0.27 mmol, 52%)

TLC (CH/EtOAc, 5:1): R_f = 0.31

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.41 (t, J = 1.7 Hz, 1H), 7.29 (d, J = 1.1 Hz, 1H), 6.29 (dd, J = 1.8, 0.9 Hz, 1H), 3.86 – 3.64 (m, 2H), 2.54 (td, J = 8.0, 5.0 Hz, 1H), 1.82 – 1.54 (m, 6H), 1.21 – 0.87 (m, 5H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 143.35, 140.51, 124.55, 110.12, 64.32, 45.05, 39.21, 31.40, 30.89, 26.61, 26.52, 26.51.

Tert-butyl 5-(1-cyclohexyl-2-hydroxyethyl)-1H-indole-1-carboxylate (22f)



Following the general procedure, compound **22f** was synthesized using: Alkene **21g** (112 mg, 0.61 mmol, 1.00 eq), 0.32 mL borane dimethyl sulfide complex (2 M in THF, 0.64 mmol, 1.05 eq.), 0.30 mL NaOH (3 M in water, 0.91 mmol, 1.50 eq.) and 0.21 mL aq. H_2O_2 (30 wt.-% 1.82 mmol, 3.00 eq.) in 2.00 mL THF. After purification via column chromatography (CH/EtOAc, 5:1), compound **22f** was obtained as a colorless oil.

Yield: 112 mg (0.33 mmol, 54%)

TLC (CH/EtOAc, 5:1): R_f = 0.47

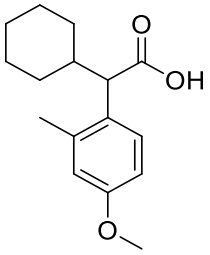
HPLC (50 – 100 % solvent B, 3 min) R_t = 1.558 min, purity (220 nm): 99%

Mass (ESI$^+$): m/z: calculated 344.21 $[M+H]^+$, found 344.20 $[M+H]^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.09 (d, J = 8.5 Hz, 1H), 7.59 (d, J = 3.7 Hz, 1H), 7.37 (s, 1H), 7.15 (dd, J = 8.5, 1.8 Hz, 1H), 6.53 (dd, J = 3.6, 0.7 Hz, 2H), 4.00 – 3.94 (m, 1H), 3.90 – 3.83 (m, 1H), 2.72 – 2.60 (m, 1H), 1.96 – 1.88 (m, 1H), 1.80 – 1.73 (m, 1H), 1.67 (s, 9H), 1.65 – 1.58 (m, 3H), 1.44 – 1.38 (m, 1H), 1.28 – 1.25 (m, 1H), 1.13 – 1.07 (m, 2H), 1.06 – 0.99 (m, 1H), 0.87 – 0.71 (m, 1H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 149.89, 135.90, 134.29, 131.02, 126.32, 124.99, 121.15, 115.31, 107.31, 83.76, 65.16, 54.79, 40.25, 31.59, 31.40, 28.32, 26.62, 26.53, 26.48.

2-Cyclohexyl-2-(4-methoxy-2-methylphenyl)acetic acid (23a)



In a 10 mL flask **22a** (89 mg, 0.36 mmol, 1.00 eq.), 4-methylmorpholine-N-oxide (484 mg, 3.58 mmol, 10.00 eq.) and tetrapropylammonium perruthenate (13 mg, 0.04 mmol, 0.10 eq.) was dissolved in 3.60 mL acetonitrile. The resulting mixture was stirred for 3 h at rt before 1 M HCl was added. The resulting mixture was then extracted three times with EtOAc and the combined org. layers were dried over $MgSO_4$. After the solvent was removed under reduced pressure, the obtained crude was purified via column chromatography (CH/EtOAc + 1% FA, 5:1). The title compound was obtained as a colorless solid.

Yield: 67 mg (0.26 mmol, 72%)

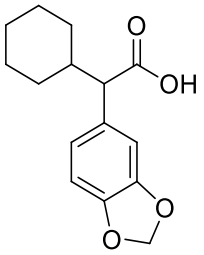
TLC (CH/EtOAc + 1% FA, 5:1): R_f = 0.22

Mass (ESI$^+$): m/z: calculated 263.16 $[M+H]^+$, found 263.10 $[M+H]^+$

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34 (d, J = 8.5 Hz, 1H), 6.74 (d, J = 8.4 Hz, 1H), 6.70 (s, 1H), 3.77 (s, 3H), 3.49 (d, J = 10.8 Hz, 1H), 2.35 (s, 3H), 2.06 – 1.89 (m, 2H), 1.80 – 1.71 (m, 1H), 1.69 – 1.55 (m, 2H), 1.42 – 1.26 (m, 2H), 1.21 – 1.01 (m, 3H), 0.81 – 0.61 (m, 1H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 180.46, 158.41, 138.56, 128.53, 128.10, 115.88, 111.86, 55.25, 52.70, 40.87, 32.26, 30.01, 26.51, 26.23, 26.17, 20.51.

2-(Benzo[d][1,3]dioxol-5-yl)-2-cyclohexylacetic acid (23b)



In a 5 mL flask, **22b** (60 mg, 0.24 mmol, 1.00 eq.) was dissolved in 5 mL acetone, and the solution was cooled to 0°C. Then 0.24 mL Jones reagent (2 M CrO_3 in aq. H_2SO_4, 0.48 mmol, 2.00 eq.) was added dropwise. The reaction mixture was then stirred for 1 h at 0°C. After the complete conversion of the starting material, isopropanol was added dropwise, and the solvent was removed. The obtained residue was dissolved in DCM and was washed with water, brine and dried over $MgSO_4$. After the removal of the solvent, the crude was purified via column chromatography (CH/EtOAc + 1% FA, 5:1). The title compound was obtained as a colorless solid.

Yield: 48 mg (0.18 mmol, 76%)

TLC (CH/EtOAc + 1% FA, 5:1): R_f = 0.21

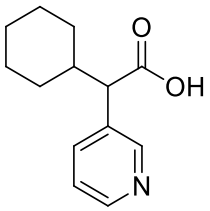
HPLC (30 – 100 % solvent B, 3 min) R_t = 1.634 min, purity (220 nm): 97%

Mass (ESI^+): m/z: calculated 263.12 $[M+H]^+$, found 263.20 $[M+H]^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ 6.87 (s, 1H), 6.73 (d, J = 0.7 Hz, 2H), 5.94 – 5.92 (m, 2H), 3.13 (d, J = 10.6 Hz, 1H), 1.93 – 1.85 (m, 2H), 1.76 – 1.71 (m, 1H), 1.64 – 1.61 (m, 1H), 1.40 – 1.35 (m, 1H), 1.32 – 1.28 (m, 1H), 1.17 – 1.12 (m, 2H), 1.08 – 1.03 (m, 1H), 0.91 – 0.81 (m, 1H), 0.77 – 0.70 (m, 1H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 180.18, 147.96, 147.05, 131.13, 122.41, 108.74, 108.25, 101.17, 58.48, 40.85, 32.02, 30.37, 26.40, 26.07.

2-Cyclohexyl-2-(pyridin-3-yl)acetic acid (23c)



In a 5 mL flask, alcohol **22d** (75 mg, 0.37 mmol, 1.00 eq.) was dissolved in 3.65 mL acetone, and the solution was cooled to 0°C. Then 0.37 mL Jones reagent (2 M CrO_3 in aq. H_2SO_4, 0.73 mmol, 2.00 eq.) was added dropwise. The reaction mixture was then stirred for 1 h at 0°C. After the complete conversion of the starting material, isopropanol was added dropwise, and the solvent was removed. The obtained residue was dissolved in DCM and was washed with water, brine and dried over $MgSO_4$. After the removal of the solvent, the crude was purified via column chromatography (CH/EtOAc + 1% FA, 3:1). The title compound was obtained as a colorless solid.

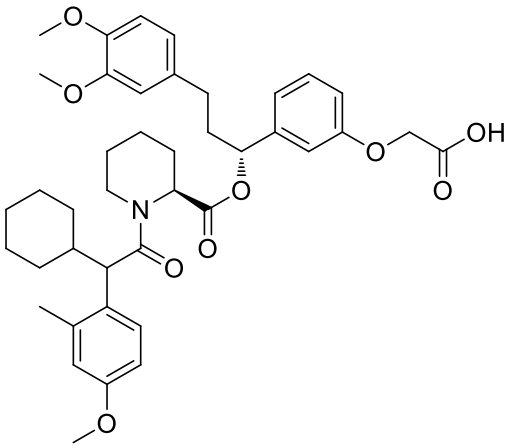
Yield: 31 mg (0.14 mmol, 39%)

TLC (EtOAc + 1% FA): R_f = 0.43

^{1}H NMR (300 MHz, $CDCl_3$) δ 11.29 (s, 1H), 8.56 (s, 1H), 8.51 – 8.47 (m, 1H), 7.88 (d, J = 8.0 Hz, 1H), 7.32 (dd, J = 8.0, 4.9 Hz, 1H), 3.30 (d, J = 10.2 Hz, 1H), 2.06 – 1.90 (m, 2H), 1.82 – 1.69 (m, 1H), 1.66 – 1.58 (m, 2H), 1.33 – 1.22 (m, 2H), 1.20 – 1.07 (m, 3H), 0.86 – 0.72 (m, 1H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 176.36, 148.32, 146.53, 137.97, 135.42, 124.15, 56.88, 41.10, 32.01, 30.54, 26.28, 26.07, 26.03.

2-(3-((1R)-1-(((2S)-1-(2-Cyclohexyl-2-(4-methoxy-2-methylphenyl)acetyl)piperidine-2-carbonyl)oxy)-3-(3,4-dimethoxyphenyl)propyl)phenoxy)acetic acid (24a)



Following the general procedure A, compound **24a** was synthesized using: Resin **7** (90 mg, 0.06 mmol, 1.50 eq, loading: 0.63 mmol/g), **23a** (10 mg, 0.04 mmol, 1.00 eq.), HATU (29 mg, 0.08 mmol, 2.00 eq.), HOAt (10 mg, 0.08 mmol, 2.00 eq.) DIPEA (33 µL, 0.19 mmol, 5.00 eq.) and 2 mL DMF. After purification via column chromatography (EtOAc + 1% FA, 1:1), compound **24a** was obtained as a colorless solid.

Yield: 12 mg (17.09 µmol, 44%)

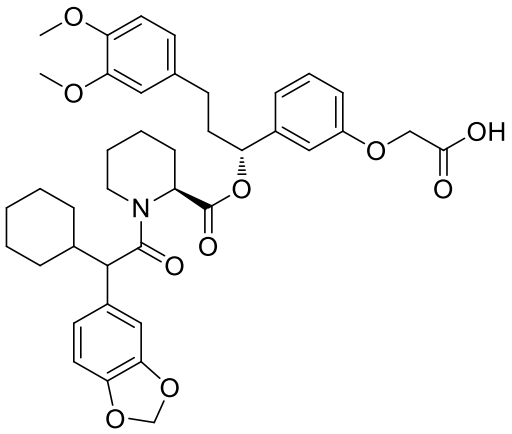
HPLC (50 – 100 % solvent B, 3 min) R_t = 1.840 min, purity (220 nm): 99 %

HRMS (ESI$^+$): m/z: calculated 702.36366 $[M+H]^+$, 702.36382 found $[M+H]^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35 (d, J = 8.6 Hz, 2H), 7.28 – 7.15 (m, 1H), 6.95 – 6.85 (m, 2H), 6.81 (d, J = 8.0 Hz, 1H), 6.77 – 6.72 (m, 1H), 6.71 – 6.65 (m, 3H), 5.66 (ddd, J = 32.0, 8.9, 4.9 Hz, 1H), 5.54 – 5.43 (m, 1H), 4.81 – 4.59 (m, 2H), 3.92 – 3.84 (m, 6H), 3.80 (s, 3H), 3.70 – 3.64 (m, 1H), 3.33 (td, 1H), 2.75 – 2.57 (m, 1H), 2.56 – 2.48 (m, 1H), 2.35 (s, 3H), 2.31 – 2.19 (m, 2H), 2.15 – 2.03 (m, 2H), 2.02 – 1.81 (m, 1H), 1.79 – 1.41 (m, 7H), 1.39 – 1.19 (m, 4H), 1.17 – 1.03 (m, 2H), 0.95 – 0.73 (m, 2H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 174.89, 174.01, 171.12, 170.98, 170.55, 170.30, 158.18, 158.13, 157.99, 157.82, 149.08, 149.02, 147.56, 147.49, 142.39, 142.37, 137.00, 136.84, 133.65, 133.56, 129.81, 129.75, 129.24, 129.21, 128.35, 122.41, 120.96, 119.92, 119.66, 116.09, 115.96, 115.79, 115.39, 111.97, 111.90, 111.83, 111.50, 111.47, 76.51, 76.03, 65.70, 65.53, 56.09, 56.04, 55.24, 55.18, 52.96, 52.59, 49.38, 49.32, 43.46, 43.43, 42.65, 38.27, 38.14, 33.15, 33.05, 31.67, 31.50, 30.14, 27.25, 27.11, 26.74, 26.72, 26.65, 26.51, 26.40, 25.63, 24.97, 21.13, 20.54, 20.36.

2-(3-((R)-1-(((S)-1-(2-(Benzo[d][1,3]dioxol-5-yl)-2-cyclohexylacetyl)piperidine-2-carbonyl)-oxy)-3-(3,4-dimethoxyphenyl)propyl)phenoxy)acetic acid (24b)



Following the general procedure A, compound **24b** was synthesized using: Resin **7** (154 mg, 0.10 mmol, 1.50 eq, loading: 0.63 mmol/g), **23b** (17 mg, 0.06 mmol, 1.00 eq.), HATU (49 mg, 0.24 mmol, 2.00 eq.), HOAt (18 mg, 0.14 mmol, 2.00 eq.) DIPEA (56 µL, 0.32 mmol, 5.00 eq.) and 2 mL DMF. After purification via column chromatography (CH:EtOAc + 1% FA, 1:1), compound **24b** was obtained as a colorless solid.

Yield: 30 mg (42.75 µmol, 67%)

TLC (EtOAc + 1% FA): R_f = 0.43

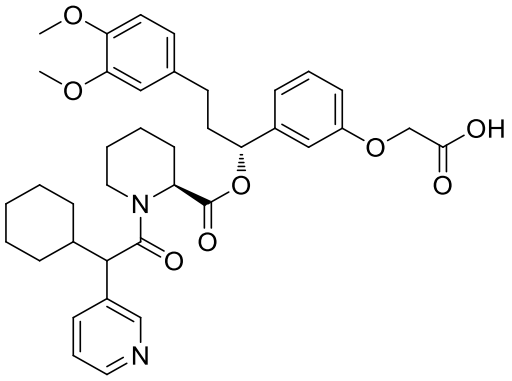
HPLC (50 – 100% solvent B, 3 min) $R_{t,d1}$ = 1.708 min, $R_{t,d2}$ = 1.747 min, purity (220 nm): 99%

HRMS (ESI$^+$): m/z: calculated 702.32727 $[M+H]^+$, 702.32728 found $[M+H]^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.33 – 7.16 (m, 1H), 6.95 – 6.83 (m, 3H), 6.80 (dd, J = 8.4, 5.6 Hz, 1H), 6.73 (t, J = 8.0 Hz, 1H), 6.71 – 6.67 (m, 3H), 6.67 – 6.53 (m, 1H), 5.92 (d, J = 37.8 Hz, 2H), 5.75 – 5.62 (m, 1H), 5.52 (dd, J = 15.4, 5.5 Hz, 1H), 4.78 – 4.59 (m, 2H), 4.00 (d, J = 13.3 Hz, 1H), 3.88 (dd, J = 6.1, 2.6 Hz, 6H), 3.41 (d, J = 9.8 Hz, 1H), 3.34 – 3.10 (m, 1H), 2.67 (td, J = 9.4, 4.9 Hz, 0H), 2.63 – 2.54 (m, 1H), 2.51 – 2.42 (m, 0H), 2.37 – 2.18 (m, 2H), 2.15 – 2.01 (m, 2H), 1.96 – 1.76 (m, 1H), 1.73 – 1.56 (m, 5H), 1.53 – 1.38 (m, 1H), 1.37 – 1.21 (m, 4H), 1.20 – 1.06 (m, 2H), 0.99 – 0.82 (m, 2H), 0.77 – 0.68 (m, 1H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 173.95, 173.03, 171.81, 171.68, 170.58, 170.39, 158.08, 157.74, 149.03, 148.95, 148.00, 147.86, 147.51, 147.41, 146.62, 146.59, 142.28, 142.23, 133.65, 133.54, 132.36, 131.54, 129.82, 129.77, 122.27, 121.90, 120.36, 120.32, 119.87, 119.81, 115.49, 114.79, 111.95, 111.92, 111.82, 111.48, 111.43, 110.67, 108.96, 108.60, 108.21, 101.11, 100.99, 76.46, 75.93, 65.48, 65.33, 56.07, 56.05, 56.00, 55.98, 54.59, 54.53, 52.65, 52.44, 43.92, 43.73, 41.33, 41.28, 38.21, 37.99, 32.79, 32.66, 31.55, 31.29, 30.73, 27.03, 26.83, 26.64, 26.60, 26.26, 26.22, 26.14, 25.63, 25.16, 21.12, 21.02.

2-(3-((R)-1-(((S)-1-(2-Cyclohexyl-2-(pyridin-3-yl)acetyl)piperidine-2-carbonyl)-oxy)-3-(3,4-dimethoxyphenyl)propyl)phenoxy)acetic acid (24c)



Following the general procedure A, compound **24c** was synthesized using: Resin **7** (69 mg, 0.04 mmol, 1.20 eq, loading: 0.63 mmol/g), **23d** (8 mg, 0.04 mmol, 1.00 eq.), HATU (28 mg, 0.07 mmol, 2.00 eq.), HOAt (10 mg, 0.07 mmol, 2.00 eq.) DIPEA (32 µL, 0.18 mmol, 5.00 eq.) and 1 mL DMF. After purification via column chromatography (EtOAc + 1% FA, 1:1), compound **24c** was obtained as a colorless solid.

Yield: 15 mg (37.95 µmol, 63%)

HPLC (30 – 100 % solvent B, 3 min) $R_{t,d1}$ = 1.498 min, $R_{t,d2}$ = 1.605 min, purity (220 nm): 97 %

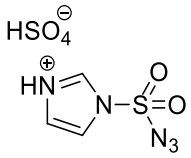
HRMS (ESI$^+$): m/z: calculated 659.33261 $[M+H]^+$, 659.33269 found $[M+H]^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.48 – 8.40 (m, 1H), 8.08 (d, J = 8.1 Hz, 1H), 7.31 (dt, J = 17.5, 7.3 Hz, 1H), 7.22 (t, J = 8.1 Hz, 1H), 7.01 (t, J = 5.8 Hz, 1H), 6.82 – 6.76 (m, 3H), 6.66 – 6.60 (m, 4H), 5.81 (s, 1H), 5.74 (t, J = 7.1 Hz, 1H), 5.65 (t, J = 6.8 Hz, 1H), 5.50 (d, J = 5.4 Hz, 1H), 4.77 – 4.60 (m, 2H), 3.99 (d, J = 13.9 Hz, 1H), 3.87 (s, 3H), 3.86 (s, 3H), 3.66 (d, J = 10.2 Hz, 1H), 2.67 (t, J = 13.1 Hz, 1H), 2.57 – 2.51 (m, 1H), 2.49 – 2.39 (m, 1H), 2.36 – 2.31 (m, 3H), 2.26 – 2.20 (m, 2H), 2.14 – 2.07 (m, 2H), 1.95 – 1.90 (m, 1H), 1.88 – 1.75 (m, 1H), 1.74 – 1.57 (m, 3H), 1.48 – 1.37 (m, 3H), 1.37 – 1.29 (m, 3H), 1.23 – 1.08 (m, 3H), 1.00 – 0.77 (m, 4H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 171.26, 169.97, 169.35, 148.87, 147.35, 147.12, 145.45, 141.38, 138.83, 135.76, 133.50, 133.22, 130.16, 129.79, 124.74, 124.58, 120.19, 120.09, 118.84, 116.52, 113.04, 112.67, 111.72, 111.65, 111.35, 111.28, 75.50, 66.20, 65.84, 55.94, 55.88, 52.11, 51.45, 50.72, 43.96, 41.85, 41.13, 40.01, 37.82, 36.75, 32.33, 31.87, 31.72, 30.94, 30.75, 30.59, 30.59, 29.71, 28.04, 27.62, 26.30, 26.26, 26.13, 26.00, 25.91, 25.89, 25.50, 24.95, 22.70, 21.37, 20.76.

11.6 Synthesis of 1,2,3-triazole containing SAFit1 analogs

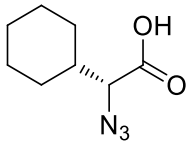
Imidazole-1-sulfonyl azide hydrogen sulfate (25)



The azide transfer reagent **25** was synthesized, following the general procedure developed by Potter et al.[122] using NaN_3 (2.13 g, 32.83, 1.00 eq.), sulfuryl chloride (2.65 mL, 32.83 mmol, 1.00 eq.), imidazole (4.24 g, 62.37 mmol, 1.90 eq.) and 32.83 mL EtOAc. After the product was precipitated using 1.76 mL conc. H_2SO_4, the title compound, was obtained as a colorless solid.

Yield: 2.96 g (10.91 mmol, 43%)

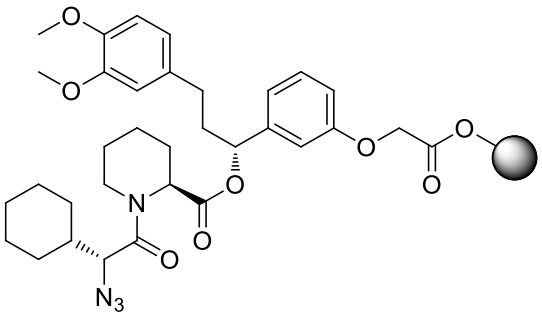
(R)-2-Azido-2-cyclohexylacetic acid (26)



A 100 mL flask was charged with (*R*)-α-aminocyclohexylacetic acid (1.19 g, 7.57 mmol, 1.00 eq.), K_2CO_3 (4.08 g, 29.52 mmol, 3.90 eq.), copper(II)sulfate pentahydrate (189 mg, 0.76 mmol, 0.10 eq.) and 37.85 mL methanol. The solution was then cooled to 0°C before the azide transfer reagent **25** (2.46 g, 9.08 mmol, 1.20 eq.) was added in small portions. The reaction mixture was then allowed to warm to rt overnight and diluted with water. By the careful addition of 1 M HCl, the aq. solution was acidified (pH < 4) and extracted with EtOAc. The combined org. layers were washed with brine, dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The crude product was then purified by column chromatography (CH/EtOAc + 1% FA, 0 – 75%). The title compound **26** was obtained as a colorless oil.

Yield: 1.03 g (5.62 mmol, 74%)
TLC (CH/EtOAc + 1% FA, 3:1): R_f = 0.24
HPLC (5 – 100 % solvent B, 3 min) R_t = 1.856 min, purity (220 nm): 96%
Mass (ESI^-): m/z: calculated 182.10 $[M-H]^-$, found 182.00 $[M-H]^-$
^{1}H NMR (500 MHz, $CDCl_3$) δ 3.77 (d, J = 6.1 Hz, 1H), 1.96 – 1.88 (m, 1H), 1.84 – 1.75 (m, 3H), 1.73 – 1.67 (m, 2H), 1.35 – 1.15 (m, 5H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 176.35, 67.47, 40.25, 28.30, 25.98, 25.94, 25.84.

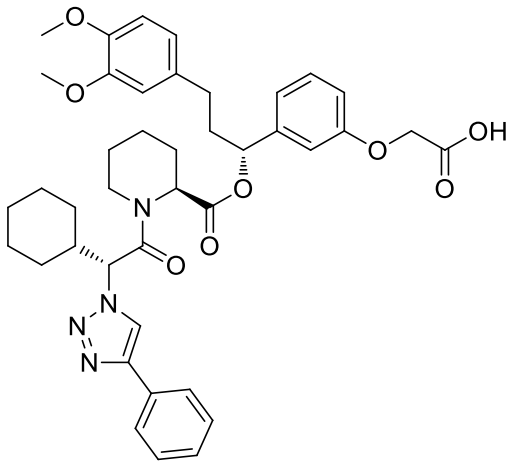
2-(3-((R)-1-(((S)-1-((R)-2-Azido-2-cyclohexylacetyl)piperidine-2-carbonyl)oxy)-3-(3,4-dimethoxyphenyl)propyl)phenoxy)acetic acid (27)



In a syringe, resin **7** (2.99 g, 1.69 mmol, 1.00eq.) was first Fmoc-deprotected following the general procedure and was then treated with a solution containing **26** (0.62 g, 3.38 mmol, 2.00 eq.), HATU (1.29 g, 3.38 mmol, 2.00 eq., HOAt (0.46 g, 3.38 mmol, 2.00 eq.), DIPEA (1.47 mL, 8.46 mmol, 5.00 eq.) and 6.77 mL DMF. The resin was shaken overnight and washed twice with 10 mL NMP and 10 mL DCM. Afterward, the resin was dried under a vacuum.

Yield: 3.04 g (1.69 mmol, quant.)
HPLC (5 – 100 % solvent B, 3 min) R_t = 2.309 min, purity (220 nm): 98%
Mass (ESI^-): m/z: calculated 621.71 $[M-H]^-$, found 621.20 $[M-H]^-$

2-(3-((R)-1-(((S)-1-((R)-2-Cyclohexyl-2-(4-phenyl-1H-1,2,3-triazol-1-yl)acetyl)piperidine-2-carbonyl)oxy)-3-(3,4-dimethoxyphenyl)propyl)phenoxy)acetic acid (28a)



In a syringe, resin **27** (217 mg, 0.12 mmol, 1.00eq.) was swelled for 10 min using 5 mL of DCM. The resin was then transferred into a 10 mL flask. Then $CuSO_4 \cdot 5\ H_2O$ (30 mg, 0.12 mmol, 1.00 eq.) and sodium ascorbate (24 mg, 0.12 mmol, 1.00 eq.) were added. The flask was then evacuated and backfilled with argon. Then 2.46 mL dry DMF was added, followed by DIPEA (169 µL, 0.97 mmol, 8.00 eq.) and phenylacetylene (67 µL, 0.61 mmol, 5.00 eq.). The resin was then stirred at rt overnight. After the complete conversion of the starting material, the resin was filtered and washed with DMF, followed by DCM. The product was then cleaved of the resin using a 20 vol.-% solution of HFIP in DCM. The crude was then purified by column chromatography (CH/EtOAc + 1% FA, 0 – 100%). The title compound was obtained as a colorless solid.

Yield: 50 mg (1.69 mmol, 57%)

TLC (CH/EtOAc + 1% FA, 1:1): R_f = 0.24

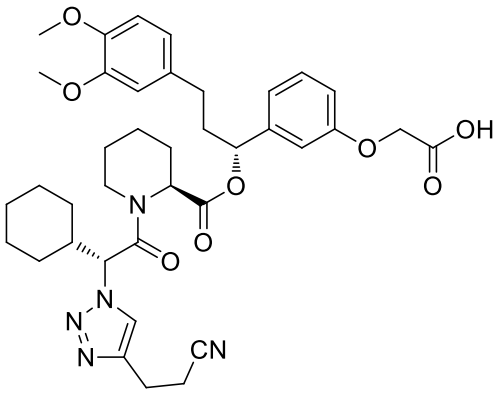
HPLC (50 – 100 % solvent B, 3 min) R_t = 1.661 min, purity (220 nm): 99%

HRMS (ESI$^+$): m/z: calculated 725.35449 $[M+H]^+$, found 725.35457 $[M+H]^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.20 (s, 1H), 7.59 (d, J = 3.6 Hz, 2H), 7.47 – 7.33 (m, 1H), 7.31 – 7.26 (m, 3H), 7.19 (t, J = 8.0 Hz, 1H), 6.79 (d, J = 5.9 Hz, 1H), 6.76 – 6.69 (m, 2H), 6.59 – 6.50 (m, 2H), 6.06 (s, 1H), 5.72 (t, J = 6.7 Hz, 1H), 5.61 (d, J = 10.3 Hz, 1H), 5.48 (d, J = 5.4 Hz, 1H), 4.67 – 4.58 (m, 2H), 4.16 (d, J = 14.1 Hz, 1H), 3.86 (d, J = 5.1 Hz, 2H), 3.84 (s, 3H), 3.80 (s, 3H), 2.75 (td, J = 13.5, 2.6 Hz, 1H), 2.43 – 2.36 (m, 2H), 2.33 – 2.28 (m, 2H), 1.90 – 1.76 (m, 3H), 1.74 – 1.65 (m, 4H), 1.50 – 1.40 (m, 1H), 1.38 – 1.30 (m, 1H), 1.27 (d, J = 14.1 Hz, 1H), 1.21 – 1.12 (m, 2H), 1.08 – 0.98 (m, 2H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 170.25, 169.39, 167.28, 157.67, 148.95, 147.43, 141.24, 133.51, 129.94, 129.79, 129.33, 129.08, 128.74, 125.85, 120.91, 120.27, 113.69, 112.31, 111.80, 111.39, 76.01, 65.54, 64.14, 56.06, 55.93, 52.82, 44.75, 41.06, 38.01, 30.82, 30.00, 28.60, 26.33, 26.03, 25.59, 25.42, 25.39, 20.72.

2-(3-((R)-1-(((S)-1-((R)-2-(4-(2-Cyanoethyl)-1H-1,2,3-triazol-1-yl)-2-cyclohexyl-acetyl)-piperidine-2-carbonyl)oxy)-3-(3,4-dimethoxyphenyl)propyl)phenoxy)-acetic acid (28b)



In a syringe, resin **27** (100 mg, 0.06 mmol, 1.00eq.) was swelled for 10 min using 5 mL of DCM. The resin was then transferred into a 10 mL flask. Then $CuSO_4 \cdot 5\ H_2O$ (14 mg, 0.06 mmol, 1.00 eq.) and sodium ascorbate (11 mg, 0.06 mmol, 1.00 eq.) were added. The flask was then evacuated and backfilled with argon. Then 1 mL dry DMF was added, followed by DIPEA (78 µL, 0.45 mmol, 8.00 eq.) and 4-pentynenitrile (17 µL, 0.28 mmol, 5.00 eq.). The resin was then stirred at rt overnight. After the complete conversion of the starting material, the resin was filtered and washed with DMF, followed by DCM. The product was then cleaved of the resin using a 20 vol.-% solution of HFIP in DCM. The crude was then purified by column chromatography (CH/EtOAc + 1% FA, 0 – 100%). The title compound was obtained as a colorless solid.

Yield: 11 mg (15.9 µmol, 28 %)

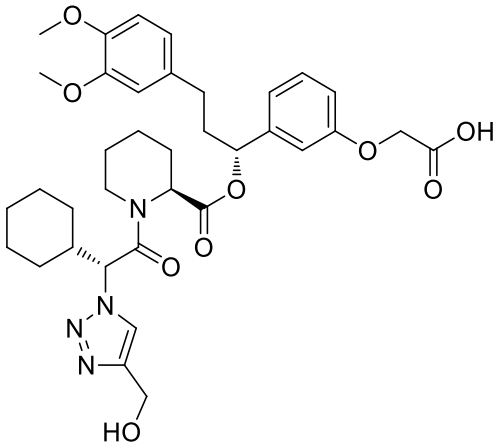
HPLC (30 – 100 % solvent B, 3 min) R_t = 1.783 min, purity (220 nm): 99%

HRMS (ESI$^+$): m/z: calculated 702.34971 $[M+H]^+$, found 702.34974 $[M+H]^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.87 (s, 1H), 7.24 (t, 1H), 6.88 – 6.84 (m, 1H), 6.79 (t, J = 8.0 Hz, 2H), 6.64 – 6.60 (m, 2H), 6.41 – 6.36 (m, 1H), 5.68 (t, J = 6.7 Hz, 1H), 5.57 (d, J = 10.2 Hz, 1H), 5.45 – 5.39 (m, 1H), 4.73 – 4.66 (m, 2H), 4.06 (d, J = 13.8 Hz, 1H), 3.86 (s, 6H), 2.96 – 2.87 (m, 2H), 2.58 – 2.51 (m, 2H), 2.42 – 2.21 (m, 4H), 1.99 – 1.91 (m, 1H), 1.89 – 1.63 (m, 8H), 1.58 – 1.39 (m, 2H), 1.37 – 1.23 (m, 3H), 1.20 – 1.07 (m, 2H), 1.06 – 0.93 (m, 2H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 170.78, 169.35, 167.57, 157.88, 149.04, 147.56, 144.22, 141.47, 133.39, 130.00, 121.70, 120.60, 120.27, 118.71, 114.71, 111.89, 111.54, 111.47, 76.43, 65.55, 64.29, 56.07, 53.03, 44.66, 40.93, 38.09, 30.99, 29.96, 28.67, 26.63, 25.98, 25.60, 25.38, 21.80, 20.72, 17.16.

2-(3-((R)-1-(((S)-1-((R)-2-Cyclohexyl-2-(4-(hydroxymethyl)-1H-1,2,3-triazol-1-yl)acetyl)-piperidine-2-carbonyl)oxy)-3-(3,4-dimethoxyphenyl)propyl)phenoxy)-acetic acid (28c)

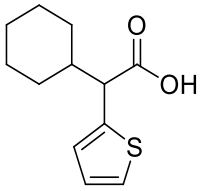


In a syringe, resin **27** (100 mg, 0.06 mmol, 1.00eq.) was swelled for 10 min using 5 mL of DCM. The resin was then transferred into a 10 mL flask. Then $CuSO_4 \cdot 5\ H_2O$ (14 mg, 0.06 mmol, 1.00 eq.) and sodium ascorbate (11 mg, 0.06 mmol, 1.00 eq.) were added. The flask was then evacuated and backfilled with argon. Then 1 mL dry DMF was added, followed by DIPEA (78 µL, 0.45 mmol, 8.00 eq.) and propargyl alcohol (16 µL, 0.28 mmol, 5.00 eq.). The resin was then stirred at rt overnight. After the complete conversion of the starting material, the resin was filtered and washed with DMF, followed by DCM. The product was then cleaved of the resin using a 20 vol.-% solution of HFIP in DCM. The crude was then purified by preparative HPLC (H_2O/ACN + 0.1% TFA, 50 – 80%). The title compound was obtained as a colorless solid.

Yield: 19 mg (27.9 µmol, 50%)
HPLC (30 – 100 % solvent B, 3 min) R_t = 1.640 min, purity (220 nm): 97%
HRMS (ESI$^+$): m/z: calculated 679.33376 $[M+H]^+$, found 679.33348 $[M+H]^+$
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.93 (s, 1H), 7.23 (t, J = 7.9 Hz, 1H), 6.85 (dd, J = 8.3, 2.5 Hz, 1H), 6.79 (dd, J = 14.0, 7.9 Hz, 2H), 6.65 – 6.58 (m, 2H), 6.55 – 6.48 (m, 1H), 5.68 – 5.63 (m, 1H), 5.53 (d, J = 10.1 Hz, 1H), 5.36 (d, J = 5.5 Hz, 1H), 4.66 (s, 3H), 4.04 (d, J = 13.5 Hz, 1H), 3.87 – 3.85 (m, 1H), 3.85 (s, 3H), 3.84 (s, 3H), 2.98 (t, J = 13.2 Hz, 1H), 2.44 – 2.39 (m, 1H), 2.39 – 2.32 (m, 2H), 2.27 – 2.20 (m, 1H), 2.03 – 1.94 (m, 1H), 1.94 – 1.86 (m, 1H), 1.84 – 1.75 (m, 3H), 1.75 – 1.66 (m, 4H), 1.52 – 1.40 (m, 2H), 1.35 – 1.25 (m, 2H), 1.21 – 1.13 (m, 2H), 1.10 – 0.71 (m, 3H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 171.37, 169.51, 167.32, 157.90, 149.01, 147.53, 147.10, 141.41, 133.43, 129.96, 122.11, 120.29, 114.79, 112.25, 111.91, 111.47, 76.49, 65.46, 64.56, 56.06, 56.04, 55.26, 53.06, 44.56, 41.02, 37.71, 31.07, 29.93, 28.66, 26.61, 25.95, 25.59, 25.38, 25.33, 20.68.

11.7 Synthesis of 2-thiophene acetic acids as bottom groups for SAFit1

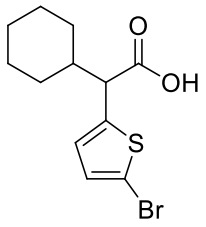
2-Cyclohexyl-2-(thiophen-2-yl)acetic acid (31a)



In a 25 mL dried flask, 2-thiopheneacetic acid (1.30 g, 9.14 mmol, 1.00 eq.) was dissolved in 46 mL THF. The resulting solution was cooled to -78°C, and 20.12 mL LiHMDS (1 M in THF, 20.12 mmol, 2.20 eq.) was added dropwise. The mixture was stirred for 1h at this temperature before iodocyclohexane (2.37 mL, 18.29 mmol, 2.00 eq.) was added. The reaction was then allowed to warm to rt overnight. After the complete conversion of the starting material, the reaction was quenched by the addition of sat. NH_4Cl solution and was extracted with EtOAc. The org. layer was then dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The title compound **31a** was obtained as a pale yellowish solid after purification via column chromatography (DCM/MeOH + 1% FA, 50:1).

Yield: 1.62 g (7.22 mmol, 79%)
TLC (DCM/MeOH + 1% FA, 100:1): R_f = 0.23
HPLC (50 – 100 % solvent B, 3 min) R_t = 0.695 min, purity (220 nm): 98%
Mass (ESI$^+$): m/z: calculated 225.09 $[M+H]^+$, found 225.20 $[M+H]^+$
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.24 – 7.20 (m, 1H), 6.98 – 6.93 (m, 2H), 3.58 (d, J = 10.0 Hz, 1H), 1.97 – 1.83 (m, 2H), 1.78 – 1.72 (m, 1H), 1.70 – 1.61 (m, 2H), 1.57 – 1.49 (m, 1H), 1.30 – 1.08 (m, 4H), 0.91 – 0.80 (m, 1H).
^{13}C NMR (75 MHz, $CDCl_3$) δ 179.26, 139.49, 126.65, 125.06, 53.90, 42.44, 31.79, 30.46, 26.30, 26.02.

2-(5-Bromothiophen-2-yl)-2-cyclohexylacetic acid (31b)



In a 10 mL flask, **31a** (401 mg, 1.79 mmol, 1.00 eq.) was dissolved in 5.97 mL acetic acid. Then NBS was added (334 mg, 1.88 mmol, 1.05 eq.), and the resulting solution was stirred at rt overnight. After the complete conversion of the starting material, water was added, and the resulting solution was extracted three times with diethyl ether. The combined org. layers were washed with brine, dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The crude was purified by column chromatography (CH/EtOAc + 1% FA, 3:1). After the removal of the solvent, the title compound was obtained as a colorless solid.

Yield: 453 mg (1.49 mmol, 84%)

TLC (DCM/MeOH + 1% FA, 100:1): R_f = 0.20

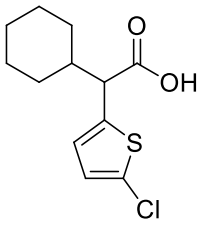
HPLC (50 – 100 % solvent B, 3 min) R_t = 0.695 min, purity (220 nm): 98%

Mass (ESI^+): m/z: calculated 304.22 $[M+H]^+$, found 304.00 $[M+H]^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ 6.89 (d, J = 3.7 Hz, 1H), 6.70 (d, J = 3.8 Hz, 1H), 3.50 (d, J = 10.0 Hz, 1H), 1.90 – 1.80 (m, 2H), 1.77 – 1.71 (m, 1H), 1.70 – 1.62 (m, 2H), 1.59 – 1.53 (m, 1H), 1.31 – 1.25 (m, 1H), 1.22 – 1.12 (m, 2H), 1.12 – 1.04 (m, 1H), 0.88 – 0.80 (m, 1H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 178.27, 140.93, 127.00, 111.62, 54.29, 42.26, 31.53, 30.22, 26.05, 25.80.

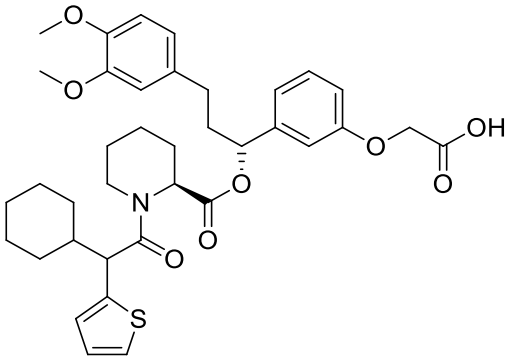
2-(5-Chlorothiophen-2-yl)-2-cyclohexylacetic acid (31c)



In a 5 mL flask, **31a** (100 mg, 0.45 mmol, 1.00 eq.) was dissolved in 0.89 mL acetic acid. Then NCS (87 mg, 0.49 mmol, 1.10 eq.) was added, and the solution was stirred at rt overnight. After the complete conversion of the starting material, water was added, and the resulting mixture was extracted three times with DCM. The combined org. layers were washed with brine, dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. After purification via preparative HPLC (H_2O/ACN + 0.1% TFA, 70-100%), the title compound was obtained as a colorless solid.

Yield: 63 mg (24.35 µmol, 55%)
TLC (DCM/MeOH + 1% FA, 50:1): R_f = 0.34
HPLC (50 – 100 % solvent B, 3 min) R_t = 1.197 min, purity (220 nm): 99%
Mass (ESI^+): m/z: calculated 259.05 $[M+H]^+$, found 259.00 $[M+H]^+$
^{1}H NMR (500 MHz, $CDCl_3$) δ 6.77 (d, J = 3.7 Hz, 1H), 6.74 (d, J = 3.7 Hz, 1H), 3.48 (d, J = 9.9 Hz, 1H), 1.93 – 1.82 (m, 2H), 1.80 – 1.65 (m, 3H), 1.63 – 1.57 (m, 1H), 1.35 – 1.07 (m, 4H), 0.91 – 0.82 (m, 1H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 179.14, 138.13, 129.56, 126.16, 125.52, 54.55, 42.39, 31.66, 30.35, 26.19, 25.94.

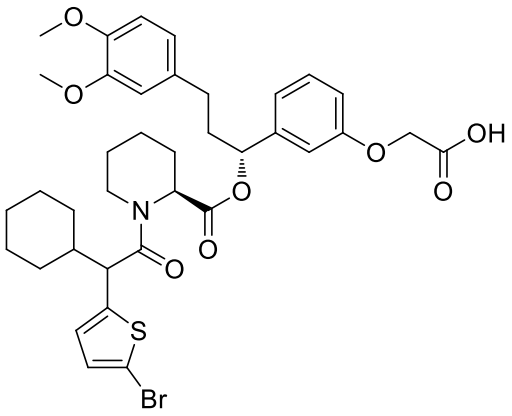
2-(3-((1R)-1-(((2S)-1-(2-Cyclohexyl-2-(thiophen-2-yl)acetyl)piperidine-2-carbonyl)oxy)-3-(3,4-dimethoxyphenyl)propyl)phenoxy)acetic acid (32a)



Following the general procedure A, compound **32a** was synthesized using: Resin **7** (107 mg, 0.08 mmol, 1.00 eq., loading: 0.73 mmol/g), **31a** (35 mg, 0.16 mmol, 2.00 eq.), HATU (59 mg, 0.16 mmol, 2.00 eq.), HOAt (21 mg, 0.16 mmol, 2.00 eq.) DIPEA (70 µL, 0.39 mmol, 5.00 eq.) and 1.60 mL DMF. After purification via preparative HPLC (H_2O/ACN + 0.1% TFA, 50 - 100%), compound **32a** was obtained as a colorless solid.

Yield: 28 mg (42.18 mmol, 54%)
HPLC (50 – 100 % solvent B, 3 min) R_t = 1.737 min, purity (220 nm): 97%
HRMS (ESI$^+$): m/z: 664.29386 calculated $[M+H]^+$, found 664.29417 $[M+H]^+$
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.26 – 7.21 (m, 1H), 7.19 – 7.15 (m, 1H), 6.93 – 6.90 (m, 1H), 6.89 – 6.86 (m, 2H), 6.84 – 6.81 (m, 1H), 6.80 – 6.76 (m, 1H), 6.71 – 6.64 (m, 3H), 5.73 – 5.59 (m, 1H), 5.54 – 5.48 (m, 1H), 4.72 – 4.58 (m, 2H), 4.07 – 3.99 (m, 1H), 3.86 (s, 3H), 3.85 (s, 3H), 3.40 – 2.95 (m, 1H), 2.70 – 2.51 (m, 2H), 2.35 – 2.18 (m, 2H), 2.10 – 1.99 (m, 2H), 1.91 – 1.75 (m, 1H), 1.71 – 1.49 (m, 5H), 1.49 – 1.32 (m, 3H), 1.20 – 1.03 (m, 4H), 0.92 – 0.66 (m, 3H).
^{13}C NMR (125 MHz, $CDCl_3$) δ 173.32, 172.56, 171.69, 171.49, 170.54, 170.25, 158.08, 157.81, 149.04, 148.96, 147.53, 147.43, 142.23, 142.20, 141.06, 140.36, 133.67, 133.53, 129.83, 129.80, 126.50, 126.47, 125.95, 125.75, 124.81, 124.78, 120.38, 120.32, 119.94, 119.82, 115.53, 115.06, 111.96, 111.83, 111.55, 111.48, 111.42, 110.69, 76.45, 76.12, 65.47, 65.37, 56.07, 56.02, 52.75, 52.58, 49.72, 49.66, 44.14, 44.11, 42.69, 42.58, 38.23, 37.99, 32.43, 32.24, 31.56, 31.37, 30.69, 29.84, 27.14, 26.84, 26.53, 26.50, 26.15, 26.06, 25.65, 25.22, 21.12, 21.03.

2-(3-((1R)-1-(((2S)-1-(2-(5-Bromothiophen-2-yl)-2-cyclohexylacetyl)piperidine-2-carbonyl)-oxy)-3-(3,4-dimethoxyphenyl)propyl)phenoxy)acetic acid (32b)



Following the general procedure A, compound **32b** was synthesized using: Resin **7** (63 mg, 0.02 mmol, 1.00 eq., loading: 0.39 mmol/g), **31b** (15 mg, 0.05 mmol, 2.00 eq.), HATU (19 mg, 0.05 mmol, 2.00 eq.), HOAt (7 mg, 0.05 mmol, 2.00 eq.) DIPEA (22 µL, 0.12 mmol, 5.00 eq.) and 1.00 mL DMF. After purification via flash chromatography (CH/EtOAc + 1% FA, 5 - 50%), compound **32b** was obtained as a colorless solid.

Yield: 12 mg (16.16 mmol, 67%)

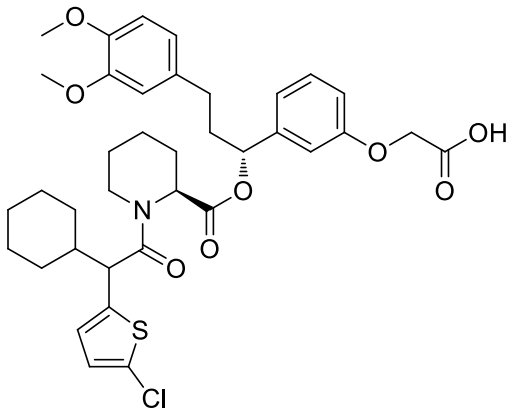
HPLC (50 – 100 % solvent B, 3 min) R_t = 1.940 min, purity (220 nm): 99%

HRMS (ESI$^+$): m/z: calculated 742.20438 $[M+H]^+$, found 742.20442 $[M+H]^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.22 (dd, J = 14.8, 7.1 Hz, 1H), 6.89 (d, J = 7.8 Hz, 1H), 6.87 – 6.83 (m, 1H), 6.82 – 6.77 (m, 2H), 6.74 – 6.69 (m, 1H), 6.69 – 6.64 (m, 2H), 6.63 – 6.59 (m, 1H), 5.70 – 5.60 (m, 1H), 5.52 – 5.46 (m, 1H), 4.73 – 4.60 (m, 2H), 4.01 – 3.90 (m, 1H), 3.87 (s, 3H), 3.86 (s, 3H), 3.83 – 3.78 (m, 1H), 3.39 – 3.07 (m, 1H), 2.72 – 2.46 (m, 2H), 2.40 – 2.16 (m, 2H), 2.14 – 1.90 (m, 2H), 1.77 – 1.57 (m, 6H), 1.53 – 1.41 (m, 2H), 1.39 – 1.25 (m, 2H), 1.22 – 1.03 (m, 3H), 0.93 – 0.75 (m, 2H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 173.07, 172.21, 171.20, 171.06, 170.35, 170.07, 158.09, 157.84, 149.08, 149.01, 147.58, 147.50, 142.84, 142.24, 142.21, 142.06, 133.57, 133.49, 129.89, 129.85, 129.12, 128.95, 126.43, 126.17, 120.41, 120.34, 119.91, 119.79, 115.83, 115.33, 111.95, 111.85, 111.51, 111.49, 111.12, 110.19, 76.58, 76.30, 65.55, 65.46, 56.09, 56.09, 56.06, 56.05, 52.88, 52.71, 50.35, 50.34, 44.25, 44.19, 42.98, 42.72, 38.23, 38.01, 32.28, 31.97, 31.62, 31.44, 30.62, 27.22, 26.95, 26.43, 26.40, 26.14, 26.12, 26.10, 26.01, 25.64, 25.36, 21.10, 21.03.

2-(3-((R)-1-(((S)-1-(2-(5-Chlorothiophen-2-yl)-2-cyclohexylacetyl)piperidine-2-carbonyl)oxy)-3-(3,4-dimethoxyphenyl)propyl)phenoxy)acetic acid (32c)



Following the general procedure A, compound **32c** was synthesized using: Resin **7** (99 mg, 0.04 mmol, 1.00 eq., loading: 0.39 mmol/g), **31c** (20 mg, 0.08 mmol, 2.00 eq.), HATU (29 mg, 0.08 mmol, 2.00 eq.), HOAt (11 mg, 0.08 mmol, 2.00 eq.) DIPEA (34 µL, 0.19 mmol, 5.00 eq.) and 1.00 mL DMF. After purification via column chromatography (CH/EtOAc + 1% FA, 2:1), compound **32c** was obtained as a colorless solid.

Yield: 18 mg (25.78 µmol, 99%)

HPLC (50 – 100 % solvent B, 3 min) R_t = 1.563 min, purity (220 nm): 95%

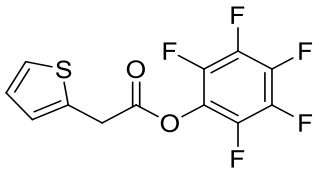
HRMS (ESI$^+$): m/z: 698.2552 calculated $[M+H]^+$, found 698.2552 $[M+H]^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.26 – 7.17 (m, 1H), 6.93 – 6.86 (m, 1H), 6.85 – 6.82 (m, 1H), 6.78 (t, J = 3.9 Hz, 1H), 6.74 – 6.70 (m, 1H), 6.69 – 6.67 (m, 1H), 6.65 (s, 1H), 6.64 – 6.60 (m, 1H), 5.72 – 5.62 (m, 1H), 5.49 (t, J = 7.5 Hz, 1H), 4.71 – 4.62 (m, 2H), 4.00 – 3.91 (m, 1H), 3.86 (s, 3H), 3.85 (s, 3H), 3.81 – 3.74 (m, 1H), 3.32 (td, J = 13.1, 3.0 Hz, 1H), 2.68 – 2.60 (m, 1H), 2.60 – 2.52 (m, 1H), 2.37 – 2.26 (m, 1H), 2.25 – 2.17 (m, 1H), 2.12 – 2.02 (m, 1H), 2.00 – 1.90 (m, 0H), 1.82 – 1.72 (m, 1H), 1.71 – 1.57 (m, 6H), 1.54 – 1.41 (m, 2H), 1.37 – 1.21 (m, 4H), 1.20 – 1.06 (m, 2H), 0.96 – 0.73 (m, 2H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 172.82, 172.05, 171.54, 171.35, 170.28, 170.02, 157.94, 157.71, 148.95, 148.88, 147.44, 147.35, 142.03, 141.99, 139.90, 139.19, 133.39, 129.71, 125.24, 125.18, 125.03, 124.98, 120.26, 120.21, 119.82, 119.74, 115.34, 114.87, 111.85, 111.77, 111.42, 111.39, 110.77, 76.37, 76.12, 65.32, 55.96, 55.90, 52.68, 52.51, 50.18, 44.11, 44.07, 42.83, 42.56, 38.05, 37.84, 32.12, 31.84, 31.40, 31.22, 30.49, 26.96, 26.71, 26.29, 26.27, 25.97, 25.89, 25.50, 25.23, 20.92, 20.88.

11.8 Asymmetric alkylation of 2-thiophene acetic acid

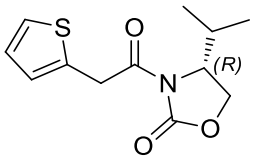
Perfluorophenyl 2-(thiophen-2-yl)acetate (33)



A 100 mL flask was charged with 2-thiopheneacetic acid (2.00 g, 14.07 mmol, 1.00 eq.), pentafluorophenol (3.37 g, 18.29 mmol, 1.30 eq.), DMAP (0.34 g, 2.81 mmol, 0.30 eq.) and 70 mL DCM. The solution was cooled to 0°C, and EDC·HCl (3.51 g, 18.29 mmol, 1.50 eq.) was added in small portions. After the complete addition, the ice bath was removed, and the reaction mixture was stirred overnight at rt. The reaction mixture was quenched by adding brine and extracted three times with DCM. The combined org. layers were washed with 1 M HCl, dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The crude was used without further purification as a yellowish oil.

Yield: 4.17 g (13.53 mmol, 96%)
TLC (CH/EtOAc + 1% FA, 1:1): R_f = 0.90
HPLC (50 – 100 % solvent B, 3 min) R_t = 1.144 min, purity (220 nm): 98%
Mass (ESI^+): m/z: calculated 309.22 $[M+H]^+$, found 309.00 $[M+H]^+$
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.20 (dd, J = 5.1, 1.2 Hz, 1H), 6.97 (dt, J = 3.3, 1.1 Hz, 1H), 6.92 (dd, J = 5.2, 3.5 Hz, 1H), 4.10 (s, 2H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 166.42, 142.06, 140.65, 140.05, 138.91, 136.88, 132.61, 127.66, 127.13, 125.79, 34.28.

(R)-4-Isopropyl-3-(2-(thiophen-2-yl)acetyl)oxazolidin-2-one (34)



In a 100 mL dried flask (R)-4-isopropyl-2-oxazolidinone (800 mg, 6.81 mmol, 1.05 eq.) was dissolved in 32 mL THF and was cooled to 0°C. Then 2.73 mL nBuLi (2.35 M in THF, 6.81 mmol, 1.05 eq.) was added dropwise, and the resulting suspension was stirred for 1 h at this temperature. Then ester **33** (2.00 g, 6.49 mmol, 1.00 eq.) was added dropwise as a solution in 20 mL THF. Finally, the solution was allowed to warm to rt overnight. The reaction mixture was quenched by the addition of sat. NH_4Cl solution and was extracted three times with diethyl ether. The combined org. layers were washed with brine, dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The crude was purified using flash chromatography (CH/EtOAc, 5- 30%) to obtain the title compound as a colorless solid.

Yield: 529 mg (2.09 mmol, 32%)

TLC (CH/EtOAc, 3:1): R_f = 0.31

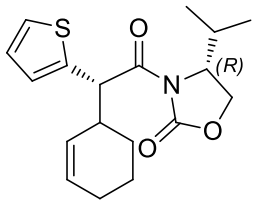
HPLC (5 – 100 % solvent B, 3 min) R_t = 1.901 min, purity (220 nm): 92%

Mass (ESI^+): m/z: calculated 254.08 $[M+H]^+$, found 254.20 $[M+H]^+$

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.25 (dd, J = 5.0, 1.3 Hz, 1H), 7.03 – 7.00 (m, 1H), 7.00 – 6.95 (m, 1H), 4.64 – 4.57 (m, 1H), 4.50 – 4.42 (m, 2H), 4.34 – 4.20 (m, 2H), 2.45 – 2.32 (m, 1H), 0.91 (d, J = 7.1 Hz, 3H), 0.84 (d, J = 6.9 Hz, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 170.00, 153.98, 134.71, 127.39, 126.72, 125.35, 63.48, 58.59, 36.01, 28.28, 17.90, 14.57.

(4R)-3-((2S)-2-(Cyclohex-2-en-1-yl)-2-(thiophen-2-yl)acetyl)-4-isopropyloxazolidin-2-one (35)



In a 50 mL dried flask, **34** (301 mg, 1.19 mmol, 1.00 eq.) was dissolved in 11.88 mL THF. The solution was cooled to -78°C, and 1.78 mL LiHMDS (1.00 M in THF, 1.78 mmol, 1.50 eq.) was added dropwise. The mixture was stirred for 1 h at this temperature before 3-bromcyclohexene (0.21 mL, 1.78 mmol, 1.50 eq.) was added. The ice bath was removed, and the reaction mixture was stirred for 3 h. After the complete conversion of the starting material, the reaction was quenched by adding sat. NH_4Cl solution and was extracted three times with EtOAc. The combined org. layers were washed with brine, dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The crude was purified using flash chromatography (CH/EtOAc, 0 - 20%) to obtain the title compound as a yellow oil.

Yield: 175 mg (0.52 mmol, 44%)

TLC (CH/EtOAc, 9:1): R_f = 0.19

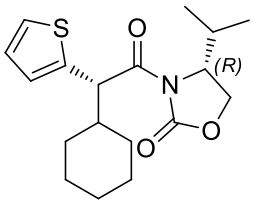
HPLC (50 – 100 % solvent B, 3 min) R_t = 1.485 min, purity (220 nm): 95%

Mass (ESI^+): m/z: calculated 334.14 $[M+H]^+$, found 334.20 $[M+H]^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.25 – 7.21 (m, 1H), 7.05 (dd, J = 3.6, 1.2 Hz, 1H), 6.94 (dd, J = 5.1, 3.5 Hz, 1H), 5.82 – 5.76 (m, 1H), 5.57 (dd, J = 10.1, 2.6 Hz, 1H), 5.44 – 5.38 (m, 1H), 4.45 (td, J = 5.6, 3.7 Hz, 1H), 4.18 (dd, J = 7.1, 5.5 Hz, 2H), 3.00 – 2.91 (m, 1H), 2.53 – 2.43 (m, 1H), 2.03 – 1.98 (m, 2H), 1.76 – 1.68 (m, 1H), 1.57 – 1.49 (m, 2H), 1.33 – 1.25 (m, 1H), 0.95 (dd, J = 7.0, 4.6 Hz, 6H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 173.15, 153.55, 139.79, 129.89, 127.96, 127.12, 126.40, 125.26, 62.82, 58.85, 48.66, 40.31, 28.35, 26.92, 25.90, 25.28, 20.26, 17.99, 14.54.

(R)-3-((S)-2-Cyclohexyl-2-(thiophen-2-yl)acetyl)-4-isopropyloxazolidin-2-one (36a)



In a 10 mL flask, **35** (173 mg, 0.52 mmol, 1.00 eq.) and 28 mg Pd/C (10 wt.-% on carbon, 0.03 mmol, 0.05 eq.) were dissolved in 5.20 mL methanol. The solution was degassed for 10 min using argon, and a hydrogen atmosphere (1 atm) was applied. The reaction mixture was then stirred for 2 h at rt and filtered through a silica plug. After the removal of the solvent, the obtained crude was purified via column chromatography (CH/EtOAc, 5:1). The title compound was obtained as a colorless oil.

Yield: 106 mg (0.32 mmol, 61%)

TLC (CH/EtOAc, 5:1): R_f = 0.38

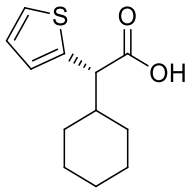
HPLC (50 – 100 % solvent B, 3 min) R_t = 1.598 min, purity (220 nm): 97%

Mass (ESI^+): m/z: calculated 336.16 $[M+H]^+$, found 336.20 $[M+H]^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.14 (d, J = 5.0 Hz, 1H), 6.92 (dd, J = 3.3, 1.0 Hz, 1H), 6.85 (dd, J = 5.2, 3.5 Hz, 1H), 5.24 (d, J = 10.1 Hz, 1H), 4.35 (td, J = 5.7, 3.8 Hz, 1H), 4.09 (dd, J = 5.7, 1.1 Hz, 2H), 2.41 – 2.32 (m, 1H), 2.07 – 1.98 (m, 1H), 1.70 – 1.61 (m, 2H), 1.60 – 1.53 (m, 2H), 1.42 – 1.35 (m, 1H), 1.26 – 1.18 (m, 2H), 1.13 – 1.04 (m, 3H), 0.85 (t, J = 7.1 Hz, 6H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 173.55, 153.72, 140.06, 127.01, 126.23, 125.21, 62.79, 58.89, 49.63, 43.12, 31.44, 30.09, 28.37, 26.20, 25.92, 25.86, 18.00, 14.51.

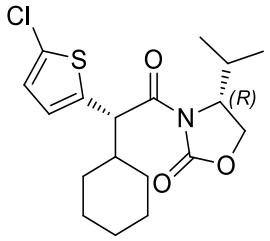
(S)-2-Cyclohexyl-2-(thiophen-2-yl)acetic acid (37a)



In a 5 mL flask, **36a** (42 mg, 0.13 mmol, 1.00 eq.) was dissolved in 2.50 mL THF/H_2O (4:1) and was cooled to 0°C. Then 51 µL aq. H_2O_2 (30 wt.-%, 0.50 mmol, 4.00 eq.) and LiOH (6 mg, 0.25 mmol, 2.00 eq.) were added. The resulting solution was then allowed to warm to rt overnight. The reaction mixture was diluted with water and acidified with 1 M HCl. The aq. phase was extracted three times with diethyl ether and the combined org. layers were dried over $MgSO_4$. After the solvent was removed, the crude was purified via column chromatography (CH/EtOAc + 1% FA, 4:1), and the title compound was obtained as a colorless solid.

Yield: 18 mg (0.08 mmol, 64%)
TLC (CH/EtOAc + 1% FA, 3:1): R_f = 0.56
HPLC (50 – 100 % solvent B, 3 min) R_t = 1.458 min, purity (220 nm): 95%
Mass (ESI$^+$): m/z: calculated 225.09 $[M+H]^+$, found 225.00 $[M+H]^+$
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.14 (dd, J = 4.8, 1.0 Hz, 1H), 6.90 – 6.86 (m, 2H), 3.50 (d, J = 10.1 Hz, 1H), 1.91 – 1.82 (m, 1H), 1.81 – 1.76 (m, 1H), 1.70 – 1.65 (m, 1H), 1.61 – 1.55 (m, 2H), 1.48 – 1.42 (m, 1H), 1.28 – 1.19 (m, 1H), 1.14 – 0.97 (m, 3H), 0.83 – 0.72 (m, 1H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 177.92, 138.30, 125.47, 123.89, 52.71, 41.26, 30.62, 29.28, 25.12, 24.83.

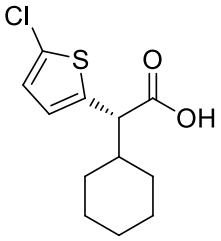
(R)-3-((S)-2-(5-Chlorothiophen-2-yl)-2-cyclohexylacetyl)-4-isopropyloxazolidin-2-one (36b)



In a 5 mL flask, **36a** (63 mg, 0.19 mmol, 1.00 eq.) was dissolved in 0.75 mL acetic acid. Then NCS (26 mg, 0.20 mmol, 1.10 eq.) was added, and the resulting solution was stirred at rt overnight. After the complete conversion of the starting material, the reaction mixture was diluted by adding water and neutralized with sat. $NaHCO_3$ solution. The resulting aq. solution was then extracted with DCM and the combined org. layers were washed with brine, dried over $MgSO_4$, and the solvent was removed under reduced pressure. The title compound was obtained after purification via flash chromatography (CH/EtOAc, 0 – 30%) as a colorless oil.

Yield: 58 mg (0.16 mmol, 84%)
TLC (CH/EtOAc , 5:1): R_f = 0.34
HPLC (70 – 100 % solvent B, 3 min) R_t = 1.189 min, purity (220 nm): 96%
Mass (ESI^+): m/z: calculated 370.12 $[M+H]^+$, found 370.00 $[M+H]^+$
^{1}H NMR (500 MHz, $CDCl_3$) δ 6.65 (dd, 2H), 5.13 (d, J = 10.0 Hz, 1H), 4.40 – 4.35 (m, 1H), 4.19 – 3.99 (m, 2H), 2.43 – 2.28 (m, 1H), 1.97 – 1.91 (m, 1H), 1.70 – 1.53 (m, 4H), 1.48 – 1.41 (m, 1H), 1.25 – 1.16 (m, 1H), 1.12 – 1.04 (m, 3H), 0.86 (d, J = 7.0 Hz, 3H), 0.83 (d, J = 7.0 Hz, 4H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 173.10, 153.70, 138.73, 129.56, 126.49, 125.17, 62.88, 58.86, 50.34, 43.02, 31.31, 29.96, 28.36, 26.11, 25.87, 25.82, 17.98, 14.49.

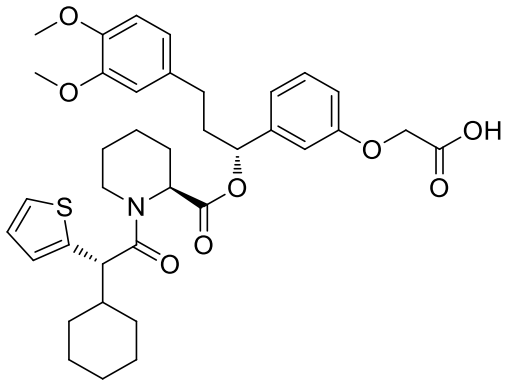
(S)-2-(5-Chlorothiophen-2-yl)-2-cyclohexylacetic acid (37b)



In a 5 mL flask, **36a** (58 mg, 0.16 mmol, 1.00 eq.) was dissolved in 3.14 mL THF/H_2O (4:1) and was cooled to 0°C. Then 64 µL aq. H_2O_2 (30 wt.-%, 0.63 mmol, 4.00 eq.) and LiOH (8 mg, 0.31 mmol, 2.00 eq.) were added. The resulting solution was then allowed to warm to rt overnight. The reaction mixture was diluted with water and acidified with 1 M HCl. The aq. phase was extracted three times with diethyl ether and the combined org. layers were dried over $MgSO_4$. After the solvent was removed, the crude was purified via flash chromatography (CH/EtOAc + 1% FA, 5 – 15%), and the title compound was obtained as a colorless solid.

Yield: 31 mg (0.12 mmol, 76%)
TLC (CH/EtOAc + 1% FA, 3:1): R_f = 0.39
HPLC (30 – 100 % solvent B, 3 min) R_t = 1.100 min, purity (220 nm): 93%
Mass (ESI^+): m/z: calculated 259.05 $[M+H]^+$, found 259.00 $[M+H]^+$
^{1}H NMR (500 MHz, $CDCl_3$) δ 6.77 (d, J = 3.7 Hz, 1H), 6.74 (d, J = 3.7 Hz, 1H), 3.48 (d, J = 9.9 Hz, 1H), 1.93 – 1.82 (m, 2H), 1.80 – 1.65 (m, 3H), 1.63 – 1.57 (m, 1H), 1.35 – 1.07 (m, 4H), 0.91 – 0.82 (m, 1H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 179.14, 138.13, 129.56, 126.16, 125.52, 54.55, 42.39, 31.66, 30.35, 26.19, 25.94.

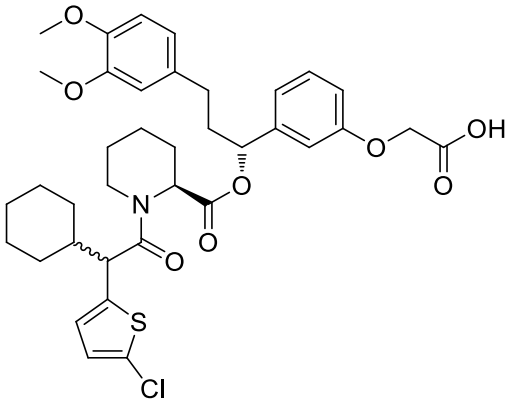
2-(3-((R)-1-(((S)-1-((R)-2-Cyclohexyl-2-(thiophen-2-yl)acetyl)piperidine-2-carbonyl)oxy)-3-(3,4-dimethoxyphenyl)propyl)phenoxy)acetic acid (38a)



Following the general procedure A, compound **38a** was synthesized using: Resin **7** (99 mg, 0.07 mmol, 1.00 eq., loading: 0.73 mmol/g), **37a** (18 mg, 0.08 mmol, 1.10 eq.), HATU (31 mg, 0.08 mmol, 1.10 eq.), HOAt (11 mg, 0.08 mmol, 1.10 eq.) DIPEA (35 µL, 0.20 mmol, 2.85 eq.) and 0.80 mL DMF. After purification via column chromatography (CH/EtOAc + 1% FA, 1:1), the title compound was obtained as a colorless solid.

Yield: 40 mg (28.62 µmol, 40%)
HPLC (50 – 100 % solvent B, 3 min) R_t = 1.563 min, purity (220 nm): 95%
HRMS (ESI$^+$): m/z: 664.2939 calculated $[M+H]^+$, found664.2936 $[M+H]^+$
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.24 (d, J = 7.9 Hz, 1H), 7.18 (dd, J = 5.1, 1.2 Hz, 1H), 6.92 (dd, J = 3.8, 1.2 Hz, 1H), 6.91 – 6.87 (m, 2H), 6.84 – 6.82 (m, 1H), 6.79 – 6.75 (m, 1H), 6.70 – 6.64 (m, 2H), 5.71 (dd, J = 8.5, 5.1 Hz, 1H), 5.51 (d, J = 6.0 Hz, 2H), 4.73 – 4.63 (m, 2H), 4.02 (d, J = 13.3 Hz, 2H), 3.86 (s, 3H), 3.85 (s, 3H), 3.49 (s, 2H), 3.36 – 3.27 (m, 1H), 2.69 – 2.62 (m, 1H), 2.60 – 2.52 (m, 1H), 2.30 – 2.25 (m, 1H), 2.25 – 2.17 (m, 1H), 2.10 – 2.00 (m, 2H), 1.81 – 1.74 (m, 1H), 1.68 – 1.63 (m, 2H), 1.61 – 1.56 (m, 3H), 1.41 (d, J = 13.8 Hz, 2H), 1.36 – 1.22 (m, 2H), 1.20 – 1.00 (m, 3H), 0.93 – 0.72 (m, 2H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 173.19, 170.97, 170.39, 158.00, 129.68, 126.33, 125.62, 124.63, 120.21, 119.66, 115.53, 111.78, 111.43, 110.41, 76.31, 65.41, 55.96, 55.91, 52.63, 49.54, 43.96, 42.56, 38.10, 32.11, 31.43, 30.56, 26.72, 26.37, 26.02, 25.93, 25.09, 20.90.

2-(3-((1R)-1-(((2S)-1-(2-(5-Chlorothiophen-2-yl)-2-cyclohexylacetyl)piperidine-2-carbonyl)-oxy)-3-(3,4-dimethoxyphenyl)propyl)phenoxy)acetic acid (38b)



Following the general procedure A, compound **38b** was synthesized using: Resin **7** (149 mg, 0.06 mmol, 1.00 eq., loading: 0.39 mmol/g), **37b** (30 mg, 0.12 mmol, 2.00 eq.), HATU (44 mg, 0.12 mmol, 2.00 eq.), HOAt (16 mg, 0.12 mmol, 2.00 eq.) DIPEA (50 µL, 0.29 mmol, 5.00 eq.) and 1 .00 mL DMF. After purification via column chromatography (CH/EtOAc + 1% FA, 1:1), the title compound was obtained as a colorless solid (epimerization ~ 50% in ^{1}H NMR).

Yield: 33 mg (47.26 µmol, 83%)

HPLC (50 – 100 % solvent B, 3 min) R_t = 1.777 min, purity (220 nm): 96%

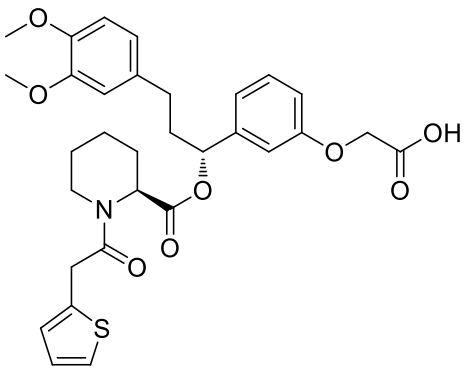
HRMS (ESI$^+$): m/z: 698.2549 calculated $[M+H]^+$, found 698.2548 $[M+H]^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.26 – 7.17 (m, 1H), 6.93 – 6.86 (m, 1H), 6.85 – 6.82 (m, 1H), 6.78 (t, J = 3.9 Hz, 1H), 6.74 – 6.70 (m, 1H), 6.69 – 6.67 (m, 1H), 6.65 (s, 1H), 6.64 – 6.60 (m, 1H), 5.72 – 5.62 (m, 1H), 5.49 (t, J = 7.5 Hz, 1H), 4.71 – 4.62 (m, 2H), 4.00 – 3.91 (m, 1H), 3.86 (s, 3H), 3.85 (s, 3H), 3.81 – 3.74 (m, 1H), 3.32 (td, J = 13.1, 3.0 Hz, 1H), 2.68 – 2.60 (m, 1H), 2.60 – 2.52 (m, 1H), 2.37 – 2.26 (m, 1H), 2.25 – 2.17 (m, 1H), 2.12 – 2.02 (m, 1H), 2.00 – 1.90 (m, 0H), 1.82 – 1.72 (m, 1H), 1.71 – 1.57 (m, 6H), 1.54 – 1.41 (m, 2H), 1.37 – 1.21 (m, 4H), 1.20 – 1.06 (m, 2H), 0.96 – 0.73 (m, 2H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 172.96, 172.19, 171.69, 171.49, 170.42, 170.16, 158.08, 157.85, 149.09, 149.02, 147.58, 147.49, 142.17, 142.14, 140.04, 139.33, 133.53, 129.86, 125.38, 125.33, 125.17, 125.12, 120.41, 120.35, 119.96, 119.88, 115.48, 115.01, 111.99, 111.91, 111.56, 111.54, 110.91, 76.51, 76.26, 65.46, 56.10, 56.05, 52.82, 52.65, 50.32, 44.26, 44.21, 42.97, 42.70, 38.19, 37.98, 32.26, 31.98, 31.55, 31.36, 30.63, 27.10, 26.85, 26.44, 26.41, 26.11, 26.03, 25.64, 25.38, 21.06, 21.02.

11.9 Synthesis of smaller 2-thiophene-containing bottom groups

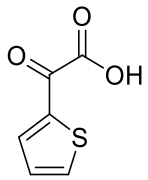
2-(3-((R)-3-(3,4-Dimethoxyphenyl)-1-(((S)-1-(2-(thiophen-2-yl)acetyl)piperidine-2-carbonyl)-oxy)propyl)phenoxy)acetic acid (39)



Following the general procedure A, compound **39** was synthesized using: Resin **7** (108 mg, 0.04 mmol, 1.00 eq., loading: 0.37 mmol/g), 2-thiopheneacetic acid (12 mg, 0.08 mmol, 2.00 eq.), HATU (32 mg, 0.08 mmol, 2.00 eq.), HOAt (11 mg, 0.08 mmol, 2.00 eq.) DIPEA (37 µL, 0.19 mmol, 5.00 eq.) and 1 mL DMF. After purification via column chromatography (CH/EtOAc + 1% FA, 1:1), compound **39** was obtained as a colorless solid.

Yield: 10 mg (17.19 µmol, 40%)
HPLC (30 – 100 % solvent B, 3 min) R_t = 1.487 min, purity (220 nm): 98%
HRMS (ESI$^+$): m/z: calculated 582.21561 $[M+H]^+$, found 582.21535 $[M+H]^+$
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.16 (t, J = 7.9 Hz, 1H), 7.11 (dd, J = 5.2, 1.2 Hz, 1H), 6.86 – 6.84 (m, 1H), 6.83 – 6.80 (m, 2H), 6.79 (d, J = 7.6 Hz, 1H), 6.74 – 6.70 (m, 2H), 6.64 – 6.60 (m, 2H), 5.59 (dd, J = 8.8, 4.9 Hz, 1H), 5.38 (d, J = 5.7 Hz, 1H), 4.63 – 4.51 (m, 2H), 3.92 – 3.81 (m, 2H), 3.80 (s, 3H), 3.79 (s, 3H), 3.77 – 3.72 (m, 1H), 3.35 – 3.26 (m, 1H), 2.64 – 2.56 (m, 1H), 2.53 – 2.44 (m, 1H), 2.30 – 2.24 (m, 1H), 2.18 – 2.12 (m, 1H), 2.03 – 1.95 (m, 1H), 1.72 – 1.65 (m, 1H), 1.61 – 1.57 (m, 1H), 1.33 – 1.26 (m, 2H), 0.84 – 0.77 (m, 1H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 170.97, 170.61, 170.20, 158.05, 149.00, 147.48, 142.12, 135.62, 133.39, 129.73, 126.89, 126.39, 124.82, 120.22, 119.54, 115.88, 111.77, 111.43, 109.78, 65.44, 55.96, 55.92, 52.59, 44.19, 38.07, 35.27, 31.49, 26.94, 25.08, 20.75.

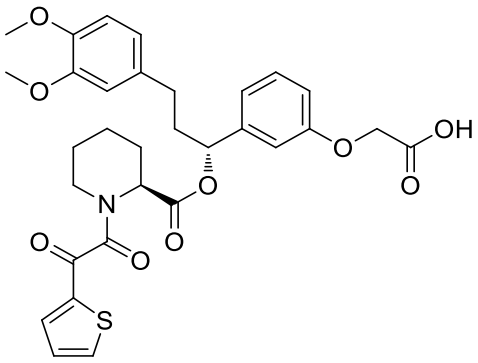
2-Oxo-2-(thiophen-2-yl)acetic acid (41)



In a 5 mL flask, ethyl thiophene-2-glyoxylate (200 µL, 1.36 mmol, 1.00 eq.) was dissolved in 4.52 mL EtOH/H_2O (1:1), and NaOH (217 mg, 5.43 mmol, 4.00 eq.) was added. The reaction mixture was then refluxed for 1 h. After cooling to rt, the mixture was acidified with 1 M HCl and was extracted with diethyl ether. The combined org. phases were then dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. After purification via flash chromatography (CH/EtOAc + 1% FA, 0 – 100%), compound **41** was obtained as a brownish solid.

Yield: 209 mg (1.34 µmol, 99%)
TLC (CH/EtOAc + 1% FA, 1:1): R_f = 0.09
HPLC (5 – 100 % solvent B, 3 min) R_t = 0.707 min, purity (220 nm): 99%
Mass (ESI$^+$): m/z: calculated 159.99 $[M+H]^+$, found 157.00 $[M+H]^+$
^{1}H NMR (500 MHz, $CDCl_3$) δ 8.50 (dd, J = 4.0, 1.2 Hz, 1H), 8.39 (s, 2H), 7.95 (dd, J = 4.9, 1.2 Hz, 1H), 7.25 (dd, J = 4.9, 4.0 Hz, 1H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 175.42, 159.83, 140.28, 140.03, 136.43, 129.41.

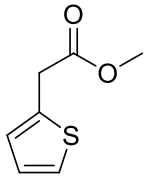
2-(3-((R)-3-(3,4-Dimethoxyphenyl)-1-(((S)-1-(2-oxo-2-(thiophen-2-yl)acetyl)-piperidine-2-carbonyl)oxy)propyl)phenoxy)acetic acid (42)



Following the general procedure B, compound **42** was synthesized using: Resin **7** (136 mg, 0.08 mmol, 1.00 eq., loading: 0.59 mmol/g), **41** (24 mg, 0.15 mmol, 2.00 eq.), PPh_3 (81 mg, 0.31 mmol, 4.00 eq.), trichloroacetonitrile (31 µL, 0.31 mmol, 4.00 eq.) DIPEA (134 µL, 0.77 mmol, 10.00 eq.) and 1.54 mL DCM. After purification via preparative HPLC (H_2O/ACN + 0.1% TFA, 5 - 100%), the title compound **42** was obtained as a colorless solid.

Yield: 19 mg (31.89 µmol, 42%)
HPLC (50 – 100 % solvent B, 3 min) R_t = 2.039 min, purity (220 nm): 99%
HRMS (ESI$^+$): m/z: 596.1949calculated $[M+H]^+$, found 596.1948 $[M+H]^+$
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.85 (dd, J = 3.9, 1.1 Hz, 1H), 7.78 (dd, J = 4.9, 1.1 Hz, 1H), 7.30 – 7.26 (m, 1H), 7.14 (dd, J = 5.0, 3.9 Hz, 1H), 6.95 (dt, J = 7.6, 1.2 Hz, 1H), 6.91 – 6.88 (m, 2H), 6.81 – 6.77 (m, 1H), 6.72 – 6.68 (m, 2H), 5.79 (dd, J = 8.3, 5.3 Hz, 1H), 5.41 (d, J = 4.5 Hz, 1H), 4.67 – 4.65 (m, 2H), 3.86 (s, 3H), 3.85 (s, 3H), 3.64 – 3.59 (m, 1H), 3.25 (td, J = 13.2, 3.1 Hz, 1H), 2.69 – 2.63 (m, 1H), 2.62 – 2.55 (m, 1H), 2.48 – 2.42 (m, 1H), 2.33 – 2.23 (m, 1H), 2.15 – 2.06 (m, 1H), 1.86 – 1.80 (m, 2H), 1.78 – 1.71 (m, 1H), 1.67 – 1.62 (m, 1H), 1.57 – 1.50 (m, 1H), 1.46 – 1.36 (m, 1H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 183.39, 171.58, 169.56, 166.47, 158.02, 149.12, 147.62, 141.84, 140.19, 137.34, 136.94, 133.45, 130.04, 129.16, 120.36, 120.01, 115.39, 111.95, 111.59, 77.02, 65.25, 56.10, 56.05, 52.32, 44.78, 38.26, 31.50, 26.69, 25.06, 21.23.

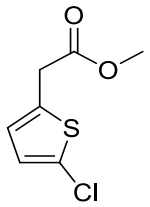
Methyl 2-(thiophen-2-yl)acetate (43)



In a 100 mL flask, 2-thiopheneacetic acid (6.59 g, 46.34 mmol, 1.00 eq.) was dissolved in 47 mL DCM, and the solution was cooled to 0°C. Then thionyl chloride (6.72 mL, 92.69 mmol, 2.00 eq.) was added dropwise, and the mixture was stirred for 1 h at rt. The reaction mixture was again cooled to 0°C, and 15 mL methanol was added dropwise. After complete addition, the reaction was allowed to warm to rt overnight and quenched by adding water. The org. phase was then washed with sat. $NaHCO_3$ solution, followed by brine and was dried over $MgSO_4$. After removing the solvent under reduced pressure, the title compound was obtained as a pale-yellow liquid.

Yield: 7.23 g (46.28 mmol, 99%)
TLC (CH/EtOAc, 3:1): R_f = 0.50
HPLC (30 – 100 % solvent B, 3 min) R_t = 1.595 min, purity (220 nm): 99%
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.21 (dd, J = 5.0, 1.5 Hz, 1H), 6.98 – 6.93 (m, 2H), 3.84 (s, 2H), 3.72 (s, 3H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 170.86, 135.00, 126.83, 126.80, 125.02, 52.20, 35.15.

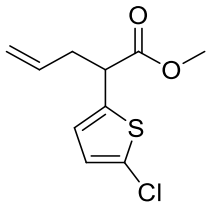
Methyl 2-(5-chlorothiophen-2-yl)acetate (44)



In a 100 mL flask, **43** (5.91, 37.83 mmol, 1.00 eq.) was dissolved in 19 mL acetic acid, and NCS (5.30 g, 39.72 mmol, 1.05 eq.) was added in one portion. The reaction mixture was stirred at rt overnight and quenched by adding water. The aq. phase was then extracted three times with DCM, and the combined organic layers were washed with sat. $NaHCO_3$ solution and brine. Finally, the org. phase was dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. Compound **44** was obtained as a yellowish liquid after purification via column chromatography (CH/EtOAc, 0 – 10%).

Yield: 6.41 g (33.62 mmol, 89%)
TLC (CH/EtOAc, 9:1): R_f = 0.30
HPLC (50 – 100 % solvent B, 3 min) R_t = 1.774 min, purity (220 nm): 94%
^{1}H NMR (300 MHz, $CDCl_3$) δ 6.74 (d, J = 3.7 Hz, 1H), 6.68 (d, J = 3.5 Hz, 2H), 3.73 (s, 2H), 3.72 (s, 3H).
^{13}C NMR (75 MHz, $CDCl_3$) δ 170.39, 133.82, 129.22, 126.23, 125.76, 52.38, 35.57.

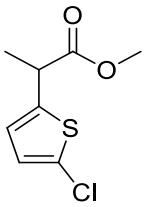
Methyl 2-(5-chlorothiophen-2-yl)pent-4-enoate (45a)



In a 25 mL dried flask, compound **44** (210 mg, 1.10 mmol, 1.00 eq.) was dissolved in 11 mL THF. The solution was cooled to -78°C, and 1.32 mL LiHMDS (1 M in THF, 1.32 mmol, 1.20 eq.) was added dropwise. The reaction mixture was stirred for 1 h at this temperature before allyl bromide (0.10 mL, 1.16 mmol, 1.05 eq.) in 10 mL THF was added dropwise, and the solution was allowed to warm to rt. After 3 h, the reaction was quenched by the addition of sat. NH_4Cl solution and the aq. phase was extracted three times with diethyl ether. The combined org. layers were then washed with brine, dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. After purification via column chromatography (CH/EtOAc, 0 - 10%), compound **45a** was obtained as a colorless oil.

Yield: 147 mg (0.64 mmol, 58%)
TLC (CH/EtOAc, 9:1): R_f = 0.43
HPLC (50 – 100 % solvent B, 3 min) R_t = 2.126 min, purity (220 nm): 96%
^{1}H NMR (300 MHz, $CDCl_3$) δ 6.74 (d, J = 3.8 Hz, 1H), 6.70 (d, J = 3.8 Hz, 1H), 5.72 (ddt, J = 17.1, 10.2, 6.9 Hz, 1H), 5.16 – 5.00 (m, 2H), 3.83 (t, J = 7.6 Hz, 1H), 3.70 (s, 3H), 2.81 – 2.67 (m, 1H), 2.60 – 2.49 (m, 1H).
^{13}C NMR (75 MHz, $CDCl_3$) δ 172.56, 139.48, 134.21, 125.67, 125.04, 117.99, 52.42, 47.28, 38.57.

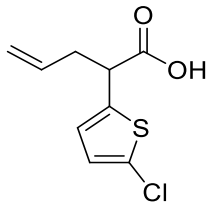
Methyl 2-(5-chlorothiophen-2-yl)propanoate (45c)



In a 25 mL dried flask, compound **44** (661 mg, 3.47 mmol, 1.00 eq.) was dissolved in 17.34 mL THF. The solution was cooled to -78°C, and 4.16 mL LiHMDS (1 M in THF, 4.16 mmol, 1.20 eq.) was added dropwise. The reaction mixture was stirred for 30 min at this temperature before methyl iodide (0.23 mL, 3.64 mmol, 1.05 eq.) was added dropwise, and the solution was allowed to warm to rt. After 2 h, the reaction was quenched by the addition of sat. NH_4Cl solution and the aq. phase was extracted three times with diethyl ether. The combined org. layers were then washed with brine, dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. After purification via column chromatography (CH/EtOAc, 0 - 5%), compound **45c** was obtained as a colorless oil.

Yield: 233 mg (1.14 mmol, 38%)
TLC (CH/EtOAc, 9:1): R_f = 0.37
HPLC (50 – 100 % solvent B, 3 min) R_t = 2.005 min, purity (220 nm): 95%
^{1}H NMR (300 MHz, $CDCl_3$) δ 6.74 (d, J = 3.8 Hz, 1H), 6.70 (dd, J = 3.8, 0.9 Hz, 1H), 3.71 (s, 3H), 1.54 (d, J = 7.2 Hz, 3H).
^{13}C NMR (75 MHz, $CDCl_3$) δ 173.45, 141.46, 128.76, 125.56, 124.15, 52.40, 41.20, 19.13.

2-(5-Chlorothiophen-2-yl)pent-4-enoic acid (46a)



In a 10 mL flask, compound **45a** (134 mg, 0.58 mmol, 1.00 eq.) was dissolved in 2.90 mL THF/H_2O/MeOH (3:1:1), and LiOH (70 mg, 2.90 mmol, 5.00 eq.) was added. The resulting solution was then stirred at rt overnight. After the complete conversion of the starting material, the reaction mixture was diluted with water and was acidified using 1 M HCl. The aq. phase was then extracted three times with EtOAc, the combined org. layers were dried over $MgSO_4$, and the solvent was removed under reduced pressure. After purification via column chromatography (CH/EtOAc + 1% FA, 0 - 30%), compound **46a** was obtained as a colorless solid.

Yield: 125 mg (0.58 mmol, 99%)

TLC (CH/EtOAc + 1% FA, 5:1): R_f = 0.31

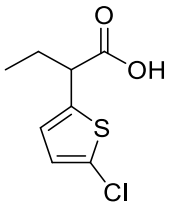
HPLC (30 – 100 % solvent B, 3 min) R_t = 1.487 min, purity (220 nm): 95%

Mass (ESI^+): m/z: calculated 217.00 $[M+H]^+$, found 217.00 $[M+H]^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ 6.75 (d, J = 4.4 Hz, 2H), 5.73 (ddt, J = 17.1, 10.5, 7.0 Hz, 1H), 5.18 – 5.06 (m, 2H), 3.84 (t, J = 7.7 Hz, 1H), 2.77 (dt, J = 14.7, 7.5 Hz, 1H), 2.57 (dt, J = 14.3, 7.1 Hz, 1H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 178.19, 138.50, 133.83, 129.60, 125.80, 125.58, 118.42, 47.18, 38.16.

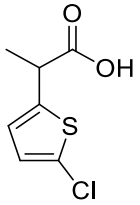
2-(5-Chlorothiophen-2-yl)butanoic acid (46b)



In a 25 mL dried flask, compound **44** (187 mg, 0.98 mmol, 1.00 eq.) was dissolved in 3.92 mL THF. The solution was cooled to -78°C, and 0.98 mL LiHMDS (1 M in THF, 0.98 mmol, 1.00 eq.) was added dropwise. The reaction mixture was stirred for 1 h at this temperature before iodoethane (0.87 µL, 1.08 mmol, 1.10 eq.) in 0.92 mL THF was added dropwise, and the solution was allowed to warm to rt. After 2 h, the reaction was quenched by the addition of sat. NH_4Cl solution and the aq. phase was extracted three times with diethyl ether. The combined org. layers were then washed with brine, dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The obtained crude (**45b**) was dissolved in 4.92 mL THF/H_2O/MeOH (3:1:1), and LiOH (118 mg, 4.92 mmol, 5.00 eq.) was added. The resulting solution was then stirred overnight at rt. After complete conversion, the reaction was diluted with water and acidified using 1 M HCl. The aq. phase was then extracted three times with EtOAc, the combined org. layers were dried over $MgSO_4$, and the solvent was removed under reduced pressure. After purification via column chromatography (CH/EtOAc + 1% FA, 0 - 30%), compound **46b** was obtained as a colorless oil.

Yield: 116 mg (0.57 mmol, 58% over 2 steps)
TLC (CH/EtOAc + 1% FA, 3:1): R_f = 0.26
HPLC (50 – 100 % solvent B, 3 min) R_t = 1.714 min, purity (220 nm): 96%
Mass (ESI^+): m/z: calculated 205.00 $[M+H]^+$, found 205.00 $[M+H]^+$
^{1}H NMR (500 MHz, $CDCl_3$) δ 6.76 (d, J = 3.8 Hz, 1H), 6.74 (d, J = 3.8 Hz, 1H), 3.67 (t, J = 7.6 Hz, 1H), 2.08 (dt, J = 13.7, 7.3 Hz, 1H), 1.85 (dt, J = 13.7, 7.4 Hz, 1H), 0.98 (t, J = 7.4 Hz, 3H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 179.31, 139.06, 129.26, 125.60, 125.34, 48.96, 27.58, 11.89.

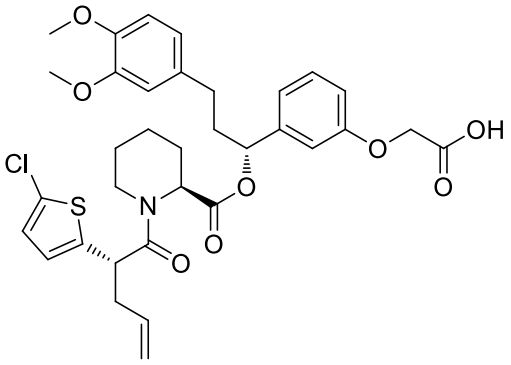
2-(5-Chlorothiophen-2-yl)propanoic acid (46c)



In a 10 mL flask, compound **45c** (203 mg, 0.99 mmol, 1.00 eq.) was dissolved in 4.96 mL THF/H_2O/MeOH (3:1:1), and LiOH (119 mg, 4.96 mmol, 5.00 eq.) was added. The resulting solution was then stirred at rt overnight. After the complete conversion of the starting material, the reaction mixture was diluted with water and was acidified using 1 M HCl. The aq. phase was then extracted three times with EtOAc, the combined org. layers were dried over $MgSO_4$, and the solvent was removed under reduced pressure. After the purification via column chromatography (CH/EtOAc + 1% FA, 0 - 30%), the title compound **46c** was obtained as a colorless solid.

Yield: 187 mg (0.98 mmol, 99%)
TLC (CH/EtOAc + 1% FA, 3:1): R_f = 0.25
HPLC (50 – 100 % solvent B, 3 min) R_t = 1.556 min, purity (220 nm): 98%
Mass (ESI^+): m/z: calculated 190.99 $[M+H]^+$, found 191.00 $[M+H]^+$
^{1}H NMR (300 MHz, $CDCl_3$) δ 6.76 (dd, J = 5.1 Hz, 2H), 3.92 (q, J = 7.2 Hz, 1H), 1.57 (d, J = 7.2 Hz, 3H).
^{13}C NMR (75 MHz, $CDCl_3$) δ 179.46, 140.59, 129.24, 125.79, 124.77, 41.26, 18.94.

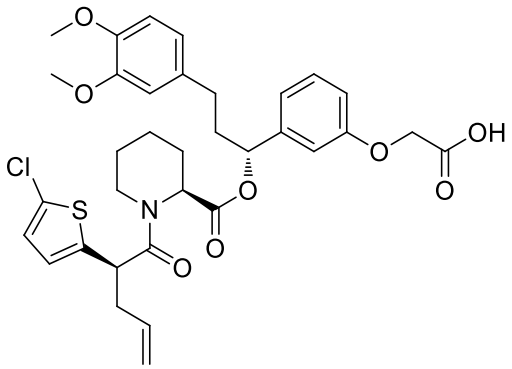
2-(3-((R)-1-(((S)-1-((S)-2-(5-Chlorothiophen-2-yl)pent-4-enoyl)piperidine-2-carbonyl)-oxy)-3-(3,4-dimethoxyphenyl)propyl)phenoxy)acetic acid (47a)



Following the general procedure A, compound **47a** was synthesized using: Resin **7** (201 mg, 0.08 mmol, 1.00 eq., loading: 0.39 mmol/g), **46a** (34 mg, 0.16 mmol, 2.00 eq.), HATU (60 mg, 0.16 mmol, 2.00 eq.), HOAt (21 mg, 0.16 mmol, 2.00 eq.) DIPEA (68 µL, 0.39 mmol, 5.00 eq.) and 1.57 mL DMF. After purification via preparative HPLC (H_2O/ACN + 0.1% TFA, 50 - 80%), diastereomers **47a** and **d47a** were obtained as colorless solids.

Yield: 20 mg (30.48 µmol, 39%)
HPLC (50 – 100 % solvent B, 3 min) R_t = 1.606 min, purity (220 nm): 99%
HRMS (ESI$^+$): m/z: calculated 656.20794 $[M+H]^+$, found 656.20771 $[M+H]^+$
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.31 – 7.27 (m, 2H), 6.91 (dt, J = 7.7, 2.2 Hz, 2H), 6.83 – 6.78 (m, 2H), 6.75 (d, J = 3.7 Hz, 1H), 6.72 (d, J = 1.9 Hz, 1H), 6.70 (t, J = 2.9 Hz, 1H), 6.67 (d, J = 3.8 Hz, 1H), 5.67 (ddd, J = 17.2, 9.3, 3.9 Hz, 2H), 5.50 (d, J = 5.5 Hz, 1H), 5.05 – 4.93 (m, 2H), 4.76 – 4.62 (m, 2H), 4.07 (t, J = 7.3 Hz, 1H), 3.89 (s, 3H), 3.88 (s, 3H), 3.39 (td, J = 13.2, 3.0 Hz, 1H), 2.80 – 2.65 (m, 2H), 2.66 – 2.55 (m, 1H), 2.53 – 2.44 (m, 1H), 2.35 (d, J = 13.8 Hz, 1H), 2.31 – 2.20 (m, 1H), 2.16 – 1.97 (m, 1H), 1.78 – 1.67 (m, 2H), 1.63 (d, J = 13.6 Hz, 1H), 1.45 – 1.29 (m, 1H), 1.24 – 1.11 (m, 1H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 172.21, 171.30, 170.22, 158.13, 149.09, 147.59, 142.24, 140.36, 134.77, 133.45, 129.90, 129.28, 125.57, 124.81, 120.33, 119.73, 117.81, 115.94, 111.82, 111.51, 109.93, 65.52, 56.09, 56.05, 52.92, 44.29, 43.92, 39.51, 38.25, 31.64, 27.02, 25.14, 21.00.

2-(3-((R)-1-(((S)-1-((R)-2-(5-Chlorothiophen-2-yl)pent-4-enoyl)piperidine-2-carbonyl)oxy)-3-(3,4-dimethoxyphenyl)propyl)phenoxy)acetic acid (d47a)



Yield: 14 mg (21.34 µmol, 27%)

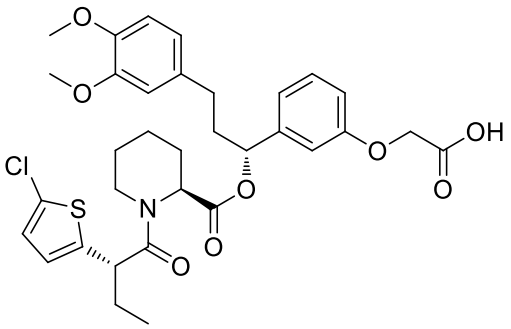
HPLC (50 – 100 % solvent B, 3 min) R_t = 1.586 min, purity (220 nm): 99%

HRMS (ESI^+): m/z: calculated 656.20794 $[M+H]^+$, found 656.20752 $[M+H]^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.30 – 7.19 (m, 1H), 6.88 (dd, J = 8.3, 2.6 Hz, 1H), 6.80 (dd, J = 7.9, 4.4 Hz, 2H), 6.78 – 6.75 (m, 1H), 6.71 – 6.67 (m, 3H), 6.66 (d, J = 3.9 Hz, 1H), 5.80 – 5.66 (m, 2H), 5.46 (d, J = 5.4 Hz, 1H), 5.12 – 5.02 (m, 2H), 4.74 – 4.60 (m, 2H), 4.08 (t, J = 7.2 Hz, 1H), 3.88 (s, 3H), 3.87 (s, 3H), 3.11 (td, J = 13.4, 3.0 Hz, 1H), 2.80 (dt, J = 14.4, 7.3 Hz, 1H), 2.66 – 2.59 (m, 2H), 2.58 – 2.50 (m, 2H), 2.37 (d, J = 13.5 Hz, 1H), 2.26 – 2.05 (m, 1H), 2.08 – 1.95 (m, 1H), 1.79 – 1.73 (m, 1H), 1.73 – 1.67 (m, 1H), 1.52 – 1.43 (m, 1H), 1.37 – 1.27 (m, 1H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 171.64, 171.52, 170.12, 157.90, 149.03, 147.52, 142.14, 139.71, 135.09, 133.52, 129.89, 129.00, 125.83, 125.07, 120.36, 119.90, 117.73, 115.35, 111.89, 111.49, 111.10, 76.42, 65.37, 56.08, 56.03, 52.82, 44.13, 44.11, 39.94, 38.09, 31.50, 27.11, 25.50, 21.04.

2-(3-((R)-1-(((S)-1-((S)-2-(5-Chlorothiophen-2-yl)butanoyl)piperidine-2-carbonyl)-oxy)-3-(3,4-dimethoxyphenyl)propyl)phenoxy)acetic acid (47b)



Following the general procedure A, compound **47b** was synthesized using: Resin **7** (129 mg, 0.07 mmol, 1.00 eq., loading: 0.56 mmol/g), **46b** (30 mg, 0.15 mmol, 2.00 eq.), HATU (56 mg, 0.15 mmol, 2.00 eq.), HOAt (20 mg, 0.15 mmol, 2.00 eq.), DIPEA (64 µL, 0.37 mmol, 5.00 eq.) and 1.47 mL DMF. After purification via preparative HPLC (H_2O/ACN + 0.1% TFA, 50 - 80%), diastereomers **47b** and **d47a** were obtained as colorless solids.

Yield: 10 mg (15.06 µmol, 21%)

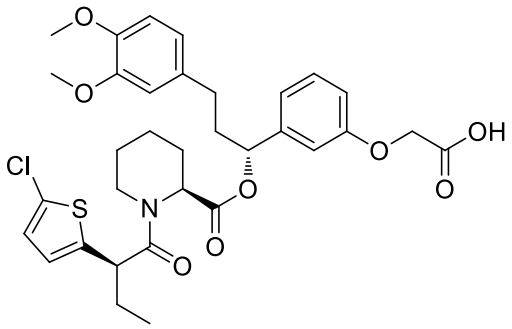
HPLC (50 – 100 % solvent B, 3 min) R_t = 1.600 min, purity (220 nm): 99%

HRMS (ESI$^+$): m/z: calculated 644.20794 $[M+H]^+$, found 644.20792 $[M+H]^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.23 (s, 1H), 6.87 (d, J = 7.7 Hz, 1H), 6.85 – 6.82 (m, 1H), 6.79 (d, J = 8.1 Hz, 1H), 6.77 (s, 1H), 6.74 (d, J = 3.7 Hz, 1H), 6.71 – 6.65 (m, 3H), 5.66 (dd, J = 8.8, 5.0 Hz, 1H), 5.52 – 5.44 (m, 1H), 4.76 – 4.61 (m, 2H), 3.92 – 3.88 (m, 1H), 3.87 (s, 3H), 3.86 (s, 3H), 3.41 – 3.31 (m, 1H), 2.71 – 2.65 (m, 1H), 2.63 – 2.54 (m, 1H), 2.35 (d, J = 14.3 Hz, 1H), 2.27 – 2.19 (m, 1H), 2.10 – 2.04 (m, 1H), 1.98 – 1.90 (m, 1H), 1.82 – 1.55 (m, 5H), 1.40 – 1.29 (m, 1H), 1.23 – 1.11 (m, 1H), 0.79 (t, J = 7.4 Hz, 3H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 172.93, 170.14, 158.21, 149.09, 147.58, 142.23, 140.79, 133.46, 129.85, 129.01, 125.63, 124.74, 120.33, 119.70, 116.13, 111.81, 111.51, 109.73, 76.81, 65.60, 56.09, 56.04, 53.04, 45.83, 43.99, 38.27, 31.67, 28.61, 26.94, 25.17, 21.07, 11.97.

2-(3-((R)-1-(((S)-1-((R)-2-(5-Chlorothiophen-2-yl)butanoyl)piperidine-2-carbonyl)-oxy)-3-(3,4-dimethoxyphenyl)propyl)phenoxy)acetic acid (d47b)



Yield: 12 mg (18.07 µmol, 26%)

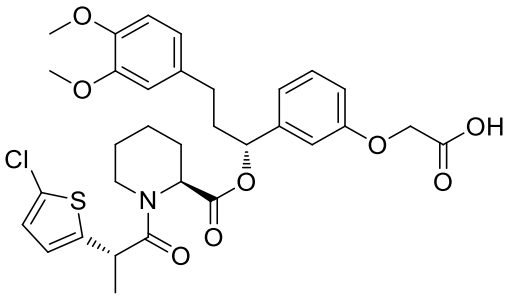
HPLC (50 – 100 % solvent B, 3 min) R_t = 1.539 min, purity (220 nm): 98%

HRMS (ESI$^+$): calculated 644.20794 $[M+H]^+$, found 644.20839 $[M+H]^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.23 (t, J = 7.9 Hz, 1H), 6.86 (dd, J = 8.3, 2.5 Hz, 1H), 6.79 (dd, J = 7.8, 4.9 Hz, 2H), 6.76 – 6.74 (m, 1H), 6.69 – 6.65 (m, 3H), 6.62 (d, J = 3.8 Hz, 1H), 5.66 (dd, J = 8.6, 5.1 Hz, 1H), 5.46 (d, J = 5.5 Hz, 1H), 4.71 – 4.57 (m, 2H), 3.91 (t, J = 7.2 Hz, 1H), 3.86 (s, 3H), 3.85 (s, 3H), 3.16 – 3.08 (m, 1H), 2.67 – 2.58 (m, 1H), 2.57 – 2.48 (m, 1H), 2.43 – 2.28 (m, 1H), 2.23 – 2.11 (m, 1H), 2.10 – 1.95 (m, 2H), 1.87 – 1.65 (m, 4H), 1.49 – 1.42 (m, 1H), 1.37 – 1.23 (m, 2H), 0.91 (t, J = 7.3 Hz, 3H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 172.23, 171.56, 170.15, 157.93, 149.03, 147.52, 142.17, 140.39, 133.53, 129.88, 128.85, 125.69, 124.90, 120.36, 119.87, 115.46, 111.89, 111.49, 110.88, 76.43, 65.42, 56.08, 56.03, 52.79, 45.81, 44.09, 38.10, 31.52, 29.10, 27.16, 25.55, 21.08, 12.24.

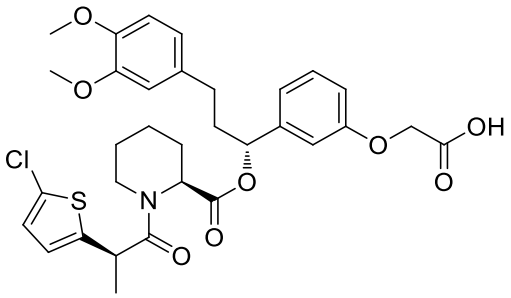
2-(3-((R)-1-(((S)-1-((S)-2-(5-Chlorothiophen-2-yl)propanoyl)piperidine-2-carbonyl)oxy)-3-(3,4-dimethoxyphenyl)propyl)phenoxy)acetic acid (47c)



Following the general procedure A, compound **47c** was synthesized using: Resin **7** (282 mg, 0.11 mmol, 1.00 eq., loading: 0.56 mmol/g), **46c** (21 mg, 0.11 mmol, 1.00 eq.), HATU (42 mg, 0.11 mmol, 1.00 eq.), HOAt (15 mg, 0.11 mmol, 1.00 eq.), DIPEA (48 µL, 0.28 mmol, 2.50 eq.) and 1.10 mL DMF. After purification via preparative HPLC (H_2O/ACN + 0.1% TFA, 70 - 100%), diastereomers **47c** and **d47c** were obtained as colorless solids.

Yield: 14 mg (22.21 µmol, 20%)
HPLC (50 – 100 % solvent B, 3 min) R_t = 2.239 min, purity (220 nm): 98%
HRMS (ESI$^+$): m/z: calculated 630.1923 $[M+H]^+$, found 630.1924 $[M+H]^+$
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.25 – 7.21 (m, 1H), 6.92 – 6.85 (m, 2H), 6.82 – 6.77 (m, 2H), 6.75 – 6.68 (m, 3H), 6.64 (d, J = 3.8 Hz, 1H), 5.65 (dd, J = 8.8, 4.9 Hz, 1H), 5.53 – 5.42 (m, 1H), 4.73 – 4.59 (m, 2H), 3.87 (s, 3H), 3.86 (s, 3H), 3.85 – 3.84 (m, 1H), 3.39 (td, J = 13.2, 3.1 Hz, 1H), 2.70 – 2.64 (m, 1H), 2.62 – 2.56 (m, 1H), 2.38 – 2.30 (m, 1H), 2.28 – 2.19 (m, 1H), 2.11 – 2.02 (m, 1H), 1.76 – 1.66 (m, 2H), 1.62 (d, J = 13.8 Hz, 1H), 1.43 (d, J = 6.8 Hz, 3H), 1.39 – 1.30 (m, 1H), 1.26 – 1.15 (m, 1H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 173.27, 171.36, 170.31, 158.22, 149.16, 147.66, 142.79, 142.30, 133.50, 129.92, 128.89, 125.68, 123.94, 120.38, 119.69, 116.15, 111.92, 111.61, 109.79, 76.90, 65.65, 56.12, 52.97, 43.85, 38.65, 38.24, 31.66, 27.09, 25.11, 20.99.

2-(3-((R)-1-(((S)-1-((R)-2-(5-Chlorothiophen-2-yl)propanoyl)piperidine-2-carbonyl)oxy)-3-(3,4-dimethoxyphenyl)propyl)phenoxy)acetic acid (d47c)



Yield: 18 mg (28.56 µmol, 26%)

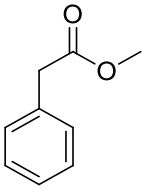
HPLC (50 – 100 % solvent B, 3 min) R_t = 2.214 min, purity (220 nm): 99%

HRMS (ESI$^+$): calculated 630.1923[M+H]$^+$, found 630.1926 [M+H]$^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.19 – 7.12 (m, 1H), 6.82 – 6.77 (m, 1H), 6.77 – 6.74 (m, 1H), 6.71 (d, J = 8.0 Hz, 1H), 6.69 – 6.67 (m, 1H), 6.62 – 6.57 (m, 3H), 6.56 – 6.54 (m, 1H), 5.60 (dd, J = 8.7, 5.1 Hz, 1H), 5.39 – 5.34 (m, 1H), 4.68 – 4.50 (m, 2H), 4.06 (q, J = 6.8 Hz, 1H), 3.78 (s, 3H), 3.77 (s, 3H), 3.74 – 3.70 (m, 1H), 3.04 (td, J = 13.6, 3.5 Hz, 1H), 2.60 – 2.51 (m, 1H), 2.50 – 2.42 (m, 1H), 2.29 (d, J = 13.6 Hz, 1H), 2.18 – 2.06 (m, 1H), 2.02 – 1.91 (m, 1H), 1.70 – 1.65 (m, 1H), 1.63 – 1.58 (m, 1H), 1.43 – 1.38 (m, 3H), 1.37 – 1.32 (m, 1H), 1.29 – 1.18 (m, 2H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 172.63, 171.38, 170.02, 157.87, 148.97, 147.46, 142.00, 141.79, 133.41, 129.76, 128.51, 125.79, 124.28, 120.25, 119.75, 115.49, 111.84, 111.47, 110.60, 76.40, 65.31, 55.97, 55.92, 52.79, 44.06, 38.45, 37.96, 31.42, 26.91, 25.33, 21.24, 20.92.

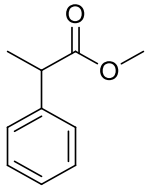
Methyl 2-phenylacetate (49)



In a 25 mL flask, phenylacetic acid (250 mg, 1.84 mmol, 1.00 eq.) was dissolved in 7.34 mL DCM, one drop of DMF was added, and the solution was cooled to 0°C. Then thionyl chloride (0.40 mL, 5.51 mmol, 3.00 eq.) was added dropwise, and the mixture was stirred for 1 h at rt. The reaction mixture was again cooled to 0°C, and 2 mL methanol was added dropwise. After complete addition, the reaction was allowed to warm to rt overnight and was quenched by the addition of water. The org. phase was then washed with sat. $NaHCO_3$ solution, followed by brine and was dried over $MgSO_4$. After removing the solvent under reduced pressure, the title compound was obtained as a colorless liquid after purification by flash chromatography (CH/EtOAc, 0 – 20%).

Yield: 204 mg (1.36 mmol, 74%)
TLC (CH/EtOAc, 9:1): R_f = 0.38
HPLC (50 – 100 % solvent B, 3 min) R_t = 0.743 min, purity (220 nm): 99%
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42 – 7.28 (m, 5H), 3.73 (s, 3H), 3.68 (s, 2H).
^{13}C NMR (75 MHz, $CDCl_3$) δ 172.00, 134.04, 129.27, 128.60, 127.13, 52.01, 41.21.

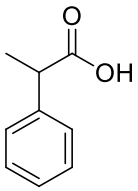
Methyl 2-phenylpropanoate (50)



In a 25 mL dried flask, compound **49** (112 mg, 0.75 mmol, 1.00 eq.) was dissolved in 7.50 mL THF. The solution was cooled to -78°C, and 0.89 mL LiHMDS (1 M in THF, 0.89 mmol, 1.20 eq.) was added dropwise. The reaction mixture was stirred for 1 h at this temperature before iodomethane (0.07 mL, 1.12 mmol, 1. 50 eq.) was added dropwise, and the solution was allowed to warm to rt. After 20 h, the reaction was quenched by the addition of sat. NH_4Cl solution and the aq. phase was extracted three times with diethyl ether. The combined org. layers were then washed with brine, dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. After purification via column chromatography (CH/EtOAc, 0 - 5%), compound **50** was obtained as a colorless oil.

Yield: 59 mg (0.36 mmol, 48%)
TLC (CH/EtOAc, 9:1): R_f = 0.58
HPLC (50 – 100 % solvent B, 3 min) R_t = 1.68 9 min, purity (220 nm): 99%
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.26 – 7.15 (m, 5H), 3.66 (q, J = 7.2 Hz, 1H), 3.58 (s, 3H), 1.43 (d, J = 7.2 Hz, 3H).
^{13}C NMR (75 MHz, $CDCl_3$) δ 174.99, 140.59, 128.65, 127.48, 127.14, 52.00, 45.45, 18.62.

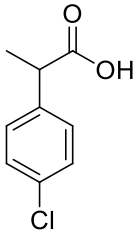
2-Phenylpropanoic acid (51)



In a 5 mL flask, compound **50** (58 mg, 0.35 mmol, 1.00 eq.) was dissolved in 1.77 mL THF/H_2O/MeOH (3:1:1), and LiOH (42 mg, 1.77 mmol, 5.00 eq.) was added. The resulting solution was then stirred at rt overnight. After the complete conversion of the starting material, the reaction mixture was diluted with water and was acidified using 1 M HCl. The aq. phase was then extracted three times with EtOAc, the combined org. layers were dried over $MgSO_4$, and the solvent was removed under reduced pressure. After purification via column chromatography (CH/EtOAc + 1% FA, 0 - 30%), compound **51** was obtained as a colorless solid.

Yield: 52 mg (0.34 mmol, 98%)
TLC (CH/EtOAc + 1% FA, 3:1): R_f = 0.58
HPLC (5 – 100 % solvent B, 3 min) R_t = 1.555 min, purity (220 nm): 99%
Mass (ESI$^+$): m/z: calculated 151.07 $[M+H]^+$, found 151.00 $[M+H]^+$
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.24 – 7.05 (m, 5H), 3.65 (q, J = 7.2 Hz, 1H), 1.43 (d, J = 7.2 Hz, 3H).
^{13}C NMR (75 MHz, $CDCl_3$) δ 181.07, 139.88, 128.81, 127.52, 45.52, 18.22.

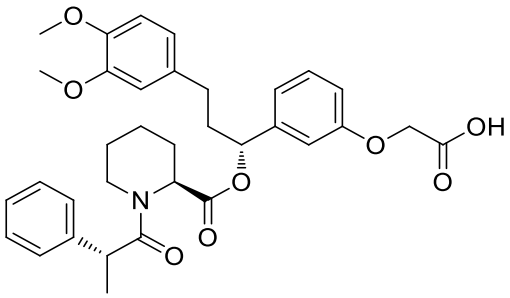
2-(4-Chlorophenyl)propanoic acid (53)



In a 25 mL dried flask, methyl-(4-chlorophenyl) acetate (200 mg, 2.00 mmol, 1.00 eq.) was dissolved in 7.50 mL THF. The solution was cooled to -78°C, and 2.40 mL LiHMDS (1 M in THF, 2.40 mmol, 1.20 eq.) was added dropwise. The reaction mixture was stirred for 1 h at this temperature before iodomethane (0.14 mL, 2.20 mmol, 1.20 eq.) was added dropwise, and the solution was allowed to warm to rt. After 3 h, the reaction was quenched by the addition of sat. NH_4Cl solution and the aq. phase was extracted three times with diethyl ether. The combined org. layers were then washed with brine, dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The obtained crude was dissolved in 3.95 mL THF/H_2O/MeOH (3:1:1), and LiOH (95 mg, 3.95 mmol, 5.00 eq.) was added. The resulting solution was then stirred overnight at rt. After complete conversion, the reaction was diluted with water and acidified using 1 M HCl. The aq. phase was then extracted three times with EtOAc, the combined org. layers were dried over $MgSO_4$, and the solvent was removed under reduced pressure. After purification via preparative HPLC (H_2O/ACN + 0.1% TFA, 40 - 100%), compound **53** was obtained as a colorless solid.

Yield: 20 mg (10.83 µmol, 14% over 2 steps)
TLC (CH/EtOAc + 1% FA, 5:1): R_f = 0.34
HPLC (50 – 100 % solvent B, 3 min) R_t = 1.730 min, purity (220 nm): 99%
Mass (ESI^+): m/z: calculated 185.03 $[M+H]^+$, found 185.00 $[M+H]^+$
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38 – 7.25 (m, 4H), 3.75 (q, J = 7.2 Hz, 1H), 1.53 (d, J = 7.2 Hz, 3H).
^{13}C NMR (75 MHz, $CDCl_3$) δ 180.37, 138.27, 133.48, 129.15, 128.98, 44.88, 18.20.

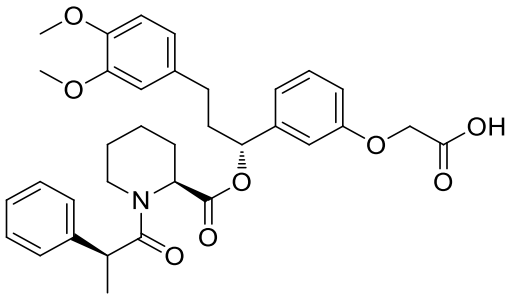
2-(3-((R)-3-(3,4-Dimethoxyphenyl)-1-(((S)-1-((R)-2-phenylpropanoyl)piperidine-2-carbonyl)-oxy)propyl)phenoxy)acetic acid (54a)



Following the general procedure B, compound **54a** was synthesized using: Resin **7** (118 mg, 0.07 mmol, 1.00 eq., loading: 0.56 mmol/g), **51** (20 mg, 0.13 mmol, 2.00 eq.), PPh_3 (70 mg, 0.27 mmol, 4.00 eq.), trichloroacetonitrile (27 µL, 0.27 mmol, 4.00 eq.), DIPEA (116 µL, 0.57 mmol, 10.00 eq.) and 1.33 mL DCM. After purification via preparative HPLC (H_2O/ACN + 0.1% TFA, 50 - 80%), diastereomers **54a** and **d54a** were obtained as colorless solids.

Yield: 6 mg (10.18 µmol, 15%)
HPLC (50 – 100 % solvent B, 3 min) R_t = 1.335 min, purity (220 nm): 98%
HRMS (ESI$^+$): m/z: calculated 590.27484 $[M+H]^+$, found 590.27498 $[M+H]^+$
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.32 (t, J = 7.5 Hz, 2H), 7.26 – 7.20 (m, 5H), 6.88 – 6.75 (m, 4H), 6.73 – 6.66 (m, 2H), 5.62 (dd, J = 8.9, 4.8 Hz, 1H), 5.51 (d, J = 5.6 Hz, 1H), 4.77 – 4.60 (m, 2H), 3.87 (s, 3H), 3.86 (s, 3H), 3.74 (d, J = 13.4 Hz, 1H), 3.29 (td, J = 13.2, 3.0 Hz, 1H), 2.74 – 2.66 (m, 1H), 2.63 – 2.54 (m, 1H), 2.31 – 2.17 (m, 2H), 2.11 – 2.03 (m, 1H), 1.63 – 1.53 (m, 2H), 1.36 – 1.22 (m, 6H), 0.67 – 0.60 (m, 1H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 174.69, 171.67, 170.48, 158.27, 149.09, 147.57, 142.40, 141.82, 133.49, 129.83, 129.12, 127.29, 127.06, 120.35, 119.64, 116.37, 111.81, 111.52, 109.16, 76.87, 65.63, 56.09, 56.03, 52.85, 43.84, 43.41, 38.26, 31.68, 27.12, 24.58, 20.98, 20.52.

2-(3-((R)-3-(3,4-Dimethoxyphenyl)-1-(((S)-1-((S)-2-phenylpropanoyl)piperidine-2-carbonyl)-oxy)propyl)phenoxy)acetic acid (d54a)



Yield: 4 mg (6.78 µmol, 10%)

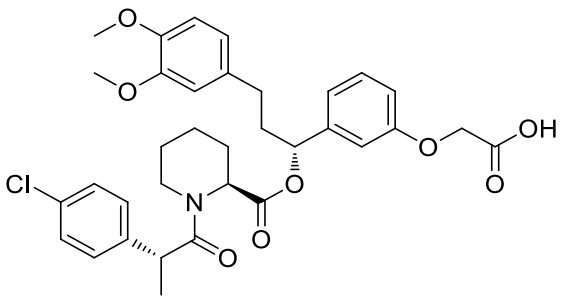
HPLC (50 – 100 % solvent B, 3 min) R_t = 1.268 min, purity (220 nm): 97%

HRMS (ESI$^+$): m/z: calculated 590.27484 $[M+H]^+$, found 590.27429 $[M+H]^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.28 – 7.20 (m, 1H), 7.18 – 7.15 (m, 1H), 7.15 – 7.13 (m, 1H), 7.12 (dd, J = 5.6, 1.6 Hz, 1H), 7.11 – 7.08 (m, 2H), 6.80 (ddd, J = 8.3, 2.6, 0.9 Hz, 1H), 6.71 (d, J = 8.1 Hz, 1H), 6.67 (d, J = 7.6 Hz, 1H), 6.66 – 6.64 (m, 1H), 6.62 (d, J = 2.1 Hz, 1H), 6.59 (t, J = 2.6 Hz, 1H), 5.58 (dd, J = 8.8, 4.9 Hz, 1H), 5.41 (d, J = 3.9 Hz, 1H), 4.62 – 4.48 (m, 2H), 3.86 (q, J = 6.8 Hz, 1H), 3.77 (s, 3H), 3.77 (s, 3H), 3.69 – 3.64 (m, 1H), 2.71 (td, J = 13.5, 3.2 Hz, 1H), 2.61 – 2.53 (m, 1H), 2.51 – 2.44 (m, 1H), 2.32 – 2.23 (m, 1H), 2.10 (m, 1H), 1.98 – 1.90 (m, 1H), 1.71 – 1.60 (m, 2H), 1.53 – 1.47 (m, 1H), 1.35 (d, J = 6.8 Hz, 3H), 1.25 – 1.10 (m, 2H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 174.18, 171.27, 170.20, 157.97, 149.05, 147.54, 142.30, 140.97, 133.58, 129.82, 129.02, 127.50, 126.98, 120.35, 119.84, 115.78, 111.88, 111.51, 110.30, 76.28, 65.51, 56.08, 56.02, 52.67, 43.89, 43.73, 38.24, 31.61, 27.09, 25.35, 21.05, 20.99.

2-(3-((R)-1-(((S)-1-((R)-2-(4-Chlorophenyl)propanoyl)piperidine-2-carbonyl)oxy)-3-(3,4-dimethoxyphenyl)propyl)phenoxy)acetic acid (54b)



Following the general procedure B, compound **54b** was synthesized using: Resin **7** (107 mg, 0.06 mmol, 1.00 eq., loading: 0.56 mmol/g), **53** (14 mg, 0.08 mmol, 1.25 eq.), PPh_3 (70 mg, 0.15 mmol, 4.00 eq.), trichloroacetonitrile (15 µL, 0.15 mmol, 4.00 eq.), DIPEA (61 µL, 0.38 mmol, 10.00 eq.) and 1.33 mL DCM. After purification via preparative HPLC (H_2O/ACN + 0.1% TFA, 60 - 80%), diastereomers **54b** and **d54b** were obtained as colorless solids.

Yield: 5 mg (8.01 µmol, 13%)

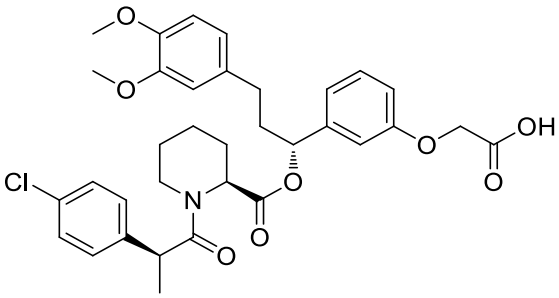
HPLC (50 – 100 % solvent B, 3 min) R_t = 2.247 min, purity (220 nm): 99%

HRMS (ESI$^+$): m/z: calculated 624.23587 $[M+H]^+$, found 624.23626 $[M+H]^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.32 (d, J = 8.4 Hz, 2H), 7.28 – 7.23 (m, 1H), 7.19 (d, J = 8.5 Hz, 2H), 6.97 – 6.86 (m, 2H), 6.82 (d, J = 8.1 Hz, 1H), 6.80 – 6.77 (m, 1H), 6.75 – 6.69 (m, 2H), 5.63 (dd, J = 8.9, 4.8 Hz, 1H), 5.52 (d, J = 5.6 Hz, 1H), 4.81 – 4.61 (m, 2H), 3.89 (s, 3H), 3.88 (s, 3H), 3.79 – 3.66 (m, 1H), 3.35 (t, J = 13.2 Hz, 1H), 2.78 – 2.68 (m, 1H), 2.65 – 2.55 (m, 1H), 2.40 – 2.29 (m, 1H), 2.25 (ddt, J = 14.4, 9.2, 4.6 Hz, 1H), 2.14 – 2.01 (m, 1H), 1.70 – 1.57 (m, 2H), 1.52 – 1.25 (m, 6H), 0.84 – 0.72 (m, 1H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 174.18, 171.34, 170.37, 158.28, 149.11, 147.60, 142.42, 140.35, 133.44, 132.91, 129.85, 129.26, 128.72, 120.34, 119.60, 116.44, 111.80, 111.51, 109.02, 77.41, 65.68, 56.09, 56.04, 52.84, 43.40, 43.10, 38.27, 31.70, 27.18, 24.77, 20.96, 20.49.

2-(3-((R)-1-(((S)-1-((S)-2-(4-Chlorophenyl)propanoyl)piperidine-2-carbonyl)oxy)-3-(3,4-dimethoxyphenyl)propyl)phenoxy)acetic acid (d54b)



Yield: 5 mg (8.01 µmol, 13%)

HPLC (50 – 100 % solvent B, 3 min) R_t = 2.215 min, purity (220 nm): 99%

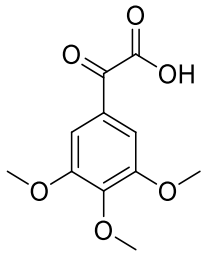
HRMS (ESI$^+$): m/z: calculated 624.23587 $[M+H]^+$, found 624.23586 $[M+H]^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.30 – 7.23 (m, 1H), 7.23 (d, J = 8.4 Hz, 2H), 7.12 (d, J = 8.5 Hz, 2H), 6.95 – 6.90 (m, 1H), 6.84 – 6.79 (m, 2H), 6.78 – 6.76 (m, 1H), 6.73 – 6.68 (m, 2H), 5.68 (dd, J = 8.8, 4.9 Hz, 1H), 5.53 – 5.45 (m, 1H), 4.77 – 4.60 (m, 2H), 3.94 (q, J = 6.8 Hz, 1H), 3.88 (s, 6H), 3.74 – 3.69 (m, 1H), 2.86 (td, J = 13.4, 3.1 Hz, 1H), 2.72 – 2.63 (m, 1H), 2.61 – 2.54 (m, 2H), 2.42 – 2.36 (m, 1H), 2.25 – 2.16 (m, 1H), 2.11 – 2.02 (m, 1H), 1.83 – 1.73 (m, 2H), 1.67 – 1.59 (m, 1H), 1.42 (d, J = 6.9 Hz, 3H), 1.32 – 1.26 (m, 1H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 173.73, 171.34, 170.11, 158.03, 149.07, 147.56, 142.25, 139.43, 133.51, 132.77, 129.84, 129.14, 128.94, 120.32, 119.74, 115.90, 111.83, 111.51, 109.99, 76.39, 65.55, 56.09, 56.02, 52.73, 43.87, 43.09, 38.28, 31.63, 27.11, 25.35, 21.05, 20.95.

11.10 Synthesis of 3,4,5-trimethoxyphenyl-containing SAFit1 analogs with different residues in R_2

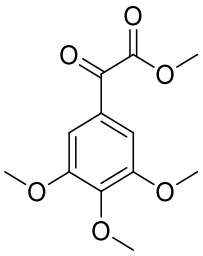
2-Oxo-2-(3,4,5-trimethoxyphenyl)acetic acid (56a)



A 50 mL dried flask with a reflux condenser on top was charged with 3,4,5-Trimethoxyacetophenone (13.20 g, 62.79 mmol, 1.00 eq.) and selenium dioxide (9.06 g, 81.62 mmol, 1.5 eq.). Then 41.86 mL dry Pyridine was added, and the apparatus was flushed with argon for 10 min. The reaction mixture was then heated to 120 °C for 5 h. After cooling to room temperature, the reaction mixture was filtered, and the solvent was removed by co-evaporation with toluene. The resulting oily residue was dissolved in 250 mL EtOAc and washed three times with 1 M HCl (100 mL). The combined aq. layers were extracted twice with 200 mL EtOAc, and the resulting organic phases were washed with brine, dried over $MgSO_4$, filtered, and the solvent was removed almost to dryness. Finally, the product was precipitated by the addition of Diethyl ether and was filtered. The product was washed twice with 5 mL ether and dried under a vacuum. Compound **56a** was obtained as a colorless solid

Yield: 8.26 g (34.39 mmol, 55%).
TLC (EtOAc + 1 % FA): R_f = 0.29
HPLC (5 – 100 % solvent B, 3 min) R_t = 1.181 min, purity (220 nm): 95%
Mass (ESI^+): m/z: calculated 241.06 $[M+H]^+$, found 241.00 $[M+H]^+$
^{1}H NMR (500 MHz, DMSO-d_6) δ 7.22 (s, 2H), 3.86 (s, 6H), 3.80 (s, 3H).
^{13}C NMR (126 MHz, DMSO-d_6) δ 187.83, 166.46, 153.53, 144.04, 127.39, 107.26, 60.78, 56.56.

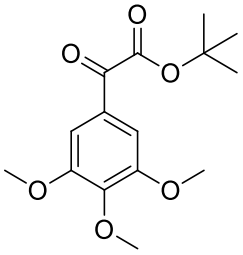
Methyl 2-oxo-2-(3,4,5-trimethoxyphenyl)acetate (56b)



In a 50 mL flask, **56a** (1.50 g, 6.25 mmol, 1.00 eq.) was dissolved in 12.50 mL MeOH. Then conc. H_2SO_4 (84 µL, 1.56 mmol, 0.25 eq.) was added, and the resulting mixture was refluxed overnight. After cooling to rt, the mixture was quenched by adding sat. $NaHCO_3$ solution and was extracted three times with EtOAc. The combined org. layers were then washed with brine, dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The compound **56b** was obtained as a colorless solid after purification via flash chromatography (CH/EtOAc, 0 – 40%).

Yield: 1.26 g (4.96 mmol, 79%).
TLC (CH/EtOAc, 1:1): R_f = 0.47
HPLC (30 – 100 % solvent B, 3 min) R_t = 1.225 min, purity (220 nm): 98%
Mass (ESI^+): m/z: calculated 255.08 $[M+H]^+$, found 255.00 $[M+H]^+$
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.28 (s, 2H), 3.96 (s, 3H), 3.93 (s, 3H), 3.89 (s, 6H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 184.63, 164.02, 153.30, 144.54, 127.40, 107.63, 61.14, 56.43, 52.90.

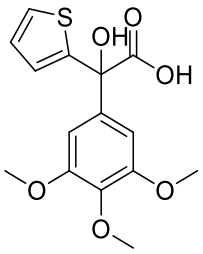
Tert-butyl 2-oxo-2-(3,4,5-trimethoxyphenyl)acetate (56c)



A 250 mL dried flask was charged with **56a** (8.24 g, 34.32 mmol, 1.00 eq.), and the flask was evacuated and backfilled with Argon. Then 69 mL dry DCM was added, and the resulting suspension was cooled to 0°C. After stirring for 5 min at this temperature, three drops of dry DMF were added, followed by the dropwise addition of oxalyl chloride (3.24 mL, 37.75 mmol, 1.30 eq.). The resulting suspension was allowed to warm to rt overnight. The resulting solution was again cooled to 0°C, and pyridine (11.06 mL, 137.26 mmol, 5.00 eq.) was added dropwise, followed by dry tert butanol (11.40 mL, 120.11 mmol, 3.50 eq.). The resulting mixture was stirred at 0°C for 15 min. Then it was poured into ice-cold water, and the org. phase was separated. The aq. the phase was then extracted with 200 mL DCM three times, and the combined organic layers were washed with 200mL 1 M HCl, followed by 200 mL brine. The combined organic layers were then dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography (CH/EtOAc, 0 - 30%). After the removal of the solvent under reduced pressure, the product was obtained as a colorless crystalline solid.

Yield: 8.67 g (29.27 mmol, 85%)
TLC (CH/EtOAc, 3:1): R_f = 0.38
HPLC (30 – 100 % solvent B, 3 min) R_t = 1.770 min, purity (220 nm): 98%
Mass (ESI^+): m/z: calculated 319.12 $[M+Na]^+$, found 319.80 $[M+Na]^+$
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.23 (s, 2H), 3.94 (s, 3H), 3.90 (s, 6H), 1.63 (s, 9H).
^{13}C NMR (75 MHz, $CDCl_3$) δ 185.80, 163.96, 153.35, 144.17, 127.55, 107.36, 84.80, 61.17, 56.37, 28.21.

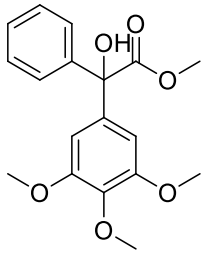
2-Hydroxy-2-(thiophen-2-yl)-2-(3,4,5-trimethoxyphenyl)acetic acid (57a)



In a 25 mL flask, 2-bromothiophene (0.40 mL, 4.16 mmol, 2.00 eq.) was dissolved in 5.21 mL THF, and the solution was cooled to 0°C. Then 3.52 mL isopropylmagnesiumchloride-lithiumchloride-complex (1.3 M in THF, 4.58 mmol, 2.20 eq.) was added dropwise, and the resulting solution was stirred for 1 h. Afterward, a solution of **56a** (500 mg, 2.08 mmol, 1.00 eq.) in 5.00 mL THF was added dropwise, and the solution was allowed to warm to rt overnight. The reaction was quenched by the addition of sat. NH_4Cl solution and was then extracted three times with EtOAc. The combined org. layers were then washed with brine, dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. After purification via flash chromatography (CH:EtOAc + 1% FA, 50 – 100%), the title compound was obtained as a colorless solid.

Yield: 378 mg (1.17 mmol, 56%).
TLC (CH/EtOAc + 1% FA, 1:1): R_f = 0.41
HPLC (5 – 100 % solvent B, 3 min) R_t = 1.703 min, purity (220 nm): 96%
Mass (ESI^+): m/z: calculated 307.06 $[M\text{-}OH]^+$, found 307.00 $[M\text{-}OH]^+$
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34 (dd, J = 5.1, 1.2 Hz, 1H), 7.21 (dd, J = 3.7, 1.2 Hz, 1H), 7.02 (dd, J = 5.1, 3.7 Hz, 1H), 6.86 (s, 2H), 3.86 (s, 3H), 3.81 (s, 6H).
^{13}C NMR (75 MHz, $CDCl_3$) δ 176.29, 164.94, 152.83, 145.22, 138.01, 136.28, 126.76, 126.69, 126.33, 104.15, 60.86, 56.15.

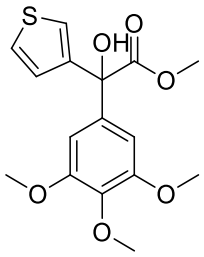
Methyl 2-hydroxy-2-phenyl-2-(3,4,5-trimethoxyphenyl)acetate (57b)



In a 10 mL flask, **56b** (82 mg, 0.32 mmol, 1.00 eq.) was dissolved in 5 mL THF and the solution was cooled to -78°C. Then 0.18 mL phenyl lithium (1.9 M in di-n-butylether, 0.34 mmol, 1.05 eq.) was added dropwise, and the resulting solution was stirred for 1 h. The reaction was quenched by the addition of sat. NH_4Cl solution and was then extracted three times with EtOAc. The combined org. layers were washed with brine, dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. After purification via flash chromatography (CH:EtOAc, 0 – 40%), the title compound was obtained as a colorless solid.

Yield: 83 mg (0.25 mmol, 78%).
TLC (CH/EtOAc, 3:1): R_f = 0.24
HPLC (5 – 100 % solvent B, 3 min) R_t = 1.794 min, purity (220 nm): 95%
Mass (ESI^+): m/z: calculated 325.12 $[M\text{-}OH]^+$, found 315.20 $[M\text{-}OH]^+$
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.45 – 7.38 (m, 2H), 7.37 – 7.31 (m, 3H), 6.70 (s, 2H), 3.86 (s, 3H), 3.85 (s, 3H), 3.79 (s, 6H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 174.90, 152.86, 141.85, 137.89, 137.04, 128.30, 128.25, 127.41, 104.91, 81.17, 60.89, 56.20, 53.67.

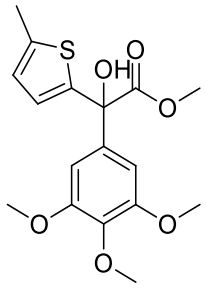
Methyl 2-hydroxy-2-(thiophen-3-yl)-2-(3,4,5-trimethoxyphenyl)acetate (57c)



In a 10 mL flask, 3-bromothiophene (55 µL, 0.59 mmol, 1.00 eq.) was dissolved in 1.90 mL THF, and the solution was cooled to 0°C. Then 0.48 mL isopropylmagnesiumchloride-lithiumchloride-complex (1.7 M in THF, 0.62 mmol, 1.05 eq.) was added dropwise, and the resulting solution was allowed to warm to rt overnight. In a second flask, **56b** (150 mg, 0.59 mmol, 1.00 eq.) was dissolved in 5.00 mL THF, and the solution was cooled to 0°C. Then the freshly prepared thiophen-3-ylmagnesium bromide solution was added dropwise, and the reaction mixture was stirred for 20 min at 0°C. The reaction was quenched by the addition of sat. NH_4Cl solution and was then extracted three times with EtOAc. The combined org. layers were then washed with brine, dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. After purification via flash chromatography (CH:EtOAc, 0 – 40%), the title compound was obtained as a colorless solid.

Yield: 127 mg (0.38 mmol, 64%).
TLC (CH/EtOAc, 1:1): R_f = 0.37
HPLC (5 – 100 % solvent B, 3 min) R_t = 1.244 min, purity (220 nm): 96%
Mass (ESI^+): m/z: calculated 321.08 $[M\text{-}OH]^+$, found 321.00 $[M\text{-}OH]^+$
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37 – 7.28 (m, 2H), 7.11 (d, J = 5.0 Hz, 1H), 6.68 (s, 2H), 3.86 (s, 3H), 3.84 (s, 3H), 3.79 (s, 6H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 174.46, 152.99, 142.62, 138.00, 137.06, 127.37, 125.97, 123.62, 104.24, 78.82, 60.90, 56.22, 53.79.

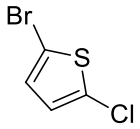
Methyl 2-hydroxy-2-(5-methylthiophen-2-yl)-2-(3,4,5-trimethoxyphenyl)acetate (57c)



In a 10 mL flask, 2-bromo-2-methyl thiophene (140 mg, 0.79 mmol, 1.00 eq.) was dissolved in 5 mL THF, and the solution was cooled to -78°C. Then 0.98 mL tert-butyllithium (1.7 M in pentane, 1.66 mmol, 2.10 eq.) was added dropwise, and the resulting solution was stirred for 1 h. In a second flask, **56b** (201 mg, 0.79 mmol, 1.00 eq.) was dissolved in 2.91 mL THF and the solution was cooled to -78°C. Then the freshly prepared (5-methylthiophen-2-yl) lithium solution was added dropwise, and the reaction mixture was stirred for 2 h at -78°C. The reaction was quenched by the addition of sat. NH_4Cl solution and was then extracted three times with EtOAc. The combined org. layers were then washed with brine, dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. After purification via flash chromatography (CH:EtOAc, 0 – 33%), the title compound was obtained as a pale yellow solid.

Yield: 196 mg (0.56 mmol, 70%).
TLC (CH/EtOAc, 3:1): R_f = 0.21
HPLC (5 – 100 % solvent B, 3 min) R_t = 1.884 min, purity (220 nm): 94%
Mass (ESI$^+$): m/z: calculated 335.09 $[M\text{-}OH]^+$, found 335.00 $[M\text{-}OH]^+$
^{1}H NMR (500 MHz, $CDCl_3$) δ 6.85 (d, J = 3.6 Hz, 1H), 6.79 (s, 2H), 6.62 (dd, J = 3.4, 1.3 Hz, 1H), 4.35 (s, 1H), 3.87 (s, 3H), 3.84 (s, 3H), 3.81 (s, 6H), 2.45 (s, 3H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 173.84, 152.91, 143.10, 140.83, 138.09, 136.66, 126.44, 124.79, 104.17, 78.69, 60.88, 56.23, 53.92, 15.40.

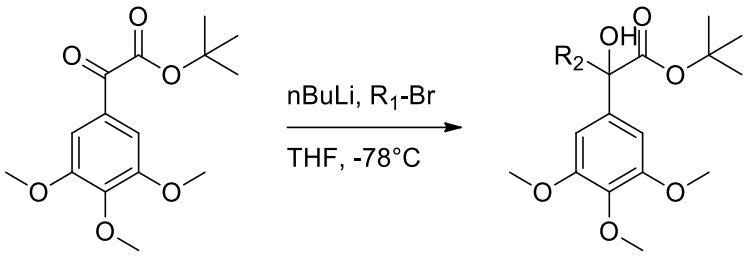
2-Bromo-5-chlorothiophene (l)



A 10 mL flask was charged with 2-chlorothiophene (500 mg, 422 mmol, 1.00 eq.), and 4.22 mL acetic acid was added. Then NBS (788 mg, 4.43 mmol, 1.05 eq.) was added in small portions over 5 min, and the solution was stirred at rt until completion. The reaction mixture is quenched by the addition of water and is then extracted three times with 20 mL n-pentane. The combined org. layers are then washed with brine, dried over $MgSO_4$, filtered, and the solvent is removed under reduced pressure. The product is obtained as a pale-yellow liquid.

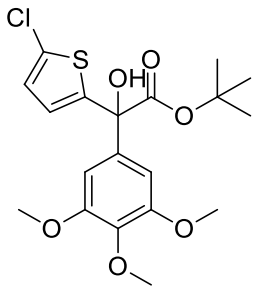
Yield: 769 mg (3.90 mmol, 92%)
Mass (EI^-): m/z: calculated 195.87 $[M]^-$, found 195.88 $[M]^-$
^{1}H NMR (300 MHz, $CDCl_3$) δ 6.86 (d, J = 3.9 Hz, 1H), 6.70 (d, J = 4.0 Hz, 1H).
^{13}C NMR (75 MHz, $CDCl_3$) δ 129.87, 129.55, 126.78, 109.31.

General procedure for the synthesis of 57e-n.



In a dried flask, the aryl bromide (1.30 eq.) is dissolved in dry diethyl ether (0.2 M), and the solution is cooled to -78°C. Then nBuLi (2.5 M in hexane, 1.43 eq.) is added dropwise, and the solution is stirred for 10 min at this temperature. Then ester **56c** (1.00 eq.), dissolved in the smallest amount of diethyl ether, is added dropwise. The resulting mixture is stirred at -78°C until completion (~15 min) and is then quenched by the addition of sat. NH_4Cl solution. After warming to rt, the mixture is extracted with diethyl ether three times, and the combined org. layers are dried over $MgSO_4$. After the solvent is removed under reduced pressure, the crude is purified via flash chromatography.

Tert-butyl 2-(5-chlorothiophen-2-yl)-2-hydroxy-2-(3,4,5-trimethoxyphenyl)-acetate (57e)



Following the general procedure, compound **57e** was synthesized using: **I** (360 mg, 1.83 mmol, 1.30 eq.), 0.80 mL nBuLi (2.5 M in hexane, 2.01 mmol, 1.43 eq.), and **56c** (416 mg, 1.40 mmol, 1.00 eq.) in 7.02 mL dry diethyl ether. After purification via flash chromatography (CH/EtOAc, 0 – 30%), the title compound was obtained as a colorless oil.

Yield: 554 mg (1.34 mmol, 95%)

TLC (CH/EtOAc, 3:1): R_f = 0.43

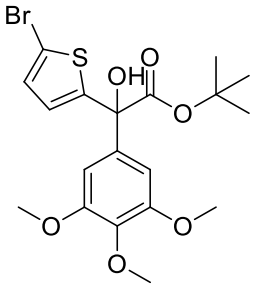
HPLC (30 – 100 % solvent B, 3 min) R_t = 1.964 min, purity (220 nm): 98%

Mass (ESI^+): m/z: calculated 397.09 $[M\text{-}OH]^+$, found 397.00 $[M\text{-}OH]^+$

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.76 (s, 2H), 6.60 (s, 2H), 5.02 (s, 1H), 3.83 (s, 3H), 3.83 (s, 6H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 176.29, 153.58, 138.84, 138.02, 132.58, 130.23, 126.07, 125.71, 105.55, 60.98, 56.33, 52.75.

Tert-butyl 2-(5-bromothiophen-2-yl)-2-hydroxy-2-(3,4,5-trimethoxyphenyl)acetate (57f)



Following the general procedure, compound **57f** was synthesized using: 2,5-dibromothiophene (0.49 mL, 4.39 mmol, 1.30 eq.), 1.93 mL nBuLi (2.5 M in hexane, 4.83 mmol, 1.43 eq.) and **56c** (1.00 g, 3.37 mmol, 1.00 eq.) in 16.87 mL dry diethyl ether. After purification via flash chromatography (CH/EtOAc, 0 – 25%), the title compound was obtained as a colorless oil.

Yield: 1.49 g (3.24 mmol, 96%)

TLC (CH/EtOAc, 3:1): R_f = 0.40

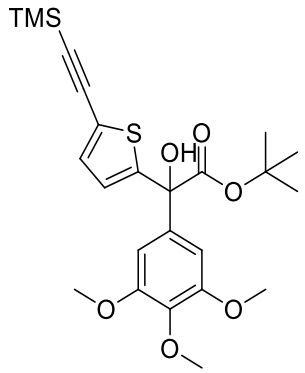
HPLC (30 – 100 % solvent B, 3 min) R_t = 2.024 min, purity (220 nm): 97%

Mass (ESI^+): m/z: calculated 441.04 $[M\text{-}OH]^+$, found 441.00 $[M\text{-}OH]^+$

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.93 (d, J = 3.9 Hz, 1H), 6.87 (d, J = 3.9 Hz, 1H), 6.78 (s, 2H), 4.56 (s, 1H), 3.84 (s, 3H), 3.82 (s, 6H), 1.50 (s, 9H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 171.67, 152.96, 147.62, 138.08, 136.84, 129.57, 126.52, 112.76, 103.91, 85.12, 78.50, 60.95, 56.23, 27.92.

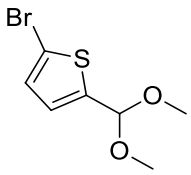
Tert-butyl 2-hydroxy-2-(3,4,5-trimethoxyphenyl)-2-(5-((trimethylsilyl)ethynyl)thiophen-2-yl)acetate (57g)



A 10 mL was charged with **57f** (150 mg, 0.33 mmol, 1.00 eq.), $Pd(dppf)Cl_2$ (13 mg, 0.02 mmol, 0.05 eq.), CuI (6 mg, 0.03 mmol, 0.10 eq.) and 1.63 mL degassed triethylamine. Then trimethylsilylacetylene (51 µL, 0.39 mmol, 1.20 eq.) was added, and the solution was heated to 75 ℃. After 2 h, the reaction was allowed to cool to rt and was filtered through a silica plug using EtOAc as the eluent. After the solvent was removed, the obtained crude was purified by flash chromatography (CH/EtOAc, 0 – 25%). The title compound **57g** was obtained as a pale orange oil.

Yield: 147mg (0.31 mmol, 94%)
TLC (CH/EtOAc, 5:1): R_f = 0.17
HPLC (50 – 100 % solvent B, 3 min) R_t = 1.947min, purity (220 nm): 97%
Mass (ESI^+): m/z: calculated 459.17 $[M\text{-}OH]^+$, found 459.20 $[M\text{-}OH]^+$
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.09 (d, J = 3.8 Hz, 1H), 6.96 (d, J = 3.8 Hz, 1H), 6.76 (s, 2H), 4.58 (s, 1H), 3.83 (s, 3H), 3.80 (s, 6H), 1.49 (s, 9H), 0.23 (s, 9H).
^{13}C NMR (75 MHz, $CDCl_3$) δ 171.73, 152.91, 147.85, 138.04, 137.08, 132.40, 126.06, 123.44, 103.98, 99.29, 97.56, 85.01, 78.57, 60.90, 56.19, 27.88, -0.05.

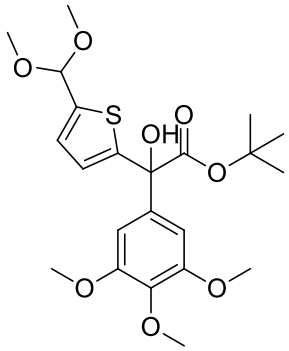
2-Bromo-5-(dimethoxymethyl)thiophene (II)



A 10 mL flask was charged with 5-bromothiophenecarbaldehyde (562 mg, 2.94 mmol, 1.00 eq.) and PTSA (5 mg, 0.03 mmol, 0.01 eq.) and was evacuated and backfilled with argon. Then 2.94 mL of methanol was added, followed by 386 µL trimethylorthoformate (3.53 mmol, 1.20 eq.). The solution was then stirred at rt for 3 h. The reaction mixture was quenched by the addition of sat. $NaHCO_3$ was then extracted three times with 10 mL DCM. The combined org. layers were washed with brine, dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The crude was then purified by flash chromatography (CH/EtOAc, 5:1), yielding compound **II** as a pale-yellow liquid.

Yield: 689 mg (2.91 mmol, 99%)
TLC (CH/EtOAc, 5:1): R_f = 0.58
Mass (EI^-): m/z: calculated 235.95 $[M]^-$, found 235.94 $[M]^-$
^{1}H NMR (300 MHz, $CDCl_3$) δ 6.95 (d, J = 3.8 Hz, 1H), 6.81 (dd, J = 3.8, 1.0 Hz, 1H), 5.53 (s, 1H), 3.34 (s, 6H).
^{13}C NMR (75 MHz, $CDCl_3$) δ 143.29, 129.71, 125.83, 112.83, 99.78, 52.66.

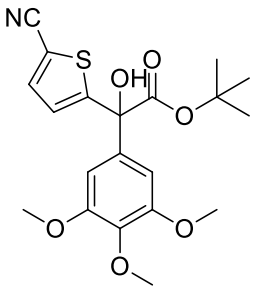
Tert-butyl 2-(5-(dimethoxymethyl)thiophen-2-yl)-2-hydroxy-2-(3,4,5-trimethoxy-phenyl)-acetate (57h)



Following the general procedure, compound **57h** was synthesized using: **II** (360 mg, 1.52 mmol, 1.30 eq.), 0.67 mL nBuLi (2.5 M in hexane, 1.67 mmol, 1.43 eq.), and **56c** (375 mg, 1.27 mmol, 1.00 eq.) in 6.33 mL dry diethyl ether. After purification via column chromatography (CH/EtOAc, 0 – 25%), the title compound **57h** was obtained as a pale-yellow oil.

Yield: 498 mg (1.09 mmol, 87%)
TLC (Cyclohexane/EtOAc, 3:1): R_f = 0.28
HPLC (30 – 100 % solvent B, 3 min) R_t = 1.749 min, purity: 93%
Mass (ESI^+): m/z: calculated 477.17 $[M+Na]^+$, found 477.00 $[M+Na]^+$
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.00 (d, J = 3.7 Hz, 1H), 6.93 (dd, J = 3.7, 0.9 Hz, 1H), 6.80 (s, 2H), 5.58 (s, 1H), 3.83 (s, 3H), 3.80 (s, 6H), 3.46 (s, 1H), 3.34 (d, J = 0.4 Hz, 6H), 1.49 (s, 9H).
^{13}C NMR (75 MHz, $CDCl_3$) δ 172.08, 152.82, 146.75, 141.68, 137.28, 127.37, 125.84, 124.99, 104.12, 100.16, 84.69, 78.53, 60.91, 56.16, 52.70, 27.91.

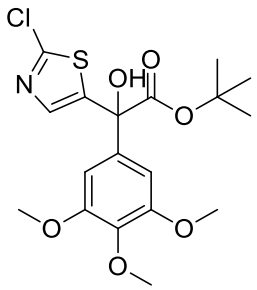
Tert-butyl 2-(5-cyanothiophen-2-yl)-2-hydroxy-2-(3,4,5-trimethoxyphenyl)-acetate (57i)



In a 10 mL flask, the starting material **57h** (455 mg, 1.00 mmol, 1.00 eq.) was dissolved in 2.50 mL THF. Then 20 µL water was added, followed by iodine (51 mg, 0.20 mmol, 0.20 eq.). The solution was stirred at rt for 1 h—then 2.50 mL aq. NH_3 (28 wt.-%) was added, followed by iodine (305 mg, 1.20 mmol, 1.20 eq.). After stirring overnight at rt, the reaction mixture was quenched by adding a sat. sodium thiosulfate solution and was extracted three times with 20 mL DCM. The combined org. layers were washed with brine, dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The crude product was purified by column chromatography (CH/EtOAc, 0 – 20%), and the title compound was obtained as a colorless solid.

Yield: 360 mg (0.89 mmol, 87%).
TLC (CH/EtOAc, 3:1): R_f = 0.21
HPLC (30 – 100 % solvent B, 3 min) R_t = 1.724 min, purity: 96%
Mass (ESI^+): m/z: calculated 428.12 $[M+Na]^+$, found 428.00 $[M+Na]^+$
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50 (d, J = 3.9 Hz, 1H), 7.14 (d, J = 3.9 Hz, 1H), 6.74 (s, 2H), 4.69 (s, 1H), 3.84 (s, 3H), 3.82 (s, 6H), 1.51 (s, 9H).
^{13}C NMR (75 MHz, $CDCl_3$) δ 170.86, 154.37, 153.17, 138.37, 137.26, 136.33, 126.53, 114.29, 109.68, 103.58, 85.85, 78.56, 60.94, 56.24, 27.89.

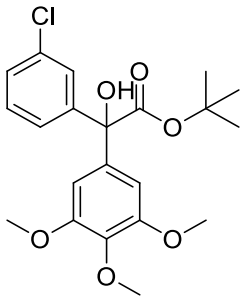
Tert-butyl-2-(2-chlorothiazol-5-yl)-2-hydroxy-2-(3,4,5-trimethoxyphenyl)acetate (57j)



Following the general procedure, compound **57j** was synthesized using: 2-chlorothiazole (424 mg, 3.54 mmol, 1.50 eq.), 1.56 mL nBuLi (2.5 M in hexane, 3.90 mmol, 1.65 eq.), and **56c** (700 mg, 2.36 mmol, 1.00 eq.) in 23.62 mL dry diethyl ether. After purification via flash chromatography (CH/EtOAc, 0 – 30%), the title compound was obtained as a colorless solid.

Yield: 873 mg (2.10 mmol, 89 %)
TLC (CH/EtOAc, 3:1): R_f = 0.24
HPLC (30 – 100 % solvent B, 3 min) R_t = 1.772 min, purity (220 nm): 99%
Mass (ESI^+): m/z: calculated 416.05 $[M+H]^+$, found 416.80 $[M+H]^+$
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.55 (s, 1H), 6.75 (s, 2H), 4.62 (s, 1H), 3.84 (s, 3H), 3.82 (s, 6H), 1.51 (s, 9H).
^{13}C NMR (75 MHz, $CDCl_3$) δ 171.00, 153.16, 152.54, 143.37, 139.51, 138.31, 136.09, 103.47, 85.80, 77.16, 60.95, 56.25, 27.90.

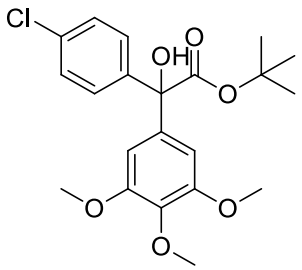
Tert-butyl 2-(3-chlorophenyl)-2-hydroxy-2-(3,4,5-trimethoxyphenyl)acetate (57k)



Following the general procedure, compound **57k** was synthesized using: 1-brom-2-chlorobenzene (113 µL, 0.88 mmol, 1.30 eq.), 0.39 mL nBuLi (2.5 M in hexane, 0.97 mmol, 1.43 eq.) and **56c** (200 mg, 0.67 mmol, 1.00 eq.) in 3.37 mL dry diethyl ether. After purification via flash chromatography (CH/EtOAc, 0 – 30%), the title compound was obtained as a colorless oil.

Yield: 271 mg (0.66 mmol, 98%)
TLC (CH/EtOAc, 3:1): R_f = 0.27
HPLC (30 – 100 % solvent B, 3 min) R_t = 1.948 min, purity (220 nm): 96%
Mass (ESI^+): m/z: calculated 391.13$[M\text{-}OH]^+$, found 391.00 $[M\text{-}OH]^+$
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45 (s, 1H), 7.33 – 7.26 (m, 3H), 6.69 (s, 2H), 4.39 (s, 1H), 3.86 (s, 3H), 3.80 (s, 6H), 1.48 (s, 9H).
^{13}C NMR (75 MHz, $CDCl_3$) δ 172.81, 152.92, 144.35, 137.87, 137.21, 134.06, 129.26, 128.17, 127.76, 125.85, 104.75, 84.64, 80.47, 60.97, 56.21, 27.97.

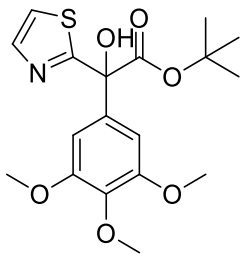
Tert-butyl 2-(4-chlorophenyl)-2-hydroxy-2-(3,4,5-trimethoxyphenyl)acetate (57l)



Following the general procedure, compound **57l** was synthesized using: 1-brom-2-chlorobenzene (168 mg, 0.88 mmol, 1.30 eq.), 0.39 mL nBuLi (2.5 M in hexane, 0.97 mmol, 1.43 eq.) and **56c** (200 mg, 0.67 mmol, 1.00 eq.) in 3.37 mL dry diethyl ether. After purification via flash chromatography (CH/EtOAc, 0 – 25%), the title compound was obtained as a colorless oil.

Yield: 79 mg (0.19 mmol, 29%)
TLC (CH/EtOAc, 3:1): R_f = 0.26
HPLC (30 – 100 % solvent B, 3 min) R_t = 1.886 min, purity (220 nm): 91%
Mass (ESI^+): m/z: calculated 391.13$[M\text{-}OH]^+$, found 391.00 $[M\text{-}OH]^+$
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42 – 7.28 (m, 4H), 6.72 (s, 2H), 3.88 (s, 3H), 3.82 (s, 6H), 1.49 (s, 9H).
^{13}C NMR (75 MHz, $CDCl_3$) δ 172.97, 152.85, 140.89, 137.77, 137.37, 133.86, 128.98, 128.13, 104.69, 84.44, 80.43, 60.90, 56.14, 27.91.

Tert-butyl 2-hydroxy-2-(thiazol-2-yl)-2-(3,4,5-trimethoxyphenyl)acetate (57m)



Following the general procedure, compound **57m** was synthesized using: 2-bromthiazole (188 mg, 1.15 mmol, 1.30 eq.), 0.50 mL nBuLi (2.5 M in hexane, 1.26 mmol, 1.43 eq.), and **56c** (200 mg, 0.67 mmol, 1.00 eq.) in 6.75 mL dry diethyl ether. After purification via flash chromatography (CH/EtOAc, 0 – 30%), the title compound was obtained as a yellowish oil.

Yield: 79 mg (0.20 mmol, 45%)

TLC (CH/EtOAc, 1:1): R_f = 0.62

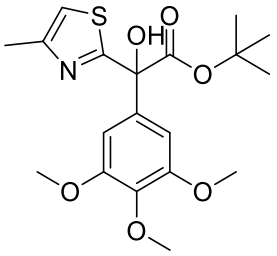
HPLC (5 – 100 % solvent B, 3 min) R_t = 1.429 min, purity (254 nm): 97%

Mass (ESI$^+$): m/z: calculated 382.12 $[M+H]^+$, found 382.20 $[M+H]^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.79 (d, J = 3.3 Hz, 1H), 7.33 (d, J = 3.3 Hz, 1H), 7.05 (s, 2H), 4.98 (s, 1H), 3.85 (s, 3H), 3.85 (s, 6H), 1.49 (s, 9H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 172.51, 170.42, 152.71, 142.76, 138.03, 134.99, 120.31, 104.21, 84.85, 79.18, 60.85, 56.11, 27.83.

Tert-butyl 2-hydroxy-2-(4-methylthiazol-2-yl)-2-(3,4,5-trimethoxyphenyl)acetate (57n)



Following the general procedure, compound **57n** was synthesized using: 4-methylthiazole (125 mg, 1.27 mmol, 1.50 eq.), 0.56 mL nBuLi (2.5 M in hexane, 1.39 mmol, 1.65 eq.), and **56c** (250 mg, 0.84 mmol, 1.00 eq.) in 8.44 mL dry diethyl ether. After purification via flash chromatography (CH/EtOAc, 0 – 30%), the title compound was obtained as a yellowish oil.

Yield: 201 mg (0.51 mmol, 60%)

TLC (CH/EtOAc, 3:1): R_f = 0.26

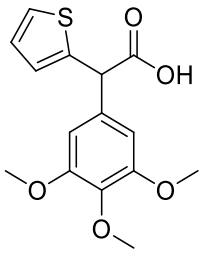
HPLC (30 – 100 % solvent B, 3 min) R_t = 1.537 min, purity (220 nm): 97%

Mass (ESI^+): m/z: calculated 396.14 $[M+H]^+$, found 396.20 $[M+H]^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.05 (s, 2H), 6.88 (s, 1H), 3.87 (s, 9H), 2.46 (s, 3H), 1.51 (s, 9H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 171.23, 170.43, 152.62, 137.94, 135.01, 123.08, 114.88, 104.19, 84.60, 79.02, 60.80, 56.04, 27.78, 17.20.

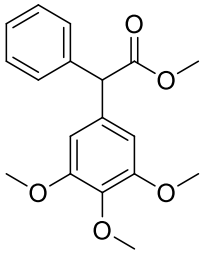
Methyl 2-(5-methylthiophen-2-yl)-2-(3,4,5-trimethoxyphenyl)acetate (58a)



A 5 mL flask was charged with **57a** (135 mg, 0.42 mmol, 1.00 eq.) and $InCl_3$ (9 mg, 0.04 mmol, 0.10 eq.). The flask was evacuated and backfilled with argon before 1.66 mL DCM and triethylsilane (100 µL, 0.62 mmol, 1.50 eq.) were added. The resulting mixture was stirred at rt overnight. After the complete conversion of the starting material, the reaction was diluted with water and extracted three times with DCM. The combined org. layers were then washed with brine, dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. After purification via flash chromatography (CH:EtOAc + 1% FA, 10 - 50%), the title compound was obtained as a brownish solid.

Yield: 66 mg (0.21 mmol, 52%).
TLC (CH/EtOAc, 3:1 + 1% FA): R_f = 0.34
HPLC (5 – 100 % solvent B, 3 min) R_t = 1.934 min, purity (220 nm): 95%
Mass (ESI$^+$): m/z: calculated 309.07 $[M+H]^+$, found 309.20 $[M+H]^+$
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.25 (dd, J = 5.0, 1.3 Hz, 1H), 7.04 – 7.02 (m, 1H), 6.97 (dd, J = 5.1, 3.6 Hz, 1H), 6.65 (s, 2H), 5.16 (s, 1H), 3.83 (s, 3H), 3.83 (s, 6H).
^{13}C NMR (75 MHz, $CDCl_3$) δ 177.16, 153.44, 140.14, 137.84, 133.25, 126.77, 126.71, 125.63, 105.70, 60.94, 56.27, 52.47.

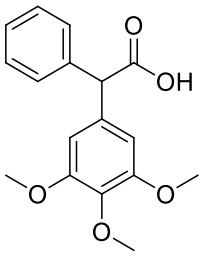
Methyl 2-phenyl-2-(3,4,5-trimethoxyphenyl)acetate (58bi)



A 10 mL flask was charged with **57b** (83 mg, 0.25 mmol, 1.00 eq.) and $InCl_3$ (6 mg, 0.02 mmol, 0.10 eq.). The flask was evacuated and backfilled with argon before 1.25 mL DCM and triethylsilane (60 µL, 0.37 mmol, 1.50 eq.) were added. The resulting mixture was then stirred at rt overnight. After the complete conversion of the starting material, the reaction was diluted with water and extracted three times with DCM. The combined org. layers were then washed with brine, dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. After purification via flash chromatography (CH:EtOAc, 0 – 40%), the title compound was obtained as a colorless oil.

Yield: 61 mg (0.19 mmol, 77%).
TLC (CH/EtOAc, 3:1): R_f = 0.53
HPLC (30 – 100 % solvent B, 3 min) R_t = 1.604 min, purity (220 nm): 96%
Mass (ESI^+): m/z: calculated 317.130 $[M+H]^+$, found 317.20 $[M+H]^+$
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35 – 7.32 (m, 4H), 7.30 – 7.26 (m, 1H), 6.57 (s, 2H), 4.97 (s, 1H), 3.84 (s, 3H), 3.82 (s, 6H), 3.76 (s, 3H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 172.97, 153.29, 138.52, 137.31, 133.98, 128.67, 128.43, 127.43, 105.86, 60.84, 57.01, 56.14, 52.40.

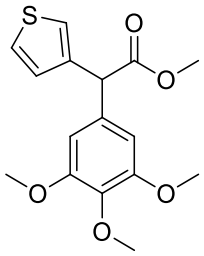
2-Phenyl-2-(3,4,5-trimethoxyphenyl)acetic acid (58b)



In a 5 mL flask, compound **58bi** (67 mg, 0.21 mmol, 1.00 eq.) was dissolved in 1.06 mL THF/H_2O/MeOH (3:1:1), and LiOH (25 mg, 1.06 mmol, 5.00 eq.) was added. The resulting solution was then stirred at rt overnight. After the complete conversion of the starting material, the reaction mixture was diluted with water and was acidified using 1 M HCl. The aq. phase was then extracted three times with EtOAc, the combined org. layers were dried over Na_2SO_4, and the solvent was removed under reduced pressure. After purification via flash chromatography (CH/EtOAc + 1% FA, 0 - 100%), compound **58b** was obtained as a pale-yellow solid.

Yield: 60 mg (0.20 mmol, 94%).
TLC (CH/EtOAc + 1% FA, 1:1): R_f = 0.37
HPLC (30 – 100 % solvent B, 3 min) R_t = 1.208 min, purity (220 nm): 98%
Mass (ESI^+): m/z: calculated 303.12 $[M+H]^+$, found 303.20 $[M+H]^+$
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35 (d, J = 4.5 Hz, 4H), 7.32 – 7.28 (m, 1H), 6.58 (s, 2H), 4.99 (s, 1H), 3.84 (s, 3H), 3.81 (s, 6H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 178.46, 153.35, 137.84, 137.50, 133.30, 128.78, 128.56, 127.71, 106.08, 60.92, 57.12, 56.23.

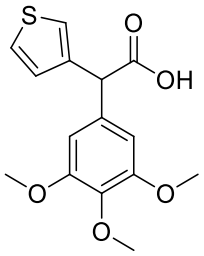
Methyl 2-(thiophen-3-yl)-2-(3,4,5-trimethoxyphenyl)acetate (58ci)



A 10 mL flask was charged with **57c** (121 mg, 0.36 mmol, 1.00 eq.) and $InCl_3$ (8 mg, 0.04 mmol, 0.10 eq.). The flask was evacuated and backfilled with argon before 3.58 mL DCM, and triethylsilane (86 µL, 0.54 mmol, 1.50 eq.) were added. The resulting mixture was then stirred at rt overnight. After the complete conversion of the starting material, the reaction was diluted with water and extracted three times with DCM. The combined org. layers were then washed with brine, dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. After purification via flash chromatography (CH:EtOAc, 0 – 40%), the title compound was obtained as a colorless oil.

Yield: 83 mg (0.26 mmol, 72%).
TLC (CH/EtOAc, 3:1): R_f = 0.51
HPLC (30 – 100 % solvent B, 3 min) R_t = 1.556 min, purity (220 nm): 99%
Mass (ESI^+): m/z: calculated 323.09 $[M+H]^+$, found 323.20 $[M+H]^+$
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.28 (dd, J = 5.0, 3.0 Hz, 1H), 7.18 – 7.16 (m, 1H), 7.02 (dd, J = 5.0, 1.4 Hz, 1H), 6.55 (s, 2H), 4.97 (s, 1H), 3.82 (s, 3H), 3.81 (s, 6H), 3.74 (s, 3H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 172.67, 153.33, 138.65, 137.40, 133.83, 127.91, 125.93, 122.79, 105.59, 60.85, 56.16, 52.72, 52.47.

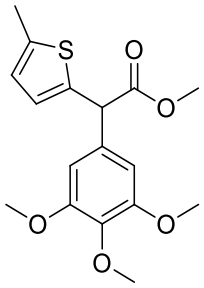
2-(Thiophen-3-yl)-2-(3,4,5-trimethoxyphenyl)acetic acid (58c)



In a 5 mL flask, compound **58ci** (67 mg, 0.21 mmol, 1.00 eq.) was dissolved in 1.04 mL THF/H_2O/MeOH (3:1:1), and LiOH (25 mg, 1.04 mmol, 5.00 eq.) was added. The resulting solution was then stirred at rt overnight. After the complete conversion of the starting material, the reaction mixture was diluted with water and was acidified using 1 M HCl. The aq. phase was then extracted three times with EtOAc, the combined org. layers were dried over Na_2SO_4, and the solvent was removed under reduced pressure. After purification via flash chromatography (CH/EtOAc + 1% FA, 0 - 50%), compound **58c** was obtained as a colorless solid.

Yield: 58 mg (0.19 mmol, 91%).
TLC (CH/EtOAc + 1% FA, 3:1): R_f = 0.18
HPLC (30 – 100 % solvent B, 3 min) R_t = 1.165 min, purity (220 nm): 94%
Mass (ESI$^+$): m/z: calculated 309.07 $[M+H]^+$, found 309.00 $[M+H]^+$
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34 (dd, J = 5.0, 3.0 Hz, 1H), 7.26 – 7.23 (m, 1H), 7.08 (dd, J = 5.1, 1.3 Hz, 1H), 6.60 (s, 2H), 5.02 (s, 1H), 3.86 (s, 3H), 3.84 (s, 6H)
^{13}C NMR (126 MHz, $CDCl_3$) δ 177.96, 153.44, 137.85, 137.66, 133.18, 127.99, 126.17, 123.23, 105.83, 60.96, 56.28, 52.81.

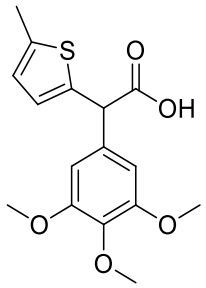
Methyl 2-(5-methylthiophen-2-yl)-2-(3,4,5-trimethoxyphenyl)acetate (58di)



A 10 mL flask was charged with **57d** (181 mg, 0.51 mmol, 1.00 eq.) and $InCl_3$ (11 mg, 0.05 mmol, 0.10 eq.). The flask was evacuated and backfilled with argon before 5.14 mL DCM and triethylsilane (123 µL, 0.77 mmol, 1.50 eq.) were added. The resulting mixture was then stirred at rt for 1 h. After the complete conversion of the starting material, the reaction was diluted with water and extracted three times with DCM. The combined org. layers were then washed with brine, dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. After purification via flash chromatography (CH:EtOAc, 0 – 20%), the title compound was obtained as a colorless oil.

Yield: 136 mg (0.40 mmol, 79%).
TLC (CH/EtOAc, 3:1): R_f = 0.35
HPLC (30 – 100 % solvent B, 3 min) R_t = 1.700 min, purity (220 nm): 96%
Mass (ESI^+): m/z: calculated 359.10 $[M+Na]^+$, found 359.00 $[M+Na]^+$
^{1}H NMR (500 MHz, $CDCl_3$) δ 6.74 (d, J = 3.4 Hz, 1H), 6.62 (s, 2H), 6.58 – 6.57 (m, 1H), 5.04 (s, 1H), 3.83 (s, 6H), 3.82 (s, 3H), 3.74 (s, 3H), 2.42 (s, 3H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 172.15, 153.28, 139.88, 138.40, 137.48, 133.87, 126.08, 124.62, 105.34, 60.78, 56.12, 52.56, 15.26.

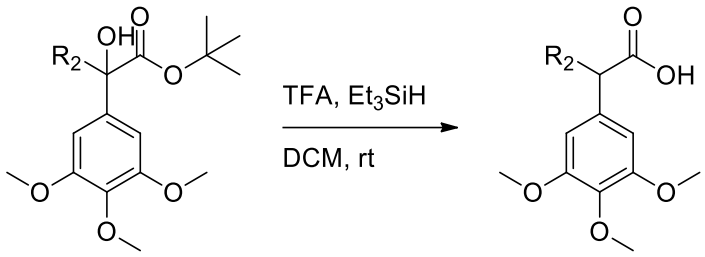
2-(5-Methylthiophen-2-yl)-2-(3,4,5-trimethoxyphenyl)acetic acid (58d)



In a 5 mL flask, compound **58di** (130 mg, 0.39 mmol, 1.00 eq.) was dissolved in 1.93 mL THF/H_2O/MeOH (3:1:1), and LiOH (46 mg, 1.93 mmol, 5.00 eq.) was added. The resulting solution was then stirred at rt overnight. After the complete conversion of the starting material, the reaction mixture was diluted with water and was acidified using 1 M HCl. The aq. phase was then extracted three times with EtOAc, the combined org. layers were dried over Na_2SO_4, and the solvent was removed under reduced pressure. After purification via flash chromatography (CH/EtOAc + 1% FA, 0 - 50%), compound **58d** was obtained as a pale-yellow solid.

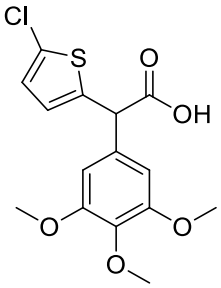
Yield: 106 mg (0.33 mmol, 85%).
TLC (CH/EtOAc + 1% FA, 3:1): R_f = 0.15
HPLC (5 – 100 % solvent B, 3 min) R_t = 1.855 min, purity (220 nm): 97%
Mass (ESI$^+$): m/z: calculated 323.09 $[M+H]^+$, found 323.00 $[M+H]^+$
^{1}H NMR (500 MHz, $CDCl_3$) δ 6.79 (d, J = 3.4 Hz, 1H), 6.64 (s, 2H), 6.60 (d, J = 3.4 Hz, 1H), 5.06 (s, 1H), 3.84 (s, 9H), 2.44 (s, 3H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 177.48, 153.41, 140.25, 137.74, 137.60, 133.27, 126.56, 124.82, 105.61, 60.94, 56.26, 52.67, 15.38.

General procedure for the synthesis of the bottom groups 58e-n.



A dried flask is charged with the hydroxy ester (1.00 eq.), and DCM (0.2 M) is added, followed by triethylsilane (3.00 eq.). Then TFA (0.2M) is added dropwise, and the reaction mixture is stirred at rt. After the complete conversion of the starting material, the solution is diluted by adding water, and the mixture is extracted three times with DCM. The combined org. layers are washed with brine, dried over MgSO4, filtered, and the solvent is removed under reduced pressure. After purification via flash chromatography, the carboxylic acids are obtained.

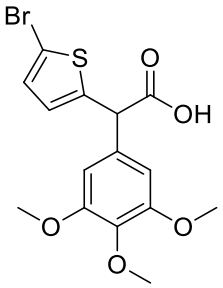
2-(5-Chlorothiophen-2-yl)-2-(3,4,5-trimethoxyphenyl)acetic acid (58e)



Following the general procedure, compound **58e** was synthesized using: **57e** (519 mg, 1.25 mmol, 1.00 eq.), triethylsilane (0.60 mL, 3.75 mmol, 3.00 eq.), and 2.50 mL TFA in 5.00 mL DCM. After purification via flash chromatography (CH/EtOAc + 1% FA, 0 – 50%), the title compound was obtained as a purple solid.

Yield: 429 mg (1.25 mmol, 99%)
TLC (CH/EtOAc + 1% FA, 1:1): R_f = 0.31
HPLC (30 – 100 % solvent B, 3 min) R_t = 1.505 min, purity (220 nm): 96%
Mass (ESI$^+$): m/z: calculated 343.03 $[M+H]^+$, found 343.00 $[M+H]^+$
^{1}H NMR (500 MHz, $CDCl_3$) δ 6.77 (s, 2H), 6.61 (s, 2H), 5.03 (s, 1H), 3.84 (s, 9H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 176.45, 153.48, 138.62, 137.93, 132.36, 130.15, 125.98, 125.60, 105.42, 60.87, 56.22, 52.62.

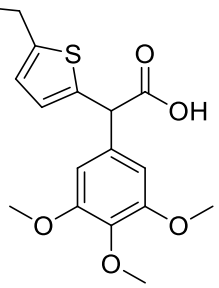
2-(5-Bromothiophen-2-yl)-2-(3,4,5-trimethoxyphenyl)acetic acid (58f)



Following the general procedure, compound **58f** was synthesized using: **57f** (1.45 g, 3.15 mmol, 1.00 eq.), triethylsilane (1.52 mL, 9.46 mmol, 3.00 eq.), and 6.30 mL TFA in 6.30 mL dry DCM. The solution was stirred for 2 h at rt. After purification via column chromatography (CH/EtOAc + 1% FA, 0 – 40%), compound **58f** was obtained as a purple solid.

Yield: 1.14 g (2.94 mmol, 93%)
TLC (CH/EtOAc, 3:1 + 1% FA): R_f = 0.18
HPLC (30 – 100 % solvent B, 3 min) R_t = 1.554 min, purity (220 nm): 96%
Mass (ESI$^+$): m/z: calculated 386.98 $[M+H]^+$, found 387.20 $[M+H]^+$
^{1}H NMR (300 MHz, $CDCl_3$) δ 6.91 (d, *J* = 3.8 Hz, 1H), 6.75 (dd, *J* = 3.8, 1.0 Hz, 1H), 6.61 (s, 2H), 5.05 (s, 1H), 3.84 (s, 6H), 3.83 (s, 3H).
^{13}C NMR (75 MHz, $CDCl_3$) δ 176.18, 153.47, 141.59, 137.94, 132.39, 129.36, 126.95, 112.38, 105.45, 60.86, 56.22, 52.62.

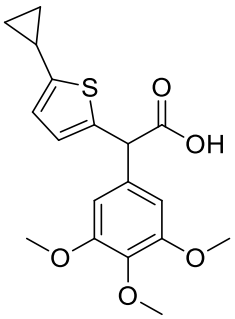
2-(5-Ethylthiophen-2-yl)-2-(3,4,5-trimethoxyphenyl)acetic acid (58g)



Following the general procedure, compound **58g** was synthesized using: **57g** (142 mg, 0.30 mmol, 1.00 eq.), triethylsilane (0.14 mL, 0.89 mmol, 3.00 eq.), and 1.19 mL TFA in 1.19 mL dry DCM. The solution was stirred overnight at rt. After purification via column chromatography (CH/EtOAc + 1% FA, 0 – 50%), compound **58g** was obtained as a yellowish solid.

Yield: 48 mg (0.14 mmol, 48%)
TLC (CH/EtOAc + 1% FA, 3:1): R_f = 0.27
HPLC (30 – 100 % solvent B, 3 min) R_t = 1.515 min, purity (220 nm): 99%
Mass (ESI$^+$): m/z: calculated 337.10 $[M+H]^+$, found 337.00 $[M+H]^+$
^{1}H NMR (500 MHz, $CDCl_3$) δ 6.81 (dd, J = 3.5, 0.9 Hz, 1H), 6.65 (s, 2H), 6.64 – 6.61 (m, 1H), 5.07 (s, 1H), 3.84 (s, 6H), 3.83 (s, 3H), 2.79 (qd, J = 7.5, 1.0 Hz, 2H), 1.28 (t, J = 7.5 Hz, 3H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 177.38, 153.40, 147.89, 137.74, 137.22, 133.29, 126.35, 122.93, 105.66, 60.95, 56.26, 52.72, 23.53, 15.86.

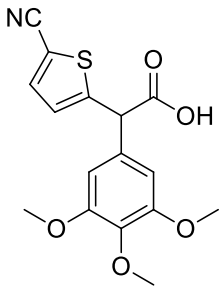
2-(5-Cyclopropylthiophen-2-yl)-2-(3,4,5-trimethoxyphenyl)acetic acid (58h)



A 4 mL vial was charged with compound **58g** (101 mg, 0.26 mmol, 1.00 eq.), $Pd(OAc)_2$ (3 mg, 0.01 mmol, 0.05 eq.), SPhos (5 mg, 0.01 mmol, 0.05 eq.), potassium phosphate (166 mg, 0.78 mmol, 3.00 eq.) and cyclopropyl boronic acid (45 mg, 0.52 mmol, 2.00 eq.). The vial was evacuated and backfilled with argon. Then 2.61 mL toluene/H_2O (20:1) was added, and the solution was heated to 100°C for 2 h. After cooling to rt, the reaction mixture was acidified with 1 M HCl and extracted with EtOAc three times. The combined org. layers were then washed with brine, dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. After purification via flash chromatography (CH/EtOAc + 1% FA, 0 – 20%), the title compound was obtained as a pale-yellow solid.

Yield: 63 mg (0.18 mmol, 70%)
TLC (CH/EtOAc, 1:1 + 1% FA): R_f = 0.31
HPLC (30 – 100 % solvent B, 3 min) R_t = 1.541 min, purity (220 nm): 94%
Mass (ESI^+): m/z: calculated 349.10 $[M+H]^+$, found 349.00 $[M+H]^+$
^{1}H NMR (300 MHz, $CDCl_3$) δ 6.77 (dd, J = 3.6, 0.9 Hz, 1H), 6.64 (s, 2H), 6.60 (dd, J = 3.6, 0.9 Hz, 1H), 5.04 (s, 1H), 3.83 (s, 10H), 2.04 – 1.96 (m, 1H), 0.99 – 0.87 (m, 2H), 0.77 – 0.61 (m, 2H).
^{13}C NMR (75 MHz, $CDCl_3$) δ 177.32, 153.38, 148.95, 137.77, 136.43, 133.23, 126.30, 122.22, 105.69, 60.91, 56.25, 52.69, 11.17, 9.67.

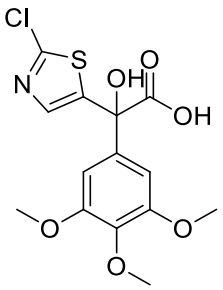
2-(5-Cyanothiophen-2-yl)-2-(3,4,5-trimethoxyphenyl)acetic acid (58i)



A 10 mL flask was charged with **57i** (312 mg, 0.77 mmol, 1.00 eq.) and $InCl_3$ (17 mg, 0.08 mmol, 0.10 eq.) and was evacuated and backfilled with Argon. Then 3.85 mL DCM was added, followed by chlorodiphenylsilane (231 µL, 1.54 mmol, 2.00 eq.). The reaction was then stirred at rt overnight. The reaction mixture was quenched by the addition of water and was extracted three times with 10 mL DCM. The combined org. layers were washed with brine, dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. After purification via flash chromatography (CH/EtOAc + 1% FA, 0 – 50%), the title compound **58i** was obtained as a pale orange foam.

Yield: 111 mg (0.33 mmol, 43%).
TLC (CH/EtOAc, 1:1 + 1% FA): R_f = 0.26
HPLC (5 – 100 % solvent B, 3 min) R_t = 1.715 min, purity: 96%
Mass (ESI^+): m/z: calculated 334.07 $[M+H]^+$, found 334.00 $[M+H]^+$
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.49 (d, J = 3.9 Hz, 1H), 7.00 (dd, J = 3.9, 0.9 Hz, 1H), 6.58 (s, 2H), 5.15 (s, 1H), 3.83 (s, 9H).
^{13}C NMR (75 MHz, $CDCl_3$) δ 175.11, 153.71, 148.42, 138.23, 137.25, 132.01, 127.20, 114.11, 109.77, 105.52, 60.99, 56.35, 52.45.

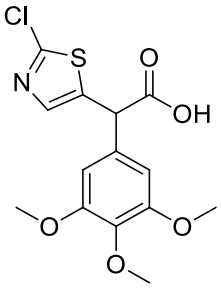
2-(2-Chlorothiazol-5-yl)-2-hydroxy-2-(3,4,5-trimethoxyphenyl)acetic acid (58j-Int.)



A 25 mL flask was charged with the starting material **57j** (793 mg, 1.91 mmol, 1.00 eq.), and 3.81 mL dry DCM was added. Then 3.81 mL TFA was added dropwise, and the resulting solution was stirred for 18 h at room temperature. The reaction mixture was quenched by adding 10 mL water and was then extracted three times with 30 mL EtOAc. The combined org. layers were washed with brine, dried over $MgSO_4$, and the solvent was removed under reduced pressure. After purification via flash chromatography (CH/EtOAc + 1% FA, 0 – 60%), the title compound was obtained as a purple solid.

Yield: 591 mg (1.64 mmol, 86 %)
TLC (CH/EtOAc, 1:1 + 1% FA): R_f = 0.10
HPLC (5 – 100 % solvent B, 3 min) R_t = 1.559 min, purity (220 nm): 99%
Mass (ESI$^+$): m/z: calculated 360.02 $[M+H]^+$, found 360.00 $[M+H]^+$
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.71 (s, 1H), 6.84 (s, 2H), 3.86 (s, 3H), 3.84 (s, 6H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 173.43, 153.76, 153.27, 143.66, 138.88, 138.41, 135.51, 103.63, 76.97, 61.04, 56.40.

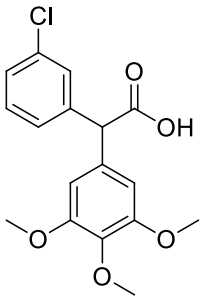
2-(2-Chlorothiazol-5-yl)-2-(3,4,5-trimethoxyphenyl)acetic acid (58j)



A 10 mL flask was charged with the starting material **58j-Int.** (462 mg, 1.28 mmol, 1.00 eq.), sodium iodide (58 mg, 0.39 mmol, 0.30 eq.), and phosphorous acid (158 mg, 1.93 mmol, 1.50 eq.). Then 5 mL of methanesulfonic acid (50 wt.-%) was added, and the resulting mixture was heated to 45°C for 5 days. After cooling to room temperature, the reaction mixture was diluted with 10 mL water and extracted three times with 10 mL EtOAc. The combined org. layers were then washed with brine, dried over $MgSO_4$, and the solvent was removed under reduced pressure. After purification via flash chromatography (CH/EtOAc + 1% FA, 0 – 50%), the title compound was obtained as a colorless solid.

Yield: 141 mg (0.41 mmol, 32 %)
TLC (CH/EtOAc, 1:1 + 1% FA): R_f = 0.29
HPLC (5 – 100 % solvent B, 3 min) R_t = 1.693 min, purity (220 nm): 98%
Mass (ESI^+): m/z: calculated 344.03 $[M+H]^+$, found 344.00 $[M+H]^+$
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.43 (s, 1H), 6.56 (s, 2H), 5.07 (s, 1H), 3.83 (s, 9H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 174.75, 153.79, 152.94, 139.60, 138.24, 137.64, 132.01, 105.29, 61.01, 56.39, 50.49.

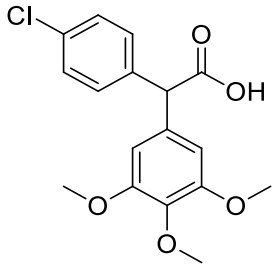
2-(3-Chlorophenyl)-2-(3,4,5-trimethoxyphenyl)acetic acid (58k)



Following the general procedure, compound **58k** was synthesized using: **57k** (241 mg, 0.59 mmol, 1.00 eq.), triethylsilane (0.28 mL, 1.77 mmol, 3.00 eq.), and 1.18 mL TFA in 1.18 mL dry DCM. The solution was stirred overnight at rt. After purification via column chromatography (CH/EtOAc + 1% FA, 0 – 30%), compound **58k** was obtained as a yellowish solid.

Yield: 82 mg (0.24 mmol, 41%)
TLC (CH/EtOAc + 1% FA, 1:1): R_f = 0.35
HPLC (30 – 100 % solvent B, 3 min) R_t = 1.521 min, purity (220 nm): 97%
Mass (ESI^+): m/z: calculated 337.08 $[M+H]^+$, found 337.00 $[M+H]^+$
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.33 – 7.31 (m, 1H), 7.25 – 7.19 (m, 3H), 6.54 (s, 2H), 4.92 (s, 1H), 3.82 (s, 3H), 3.79 (s, 6H).
^{13}C NMR (75 MHz, $CDCl_3$) δ 177.43, 153.45, 139.83, 137.70, 134.58, 132.63, 129.95, 128.74, 127.89, 126.76, 106.06, 60.92, 56.64, 56.27.

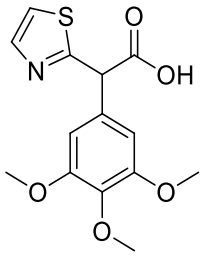
2-(4-chlorophenyl)-2-(3,4,5-trimethoxyphenyl)acetic acid (58l)



Following the general procedure, compound **58l** was synthesized using: **57l** (70 mg, 0.17 mmol, 1.00 eq.), triethylsilane (0.08 mL, 0.51 mmol, 3.00 eq.), and 0.68 mL TFA in 0.68 mL dry DCM. The solution was stirred overnight at rt. After purification via column chromatography (CH/EtOAc + 1% FA, 0 – 30%), compound **58l** was obtained as a colorless solid.

Yield: 45 mg (0.13 mmol, 78%)
TLC (CH/EtOAc + 1% FA, 1:1): R_f = 0.23
HPLC (30 – 100 % solvent B, 3 min) R_t = 1.462 min, purity (220 nm): 96%
Mass (ESI$^+$): m/z: calculated 337.08 $[M+H]^+$, found 337.00 $[M+H]^+$
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35 – 7.27 (m, 4H), 6.53 (s, 2H), 4.93 (s, 1H), 3.83 (s, 3H), 3.80 (s, 6H).
^{13}C NMR (75 MHz, $CDCl_3$) δ 177.65, 153.49, 137.72, 136.43, 133.69, 132.92, 129.99, 128.93, 106.04, 60.95, 56.43, 56.30.

2-(Thiazol-2-yl)-2-(3,4,5-trimethoxyphenyl)acetic acid (58m)



Following the general procedure, compound **58m** was synthesized using: **57m** (143 mg, 0.37 mmol, 1.00 eq.), triethylsilane (0.09 mL, 0.56 mmol, 3.00 eq.), and 0.75 mL TFA in 0.75 mL dry DCM. The solution was stirred overnight at rt. After purification via column chromatography (CH/EtOAc + 1% FA, 5 – 100%), compound **58m** was obtained as a colorless solid.

Yield: 45 mg (0.13 mmol, 78%)

TLC (CH/EtOAc + 1% FA, 1:1): R_f = 0.23

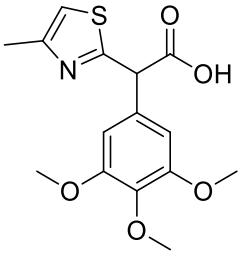
HPLC (30 – 100 % solvent B, 3 min) R_t = 1.444 min, purity (220 nm): 95%

Mass (ESI$^+$): m/z: calculated 264.07 $[M\text{-}CO_2H]^+$, found 264.20 $[M\text{-}CO_2H]^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.66 (d, J = 3.2 Hz, 1H), 7.29 (d, J = 2.5 Hz, 1H), 6.71 (s, 2H), 5.99 (s, 1H), 3.82 (s, 9H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 174.96, 163.76, 153.40, 142.14, 137.78, 137.18, 119.68, 103.54, 73.70, 60.87, 56.14

2-(4-Methylthiazol-2-yl)-2-(3,4,5-trimethoxyphenyl)acetic acid (58n)



Following the general procedure, compound **58n** was synthesized using: **57n** (171 mg, 0.43 mmol, 1.00 eq.), triethylsilane (0.21 mL, 1.30 mmol, 3.00 eq.), and 1.73 mL TFA in 1.73 mL dry DCM. The solution was stirred overnight at rt. After purification via column chromatography (CH/EtOAc + 1% FA, 0 – 100%), compound **58n** was obtained as a pale orange oil.

Yield: 56 mg (0.17 mmol, 40%)

TLC (CH/EtOAc + 1% FA, 1:1): R_f = 0.23

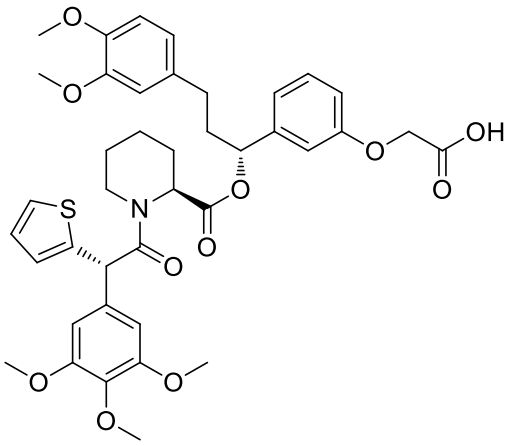
HPLC (30 – 100 % solvent B, 3 min) R_t = 1.529 min, purity (220 nm): 95%

Mass (ESI^+): m/z: calculated 278.08 $[M\text{-}CO_2H]^+$, found 278.20 $[M\text{-}CO2H]^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ 6.88 (s, 1H), 6.70 (s, 2H), 6.05 (s, 1H), 3.81 (s, 9H), 2.40 (s, 3H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 176.03, 164.41, 153.31, 151.19, 137.80, 136.17, 114.86, 103.54, 72.35, 60.82, 56.08, 15.91.

2-(3-((R)-3-(3,4-Dimethoxyphenyl)-1-(((S)-1-((S)-2-(thiophen-2-yl)-2-(3,4,5-tri-methoxy-phenyl)acetyl)piperidine-2-carbonyl)oxy)propyl)phenoxy)acetic acid (59a)



Following the general procedure B, compound **59a** was synthesized using: Resin **7** (200 mg, 0.08 mmol, 1.00 eq., loading: 0.39 mmol/g), **58a** (24 mg, 0.08 mmol, 1.00 eq.), PPh_3 (41 mg, 0.16 mmol, 2.00 eq.), trichloroacetonitrile (16 µL, 0.16 mmol, 2.00 eq.), DIPEA (68 µL, 0.39 mmol, 5.00 eq.) and 1 mL DCM. After purification via preparative HPLC (H_2O/ACN + 0.1% TFA, 50 - 70%), diastereomers **59a** and **d59a** were obtained as colorless solids.

Yield: 10 mg (13.37 µmol, 17%)

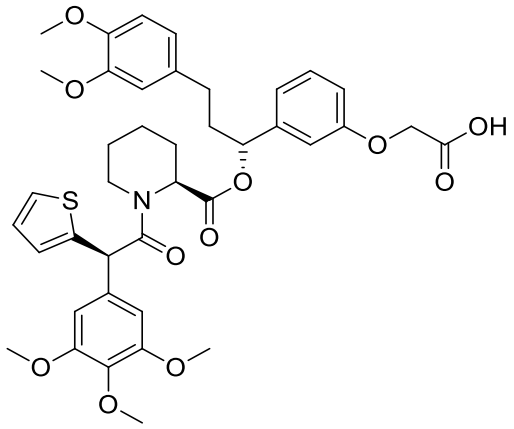
HPLC (70 – 100 % solvent B, 3 min) R_t = 2.059 min, purity (220 nm): 99%

HRMS (ESI$^+$): m/z: calculated 748.2786 $[M+H]^+$, found 748.2788 $[M+H]^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.16 – 7.14 (m, 1H), 7.13 (d, J = 7.8 Hz, 1H), 6.84 (dd, J = 5.1, 3.5 Hz, 1H), 6.80 – 6.75 (m, 2H), 6.74 – 6.69 (m, 2H), 6.66 – 6.65 (m, 1H), 6.62 – 6.58 (m, 2H), 6.26 (s, 2H), 5.45 (dd, J = 9.0, 4.7 Hz, 1H), 5.41 – 5.39 (m, 1H), 5.32 (s, 1H), 4.71 – 4.45 (m, 2H), 3.78 (s, 3H), 3.77 (s, 3H), 3.76 – 3.74 (m, 1H), 3.70 (s, 3H), 3.46 (s, 6H), 3.08 (td, J = 13.3, 3.2 Hz, 1H), 2.66 – 2.56 (m, 1H), 2.54 – 2.44 (m, 1H), 2.29 – 2.22 (m, 1H), 2.18 – 2.08 (m, 1H), 1.99 – 1.90 (m, 1H), 1.80 – 1.67 (m, 2H), 1.63 – 1.56 (m, 1H), 1.42 – 1.31 (m, 1H), 1.31 – 1.19 (m, 1H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 172.03, 170.80, 170.39, 158.30, 153.47, 149.18, 147.65, 142.68, 142.37, 137.15, 133.64, 133.46, 129.70, 126.65, 126.36, 125.92, 120.38, 119.52, 116.06, 111.88, 111.59, 108.82, 105.37, 77.05, 65.53, 60.92, 56.11, 56.07, 56.02, 52.92, 50.28, 44.18, 38.59, 31.72, 27.23, 25.20, 20.75.

2-(3-((R)-3-(3,4-Dimethoxyphenyl)-1-(((S)-1-((R)-2-(thiophen-2-yl)-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2-carbonyl)oxy)propyl)phenoxy)acetic acid (d59a)



Yield: 12 mg (16.05 µmol, 21%)

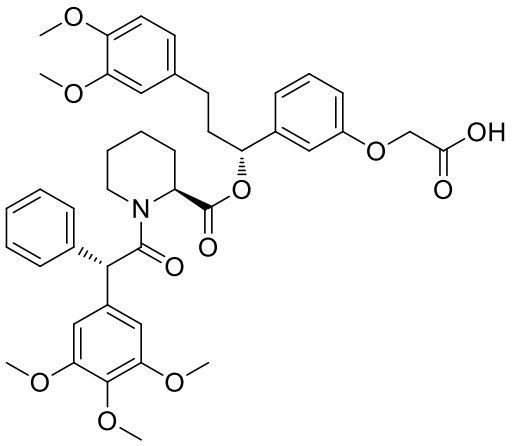
HPLC (70 – 100 % solvent B, 3 min) R_t = 2.033 min, purity (220 nm): 99%

HRMS (ESI$^+$): m/z: calculated 748.2786 $[M+H]^+$, found 748.2789 $[M+H]^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.26 – 7.22 (m, 1H), 7.19 (dd, J = 4.6, 1.8 Hz, 1H), 6.91 – 6.87 (m, 4H), 6.79 – 6.77 (m, 2H), 6.71 – 6.65 (m, 2H), 6.51 (s, 2H), 5.69 (dd, J = 8.7, 5.1 Hz, 1H), 5.54 – 5.50 (m, 1H), 5.37 (s, 1H), 4.68 – 4.56 (m, 2H), 3.86 (s, 3H), 3.86 (s, 3H), 3.84 (d, J = 2.1 Hz, 1H), 3.83 (s, 3H), 3.81 (s, 6H), 3.30 (td, J = 13.3, 3.0 Hz, 1H), 2.71 – 2.63 (m, 1H), 2.61 – 2.54 (m, 1H), 2.37 – 2.30 (m, 1H), 2.27 – 2.19 (m, 1H), 2.13 – 2.02 (m, 1H), 1.76 – 1.63 (m, 2H), 1.60 – 1.52 (m, 1H), 1.40 – 1.31 (m, 1H), 1.17 – 1.08 (m, 1H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 171.64, 170.88, 170.32, 158.13, 153.52, 149.14, 147.64, 142.22, 141.61, 137.61, 134.66, 133.53, 129.89, 126.81, 126.65, 125.73, 120.39, 119.79, 115.97, 111.97, 111.61, 105.88, 76.67, 65.47, 61.02, 56.40, 56.12, 56.08, 53.11, 50.66, 44.27, 38.19, 31.62, 27.21, 25.10, 20.93.

2-(3-((R)-3-(3,4-Dimethoxyphenyl)-1-(((S)-1-((S)-2-phenyl-2-(3,4,5-trimethoxy-phenyl)-acetyl)piperidine-2-carbonyl)oxy)propyl)phenoxy)acetic acid (59b)



A 10 mL filter syringe was charged with resin **7** (150 mg, 0.10 mmol, 1.00 eq., loading: 0.45 mmol/g). After Fmoc-deprotection following the general procedure, the resin was treated with a solution of **58b** (31 mg, 0.07 mmol, 1.50 eq.), COMU (43 mg, 0.10 mmol, 1.50 eq.), pyridine (16 µL, 0.20 mmol, 3.00 eq.) in 0.68 mL DMF. The reaction mixture was then stirred at rt overnight. After complete conversion, the resin was filtered and washed twice with 3 mL DMF and 3 mL DCM. Finally, the product was cleaved off the resin by treating it with a solution of 20 vol.-% HFIP in DCM for 1 h. After purification via preparative HPLC (H_2O/ACN + 0.1% TFA, 50 – 70%), diastereomers **59b** and **d58b** were obtained as colorless solids.

Yield: 17 mg (22.92 µmol, 34%)

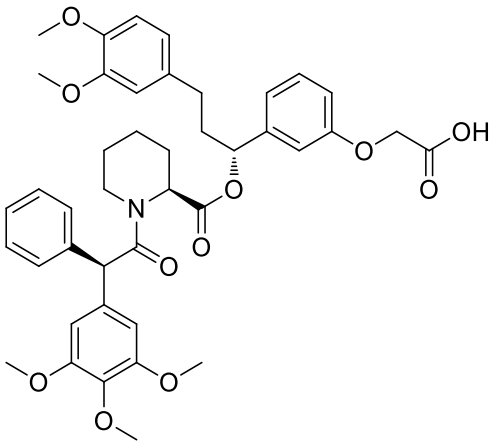
HPLC (50 – 100 % solvent B, 3 min) R_t = 1.399 min, purity (220 nm): 96%

HRMS (ESI$^+$): m/z: calculated 742.32219 $[M+H]^+$, found 742.32158 $[M+H]^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37 – 7.27 (m, 5H), 7.21 (d, J = 7.5 Hz, 2H), 6.94 – 6.90 (m, 1H), 6.86 (dd, J = 16.3, 7.7 Hz, 2H), 6.81 – 6.79 (m, 1H), 6.74 (d, J = 9.9 Hz, 2H), 6.33 (s, 2H), 5.59 (dd, J = 9.1, 4.7 Hz, 1H), 5.54 (d, J = 5.9 Hz, 1H), 5.21 (s, 1H), 4.67 (q, J = 16.2 Hz, 2H), 3.91 (s, 3H), 3.91 (s, 3H), 3.84 (s, 3H), 3.59 (s, 6H), 3.26 (t, J = 11.7 Hz, 1H), 2.79 – 2.71 (m, 1H), 2.68 – 2.59 (m, 1H), 2.43 – 2.34 (m, 1H), 2.31 – 2.23 (m, 1H), 2.12 – 2.04 (m, 1H), 1.89 – 1.77 (m, 2H), 1.70 (d, J = 10.4 Hz, 1H), 1.47 – 1.36 (m, 2H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 173.09, 171.09, 170.49, 158.24, 153.33, 149.09, 147.57, 142.69, 139.33, 136.74, 133.65, 133.42, 129.69, 129.48, 128.49, 127.35, 120.34, 119.45, 116.02, 111.77, 111.49, 108.75, 105.87, 77.07, 65.41, 60.90, 56.07, 56.03, 55.92, 55.43, 52.80, 44.09, 38.59, 31.71, 27.31, 25.18, 20.78.

2-(3-((R)-3-(3,4-Dimethoxyphenyl)-1-(((S)-1-((R)-2-phenyl-2-(3,4,5-trimethoxy-phenyl)-acetyl)piperidine-2-carbonyl)oxy)propyl)phenoxy)acetic acid (d59b)



Yield: 15 mg (20.22 µmol, 30%)

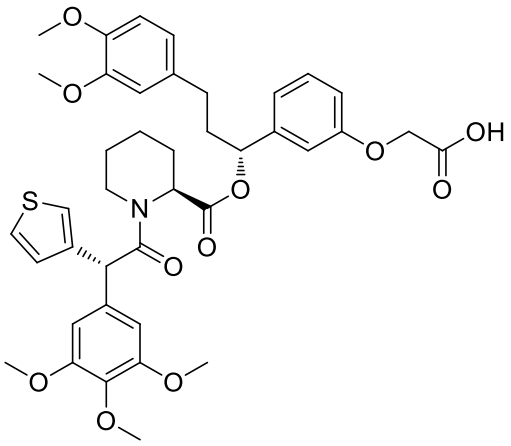
HPLC (50 – 100 % solvent B, 3 min) R_t = 1.358 min, purity (220 nm): 98%

HRMS (ESI$^+$): m/z: calculated 742.32219 $[M+H]^+$, found 742.32174 $[M+H]^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.25 – 7.20 (m, 4H), 7.18 – 7.13 (m, 2H), 6.92 – 6.83 (m, 2H), 6.82 – 6.75 (m, 2H), 6.71 – 6.66 (m, 2H), 6.43 (s, 2H), 5.69 (dd, J = 8.8, 4.9 Hz, 1H), 5.54 (d, J = 5.5 Hz, 1H), 5.16 (s, 1H), 4.62 – 4.49 (m, 2H), 3.85 (s, 6H), 3.83 (s, 3H), 3.78 (s, 6H), 3.21 (td, J = 13.2, 3.0 Hz, 1H), 2.72 – 2.62 (m, 1H), 2.62 – 2.54 (m, 1H), 2.35 (d, J = 13.3 Hz, 1H), 2.28 – 2.16 (m, 1H), 2.16 – 2.02 (m, 1H), 1.76 – 1.67 (m, 2H), 1.55 (d, J = 13.3 Hz, 1H), 1.40 – 1.15 (m, 3H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 172.58, 170.97, 170.40, 158.09, 153.34, 149.08, 147.57, 142.24, 138.84, 137.23, 134.69, 133.49, 129.84, 129.06, 128.65, 127.22, 120.33, 119.65, 115.88, 111.84, 111.50, 110.03, 106.49, 76.53, 65.40, 60.99, 56.30, 56.08, 56.03, 55.60, 52.90, 44.14, 38.26, 31.63, 27.28, 25.17, 20.94.

2-(3-((R)-3-(3,4-Dimethoxyphenyl)-1-(((S)-1-((R)-2-(thiophen-3-yl)-2-(3,4,5-tri-methoxy-phenyl)acetyl)piperidine-2-carbonyl)oxy)propyl)phenoxy)acetic acid (59c)



A 10 mL filter syringe was charged with resin **7** (150 mg, 0.07 mmol, 1.00 eq., loading: 0.45 mmol/g). After Fmoc-deprotection following the general procedure, the resin was treated with a solution containing **58c** (29 mg, 0.08 mmol, 1.00 eq.), T3P (80 µL, 0.14 mmol, 2.00 eq.), and pyridine (16 µL, 0.20 mmol, 3.00 eq.) in 0.68 mL DMF overnight. After the complete conversion of the starting material, the resin was filtered and washed twice with DMF, followed by DCM. Finally, the product was cleaved off the resin by treating it with a solution of 20 vol.-% HFIP in DCM for 1 h. After removal of the solvent under reduced pressure and purification via preparative HPLC (50 - 70%, solvent B). The respective diastereomers were obtained as colorless solids.

Yield: 8 mg (10.69 µmol, 16%)

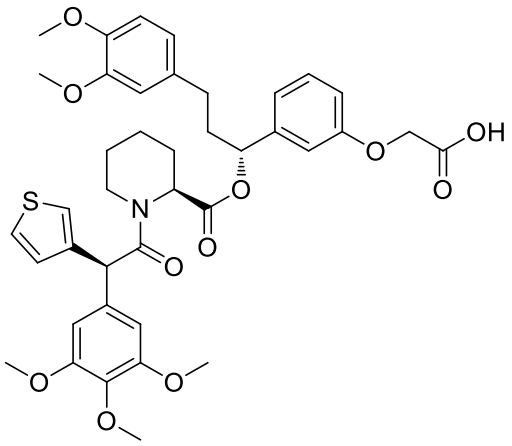
HPLC (30 – 100 % solvent B, 3 min) R_t = 1.818 min, purity (220 nm): 99%

HRMS (ESI$^+$): m/z: calculated 748.27861 $[M+H]^+$, found 748.27844 $[M+H]^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.28 (s, 1H), 7.26 – 7.21 (m, 1H), 7.03 – 6.96 (m, 2H), 6.93 – 6.87 (m, 1H), 6.82 (t, J = 7.9 Hz, 2H), 6.77 – 6.74 (m, 1H), 6.71 (d, J = 8.8 Hz, 2H), 6.31 (s, 2H), 5.53 (dt, J = 15.6, 5.2 Hz, 2H), 5.24 (s, 1H), 4.71 – 4.58 (m, 2H), 3.88 (s, 3H), 3.88 (s, 3H), 3.86 – 3.84 (m, 1H), 3.80 (s, 3H), 3.55 (s, 6H), 3.19 (td, J = 13.1, 3.0 Hz, 1H), 2.76 – 2.67 (m, 1H), 2.65 – 2.54 (m, 1H), 2.35 (d, J = 14.1 Hz, 1H), 2.29 – 2.16 (m, 1H), 2.08 – 2.01 (m, 1H), 1.87 – 1.72 (m, 2H), 1.72 – 1.60 (m, 1H), 1.49 – 1.32 (m, 2H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 172.74, 170.84, 170.44, 158.28, 153.41, 149.12, 147.59, 142.74, 139.92, 136.82, 133.50, 133.41, 129.68, 128.81, 125.67, 123.09, 120.35, 119.44, 116.20, 111.77, 111.49, 108.45, 65.49, 60.91, 56.09, 56.04, 55.95, 52.76, 50.80, 44.12, 38.64, 31.75, 27.33, 25.21, 20.74.

2-(3-((R)-3-(3,4-Dimethoxyphenyl)-1-(((S)-1-((S)-2-(thiophen-3-yl)-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2-carbonyl)oxy)propyl)phenoxy)acetic acid (d59c)



Yield: 12 mg (16.05µmol, 24%)

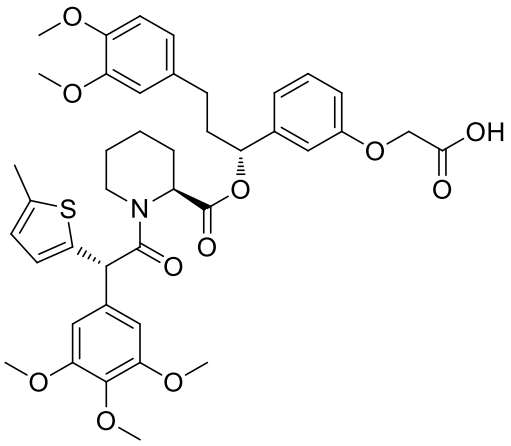
HPLC (30 – 100 % solvent B, 3 min) R_t = 1.827 min, purity (220 nm): 99%

HRMS (ESI$^+$): m/z: calculated 748.27861 $[M+H]^+$, found 748.27844 $[M+H]^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.25 (t, 1H), 7.21 (dd, J = 5.0, 3.0 Hz, 1H), 7.05 (dt, J = 2.7, 1.1 Hz, 1H), 6.92 – 6.87 (m, 3H), 6.80 (d, J = 8.0 Hz, 1H), 6.77 – 6.76 (m, 1H), 6.72 – 6.67 (m, 2H), 6.44 (s, 2H), 5.69 (dd, J = 8.9, 4.8 Hz, 1H), 5.56 – 5.43 (m, 1H), 5.18 (s, 1H), 4.61 (q, J = 16.4 Hz, 2H), 3.87 (s, 3H), 3.86 (s, 3H), 3.83 (s, 4H), 3.79 (s, 6H), 3.28 (td, J = 13.3, 3.0 Hz, 1H), 2.75 – 2.64 (m, 1H), 2.63 – 2.51 (m, 1H), 2.41 – 2.28 (m, 1H), 2.29 – 2.16 (m, 1H), 2.12 – 2.04 (m, 1H), 1.80 – 1.66 (m, 2H), 1.62 – 1.50 (m, 1H), 1.39 – 1.29 (m, 1H), 1.26 – 1.08 (m, 1H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 172.46, 170.79, 170.39, 158.15, 153.37, 149.11, 147.61, 142.23, 138.98, 137.32, 134.49, 133.46, 129.89, 128.42, 125.84, 123.32, 120.34, 119.58, 116.19, 111.84, 111.52, 109.57, 106.26, 76.70, 65.48, 61.01, 56.33, 56.10, 56.06, 53.01, 51.13, 44.20, 38.22, 31.69, 27.31, 25.17, 20.99.

2-(3-((R)-3-(3,4-Dimethoxyphenyl)-1-(((S)-1-((S)-2-(5-methylthiophen-2-yl)-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2-carbonyl)oxy)propyl)phenoxy)-acetic acid (59d)



Following the general procedure B, compound **59d** was synthesized using: Resin **7** (82 mg, 0.05 mmol, 1.00 eq., loading: 0.56 mmol/g), **58d** (30 mg, 0.09 mmol, 2.00 eq.), PPh_3 (49 mg, 0.19 mmol, 4.00 eq.), trichloroacetonitrile (19 µL, 0.19 mmol, 4.00 eq.), DIPEA (81 µL, 0.47 mmol, 10.00 eq.) and 0.93 mL DCM. After purification via preparative HPLC (H_2O/ACN + 0.1% TFA, 50 - 80%), diastereomers **59d** and **d59c** were obtained as colorless solids.

Yield: 12 mg (15.75 µmol, 34%)

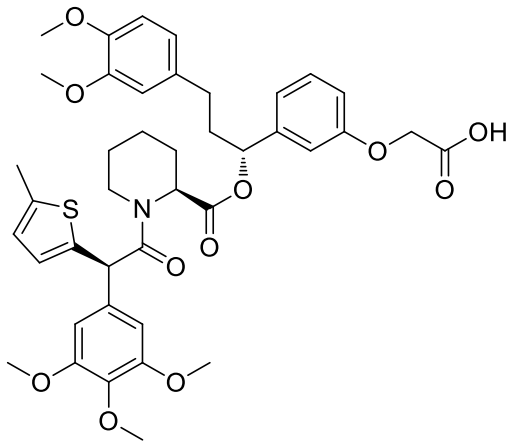
HPLC (50 – 100 % solvent B, 3 min) R_t = 1.438 min, purity (220 nm): 95%

HRMS (ESI$^+$): m/z: calculated 762.29426 $[M+H]^+$, found 762.29421 $[M+H]^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.24 (t, J = 7.9 Hz, 1H), 6.90 – 6.86 (m, 1H), 6.84 – 6.80 (m, 2H), 6.76 (d, J = 2.6 Hz, 1H), 6.71 (d, J = 9.1 Hz, 2H), 6.64 (d, J = 3.4 Hz, 1H), 6.57 (d, J = 3.4 Hz, 1H), 6.36 (s, 2H), 5.56 (dd, J = 9.0, 4.7 Hz, 1H), 5.51 (d, J = 5.9 Hz, 1H), 5.33 (s, 1H), 4.73 – 4.59 (m, 2H), 3.89 (s, 3H), 3.88 (s, 3H), 3.89 – 3.81 (m, 1H), 3.80 (s, 3H), 3.57 (s, 6H), 3.17 (td, J = 13.3, 3.0 Hz, 1H), 2.75 – 2.66 (m, 1H), 2.63 – 2.56 (m, 1H), 2.43 (s, 3H), 2.40 – 2.33 (m, 1H), 2.25 – 2.18 (m, 1H), 2.10 – 2.03 (m, 1H), 1.87 – 1.75 (m, 2H), 1.70 – 1.65 (m, 1H), 1.51 – 1.41 (m, 1H), 1.40 – 1.29 (m, 1H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 172.05, 170.94, 170.24, 158.08, 153.25, 148.96, 147.44, 142.51, 140.39, 139.60, 136.83, 133.62, 133.29, 129.57, 126.24, 124.23, 120.22, 119.37, 115.75, 111.65, 111.36, 108.84, 105.12, 76.85, 65.35, 60.78, 55.95, 55.90, 55.85, 52.76, 50.35, 44.01, 38.42, 31.57, 27.08, 25.06, 20.64, 15.35.

2-(3-((R)-3-(3,4-Dimethoxyphenyl)-1-(((S)-1-((R)-2-(5-methylthiophen-2-yl)-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2-carbonyl)oxy)propyl)phenoxy)-acetic acid (d59d)



Yield: 14 mg (18.37 µmol, 40%)

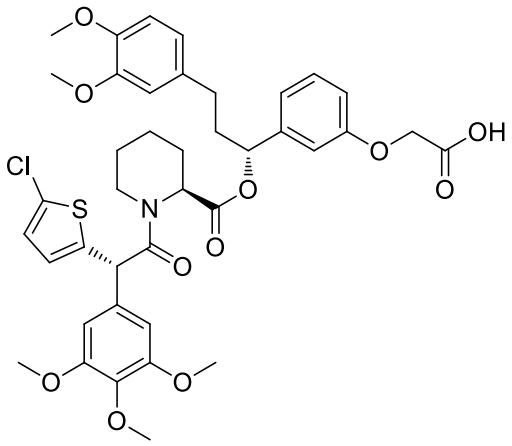
HPLC (50 – 100 % solvent B, 3 min) R_t = 1.388 min, purity (220 nm): 96%

HRMS (ESI$^+$): calculated 762.29426 $[M+H]^+$, found 762.29450 $[M+H]^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.31 – 7.27 (m, 1H), 6.90 (t, J = 6.7 Hz, 2H), 6.81 (d, J = 7.9 Hz, 2H), 6.73 – 6.67 (m, 2H), 6.55 (d, J = 3.4 Hz, 1H), 6.53 (s, 2H), 5.72 (dd, J = 8.3, 5.3 Hz, 1H), 5.53 (d, J = 5.4 Hz, 1H), 5.29 (s, 1H), 4.71 – 4.58 (m, 2H), 3.88 (s, 6H), 3.87 – 3.85 (m, 1H), 3.85 (s, 3H), 3.83 (s, 6H), 3.68 (s, 1H), 3.28 (td, J = 13.3, 2.9 Hz, 1H), 2.71 – 2.64 (m, 1H), 2.62 – 2.54 (m, 1H), 2.40 (s, 3H), 2.38 – 2.33 (m, 1H), 2.29 – 2.21 (m, 1H), 2.12 – 2.04 (m, 1H), 1.75 – 1.67 (m, 2H), 1.59 – 1.55 (m, 1H), 1.43 – 1.28 (m, 2H), 1.22 – 1.11 (m, 1H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 171.73, 171.37, 170.30, 158.01, 153.41, 149.04, 147.54, 142.17, 140.26, 138.89, 137.40, 134.72, 133.51, 129.89, 126.62, 124.68, 120.35, 119.84, 115.61, 111.87, 111.50, 110.63, 105.78, 76.60, 65.35, 61.01, 56.34, 56.08, 56.03, 53.05, 50.81, 44.29, 38.19, 31.57, 27.15, 25.10, 20.92, 15.43.

2-(3-((R)-1-(((S)-1-((S)-2-(5-Chlorothiophen-2-yl)-2-(3,4,5-trimethoxyphenyl)-acetyl)-piperidine-2-carbonyl)oxy)-3-(3,4-dimethoxyphenyl)propy)phenoxy)-acetic acid (59e)



Following the general procedure B, compound **59e** was synthesized using: Resin **7** (77 mg, 0.04 mmol, 1.00 eq., loading: 0.56 mmol/g), **58e** (15 mg, 0.04 mmol, 1.00 eq.), PPh_3 (23 mg, 0.09 mmol, 2.00 eq.), trichloroacetonitrile (9 µL, 0.09 mmol, 2.00 eq.), DIPEA (38 µL, 0.22 mmol, 5.00 eq.) and 0.5 mL DCM. After purification via preparative HPLC (H_2O/ACN + 0.1% TFA, 50 - 70%), diastereomers **59e** and **d59e** were obtained as colorless solids.

Yield: 8 mg (10.23 µmol, 24%)

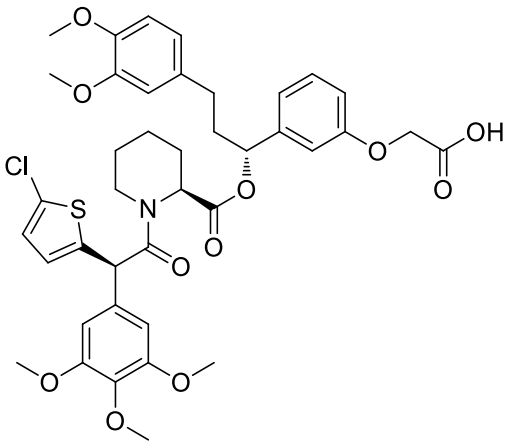
HPLC (50 – 100 % solvent B, 3 min) R_t = 2.253 min, purity (220 nm): 99%

HRMS (ESI$^+$): m/z: calculated 782.2396 $[M+H]^+$, found 782.2403 $[M+H]^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.21 (t, J = 7.8 Hz, 1H), 6.89 – 6.83 (m, 1H), 6.80 – 6.75 (m, 2H), 6.73 (s, 1H), 6.70 (dd, J = 5.4, 2.8 Hz, 2H), 6.67 (s, 1H), 6.62 (d, J = 3.8 Hz, 1H), 6.31 (s, 2H), 5.52 (dd, J = 9.0, 4.7 Hz, 1H), 5.47 (d, J = 6.6 Hz, 1H), 5.27 (s, 1H), 4.72 – 4.57 (m, 2H), 3.86 (s, 3H), 3.85 (s, 3H), 3.78 (s, 3H), 3.77 – 3.70 (m, 1H), 3.54 (s, 6H), 3.14 – 3.06 (m, 1H), 2.73 – 2.64 (m, 1H), 2.61 – 2.52 (m, 1H), 2.37 – 2.30 (m, 1H), 2.24 – 2.16 (m, 1H), 2.06 – 1.97 (m, 1H), 1.87 – 1.74 (m, 1H), 1.68 (d, J = 13.4 Hz, 1H), 1.52 – 1.42 (m, 1H), 1.37 – 1.23 (m, 2H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 171.22, 170.05, 169.26, 158.08, 153.43, 148.98, 147.46, 142.53, 141.15, 137.10, 133.24, 132.77, 130.39, 129.55, 125.40, 124.97, 120.20, 119.38, 115.85, 111.63, 111.34, 104.88, 77.23, 60.79, 55.95, 55.91, 55.88, 52.80, 50.76, 44.08, 38.45, 31.59, 29.71, 27.08, 25.09, 20.63.

2-(3-((R)-1-(((S)-1-((R)-2-(5-Chlorothiophen-2-yl)-2-(3,4,5-trimethoxyphenyl)-acetyl)-piperidine-2-carbonyl)oxy)-3-(3,4-dimethoxyphenyl)propy)phenoxy)-acetic acid (d59e)



Yield: 8 mg (10.23 µmol, 24%)

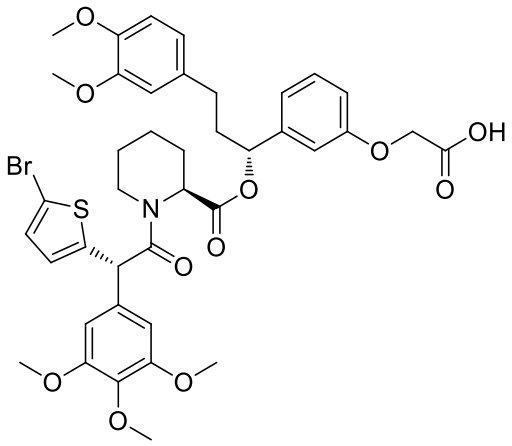
HPLC (50 – 100 % solvent B, 3 min) R_t = 2.227 min, purity (220 nm): 99%

HRMS (ESI$^+$): m/z: calculated 782.2401 $[M+H]^+$, found 782.2396 $[M+H]^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.25 – 7.23 (m, 1H), 6.88 (dd, J = 8.0, 1.8 Hz, 2H), 6.82 – 6.76 (m, 2H), 6.71 – 6.64 (m, 4H), 6.50 (s, 2H), 5.68 (dd, J = 8.6, 5.1 Hz, 1H), 5.54 – 5.48 (m, 1H), 5.23 (s, 1H), 4.69 – 4.57 (m, 2H), 3.86 (s, 3H), 3.86 (s, 3H), 3.85 – 3.84 (m, 1H), 3.83 (s, 3H), 3.82 (s, 6H), 3.32 (td, J = 13.3, 3.0 Hz, 1H), 2.70 – 2.62 (m, 1H), 2.60 – 2.53 (m, 1H), 2.34 (d, J = 13.5 Hz, 1H), 2.27 – 2.17 (m, 1H), 2.11 – 2.02 (m, 1H), 1.75 – 1.60 (m, 2H), 1.57 – 1.51 (m, 1H), 1.40 – 1.27 (m, 1H), 1.14 – 1.00 (m, 1H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 170.78, 170.11, 157.93, 153.50, 148.94, 147.45, 142.04, 140.48, 137.57, 133.95, 133.30, 130.06, 129.78, 125.67, 125.26, 120.20, 119.65, 115.65, 111.70, 111.37, 105.34, 76.63, 60.90, 56.29, 55.95, 55.91, 52.95, 50.88, 44.08, 38.06, 31.46, 27.03, 24.89, 20.76.

2-(3-((R)-1-(((S)-1-((S)-2-(5-Bromothiophen-2-yl)-2-(3,4,5-trimethoxyphenyl)-acetyl)-piperidine-2-carbonyl)oxy)-3-(3,4-dimethoxyphenyl)propyl)phenoxy)-acetic acid (59f)



Following the general procedure C, compound **59f** was synthesized using: Resin **7** (208 mg, 0.09 mmol, 1.10 eq., loading: 0.45mmol/g), **58f** (33 mg, 0.09 mmol, 1.00 eq.), oxalyl chloride (15 µL, 0.17 mmol, 2.00 eq.), DIPEA (74 µL, 0.43 mmol, 5.00 eq.) and 0.47 mL DCM. After purification via preparative HPLC (H_2O/ACN + 0.1% TFA, 50 - 80%), diastereomers **59f** and **d59f** were obtained as colorless solids.

Yield: 11 mg (14.04 µmol, 16%)

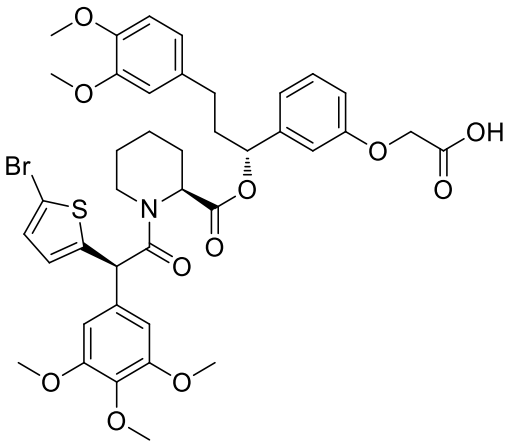
HPLC (50 – 100 % solvent B, 3 min) R_t = 1.589 min, purity (220 nm): 99%

HRMS (ESI$^+$): m/z: calculated 826.18912 $[M+H]^+$, found 826.18954 $[M+H]^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.30 – 7.23 (m, 1H), 6.91 – 6.86 (m, 2H), 6.82 (d, J = 3.8 Hz, 1H), 6.80 – 6.77 (m, 2H), 6.70 – 6.63 (m, 3H), 6.50 (s, 2H), 5.69 (dd, J = 8.5, 5.2 Hz, 1H), 5.55 – 5.47 (m, 1H), 5.26 (s, 1H), 4.69 – 4.58 (m, 2H), 3.86 (s, 3H), 3.86 (s, 3H), 3.84 – 3.83 (m, 1H), 3.83 (s, 3H), 3.82 (s, 6H), 3.31 (td, J = 13.0, 2.7 Hz, 1H), 2.69 – 2.62 (m, 1H), 2.59 – 2.52 (m, 1H), 2.38 – 2.29 (m, 1H), 2.28 – 2.19 (m, 1H), 2.13 – 2.03 (m, 1H), 1.73 – 1.61 (m, 2H), 1.57 – 1.49 (m, 1H), 1.39 – 1.23 (m, 1H), 1.11 – 1.01 (m, 1H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 171.23, 170.95, 170.24, 158.03, 153.63, 149.06, 147.57, 143.45, 142.13, 137.69, 134.04, 133.44, 129.94, 129.17, 126.84, 120.35, 119.82, 115.68, 112.57, 111.85, 111.52, 110.46, 105.49, 76.76, 65.36, 61.04, 56.42, 56.05, 53.10, 50.98, 44.23, 38.17, 31.58, 27.14, 25.02, 20.88.

2-(3-((R)-1-(((S)-1-((R)-2-(5-Bromothiophen-2-yl)-2-(3,4,5-trimethoxyphenyl)-acetyl)-piperidine-2-carbonyl)oxy)-3-(3,4-dimethoxyphenyl)propyl)phenoxy)-acetic acid (d59f)



Yield: 12 mg (15.32 µmol, 17%)

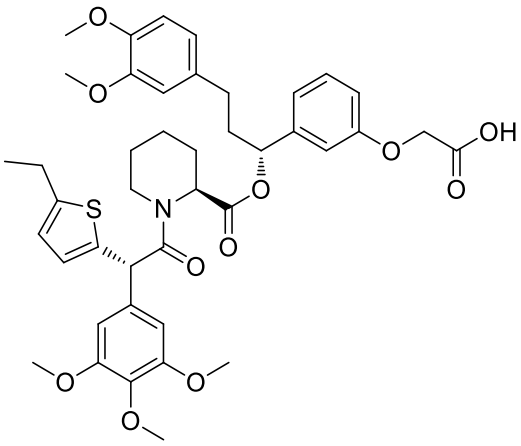
HPLC (30 – 100 % solvent B, 3 min) R_t = 1.519 min, purity (220 nm): 99%

HRMS (ESI$^+$): m/z: calculated 826.18870 $[M+H]^+$, found 826.18912 $[M+H]^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.22 (t, J = 7.9 Hz, 1H), 6.88 – 6.84 (m, 2H), 6.79 (dd, J = 7.7, 4.1 Hz, 2H), 6.73 (dd, J = 2.7, 1.4 Hz, 1H), 6.70 – 6.65 (m, 2H), 6.60 (d, J = 3.8 Hz, 1H), 6.32 (s, 2H), 5.52 (dd, J = 9.0, 4.7 Hz, 1H), 5.47 – 5.44 (m, 1H), 5.31 (s, 1H), 4.73 – 4.60 (m, 2H), 3.86 (s, 3H), 3.85 (s, 3H), 3.85 – 3.83 (m, 1H), 3.78 (s, 3H), 3.54 (s, 6H), 3.10 (td, J = 13.5, 3.1 Hz, 1H), 2.77 – 2.63 (m, 1H), 2.57 (ddd, J = 14.0, 9.2, 6.8 Hz, 1H), 2.35 (d, J = 13.7 Hz, 1H), 2.25 – 2.12 (m, 1H), 2.02 (tdd, J = 11.8, 8.7, 5.6 Hz, 1H), 1.88 – 1.74 (m, 2H), 1.68 (d, J = 13.4 Hz, 1H), 1.53 – 1.43 (m, 1H), 1.39 – 1.11 (m, 1H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 171.19, 171.03, 170.03, 158.06, 153.42, 148.96, 147.44, 143.98, 142.49, 137.06, 133.25, 132.77, 129.59, 128.73, 126.45, 120.22, 119.41, 115.77, 112.73, 111.65, 111.36, 108.77, 104.92, 65.32, 60.80, 55.95, 55.91, 55.89, 52.81, 50.72, 44.09, 38.42, 31.57, 27.07, 25.09, 20.63.

2-(3-((R)-3-(3,4-Dimethoxyphenyl)-1-(((S)-1-((S)-2-(5-ethylthiophen-2-yl)-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2-carbonyl)oxy)propyl)phenoxy)acetic acid (59g)



Following the general procedure C, compound **59g** was synthesized using: Resin **7** (291 mg, 0.13 mmol, 1.10 eq., loading: 0.45 mmol/g), **58g** (40 mg, 0.12 mmol, 1.00 eq.), oxalyl chloride (20 µL, 0.24 mmol, 2.00 eq.), DIPEA (104 µL, 0.59 mmol, 5.00 eq.) and 0.48 mL DCM. After purification via preparative HPLC (H_2O/ACN + 0.1% TFA, 50 - 80%), diastereomers **59g** and **d59g** were obtained as colorless solids.

Yield: 23 mg (29.67 µmol, 25%)

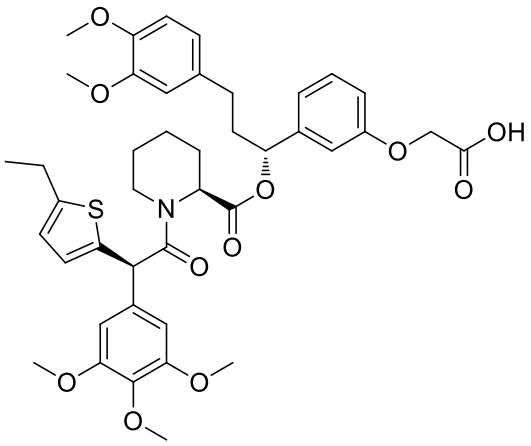
HPLC (50 – 100 % solvent B, 3 min) R_t = 1.604 min, purity (220 nm): 99%

HRMS (ESI$^+$): m/z: calculated 776.30991 $[M+H]^+$, found 776.31047 $[M+H]^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.21 (t, J = 7.9 Hz, 1H), 6.87 – 6.83 (m, 1H), 6.79 (dd, J = 7.8, 6.2 Hz, 2H), 6.74 (t, J = 1.8 Hz, 1H), 6.71 – 6.66 (m, 2H), 6.63 (d, J = 3.5 Hz, 1H), 6.60 – 6.57 (m, 1H), 6.35 (s, 2H), 5.54 (dd, J = 8.9, 4.7 Hz, 1H), 5.50 – 5.47 (m, 1H), 5.32 (s, 1H), 4.71 – 4.56 (m, 2H), 3.86 (s, 3H), 3.85 (s, 3H), 3.85 – 3.81 (m, 1H), 3.78 (s, 3H), 3.56 (s, 6H), 3.14 (td, J = 13.3, 3.1 Hz, 1H), 2.81 – 2.73 (m, 2H), 2.72 – 2.65 (m, 1H), 2.61 – 2.51 (m, 1H), 2.36 – 2.30 (m, 1H), 2.24 – 2.15 (m, 1H), 2.08 – 1.96 (m, 1H), 1.86 – 1.73 (m, 2H), 1.68 – 1.64 (m, 1H), 1.50 – 1.38 (m, 1H), 1.37 – 1.29 (m, 1H), 1.25 (t, J = 7.5 Hz, 3H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 172.18, 171.13, 170.39, 158.19, 153.36, 149.08, 148.09, 147.55, 142.60, 139.29, 136.96, 133.74, 133.42, 129.69, 126.20, 122.45, 120.34, 119.51, 115.76, 111.78, 111.48, 109.19, 105.32, 76.91, 56.07, 56.02, 55.98.

2-(3-((R)-3-(3,4-Dimethoxyphenyl)-1-(((S)-1-((R)-2-(5-ethylthiophen-2-yl)-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2-carbonyl)oxy)propyl)phenoxy)acetic acid (d59g)



Yield: 26 mg (33.54 µmol, 28%)

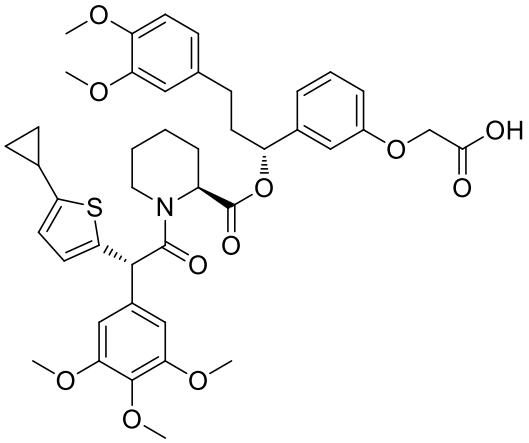
HPLC (50 – 100 % solvent B, 3 min) R_t = 1.515 min, purity (220 nm): 99%

HRMS (ESI$^+$): m/z: calculated 776.30991 $[M+H]^+$, found 776.31030 $[M+H]^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.21 (t, J = 7.9 Hz, 1H), 6.87 – 6.83 (m, 1H), 6.79 (dd, J = 7.9, 6.2 Hz, 2H), 6.74 (t, J = 1.8 Hz, 1H), 6.70 – 6.66 (m, 2H), 6.63 (d, J = 3.5 Hz, 1H), 6.58 (d, J = 3.2 Hz, 1H), 6.35 (s, 2H), 5.54 (dd, J = 8.9, 4.7 Hz, 1H), 5.50 – 5.47 (m, 1H), 5.32 (s, 1H), 4.70 – 4.56 (m, 2H), 3.86 (s, 3H), 3.85 (s, 3H), 3.84 – 3.83 (m, 1H), 3.78 (s, 3H), 3.56 (s, 6H), 3.14 (td, J = 13.3, 3.1 Hz, 1H), 2.81 – 2.72 (m, 2H), 2.71 – 2.63 (m, 1H), 2.60 – 2.51 (m, 1H), 2.35 – 2.30 (m, 1H), 2.26 – 2.15 (m, 1H), 2.10 – 1.98 (m, 1H), 1.79 (tdd, J = 17.2, 9.9, 6.3 Hz, 2H), 1.71 – 1.63 (m, 1H), 1.47 – 1.39 (m, 1H), 1.37 – 1.29 (m, 1H), 1.25 (t, J = 7.5 Hz, 3H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 172.06, 171.01, 170.27, 158.07, 153.24, 148.96, 147.97, 147.43, 142.48, 139.17, 136.84, 133.62, 133.30, 129.57, 126.08, 122.33, 120.22, 119.39, 115.64, 111.66, 111.36, 109.07, 105.20, 77.24, 65.32, 60.78, 55.95, 55.90, 55.86, 52.75, 50.33, 44.01, 38.42, 31.56, 27.05, 25.06, 23.46, 20.64, 15.72.

2-(3-((R)-1-(((S)-1-((S)-2-(5-Cyclopropylthiophen-2-yl)-2-(3,4,5-trimethoxyphenyl)-acetyl)-piperidine-2-carbonyl)oxy)-3-(3,4-dimethoxyphenyl)propyl)phenoxy)-acetic acid (59h)



Following the general procedure C, compound **59h** was synthesized using: Resin **7** (316 mg, 0.14 mmol, 1.10 eq., loading: 0.45 mmol/g), **58h** (45 mg, 0.13 mmol, 1.00 eq.), oxalyl chloride (22 µL, 0.26 mmol, 2.00 eq.), DIPEA (112 µL, 0.65 mmol, 5.00 eq.) and 1.29 mL DCM. After purification via preparative HPLC (H_2O/ACN + 0.1% TFA, 50 - 80%), diastereomers **59h** and **d59h** were obtained as colorless solids.

Yield: 32 mg (40.61 µmol, 31%)

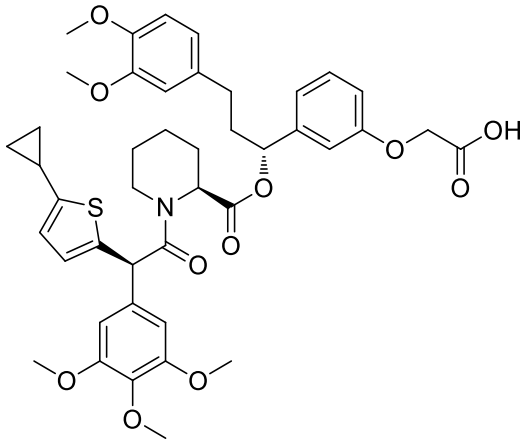
HPLC (50 – 100 % solvent B, 3 min) R_t = 1.613 min, purity (220 nm): 98%

HRMS (ESI$^+$): m/z: calculated 788.30991 $[M+H]^+$, found 788.30977 $[M+H]^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.21 (t, J = 7.9 Hz, 1H), 6.85 (dd, J = 8.3, 2.4 Hz, 1H), 6.79 (dd, J = 7.8, 5.8 Hz, 2H), 6.73 (t, J = 1.8 Hz, 1H), 6.68 (d, J = 9.1 Hz, 2H), 6.60 (d, J = 3.5 Hz, 1H), 6.55 (d, J = 3.5 Hz, 1H), 6.33 (s, 2H), 5.79 (s, 1H), 5.54 (dd, J = 9.0, 4.7 Hz, 1H), 5.47 (d, J = 5.7 Hz, 1H), 5.29 (s, 1H), 4.70 – 4.53 (m, 2H), 3.86 (s, 3H), 3.85 (s, 3H), 3.83 (d, J = 8.0 Hz, 1H), 3.78 (s, 3H), 3.55 (s, 6H), 3.13 (td, J = 13.4, 3.1 Hz, 1H), 2.71 – 2.62 (m, 1H), 2.61 – 2.49 (m, 1H), 2.36 – 2.29 (m, 1H), 2.24 – 2.14 (m, 1H), 2.07 – 1.94 (m, 2H), 1.84 – 1.74 (m, 2H), 1.69 – 1.64 (m, 1H), 1.46 – 1.26 (m, 2H), 0.95 – 0.86 (m, 2H), 0.70 – 0.64 (m, 2H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 172.12, 171.18, 170.39, 158.19, 153.35, 149.17, 149.07, 147.55, 142.60, 138.54, 136.97, 133.68, 133.42, 129.69, 126.18, 121.78, 120.34, 119.51, 115.76, 111.77, 111.48, 109.22, 109.22, 105.31, 105.31, 77.37, 65.45, 60.90, 56.07, 56.02, 55.99, 52.86, 50.45, 44.12, 38.54, 31.67, 27.16, 25.18, 20.76, 11.29, 9.71, 9.67.

2-(3-((R)-1-(((S)-1-((R)-2-(5-Cyclopropylthiophen-2-yl)-2-(3,4,5-trimethoxyphenyl)-acetyl)-piperidine-2-carbonyl)oxy)-3-(3,4-dimethoxyphenyl)propyl)phenoxy)-acetic acid (d59h)



Yield: 33 mg (41.88 µmol, 32%)

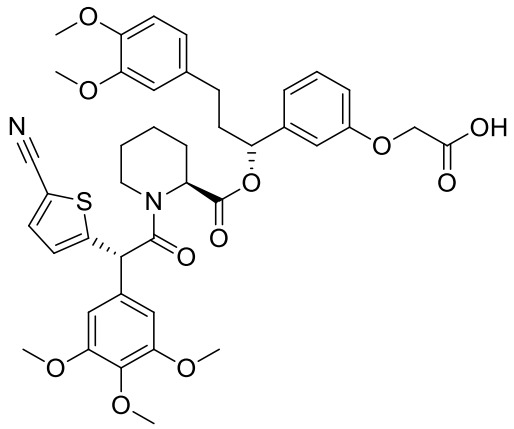
HPLC (30 – 100 % solvent B, 3 min) R_t = 1.607 min, purity (220 nm): 99%

HRMS (ESI$^+$): m/z: calculated 788.30991 $[M+H]^+$, found 788.30993 $[M+H]^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.28 (t, J = 4.0 Hz, 1H), 6.93 – 6.86 (m, 2H), 6.85 – 6.79 (m, 2H), 6.72 – 6.68 (m, 3H), 6.55 (d, J = 3.6 Hz, 1H), 6.53 (s, 2H), 5.74 (dd, J = 8.4, 5.2 Hz, 1H), 5.53 (d, J = 5.5 Hz, 1H), 5.29 (s, 1H), 4.70 – 4.57 (m, 2H), 3.88 (s, 3H), 3.87 (s, 3H), 3.86 (d, J = 2.5 Hz, 1H), 3.85 (s, 3H), 3.83 (s, 6H), 3.68 (s, 1H), 3.26 (td, J = 13.3, 2.9 Hz, 1H), 2.72 – 2.54 (m, 2H), 2.40 – 2.31 (m, 1H), 2.29 – 2.21 (m, 1H), 2.13 – 2.02 (m, 1H), 2.01 – 1.94 (m, 1H), 1.76 – 1.62 (m, 2H), 1.60 – 1.54 (m, 1H), 1.41 – 1.29 (m, 1H), 1.20 – 1.08 (m, 1H), 0.94 – 0.89 (m, 2H), 0.68 – 0.64 (m, 2H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 171.62, 170.33, 169.71, 157.98, 153.37, 149.01, 148.93, 147.50, 142.08, 137.77, 137.37, 134.67, 133.50, 129.88, 126.36, 122.01, 120.33, 119.86, 115.34, 111.86, 111.49, 105.78, 76.55, 65.27, 60.99, 56.32, 56.06, 56.01, 53.01, 50.75, 44.28, 38.18, 31.52, 27.07, 25.08, 20.91, 11.24, 9.66.

2-(3-((R)-1-(((S)-1-((S)-2-(5-cyanothiophen-2-yl)-2-(3,4,5-trimethoxyphenyl)-acetyl)-piperidine-2-carbonyl)oxy)-3-(3,4-dimethoxyphenyl)propyl)phenoxy)-acetic acid (59i)



A 10 mL filter syringe was charged with resin **7** (225 mg, 0.06 mmol, 0.50 eq., loading: 0.30 mmol/g). After complete Fmoc-deprotection following the general procedure, the resin was treated with a solution containing **58i** (45 mg, 0.12 mmol, 1.00 eq.), TCFH (45 mg, 0.14 mmol, 1.20 eq.), and NMI (38 µL, 0.42 mmol, 3.50 eq.) in 0.67 mL DCM/ACN (1:1) at rt overnight. After the complete conversion of the starting material, the resin was filtered and washed twice with DCM. Finally, the product was cleaved off the resin by treating it with a solution of 20 vol.-% HFIP in DCM for 1 h. After removal of the solvent under reduced pressure and purification via preparative HPLC. The respective compounds were obtained as colorless solids. After purification via preparative HPLC (50 - 80%, solvent B), compound **58i** (11 mg, 14.23 µmol, 21%) was obtained as a pale-yellow solid.

Yield: 11 mg (14.23 µmol, 21%)

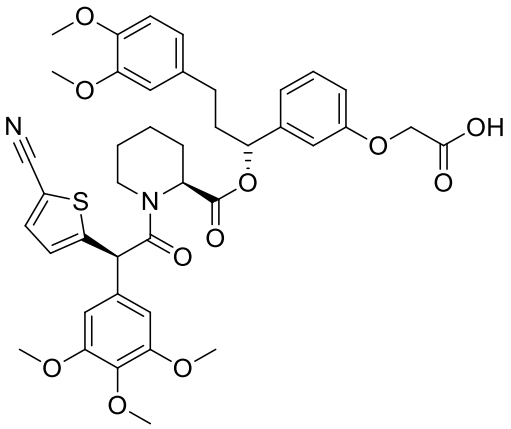
HPLC (30 – 100 % solvent B, 3 min) R_t = 1.846 min, purity (220 nm): 99%

HRMS (ESI$^+$): m/z: calculated 773.2739 $[M+H]^+$, found 773.2742 $[M+H]^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.41 (d, J = 3.8 Hz, 1H), 7.22 (t, J = 7.9 Hz, 1H), 6.88 – 6.85 (m, 2H), 6.81 – 6.76 (m, 2H), 6.76 – 6.73 (m, 1H), 6.69 – 6.65 (m, 2H), 6.33 (s, 2H), 5.52 (dd, J = 8.9, 4.8 Hz, 1H), 5.45 – 5.41 (m, 1H), 5.39 (s, 1H), 4.73 – 4.63 (m, 2H), 3.86 (s, 3H), 3.85 (s, 3H), 3.78 (s, 3H), 3.74 – 3.69 (m, 1H), 3.54 (s, 6H), 3.07 (td, J = 13.5, 3.2 Hz, 1H), 2.72 – 2.64 (m, 1H), 2.62 – 2.53 (m, 1H), 2.40 – 2.34 (m, 1H), 2.23 – 2.15 (m, 1H), 2.07 – 1.96 (m, 1H), 1.87 – 1.74 (m, 2H), 1.74 – 1.67 (m, 1H), 1.56 – 1.45 (m, 1H), 1.38 – 1.23 (m, 1H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 171.03, 170.32, 170.01, 158.20, 153.80, 151.07, 149.12, 147.62, 142.58, 137.57, 136.67, 133.33, 132.13, 129.74, 126.71, 120.34, 119.49, 115.87, 114.57, 111.77, 111.48, 110.05, 108.99, 104.93, 77.20, 65.38, 60.93, 56.08, 56.05, 56.04, 52.98, 50.78, 44.29, 38.58, 31.69, 27.17, 25.21, 20.71.

2-(3-((R)-1-(((S)-1-((R)-2-(5-cyanothiophen-2-yl)-2-(3,4,5-trimethoxyphenyl)-acetyl)-piperidine-2-carbonyl)oxy)-3-(3,4-dimethoxyphenyl)propyl)phenoxy)-acetic acid (d59i)



Yield: 22 mg (28.47 µmol, 42%)

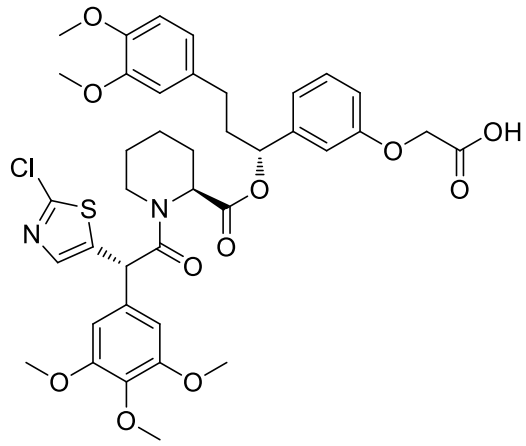
HPLC (30 – 100 % solvent B, 3 min) R_t = 1.829 min, purity (220 nm): 99%

HRMS (ESI$^+$): m/z: calculated 773.2739 $[M+H]^+$, found 773.2743 $[M+H]^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38 (d, J = 3.8 Hz, 1H), 7.29 – 7.23 (m, 1H), 6.92 – 6.85 (m, 3H), 6.79 (d, J = 8.1 Hz, 2H), 6.71 – 6.64 (m, 2H), 6.51 (s, 2H), 5.68 (dd, J = 8.4, 5.3 Hz, 1H), 5.51 (d, J = 5.6 Hz, 1H), 5.36 (s, 1H), 4.72 – 4.60 (m, 2H), 3.85 (s, 3H), 3.85 (s, 3H), 3.83 (s, 3H), 3.83 (s, 6H), 3.81 – 3.77 (m, 1H), 3.35 (td, J = 13.5, 3.5 Hz, 1H), 2.67 – 2.59 (m, 1H), 2.59 – 2.50 (m, 1H), 2.34 (d, J = 14.0 Hz, 1H), 2.26 – 2.18 (m, 1H), 2.10 – 2.04 (m, 1H), 1.73 – 1.60 (m, 2H), 1.52 (d, J = 13.4 Hz, 1H), 1.38 – 1.28 (m, 1H), 1.01 – 0.91 (m, 1H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 170.18, 158.04, 150.61, 149.06, 147.59, 142.02, 138.05, 133.37, 133.35, 129.97, 126.85, 120.31, 119.85, 109.87, 77.39, 65.29, 61.06, 56.48, 56.07, 56.04, 53.15, 50.71, 44.18, 38.14, 31.52, 27.03, 24.90, 20.79.

2-(3-((R)-1-(((S)-1-((S)-2-(2-Chlorothiazol-5-yl)-2-(3,4,5-trimethoxyphenyl)acetyl)-piperidine-2-carbonyl)oxy)-3-(3,4-dimethoxyphenyl)propyl)phenoxy)acetic acid (59j)



Following the general procedure C, compound **59j** was synthesized using: Resin **7** (213 mg, 0.10 mmol, 1.10 eq., loading: 0.45 mmol/g), **58j** (30 mg, 0.09 mmol, 1.00 eq.), oxalyl chloride (7 µL, 0.08 mmol, 0.95 eq.), DIPEA (76 µL, 0.44 mmol, 5.00 eq.) and 0.78 mL DCM. After purification via preparative HPLC (H_2O/ACN + 0.1% TFA, 50 - 80%), diastereomers **59j** and **d59j** were obtained as colorless solids.

Yield: 11 mg (14.04 µmol, 16%)

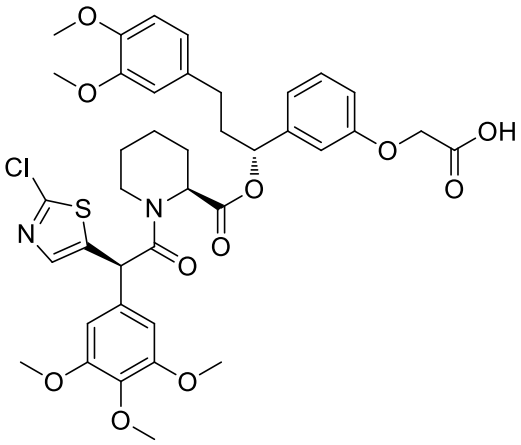
HPLC (50 – 100 % solvent B, 3 min) R_t = 1.395 min, purity (220 nm): 99%

HRMS (ESI$^+$): m/z: calculated 783.23489 $[M+H]^+$, found 783.23594 $[M+H]^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36 (s, 1H), 7.22 (t, J = 7.9 Hz, 1H), 6.93 – 6.84 (m, 1H), 6.78 (d, J = 7.5 Hz, 2H), 6.74 (s, 1H), 6.71 – 6.63 (m, 2H), 6.31 (s, 2H), 5.53 (dd, J = 9.0, 4.8 Hz, 1H), 5.44 (d, J = 5.7 Hz, 1H), 5.35 (s, 1H), 4.69 (q, J = 16.4 Hz, 2H), 3.86 (s, 3H), 3.85 (s, 3H), 3.77 (s, 3H), 3.72 (d, J = 13.9 Hz, 1H), 3.54 (s, 6H), 3.08 (td, J = 13.3, 3.1 Hz, 1H), 2.71 – 2.63 (m, 1H), 2.60 – 2.53 (m, 1H), 2.42 – 2.35 (m, 1H), 2.23 – 2.15 (m, 1H), 2.07 – 2.00 (m, 1H), 1.87 – 1.75 (m, 2H), 1.73 – 1.66 (m, 1H), 1.57 – 1.47 (m, 1H), 1.36 – 1.23 (m, 1H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 171.01, 170.24, 169.89, 158.18, 153.80, 149.12, 147.62, 142.55, 140.23, 138.24, 137.46, 133.32, 132.26, 129.75, 120.33, 119.54, 115.84, 111.77, 111.48, 108.98, 104.55, 77.28, 65.42, 60.92, 56.08, 56.06, 56.04, 53.02, 48.85, 44.32, 38.54, 31.69, 27.20, 25.20, 20.80.

2-(3-((R)-1-(((S)-1-((R)-2-(2-Chlorothiazol-5-yl)-2-(3,4,5-trimethoxyphenyl)acetyl)-piperidine-2-carbonyl)oxy)-3-(3,4-dimethoxyphenyl)propyl)phenoxy)acetic acid (d59j)



Yield: 19 mg (24.26 µmol, 28%)

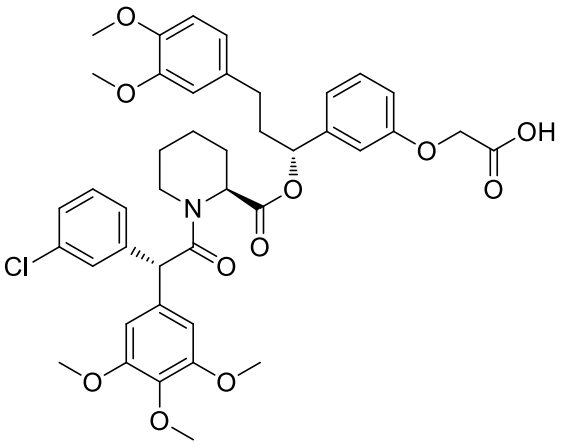
HPLC (50 – 100 % solvent B, 3 min) R_t = 1.363 min, purity (220 nm): 99%

HRMS (ESI$^+$): m/z: calculated 783.23489 $[M+H]^+$, found 783.23478 $[M+H]^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38 (s, 1H), 7.24 (s, 1H), 6.88 (t, J = 8.7 Hz, 2H), 6.78 (d, J = 8.7 Hz, 2H), 6.66 (d, J = 9.3 Hz, 2H), 6.50 (s, 2H), 5.69 (t, J = 6.9 Hz, 1H), 5.51 (d, J = 5.6 Hz, 1H), 5.33 (s, 1H), 4.74 – 4.61 (m, 2H), 3.86 (s, 7H), 3.82 (s, 9H), 3.28 (t, J = 13.3 Hz, 1H), 2.64 – 2.49 (m, 2H), 2.38 – 2.30 (m, 1H), 2.25 – 2.17 (m, 1H), 2.11 – 2.01 (m, 1H), 1.70 – 1.56 (m, 2H), 1.52 – 1.46 (m, 1H), 1.38 – 1.25 (m, 1H), 0.99 – 0.87 (m, 1H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 171.15, 170.05, 169.96, 158.03, 153.96, 153.48, 149.05, 147.56, 141.92, 139.61, 138.74, 137.94, 133.49, 133.38, 129.99, 120.31, 120.07, 115.27, 111.83, 111.49, 111.30, 104.81, 76.86, 65.38, 61.06, 56.49, 56.08, 56.04, 53.11, 48.77, 44.21, 37.96, 31.48, 26.88, 24.91, 20.75.

2-(3-((R)-1-(((S)-1-((R)-2-(3-Chlorophenyl)-2-(3,4,5-trimethoxyphenyl)acetyl)-piperidine-2-carbonyl)oxy)-3-(3,4-dimethoxyphenyl)propyl)phenoxy)acetic acid (59k)



Following the general procedure D, compound **59k** was synthesized using: Resin **7** (358 mg, 0.13 mmol, 1.10 eq., loading: 0.36 mmol/g), **58k** (40 mg, 0.12 mmol, 1.00 eq.), TCFH (40 mg, 0.14 mmol, 1.20 eq.), NMI (33 µL, 0.42 mmol, 3.50 eq.) and 1.19 mL DCM. After purification via preparative HPLC (H_2O/ACN + 0.1% TFA, 50 - 80%), diastereomers **59k** and **d59k** were obtained as colorless solids.

Yield: 13 mg (17 µmol, 14%)

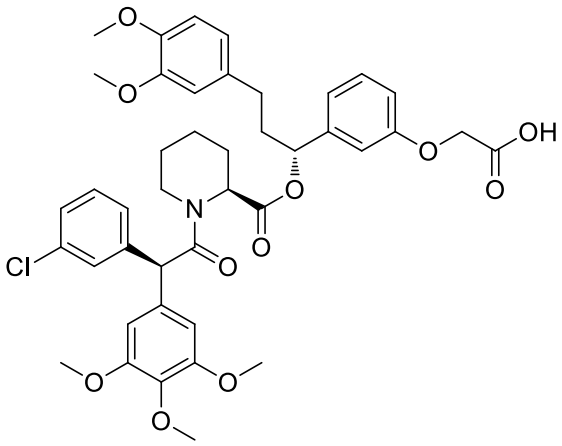
HPLC (30 – 100 % solvent B, 3 min) R_t = 2.027min, purity (220 nm): 99%

HRMS (ESI$^+$): m/z: calculated 776.28322 $[M+H]^+$, found 776.28359 $[M+H]^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.23 – 7.18 (m, 3H), 7.15 (d, J = 1.8 Hz, 1H), 7.05 – 7.00 (m, 1H), 6.89 – 6.86 (m, 1H), 6.81 (d, J = 7.6 Hz, 1H), 6.79 (d, J = 8.0 Hz, 1H), 6.74 (dd, J = 2.5, 1.4 Hz, 1H), 6.70 – 6.66 (m, 2H), 6.26 (s, 2H), 5.52 (dd, J = 9.1, 4.6 Hz, 1H), 5.48 – 5.42 (m, 1H), 5.11 (s, 1H), 4.72 – 4.54 (m, 2H), 3.86 (s, 3H), 3.85 (s, 3H), 3.79 (s, 3H), 3.53 (s, 6H), 3.18 (td, J = 13.3, 3.1 Hz, 1H), 2.77 – 2.66 (m, 1H), 2.65 – 2.52 (m, 1H), 2.34 (d, J = 14.0 Hz, 1H), 2.25 – 2.16 (m, 1H), 2.10 – 2.01 (m, 1H), 1.88 – 1.73 (m, 2H), 1.68 (d, J = 13.1 Hz, 1H), 1.47 – 1.23 (m, 3H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 172.28, 170.84, 170.38, 158.28, 153.52, 149.12, 147.60, 142.74, 141.60, 136.95, 134.13, 133.39, 132.74, 129.70, 129.63, 129.62, 127.94, 127.50, 120.34, 119.42, 116.17, 111.76, 111.49, 108.46, 105.69, 77.21, 65.42, 60.91, 56.08, 55.95, 55.15, 52.77, 44.12, 38.65, 31.74, 27.36, 25.26, 20.78.

2-(3-((R)-1-(((S)-1-((S)-2-(3-Chlorophenyl)-2-(3,4,5-trimethoxyphenyl)acetyl)-piperidine-2-carbonyl)oxy)-3-(3,4-dimethoxyphenyl)propyl)phenoxy)acetic acid (d59k)



Yield: 26 mg (33 µmol, 28%)

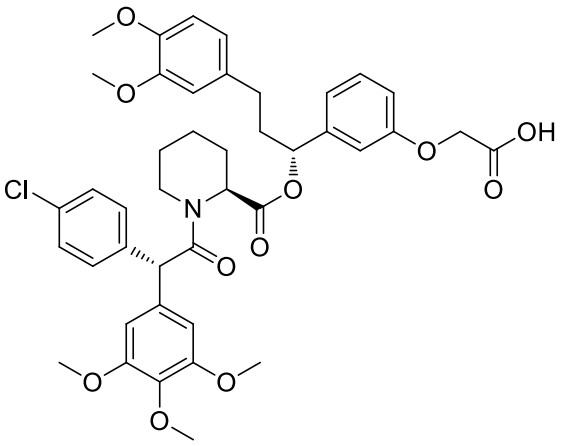
HPLC (30 – 100 % solvent B, 3 min) R_t = 1.970 min, purity (220 nm): 99%

HRMS (ESI$^+$): m/z: calculated 776.28322 $[M+H]^+$, found 776.28350 $[M+H]^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.26 – 7.23 (m, 1H), 7.19 – 7.16 (m, 2H), 7.14 – 7.12 (m, 1H), 7.09 – 7.06 (m, 1H), 6.91 – 6.83 (m, 2H), 6.81 – 6.75 (m, 2H), 6.69 – 6.64 (m, 2H), 6.43 (s, 2H), 5.70 (dd, J = 8.3, 5.1 Hz, 1H), 5.58 – 5.49 (m, 1H), 5.12 (s, 1H), 4.66 – 4.51 (m, 2H), 3.85 (s, 6H), 3.84 (s, 3H), 3.80 (s, 6H), 3.22 (dq, J = 13.3, 4.7, 3.1 Hz, 1H), 2.67 – 2.49 (m, 2H), 2.37 – 2.31 (m, 2H), 2.27 – 2.17 (m, 1H), 2.09 – 2.03 (m, 1H), 1.75 – 1.67 (m, 2H), 1.54 (d, J = 13.4 Hz, 1H), 1.45 – 1.25 (m, 1H), 1.19 – 1.07 (m, 1H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 171.89, 171.86, 170.35, 158.05, 153.51, 149.05, 147.55, 142.09, 141.02, 137.43, 134.32, 133.87, 133.46, 129.88, 129.78, 129.32, 127.45, 127.36, 120.33, 119.75, 111.84, 111.49, 76.61, 65.28, 61.01, 56.35, 56.07, 56.02, 55.07, 52.90, 44.11, 38.17, 31.54, 27.17, 27.04, 25.11, 20.89.

2-(3-((R)-1-(((S)-1-((S)-2-(4-Chlorophenyl)-2-(3,4,5-trimethoxyphenyl)acetyl)-piperidine-2-carbonyl)oxy)-3-(3,4-dimethoxyphenyl)propyl)phenoxy)acetic acid (59l)



Following the general procedure D, compound **59l** was synthesized using: Resin **7** (325 mg, 0.06 mmol, 1.00 eq., loading: 0.18 mmol/g), **58l** (40 mg, 0.12 mmol, 2.00 eq.), TCFH (40 mg, 0.14 mmol, 2.40 eq.), NMI (33 µL, 0.42 mmol, 7.00 eq.) and 1.19 mL DCM. After purification via preparative HPLC (H_2O/ACN + 0.1% TFA, 50 - 80%), diastereomers **59l** and **d59l** were obtained as colorless solids.

Yield: 6 mg (7.7 µmol, 13%)

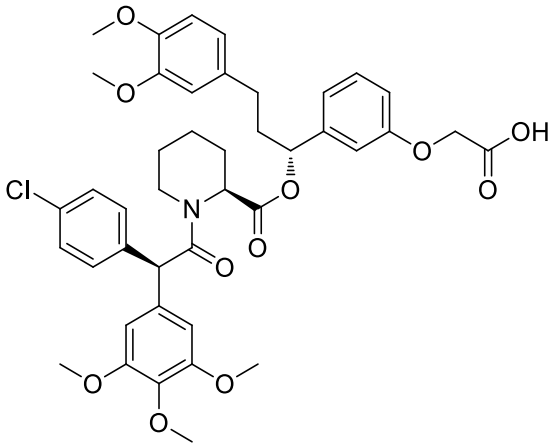
HPLC (50 – 100 % solvent B, 3 min) R_t = 1.562 min, purity (220 nm): 99%

HRMS (ESI$^+$): m/z: calculated 776.28322 $[M+H]^+$, found 776.28391 $[M+H]^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.26 – 7.19 (m, 3H), 7.12 – 7.07 (m, 2H), 6.88 (dd, J = 8.2, 2.5 Hz, 1H), 6.80 (dd, J = 9.3, 7.5 Hz, 2H), 6.74 – 6.65 (m, 3H), 6.23 (s, 2H), 5.52 – 5.44 (m, 2H), 5.10 (s, 1H), 4.70 – 4.56 (m, 2H), 3.86 (s, 3H), 3.85 (s, 3H), 3.77 (s, 3H), 3.74 (d, J = 15.5 Hz, 1H), 3.50 (s, 6H), 3.26 – 3.16 (m, 1H), 2.74 – 2.67 (m, 1H), 2.65 – 2.52 (m, 1H), 2.36 – 2.30 (m, 1H), 2.27 – 2.15 (m, 1H), 2.08 – 1.98 (m, 1H), 1.86 – 1.75 (m, 2H), 1.71 – 1.65 (m, 1H), 1.47 – 1.28 (m, 2H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 172.46, 170.80, 170.38, 158.32, 153.49, 149.14, 147.62, 142.82, 138.15, 136.90, 133.37, 133.15, 130.96, 129.66, 128.54, 120.34, 119.37, 116.34, 111.75, 111.48, 108.07, 105.58, 77.29, 65.47, 60.90, 56.09, 56.05, 55.92, 54.91, 52.75, 44.11, 38.69, 31.78, 27.41, 25.29, 20.80.

2-(3-((R)-1-(((S)-1-((R)-2-(4-Chlorophenyl)-2-(3,4,5-trimethoxyphenyl)acetyl)-piperidine-2-carbonyl)oxy)-3-(3,4-dimethoxyphenyl)propyl)phenoxy)acetic acid (d59l)



Yield: 11 mg (14.2 µmol, 24%)

HPLC (50 – 100 % solvent B, 3 min) R_t = 1.519 min, purity (220 nm): 99%

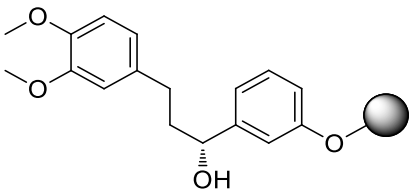
HRMS (ESI$^+$): m/z: calculated 776.28322 $[M+H]^+$, found 776.28379 $[M+H]^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.24 (t, 1H), 7.21 (d, J = 8.2 Hz, 2H), 7.09 – 7.04 (m, 2H), 6.91 – 6.86 (m, 2H), 6.79 (d, J = 8.1 Hz, 1H), 6.76 (d, J = 2.9 Hz, 1H), 6.71 – 6.64 (m, 2H), 6.41 (s, 2H), 5.69 (dd, J = 8.7, 5.0 Hz, 1H), 5.58 – 5.49 (m, 1H), 5.11 (s, 1H), 4.65 – 4.55 (m, 2H), 3.86 (s, 6H), 3.83 (s, 4H), 3.79 (s, 6H), 3.24 (td, J = 13.2, 3.1 Hz, 1H), 2.70 – 2.63 (m, 1H), 2.61 – 2.53 (m, 1H), 2.39 – 2.33 (m, 1H), 2.26 – 2.18 (m, 1H), 2.12 – 2.04 (m, 1H), 1.75 – 1.65 (m, 2H), 1.63 – 1.52 (m, 1H), 1.39 – 1.28 (m, 1H), 1.23 – 1.12 (m, 1H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 172.18, 171.07, 170.34, 158.09, 153.47, 149.08, 147.58, 142.17, 137.49, 134.20, 133.44, 133.09, 130.50, 129.88, 128.73, 120.32, 119.66, 115.82, 111.82, 111.50, 110.08, 106.30, 76.68, 65.34, 61.01, 56.34, 56.08, 56.03, 54.87, 52.94, 44.12, 38.26, 31.62, 27.25, 25.14, 20.91.

11.11 Synthesis of the thiophene/thiazole-containing SAFit2 analogs

Immobilization of (R)-3-(3-(3,4-dimethoxyphenyl)-1-hydroxypropyl)phenol on the 2-CTC resin (60)



A 25 mL flask was charged with 2-chlorotrityl resin (1.42 g, 2.43 mmol, 2.00 eq.) and 6 mL DCM. The resin was stirred for 10 minutes before being cooled down to 0°C. Then alcohol **3** (0.30 g, 1.21 mmol, 1.00 eq.) was added in 5 mL DCM, followed by DIPEA (1.06 mL, 6.07 mmol, 5.00 eq.). The reaction mixture was allowed to warm to rt and was stirred overnight. The suspension was filtered, washed three times with 10 mL DCM, and dried under a vacuum. Finally, the obtained resin was capped by treating it with a solution of DCM/MeOH/DIPEA (80/15/5 vol.-%) for 10 min. This general procedure was repeated twice. The resin was washed extensively with DCM and dried under a vacuum (m_{resin} = 1.76 g). The loading of the resin has then been calculated using the following formula:

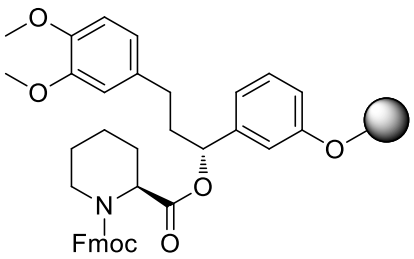
$$\frac{(m_{total} - m_{resin}) \cdot 10^3}{(MW - 36.46) \cdot m_{total}} = 0.69\ loading\ \left[\frac{mmol}{g}\right]$$

Yield: 1.76 g (quant.)

HPLC (5 – 100 % solvent B, 3 min) R_t = 1.618 min, purity (220 nm): 99 %

Mass (ESI^+): m/z: calculated 271.13 $[M\text{-}OH]^+$, found 271.20 $[M\text{-}OH]^+$

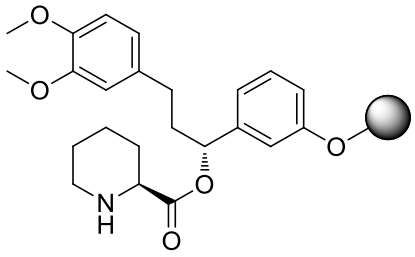
(S)-1-((9H-fluoren-9-yl)methyl)-2-((R)-3-(3,4-dimethoxyphenyl)-1-(3-hydroxyphenyl)-propyl) piperidine-1,2-dicarboxylate (61)



In a 10 mL flask, resin **60** (100 mg, 0.07 mmol, 1.00 eq., loading: 0.60 mmol/g) was swelled in 1 mL DCM. After 10 min, the solution was cooled to 0°C and (*S*)-1-Fmoc-piperidine-2-carboxylic acid (36 mg, 0.10 mmol, 1.50 eq.), DMAP (1 mg, 7 µmol, 0.10 eq.) and DIC (32 µL, 0.21 mmol, 3.00 eq.) were added. The solution was then allowed to warm to rt and was stirred overnight. After the complete conversion of the starting material, the resin was filtered, washed three times with 5 mL DCM and dried under reduced pressure.

HPLC (5 – 100 % solvent B, 3 min) R_t = 2.396 min, purity (220 nm): 95 %
Mass (ESI^+): m/z: calculated 644.17 $[M\text{-}Na]^+$, found 644.00 $[M\text{-}Na]^+$

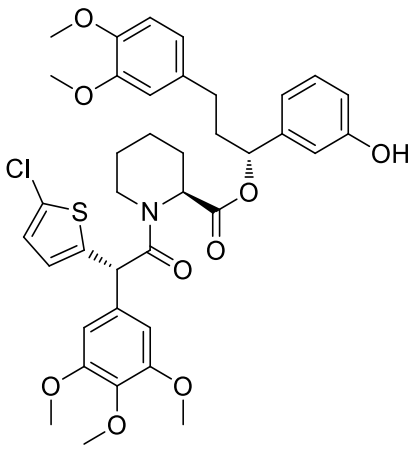
(S)-(R)-3-(3,4-Dimethoxyphenyl)-1-(3-hydroxyphenyl)propyl-piperidine-2-carboxylate (62)



The resin **61** was transferred into a filter syringe and swelled using 5 mL DCM. Then the resin was washed with DMF and treated three times with 2 mL of ice-cold 20 vol.-% 4-methylpiperidine for 5 min. Finally, the resin was washed with 3 x 2 mL DMF and 3 x 2 mL DCM. The resin was then directly used for the amid coupling.

HPLC (5 – 100 % solvent B, 3 min) R_t = 1.563 min, purity (220 nm): 94 %
Mass (ESI^+): m/z: calculated 400.20 $[M+H]^+$, found 400.00 $[M+H]^+$

(S)-(R)-3-(3,4-Dimethoxyphenyl)-1-(3-hydroxyphenyl)propyl-1-((S)-2-(5-chlorothiophen-2-yl)-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2-carboxylate (63)



Following the general procedure C, compound **63** was synthesized using: Resin **62** (0.07 mmol, 1.00 eq., loading: 0.69 mmol/g), **58e** (25 mg, 0.07 mmol, 1.00 eq.), oxalyl chloride (12 µL, 0.14 mmol, 2.00 eq.), DIPEA (37 µL, 0.21 mmol, 5.00 eq.) and 0.69 mL DCM. After purification via preparative HPLC (H_2O/ACN + 0.1% TFA, 50 - 80%), diastereomers **63** and **d63** were obtained as colorless solids.

Yield: 10 mg (13.82 µmol, 20%)

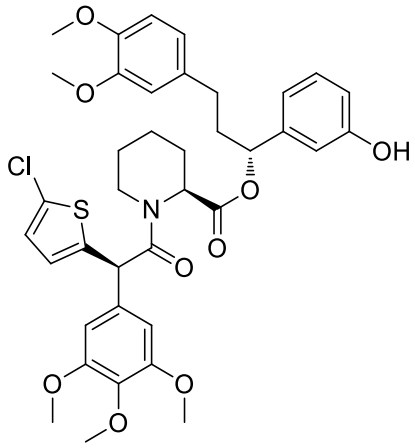
HPLC (30 – 100 % solvent B, 3 min) R_t = 2.089 min, purity (220 nm): 99 %

HRMS (ESI$^+$): m/z: calculated 724.23416 $[M+H]^+$, found 724.23522 $[M+H]^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.13 (t, J = 7.8 Hz, 1H), 6.78 (d, J = 8.0 Hz, 1H), 6.72 (d, J = 4.0 Hz, 2H), 6.69 – 6.65 (m, 3H), 6.64 – 6.59 (m, 2H), 6.44 (s, 2H), 5.63 (dd, J = 8.2, 5.3 Hz, 1H), 5.43 (d, J = 5.2 Hz, 1H), 5.30 (s, 1H), 3.86 (s, 3H), 3.85 (s, 3H), 3.85 – 3.83 (m, 1H), 3.80 (s, 3H), 3.62 (s, 6H), 2.99 (td, J = 13.3, 3.2 Hz, 2H), 2.64 – 2.45 (m, 2H), 2.34 – 2.26 (m, 1H), 2.15 – 2.05 (m, 1H), 2.03 – 1.94 (m, 1H), 1.82 – 1.71 (m, 2H), 1.69 – 1.59 (m, 1H), 1.51 – 1.39 (m, 1H), 1.34 – 1.19 (m, 1H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 171.00, 170.75, 156.59, 153.60, 149.03, 147.49, 141.70, 141.52, 137.43, 133.61, 133.22, 130.45, 129.78, 125.45, 125.06, 120.33, 118.44, 115.30, 112.38, 111.84, 111.43, 105.54, 76.36, 60.97, 56.23, 56.08, 55.99, 52.78, 50.60, 44.28, 38.04, 31.50, 26.81, 25.23, 20.55.

(S)-(R)-3-(3,4-Dimethoxyphenyl)-1-(3-hydroxyphenyl)propyl21-((R)-2-(5-chlorothiophen-2-yl)-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2-carboxylate (d63)



Yield: 10 mg (13.82 µmol, 20%)

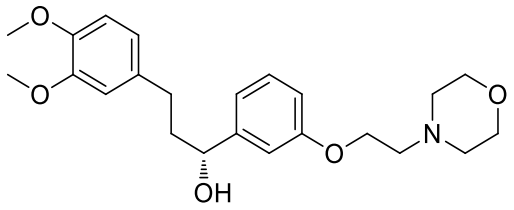
HPLC (30 – 100 % solvent B, 3 min) R_t = 2.046 min, purity (220 nm): 99 %

HRMS (ESI$^+$): m/z: calculated 724.23416 $[M+H]^+$, found 724.23501 $[M+H]^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.18 (t, J = 7.8 Hz, 1H), 6.81 – 6.78 (m, 2H), 6.77 – 6.76 (m, 2H), 6.74 (dt, J = 8.9, 1.4 Hz, 1H), 6.68 (t, J = 4.8 Hz, 1H), 6.65 (q, J = 3.4, 2.9 Hz, 2H), 6.51 (s, 2H), 5.73 (dd, J = 7.9, 5.6 Hz, 1H), 5.50 (d, J = 5.3 Hz, 1H), 5.27 (s, 1H), 3.85 (s, 6H), 3.83 (s, 3H), 3.82 (s, 6H), 3.67 (s, 1H), 3.24 (td, J = 13.2, 3.1 Hz, 1H), 2.89 – 2.79 (m, 1H), 2.63 – 2.48 (m, 2H), 2.34 – 2.29 (m, 1H), 2.27 – 2.15 (m, 1H), 2.09 – 2.01 (m, 1H), 1.73 – 1.59 (m, 2H), 1.57 – 1.52 (m, 1H), 1.43 – 1.27 (m, 1H), 1.14 – 1.04 (m, 1H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 170.73, 170.60, 156.26, 153.63, 149.02, 147.49, 141.74, 140.82, 137.68, 134.24, 133.64, 130.28, 129.91, 125.68, 125.36, 120.30, 118.59, 115.27, 113.21, 111.85, 111.47, 105.47, 76.51, 61.03, 56.39, 56.19, 56.08, 56.00, 52.88, 50.73, 44.20, 38.10, 31.39, 26.78, 25.07, 20.73.

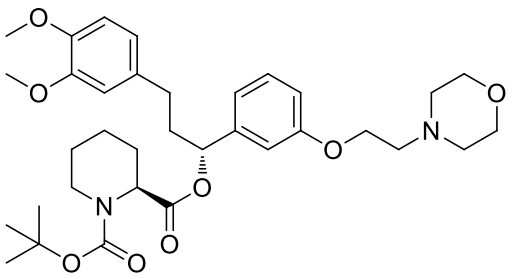
(R)-3-(3,4-Dimethoxyphenyl)-1-(3-(2-morpholinoethoxy)phenyl)propan-1-ol (65)



In a 100 mL flask, chiral alcohol **3** (1.71 g, 5.92 mmol, 1.00 eq), potassium carbonate (3.27 g, 23.68 mmol, 4.00 eq.), and 4-(2-chloethyl)-morpholin-hydrochloride (1.21 g, 6.51 mmol, 1.10 eq.) was dissolved in 30 mL acetonitrile. The resulting suspension was then heated to reflux overnight. After cooling to rt, the solvent was removed under reduced pressure, and the residue was dissolved in EtOAc. The org. phase was washed with water, followed by brine, and dried over $MgSO_4$. Then the solvent was removed, and the obtained crude was purified via flash chromatography (EtOAc/MeOH + 3% TEA, 0 – 5%). The title compound was obtained as a colorless oil.

Yield: 2.37 g (5.90 mmol, 99%)
TLC (EtOAc/MeOH + 3% TEA, 20:1): R_f = 0.29
HPLC (5 – 100 % solvent B, 3 min) R_t = 1.429 min, purity (220 nm): 94%
Mass (ESI$^+$): m/z: calculated 402.22 $[M+Na]^+$, found 402.20 $[M+Na]^+$
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.22 (t, J = 8.0 Hz, 1H), 6.91 – 6.89 (m, 2H), 6.79 – 6.75 (m, 2H), 6.73 – 6.68 (m, 2H), 4.62 (dd, J = 7.9, 5.1 Hz, 1H), 4.07 (t, J = 5.7 Hz, 2H), 3.83 (s, 3H), 3.83 (s, 3H), 3.72 – 3.66 (m, 4H), 2.75 (t, J = 5.7 Hz, 2H), 2.73 – 2.65 (m, 1H), 2.63 – 2.56 (m, 1H), 2.54 (t, J = 4.7 Hz, 4H), 2.11 – 2.01 (m, 1H), 1.99 – 1.94 (m, 1H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 158.93, 148.86, 147.21, 146.70, 134.47, 129.52, 120.24, 118.53, 113.58, 112.16, 111.84, 111.32, 73.62, 66.86, 65.71, 57.67, 55.97, 55.86, 54.08, 40.73, 31.71.

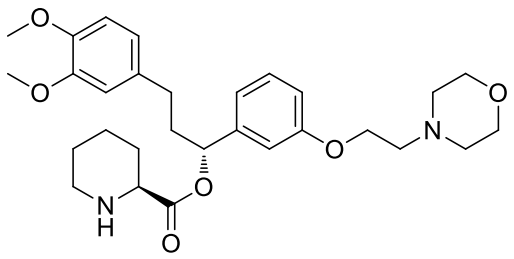
(S)-1-Tert-butyl-2-((R)-3-(3,4-dimethoxyphenyl)-1-(3-(2-morpholinoethoxy)phenyl)-propyl) piperidine-1,2-dicarboxylate (66)



In a 25 mL flask, alcohol **65** (1.59 g, 3.96 mmol, 1.00 eq.), DMAP (97 mg, 0.79 mmol, 0.20 eq.), and (S)-(−)-1-(tert-Butoxycarbonyl)-2-piperidinecarboxylic acid (0.99 g, 4.35 mmol, 1.10 eq.) was dissolved in 8 mL DCM. The solution was then cooled to 0°C, and EDC·HCl (1.14 g, 5.94 mmol, 1.50 eq.) was added in small portions over 5 min. After the complete addition of EDC, the ice bath was removed, and the solution was stirred for 1 h at rt. Finally, the solvent was removed under reduced pressure, and the crude was purified by flash chromatography (CH/EtOAc + 1% TEA, 0 – 100%). After the removal of the solvent, title compound **66** was obtained as a colorless oil.

Yield: 1.82 g (2.97 mmol, 75%)
TLC (EtOAc + 3% TEA): R_f = 0.30
HPLC (5 – 100 % solvent B, 3 min) R_t = 1.743 min, purity (220 nm): 97%
Mass (ESI^+): m/z: calculated 613.34 $[M+Na]^+$, found 613.40 $[M+Na]^+$
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.23 (td, J = 7.9, 5.2 Hz, 1H), 6.90 (q, J = 6.9 Hz, 1H), 6.85 (d, J = 1.8 Hz, 1H), 6.84 – 6.79 (m, 2H), 6.77 (d, J = 8.1 Hz, 1H), 6.68 (dd, J = 8.0, 2.0 Hz, 1H), 6.66 (d, J = 2.0 Hz, 1H), 5.74 (dt, J = 32.3, 6.9 Hz, 1H), 4.98 – 4.88 (m, 1H), 4.77 (s, 0H), 4.09 (t, J = 5.8 Hz, 2H), 3.88 – 3.82 (m, 6H), 3.75 – 3.69 (m, 4H), 2.93 (dt, J = 34.6, 13.1 Hz, 1H), 2.78 (t, J = 5.7 Hz, 2H), 2.66 – 2.47 (m, 6H), 2.22 (qd, J = 15.3, 10.0 Hz, 3H), 2.04 (ddd, J = 16.2, 12.9, 6.4 Hz, 1H), 1.72 – 1.52 (m, 3H), 1.43 (d, J = 32.0 Hz, 11H), 1.22 – 1.02 (m, 1H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 171.5, 171.3, 159.0, 156.1, 155.4, 149.0, 147.5, 142.3, 141.9, 133.8, 133.7, 129.6, 120.3, 118.8, 114.1, 113.9, 113.2, 112.6, 111.9, 111.5, 80.1, 80.0, 76.2, 76.1, 67.0, 65.9, 57.8, 56.0, 56.0, 55.0, 54.2, 53.9, 42.3, 41.2, 38.5, 38.4, 31.6, 31.4, 28.5, 26.9, 26.8, 24.9, 24.7, 20.8, 20.7.

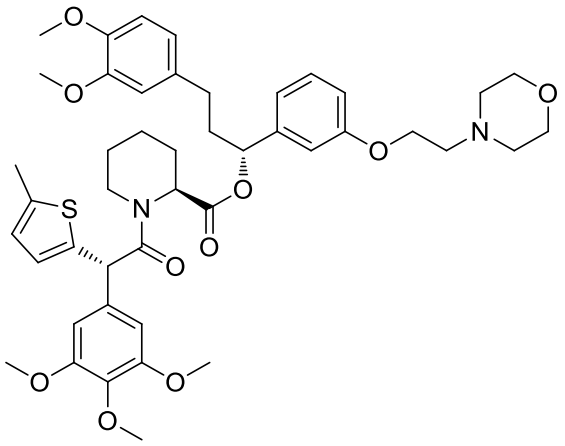
(S)-(R)-3-(3,4-Dimethoxyphenyl)-1-(3-(2-morpholinoethoxy)phenyl)propylpiperidine-2-carboxylate (67)



In 10 mL flask **66** (643 mg, 1.05 mmol, 1.00 eq.), was dissolved in 1.05 mL DCM and the resulting solution was cooled to 0°C. Then 1.05 mL TFA was added dropwise, and the mixture was stirred at rt for 30 min. After the complete conversion of the starting material, the reaction mixture was diluted with DCM and neutralized by adding sat. $NaHCO_3$ solution. The resulting aq. phase was extracted twice with DCM and the combined org. layers were dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The obtained crude was used without further purification as a colorless oil.

Yield: 507 mg (0.99 mmol, 94%)
HPLC (5 – 100 % solvent B, 3 min) R_t = 1.269 min, purity (220 nm): 97%
Mass (ESI^+): m/z: calculated 513.29 $[M+Na]^+$, found 513.40 $[M+Na]^+$
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.23 (t, J = 7.9 Hz, 1H), 6.94 – 6.88 (m, 1H), 6.86 (s, 1H), 6.82 (dd, J = 8.3, 2.5 Hz, 1H), 6.77 (d, J = 8.0 Hz, 1H), 6.68 – 6.64 (m, 2H), 5.74 (dd, J = 8.1, 5.6 Hz, 1H), 4.09 (t, J = 5.7 Hz, 2H), 3.85 (s, 3H), 3.84 (s, 3H), 3.73 (t, J = 4.6 Hz, 4H), 3.39 (dd, J = 10.0, 3.3 Hz, 1H), 3.08 (dt, J = 12.0, 3.7 Hz, 1H), 2.79 (t, J = 5.7 Hz, 2H), 2.68 – 2.61 (m, 2H), 2.60 – 2.55 (m, 4H), 2.55 – 2.49 (m, 1H), 2.27 – 2.20 (m, 1H), 2.09 – 2.01 (m, 2H), 1.84 – 1.76 (m, 1H), 1.67 – 1.57 (m, 2H), 1.51 – 1.43 (m, 2H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 172.60, 158.95, 149.00, 147.46, 141.89, 133.68, 129.70, 120.26, 119.14, 113.95, 113.19, 111.77, 111.40, 75.84, 67.03, 65.86, 58.70, 57.79, 56.04, 55.97, 54.23, 45.61, 38.05, 31.49, 29.17, 25.61, 24.12.

(S)-(R)-3-(3,4-Dimethoxyphenyl)-1-(3-(2-morpholinoethoxy)phenyl)propyl-1-((S)-2-(5-methylthiophen-2-yl)-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2-carboxylate (68a)



Following the general procedure D, compound **68a** was synthesized using: **58d** (40 mg, 0.12 mmol, 1.00 eq), amine **67** (70 mg, 0.15 mmol, 1.10 eq.), TCFH (42 mg, 0.15 mmol, 1.20 eq.), NMI (35 µL, 0.43 mmol, 3.50 eq.) and 1.24 mL MeCN. After purification via preparative HPLC (H_2O/ACN, 5 - 80%), diastereomers **68a** and **d68a** were obtained as colorless solids.

Yield: 21 mg (25 µmol, 20%)

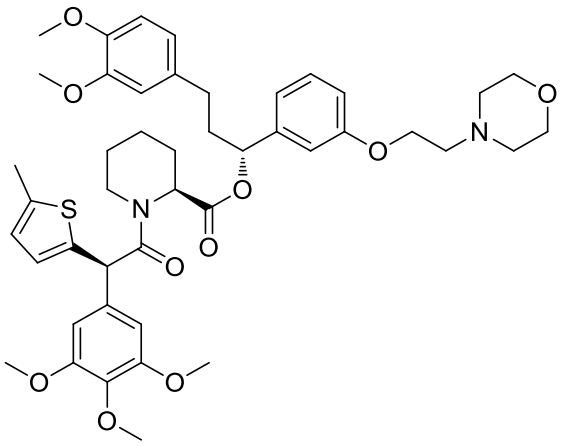
HPLC (5 – 100 % solvent B, 3 min) R_t = 1.969 min, purity (220 nm): 99%

HRMS (ESI$^+$): m/z: calculated 817.37284 $[M+H]^+$, found 817.37304 $[M+H]^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.19 (t, J = 7.9 Hz, 1H), 6.82 (t, J = 2.0 Hz, 1H), 6.81 – 6.75 (m, 3H), 6.66 (dd, J = 8.2, 2.2 Hz, 1H), 6.64 – 6.62 (m, 2H), 6.57 – 6.53 (m, 1H), 6.46 (s, 2H), 5.65 (dd, J = 8.2, 5.4 Hz, 1H), 5.52 – 5.44 (m, 1H), 5.32 (s, 1H), 4.11 (t, J = 5.7 Hz, 2H), 3.85 (s, 6H), 3.79 (s, 3H), 3.74 (t, J = 4.7 Hz, 4H), 3.67 (s, 6H), 3.11 (td, J = 13.1, 3.1 Hz, 1H), 2.81 (t, J = 5.6 Hz, 2H), 2.64 – 2.55 (m, 5H), 2.56 – 2.46 (m, 1H), 2.42 (s, 3H), 2.35 – 2.29 (m, 1H), 2.17 (dtd, J = 14.1, 8.9, 5.6 Hz, 1H), 2.06 – 1.95 (m, 1H), 1.78 – 1.70 (m, 2H), 1.67 – 1.56 (m, 2H), 1.39 – 1.17 (m, 2H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 171.21, 170.67, 158.88, 153.34, 149.02, 147.49, 141.92, 140.36, 140.29, 137.08, 134.38, 133.59, 129.70, 126.05, 124.26, 120.27, 118.79, 114.05, 111.82, 111.43, 105.52, 76.36, 66.89, 65.65, 60.88, 57.73, 56.08, 56.06, 55.98, 54.13, 52.59, 50.27, 44.03, 38.38, 31.50, 26.96, 25.32, 20.98, 15.48.

(S)-(R)-3-(3,4-Dimethoxyphenyl)-1-(3-(2-morpholinoethoxy)phenyl)propyl-1-((R)-2-(5-methylthiophen-2-yl)-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2-carboxylate (d68a)



Yield: 23 mg (28 µmol, 23%)

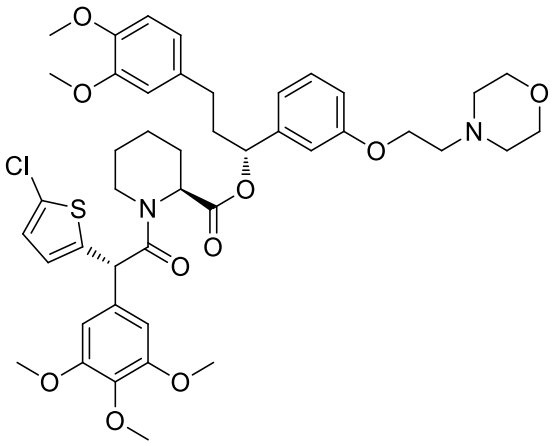
HPLC (5 – 100 % solvent B, 3 min) R_t = 1.946 min, purity (220 nm): 99%

HRMS (ESI$^+$): m/z: calculated 817.37284 $[M+H]^+$, found 817.37308 $[M+H]^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.26 – 7.21 (m, 1H), 6.87 (dd, J = 7.9, 1.4 Hz, 1H), 6.84 – 6.80 (m, 2H), 6.77 (d, J = 8.0 Hz, 1H), 6.72 (d, J = 3.5 Hz, 1H), 6.69 – 6.63 (m, 2H), 6.54 (s, 3H), 5.76 (dd, J = 7.9, 5.7 Hz, 1H), 5.53 (d, J = 5.0 Hz, 1H), 5.28 (s, 1H), 4.12 – 4.08 (m, 2H), 3.85 (s, 6H), 3.82 (s, 9H), 3.75 – 3.72 (m, 4H), 3.64 (s, 1H), 3.17 (td, J = 13.3, 3.0 Hz, 1H), 2.82 (t, J = 5.5 Hz, 2H), 2.62 – 2.56 (m, 5H), 2.50 (ddd, J = 13.9, 9.8, 6.0 Hz, 1H), 2.38 (s, 3H), 2.32 (d, J = 14.3 Hz, 1H), 2.22 (dtd, J = 9.7, 8.0, 4.7 Hz, 1H), 2.04 (ddt, J = 14.0, 10.2, 6.1 Hz, 1H), 1.69 – 1.57 (m, 2H), 1.51 (d, J = 13.3 Hz, 1H), 1.34 – 1.23 (m, 1H), 1.12 (tdd, J = 13.2, 9.0, 4.1 Hz, 1H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 170.69, 170.57, 158.87, 153.39, 148.98, 147.45, 141.70, 140.14, 139.73, 137.37, 135.16, 133.67, 129.76, 126.23, 124.45, 120.27, 119.16, 114.10, 113.21, 111.88, 111.41, 105.71, 76.45, 66.91, 65.73, 60.96, 57.74, 56.31, 56.06, 55.97, 54.15, 52.65, 50.47, 44.15, 38.22, 31.42, 26.93, 25.27, 21.08, 15.45.

(S)-(R)-3-(3,4-Dimethoxyphenyl)-1-(3-(2-morpholinoethoxy)phenyl)propyl 1-((S)-2-(5-chlorothiophen-2-yl)-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2-carboxylate (68b)



In a 5 mL flask, **58e** (40 mg, 0.12 mmol, 1.00 eq.) was dissolved in 1 mL DCM and one drop of DMF. The resulting solution was then cooled to 0°C, and oxalyl chloride (90 µL, 0.18 mmol, 1.50 eq.) was added. The resulting mixture was then stirred at rt for 1h. After the complete conversion of the carboxylic acid, the solvent was removed, and the residue was redissolved in 1.17 mL. In a second flask, amine **67** (66 mg, 0.13 mmol, 1.10 eq.) was dissolved in 1 mL DCM, and 60 µL DIPEA (0.35 mmol, 3.00 eq.) was added. The solution was cooled to 0°C, and the acid chloride solution was added dropwise. After 1h, the solvent was removed, and the crude was purified via preparative HPLC (H_2O/ACN, 5 - 80%). The title compound was obtained as a colorless solid.

Yield: 17 mg (20 µmol, 17%)

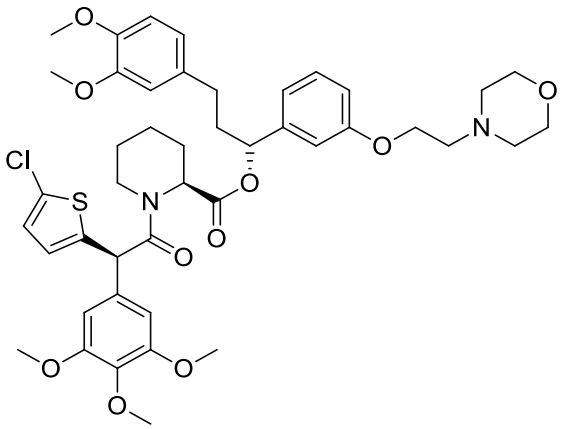
HPLC (5 – 100 % solvent B, 3 min) R_t = 1.995min, purity (220 nm): 99%

HRMS (ESI$^+$): m/z: calculated 837.31822 $[M+H]^+$, found 837.31853 $[M+H]^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.19 (t, J = 7.9 Hz, 1H), 6.90 – 6.74 (m, 4H), 6.71 (d, J = 3.7 Hz, 1H), 6.69 – 6.60 (m, 3H), 6.42 (s, 2H), 5.63 (dd, J = 8.2, 5.3 Hz, 1H), 5.44 – 5.40 (m, 1H), 5.29 (s, 1H), 4.19 – 4.05 (m, 2H), 3.85 (s, 6H), 3.79 (s, 3H), 3.77 – 3.71 (m, 4H), 3.65 (s, 6H), 3.07 (td, J = 13.3, 3.1 Hz, 1H), 2.82 (t, J = 6.1 Hz, 2H), 2.68 – 2.56 (m, 4H), 2.54 – 2.46 (m, 2H), 2.37 – 2.26 (m, 1H), 2.22 – 2.10 (m, 1H), 2.08 – 1.95 (m, 1H), 1.80 – 1.71 (m, 2H), 1.69 – 1.57 (m, 1H), 1.50 – 1.40 (m, 1H), 1.36 – 1.24 (m, 2H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 170.64, 170.54, 158.94, 153.54, 149.04, 147.52, 141.90, 141.86, 137.31, 133.56, 133.48, 130.43, 129.69, 125.17, 124.91, 120.28, 118.70, 114.13, 112.52, 111.81, 111.44, 105.21, 76.48, 66.94, 60.91, 57.79, 56.11, 56.07, 55.99, 54.17, 52.67, 50.67, 44.10, 38.40, 31.53, 26.94, 25.31, 20.89.

(S)-(R)-3-(3,4-Dimethoxyphenyl)-1-(3-(2-morpholinoethoxy)phenyl)propyl 1-((R)-2-(5-chlorothiophen-2-yl)-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2-carboxylate (d68b)



Yield: 20 mg (24 µmol, 20%)

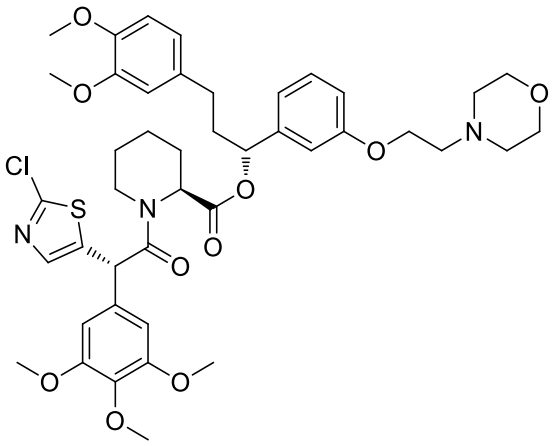
HPLC (5 – 100 % solvent B, 3 min) R_t = 1.894 min, purity (220 nm): 99%

HRMS (ESI$^+$): m/z: calculated 837.31822 $[M+H]^+$, found 837.31862 $[M+H]^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.26 – 7.21 (m, 1H), 6.87 (d, J = 7.8 Hz, 1H), 6.85 – 6.82 (m, 2H), 6.78 (d, J = 8.0 Hz, 1H), 6.72 – 6.61 (m, 4H), 6.52 (s, 2H), 5.75 (dd, J = 7.9, 5.9 Hz, 1H), 5.54 – 5.50 (m, 1H), 5.26 (s, 1H), 4.10 (t, J = 5.8 Hz, 2H), 3.85 (s, 6H), 3.83 (s, 9H), 3.74 (dd, J = 8.8, 4.3 Hz, 5H), 3.65 (s, 1H), 3.22 (td, J = 13.2, 3.0 Hz, 1H), 2.84 – 2.76 (m, 2H), 2.61 – 2.56 (m, 4H), 2.54 – 2.46 (m, 1H), 2.35 – 2.30 (m, 1H), 2.26 – 2.16 (m, 1H), 2.10 – 2.00 (m, 1H), 1.69 – 1.55 (m, 2H), 1.52 – 1.46 (m, 1H), 1.36 – 1.22 (m, 1H), 1.10 – 0.97 (m, 1H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 170.44, 170.17, 158.97, 153.63, 149.01, 147.49, 141.58, 141.24, 137.65, 134.41, 133.59, 130.23, 129.80, 125.43, 125.16, 120.27, 119.12, 114.13, 113.23, 111.84, 111.45, 105.39, 76.59, 67.01, 61.01, 57.80, 56.39, 56.07, 55.99, 54.22, 52.75, 50.72, 44.13, 38.17, 31.42, 26.88, 25.16, 20.99.

(S)-(R)-3-(3,4-Dimethoxyphenyl)-1-(3-(2-morpholinoethoxy)phenyl)propyl 1-((S)-2-(2-chlorothiazol-5-yl)-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2-carboxylate (68c)



Following the general procedure D, compound **68c** was synthesized using: **58j** (30 mg, 0.09 mmol, 1.00 eq), amine **67** (49 mg, 0.10 mmol, 1.10 eq.), TCFH (29 mg, 0.10 mmol, 1.20 eq.), NMI (24 µL, 0.31 mmol, 3.50 eq.) and 0.44 mL MeCN. After purification via preparative HPLC (H_2O/ACN, 5 - 80%), diastereomers **68c** and **d68c** were obtained as colorless solids.

Yield: 30 mg (35.78 µmol, 41%)

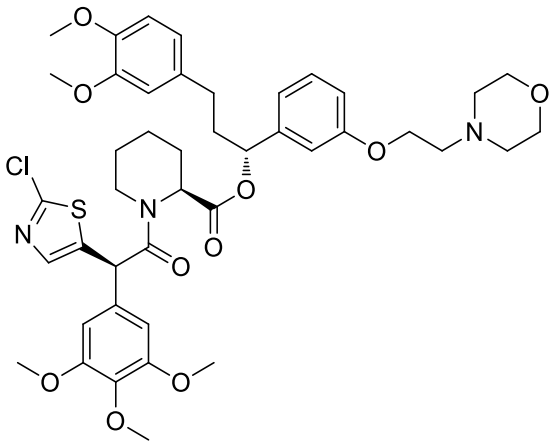
HPLC (5 – 100 % solvent B, 3 min) R_t = 1.893 min, purity (220 nm): 99%

HRMS (ESI$^+$): m/z: calculated 838.3135 $[M+H]^+$, found 838.3145 $[M+H]^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34 (s, 1H), 7.21 (t, J = 7.9 Hz, 1H), 6.84 – 6.81 (m, 1H), 6.80 – 6.75 (m, 3H), 6.67 (dd, J = 8.1, 1.9 Hz, 1H), 6.64 (d, J = 1.9 Hz, 1H), 6.40 (s, 2H), 5.64 (dd, J = 8.3, 5.3 Hz, 1H), 5.40 – 5.37 (m, 1H), 5.35 (s, 1H), 4.26 – 4.13 (m, 2H), 3.85 (s, 6H), 3.84 – 3.80 (m, 4H), 3.79 (s, 3H), 3.65 (s, 6H), 3.05 (td, J = 13.4, 3.2 Hz, 1H), 2.99 – 2.92 (m, 2H), 2.79 – 2.68 (m, 4H), 2.67 – 2.46 (m, 2H), 2.35 (d, J = 13.5 Hz, 1H), 2.23 – 2.14 (m, 1H), 2.06 – 1.97 (m, 1H), 1.82 – 1.73 (m, 2H), 1.68 (d, J = 13.2 Hz, 1H), 1.59 – 1.45 (m, 1H), 1.39 – 1.27 (m, 2H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 170.37, 169.87, 158.66, 153.79, 153.39, 149.07, 147.57, 141.99, 140.41, 138.39, 137.53, 133.49, 132.78, 129.76, 120.29, 119.02, 114.23, 112.34, 111.82, 111.46, 104.78, 76.60, 60.93, 57.68, 56.51, 56.17, 56.08, 56.01, 54.02, 52.88, 48.58, 44.25, 38.39, 31.56, 26.88, 25.24, 20.83.

(S)-(R)-3-(3,4-Dimethoxyphenyl)-1-(3-(2-morpholinoethoxy)phenyl)propyl 1-((R)-2-(2-chlorothiazol-5-yl)-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2-carboxylate (d68c)



Yield: 26 mg (31.01 µmol, 36%)

HPLC (5 – 100 % solvent B, 3 min) R_t = 1.884 min, purity (220 nm): 99%

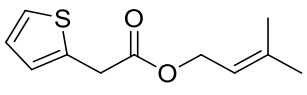
HRMS (ESI$^+$): m/z: calculated 838.3139 $[M+H]^+$, found 838.3135 $[M+H]^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37 (s, 1H), 7.25 – 7.23 (m, 1H), 6.88 (d, J = 7.6 Hz, 1H), 6.85 – 6.80 (m, 2H), 6.77 (d, J = 8.3 Hz, 1H), 6.64 (d, J = 6.9 Hz, 2H), 6.52 (s, 2H), 5.73 (dd, J = 7.9, 5.8 Hz, 1H), 5.52 (dd, J = 6.1, 2.2 Hz, 1H), 5.35 (s, 1H), 4.18 (t, J = 5.3 Hz, 2H), 3.85 (s, 3H), 3.85 (s, 3H), 3.83 (s, 6H), 3.82 (s, 3H), 3.82 – 3.78 (m, 4H), 3.26 (td, J = 13.1, 3.0 Hz, 1H), 2.97 (s, 2H), 2.77 (s, 4H), 2.59 – 2.45 (m, 2H), 2.34 (d, J = 14.0 Hz, 1H), 2.24 – 2.16 (m, 1H), 2.08 – 1.98 (m, 1H), 1.68 – 1.53 (m, 2H), 1.49 (d, J = 13.5 Hz, 1H), 1.37 – 1.25 (m, 2H), 0.99 – 0.87 (m, 1H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 170.26, 169.68, 158.65, 153.94, 153.16, 149.03, 147.53, 141.60, 139.94, 138.73, 133.74, 133.47, 129.89, 120.26, 119.45, 114.19, 112.98, 111.82, 111.46, 104.75, 76.72, 66.27, 65.02, 61.05, 57.60, 56.46, 56.07, 56.01, 53.95, 52.91, 48.61, 44.13, 38.07, 31.39, 26.77, 24.96, 20.83.

11.12 Synthesis of selective SLF analogs by Claisen Ireland rearrangement

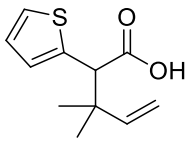
3-Methylbut-2-en-1-yl 2-(thiophen-2-yl)acetate (71)



In a 10 mL flask, 2-thienylacetic acid (250 mg, 1.76 mmol, 1.00 eq.) was dissolved in 3.52 mL DCM, and the resulting solution was cooled to 0°C. Then oxalyl chloride (0.18 mL, 2.11 mmol, 1.20 eq.) was added dropwise, and the ice bath was removed. The reaction mixture was then stirred at rt for 1 h before the solvent was removed under reduced pressure. The residue was redissolved in 1.52 mL DCM and was cooled to 0°C. A solution of 3-methyl-2-buten-1-ol (0.26 mL, 2.64 mmol, 1.50 eq.) and pyridine (0.28 mL, 3.52 mmol, 2.00 eq.) in 1.50 mL DCM was added dropwise. After 1h, the solution was diluted with 25 mL DCM and was transferred to a separatory funnel. The org. phase was then washed consecutively with sat. $NaHCO_3$, 1 M HCl, and brine. The DCM layer was then dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The title compound was obtained as a yellow oil after purification via column chromatography (CH/EtOAc, 0 – 10%).

Yield: 336 mg (1.59 mmol, 91%)
TLC (CH/EtOAc, 9:1): R_f = 0.35
HPLC (50 – 100 % solvent B, 3 min) R_t = 1.229 min, purity (220 nm): 93 %
Mass (ESI^+): m/z: calculated 211.07 $[M+H]^+$, found 211.00 $[M+H]^+$
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.21 (dd, J = 4.7, 1.8 Hz, 1H), 6.98 – 6.91 (m, 2H), 5.45 – 5.32 (m, 1H), 4.63 (d, J = 7.3 Hz, 2H), 3.83 (s, 2H), 1.77 (s, 3H), 1.71 (d, J = 1.4 Hz, 3H).
^{13}C NMR (75 MHz, $CDCl_3$) δ 170.54, 139.54, 135.27, 126.83, 125.05, 118.39, 62.17, 35.54, 25.84, 18.12.

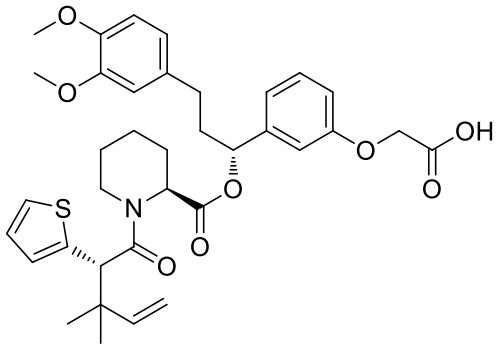
3,3-Dimethyl-2-(thiophen-2-yl)pent-4-enoic acid (72)



In a 10 mL dried flask, **71** (117 mg, 0.56 mmol, 1.00 eq.) was dissolved in 2 mL THF. The resulting solution was cooled to -78°C, and 0.67 mL LiHMDS (1 M in THF, 0.67 mmol, 1.20 eq.) was added dropwise. After 1 h at -78°C, TMSCl (78 µL, 0.61 mmol, 1.10 eq.) was added, and the solution was stirred for 15 min at this temperature. Then the dry ice bath was removed, and the reaction mixture was stirred for an additional 30 min. Then 1 M HCl was added, and the reaction mixture was extracted three times with EtOAc. The combined org. layers were then washed with brine, dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The title compound was obtained as a pale-yellow oil after purification by column chromatography (CH/EtOAc + 1% FA, 0 – 25%).

Yield: 73 mg (0.35 mmol, 62%)
TLC (CH/EtOAc + 1% FA, 3:1): R_f = 0.42
HPLC (30 – 100 % solvent B, 3 min) R_t = 1.504 min, purity (220 nm): 98 %
Mass (ESI^+): m/z: calculated 211.07 $[M+H]^+$, found 211.00 $[M+H]^+$
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.24 (dd, J = 5.2, 1.2 Hz, 1H), 7.04 (dd, J = 3.5, 1.2 Hz, 1H), 6.98 (dd, J = 5.2, 3.5 Hz, 1H), 6.04 (dd, J = 17.4, 10.7 Hz, 1H), 5.09 – 4.97 (m, 2H), 3.86 (s, 1H), 1.18 (s, 6H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 178.42, 144.48, 136.49, 128.12, 126.33, 125.28, 113.35, 57.06, 40.32, 25.85, 24.30.

2-(3-((R)-3-(3,4-dimethoxyphenyl)-1-(((S)-1-((S)-3,3-dimethyl-2-(thiophen-2-yl)-pent-4-enoyl)piperidine-2-carbonyl)oxy)propyl)phenoxy)acetic acid (73)



Following the general procedure C, compound **73** was synthesized using: resin **7** (372 mg, 0.17 mmol, 1.10 eq., loading: 0.46 mmol/g), **72** (32 mg, 0.15 mmol, 1.00 eq.), oxalyl chloride (26 µL, 0.30 mmol, 2.00 eq.), DIPEA (133 µL, 0.76 mmol, 5.00 eq.) and 1.24 mL DCM. After purification via preparative HPLC (H_2O/ACN + 0.1% TFA, 50 - 80%), diastereomers **73** and **d73** were obtained as colorless solids.

Yield: 12 mg (18.47 µmol, 12%)

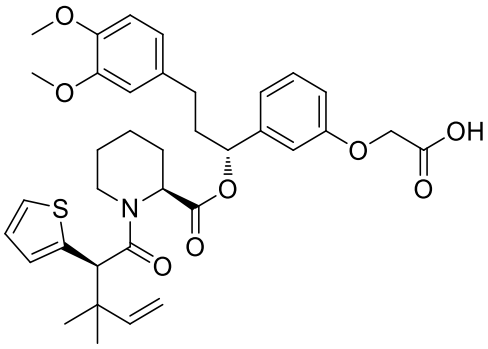
HPLC (50 – 100 % solvent B, 3 min) R_t = 1.665 min, purity (220 nm): 99%

HRMS (ESI$^+$): m/z: calculated 650.27821 $[M+H]^+$, found 650.27881 $[M+H]^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.27 (d, J = 7.7 Hz, 1H), 7.25 – 7.19 (m, 1H), 6.96 (d, J = 4.3 Hz, 2H), 6.94 – 6.90 (m, 2H), 6.86 – 6.83 (m, 1H), 6.82 – 6.80 (m, 1H), 6.74 – 6.69 (m, 2H), 6.08 (dd, J = 17.4, 10.9 Hz, 2H), 5.74 (dd, J = 8.6, 5.0 Hz, 1H), 5.55 (d, J = 5.5 Hz, 1H), 4.96 – 4.82 (m, 2H), 4.77 – 4.66 (m, 2H), 4.19 (s, 1H), 3.99 (d, J = 13.6 Hz, 1H), 3.89 (s, 3H), 3.88 (s, 3H), 3.33 (td, J = 13.3, 3.0 Hz, 1H), 2.74 – 2.65 (m, 1H), 2.64 – 2.56 (m, 1H), 2.35 – 2.29 (m, 1H), 2.29 – 2.20 (m, 1H), 2.14 – 2.05 (m, 1H), 1.72 – 1.54 (m, 4H), 1.41 – 1.28 (m, 1H), 1.14 (s, 3H), 1.13 (s, 3H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 172.26, 171.44, 170.37, 158.05, 149.05, 147.54, 145.62, 142.24, 138.41, 133.54, 129.87, 127.78, 126.12, 125.28, 120.33, 119.77, 115.61, 112.35, 111.83, 111.50, 110.33, 76.43, 65.47, 56.08, 56.03, 52.58, 51.96, 44.89, 41.15, 38.36, 31.63, 26.96, 25.43, 21.10.

2-(3-((R)-3-(3,4-dimethoxyphenyl)-1-(((S)-1-((R)-3,3-dimethyl-2-(thiophen-2-yl)-pent-4-enoyl)piperidine-2-carbonyl)oxy)propyl)phenoxy)acetic acid (d73)



Yield: 35 mg (53.86 µmol, 62%)

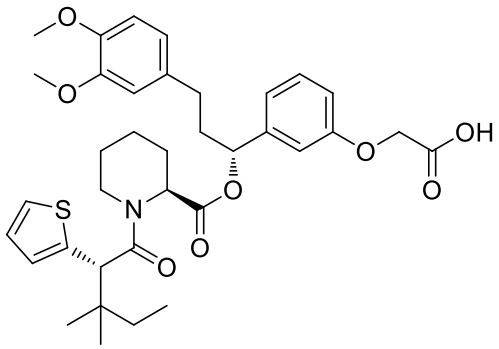
HPLC (50 – 100 % solvent B, 3 min) R_t = 1.682 min, purity (220 nm): 99%

HRMS (ESI$^+$): m/z: calculated 650.27821 $[M+H]^+$, found 650.27859 $[M+H]^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.20 – 7.10 (m, 2H), 6.95 (dd, J = 3.6, 1.4 Hz, 1H), 6.88 (dd, J = 5.1, 3.6 Hz, 1H), 6.83 – 6.73 (m, 2H), 6.71 – 6.64 (m, 4H), 6.08 (dd, J = 17.4, 10.8 Hz, 1H), 5.63 (dd, J = 8.4, 5.3 Hz, 1H), 5.49 (d, J = 5.4 Hz, 1H), 5.03 – 4.96 (m, 1H), 4.94 – 4.83 (m, 1H), 4.73 – 4.55 (m, 2H), 4.15 (s, 1H), 3.93 (d, J = 14.2 Hz, 1H), 3.85 (s, 3H), 3.84 (s, 3H), 2.99 – 2.87 (m, 1H), 2.62 – 2.50 (m, 1H), 2.47 – 2.38 (m, 1H), 2.38 – 2.27 (m, 1H), 2.08 – 1.99 (m, 1H), 1.93 – 1.83 (m, 1H), 1.72 – 1.59 (m, 3H), 1.52 – 1.41 (m, 1H), 1.30 – 1.23 (m, 1H), 1.17 (s, 3H), 1.14 (s, 3H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 172.35, 171.78, 170.29, 157.72, 148.92, 147.38, 145.95, 142.15, 137.47, 133.68, 129.81, 128.01, 126.24, 125.23, 120.34, 119.98, 114.80, 112.29, 111.95, 111.41, 76.07, 65.22, 55.99, 52.23, 51.79, 44.50, 40.84, 38.05, 31.29, 26.94, 25.89, 25.42, 24.85, 20.96.

2-(3-((R)-3-(3,4-dimethoxyphenyl)-1-(((S)-1-((S)-3,3-dimethyl-2-(thiophen-2-yl)-pentanoyl)-piperidine-2-carbonyl)oxy)propyl)phenoxy)acetic acid (74)



A 4 mL screwcap vial was charged with **73** (8.2 mg, 13 µmol, 1.00 eq.) and palladium on carbon (1.4 mg, 1 µmol, 0.10 eq., 10 wt.-% on carbon). Then 0.25 mL methanol was added, and 1 atm of hydrogen was applied. After stirring for 1 h at rt, the reaction mixture was filtered, and the solvent was removed under reduced pressure. The title compound **74** was obtained as a colorless solid without further purification.

Yield: 6.4 mg (9.82 µmol, 78%)

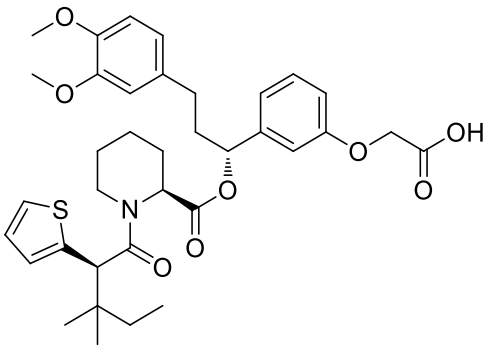
HPLC (50 – 100 % solvent B, 3 min) R_t = 1.753 min, purity (220 nm): 99%

HRMS (ESI$^+$): m/z: calculated 652.2939 $[M+H]^+$, found 652.2940 $[M+H]^+$

^{1}H NMR (**XY**, 500 MHz, $CDCl_3$) δ 7.23 (t, J = 7.9 Hz, 1H), 7.17 (dd, J = 4.6, 1.7 Hz, 1H), 6.93 (d, J = 4.8 Hz, 2H), 6.91 – 6.81 (m, 3H), 6.78 (d, J = 8.1 Hz, 1H), 6.71 – 6.64 (m, 2H), 5.72 (dd, J = 8.6, 5.1 Hz, 1H), 5.54 – 5.49 (m, 1H), 4.67 – 4.55 (m, 2H), 4.17 (s, 1H), 4.03 – 3.97 (m, 1H), 3.85 (s, 3H), 3.84 (s, 3H), 3.28 (td, J = 13.3, 2.9 Hz, 1H), 2.65 (ddd, J = 14.9, 9.8, 5.4 Hz, 1H), 2.56 (ddd, J = 14.4, 9.5, 6.4 Hz, 1H), 2.35 – 2.16 (m, 3H), 2.12 – 2.00 (m, 1H), 1.71 – 1.53 (m, 4H), 1.42 – 1.22 (m, 3H), 1.01 (s, 3H), 0.95 (s, 3H), 0.80 (t, J = 7.5 Hz, 3H).

^{13}C NMR (**XY**, 126 MHz, $CDCl_3$) δ 172.74, 172.18, 170.59, 158.22, 149.05, 147.53, 142.18, 138.90, 133.57, 129.85, 127.61, 126.18, 125.11, 120.32, 119.58, 115.35, 111.84, 111.49, 110.86, 76.43, 56.07, 56.01, 52.48, 50.40, 44.84, 38.35, 38.32, 33.04, 31.61, 26.84, 25.42, 24.68, 23.99, 21.14, 8.50.

2-(3-((R)-3-(3,4-dimethoxyphenyl)-1-(((S)-1-((R)-3,3-dimethyl-2-(thiophen-2-yl)-pentanoyl)-piperidine-2-carbonyl)oxy)propyl)phenoxy)acetic acid (d74)



A 4 mL screwcap vial was charged with **d73** (15.3 mg, 24 µmol, 1.00 eq.) and palladium on carbon (2.5 mg, 2 µmol, 0.10 eq., 10 wt.-% on carbon). Then 0.48 mL methanol was added, and 1 atm of hydrogen was applied. After stirring for 1 h at rt, the reaction mixture was filtered, and the solvent was removed under reduced pressure. The title compound **d74** was obtained as a colorless solid without further purification.

Yield: 12.1 mg (18.56 µmol, 78%)

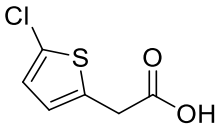
HPLC (**XY**, 50 – 100 % solvent B, 3 min) R_t = 1.818 min, purity (220 nm): 99 %

HRMS (ESI$^+$): m/z: calculated 652.29386 $[M+H]^+$, found 652.29375 $[M+H]^+$

^{1}H NMR (**XY**, 500 MHz, $CDCl_3$) δ 7.18 (t, J = 8.1 Hz, 1H), 7.12 (dd, J = 5.1, 1.2 Hz, 1H), 6.92 (dd, J = 3.6, 1.2 Hz, 1H), 6.88 (dd, J = 5.2, 3.5 Hz, 1H), 6.84 (dd, J = 8.1, 2.5 Hz, 1H), 6.78 (d, J = 8.8 Hz, 1H), 6.70 – 6.65 (m, 4H), 5.62 (dd, J = 8.6, 5.1 Hz, 1H), 5.49 (d, J = 5.4 Hz, 1H), 4.67 – 4.59 (m, 2H), 4.13 (s, 1H), 4.00 – 3.94 (m, 1H), 3.86 (s, 3H), 3.85 (s, 3H), 2.99 (td, J = 13.4, 3.0 Hz, 1H), 2.61 – 2.54 (m, 1H), 2.52 – 2.44 (m, 1H), 2.37 – 2.32 (m, 1H), 2.12 – 2.03 (m, 1H), 1.96 – 1.86 (m, 1H), 1.74 – 1.68 (m, 1H), 1.68 – 1.63 (m, 1H), 1.53 – 1.42 (m, 2H), 1.44 – 1.23 (m, 3H), 1.05 (s, 3H), 0.99 (s, 3H), 0.87 (t, J = 7.5 Hz, 3H).

^{13}C NMR (**XY**, 126 MHz, $CDCl_3$) δ 172.44, 171.47, 170.19, 157.80, 148.99, 147.47, 142.30, 137.82, 133.68, 129.84, 127.90, 126.36, 125.01, 120.37, 119.93, 115.32, 111.97, 111.45, 111.11, 76.13, 65.43, 56.07, 56.04, 52.29, 50.42, 44.45, 38.13, 37.99, 33.11, 31.45, 27.16, 25.46, 24.85, 24.02, 21.09, 8.60.

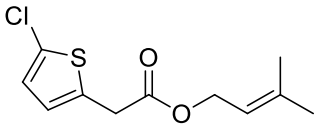
2-(5-chlorothiophen-2-yl)acetic acid (76)



In a 10 mL flask, 2-thiopheneacetic acid (750 mg, 5.28 mmol, 1.00 eq.) was dissolved in 5.28 mL chloroform and 0.5 mL acetic acid. Then NCS (719 mg, 5.38 mmol, 1.02 eq.) was added, and the reaction mixture was stirred at rt overnight. The mixture was then diluted with DCM and washed with 1 M HCl. The org. layer was dried over $MgSO_4$, and the solvent was removed under reduced pressure. The title compound was obtained as a brownish solid after purification via column chromatography (CH/EtOAc + 1% FA, 0 – 25%)

Yield: 581 mg (3.29 mmol, 62%)
TLC (CH/EtOAc + 1% FA, 3:1): R_f = 0.14
HPLC (5 – 100 % solvent B, 3 min) R_t = 1.695 min, purity (220 nm): 93 %
Mass (ESI^+): m/z: calculated 176.97 $[M+H]^+$, found 176.80 $[M+H]^+$
^{1}H NMR (300 MHz, $CDCl_3$) δ 10.75 (s, 1H), 6.80 (d, J = 3.8 Hz, 1H), 6.75 (d, J = 4.0 Hz, 1H), 3.82 (s, 2H).
^{13}C NMR (75 MHz, $CDCl_3$) δ 176.49, 132.78, 129.74, 126.78, 125.96, 35.53.

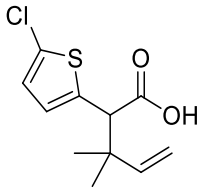
3-methylbut-2-en-1-yl 2-(5-chlorothiophen-2-yl)acetate (77)



In a 10 mL flask, **76** (228 mg, 1.29 mmol, 1.00 eq.) was dissolved in 3.52 mL DCM, and the resulting solution was cooled to 0°C. Then oxalyl chloride (0.13 mL, 1.55 mmol, 1.20 eq.) was added dropwise, and the ice bath was removed. The reaction mixture was then stirred at rt for 1 h before the solvent was removed under reduced pressure. The residue was redissolved in 1.52 mL DCM and was cooled to 0°C. A solution of 3-methyl-2-buten-1-ol (0.13 mL, 1.32 mmol, 1.02 eq.) and pyridine (0.21 mL, 2.58 mmol, 2.00 eq.) in 2.58 mL DCM was added dropwise. After 1h, the solution was diluted with 25 mL DCM and was transferred to a separatory funnel. The org. phase was then washed consecutively with sat. $NaHCO_3$, 1 M HCl, and brine. The DCM layer was then dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The title compound was obtained as a yellow oil after purification via column chromatography (CH/EtOAc, 0 – 20%).

Yield: 134 mg (0.54 mmol, 42%)
TLC (CH/EtOAc, 5:1): R_f = 0.55
HPLC (50 – 100 % solvent B, 3 min) R_t = 1.608 min, purity (220 nm): 94 %
Mass (ESI^+): m/z: calculated 245.03 $[M+H]^+$, found 245.00 $[M+H]^+$
^{1}H NMR (300 MHz, $CDCl_3$) δ 6.76 (dd, J = 3.8, 1.0 Hz, 1H), 6.71 – 6.68 (m, 1H), 5.38 – 5.31 (m, 1H), 4.63 (d, J = 7.3 Hz, 2H), 3.73 (s, 2H), 1.77 (s, 3H), 1.71 (s, 3H)
^{13}C NMR (75 MHz, $CDCl_3$) δ 170.12, 139.91, 134.07, 129.32, 126.23, 125.79, 118.25, 62.43, 36.00, 25.92, 18.21.

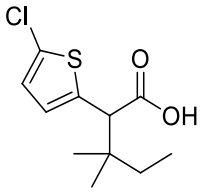
2-(5-chlorothiophen-2-yl)-3,3-dimethylpent-4-enoic acid (78)



In a 10 mL dried flask, **77** (110 mg, 0.45 mmol, 1.00 eq.) was dissolved in 1.80 mL THF. The resulting solution was cooled to -78°C, and 0.54 mL LiHMDS (1 M in THF, 0.54 mmol, 1.20 eq.) was added dropwise. After 1 h at -78°C, TMSCl (63 µL, 0.49 mmol, 1.10 eq.) was added, and the solution was stirred for 15 min at this temperature. Then the dry ice bath was removed, and the reaction mixture was stirred for an additional 30 min. Then 1 M HCl was added, and the reaction mixture was extracted three times with EtOAc. The combined org. layers were then washed with brine, dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The title compound was obtained as a pale-yellow oil after purification by column chromatography (CH/EtOAc + 1% FA, 0 – 15%).

Yield: 90 mg (0.37 mmol, 82%)
TLC (CH/EtOAc + 1% FA, 5:1): R_f = 0.26
HPLC (30 – 100 % solvent B, 3 min) R_t = 1.773 min, purity (220 nm): 97 %
Mass (ESI^+): m/z: calculated 245.03 $[M+H]^+$, found 245.00 $[M+H]^+$
^{1}H NMR (500 MHz, $CDCl_3$) δ 6.79 – 6.72 (m, 2H), 6.00 – 5.92 (m, 1H), 5.07 (d, J = 10.7 Hz, 1H), 5.00 (d, J = 17.4 Hz, 1H), 3.71 (s, 1H), 1.15 (s, 3H), 1.14 (s, 3H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 177.47, 143.97, 135.25, 129.73, 127.60, 125.24, 113.88, 57.69, 40.36, 25.77, 24.37.

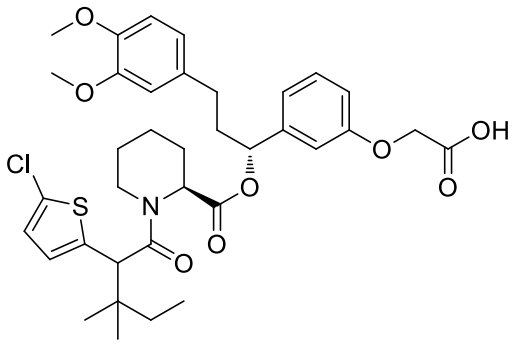
2-(5-chlorothiophen-2-yl)-3,3-dimethylpentanoic acid (79)



A 4 mL screwcap vial was charged with **78** (46 mg, 0.19 mmol, 1.00 eq.) and platinum on carbon (7 mg, 4 µmol, 0.10 eq., 10 wt.-% on carbon). Then 0.94 mL methanol was added, and 1 atm of hydrogen was applied. After stirring for 1 h at rt, the reaction mixture was filtered, and the solvent was removed under reduced pressure. The title compound **79** was obtained as a colorless solid after purification via column chromatography (CH/EtOAc, 0 – 15%).

Yield: 38 mg (0.15 mmol, 83%)
TLC (CH/EtOAc + 1% FA, 5:1): R_f = 0.31
HPLC (30 – 100 % solvent B, 3 min) R_t = 1.887 min, purity (220 nm): 95 %
Mass (ESI$^+$): m/z: calculated 269.05 $[M+Na]^+$, found 268.80 $[M+Na]^+$
^{1}H NMR (300 MHz, $CDCl_3$) δ 6.83 – 6.76 (m, 2H), 3.88 – 3.40 (m, 1H), 1.50 – 1.32 (m, 2H), 1.06 (s, 3H), 0.99 (s, 3H), 0.91 (t, J = 7.4 Hz, 3H).
^{13}C NMR (75 MHz, $CDCl_3$) δ 178.27, 135.79, 129.53, 127.50, 125.28, 56.52, 37.46, 33.02, 24.12, 24.06, 8.40.

2-(3-((R)-1-(((S)-1-(2-(5-chlorothiophen-2-yl)-3,3-dimethylpentanoyl)-piperidine-2-carbonyl)-oxy)-3-(3,4-dimethoxyphenyl)propyl)phenoxy)acetic acid (80)



Following the general procedure C, compound **80** was synthesized using: resin **7** (211 mg, 0.08 mmol, 1.00 eq., loading: 0.38 mmol/g), **79** (19 mg, 0.08 mmol, 1.00 eq.), oxalyl chloride (13 µL, 0.15 mmol, 2.00 eq.), DIPEA (34 µL, 0.19 mmol, 5.00 eq.) and 0.77 mL DCM. After purification via column chromatography (CH/EtOAc + 1% FA, 0 - 50%), compound **80** was obtained as an inseparable mixture of diastereomers.

Yield: 40 mg (58.29 µmol, 75%)
HPLC (50 – 100 % solvent B, 3 min) R_t = 2.006 min, purity (220 nm): 97 %
HRMS (ESI$^+$): m/z: calculated 686.25489 $[M+H]^+$, found 686.25458 $[M+H]^+$
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.22 – 7.16 (m, 1H), 6.94 – 6.90 (m, 1H), 6.89 – 6.80 (m, 1H), 6.81 – 6.76 (m, 1H), 6.75 – 6.72 (m, 1H), 6.72 – 6.64 (m, 4H), 5.70 (dt, J = 44.4, 6.8 Hz, 1H), 5.52 (dd, J = 25.3, 5.6 Hz, 1H), 4.69 – 4.63 (m, 2H), 4.11 – 4.04 (m, 1H), 3.96 (dd, J = 15.8, 7.6 Hz, 1H), 3.86 (s, 3H), 3.85 (s, 3H), 3.33 – 2.94 (m, 1H), 2.66 – 2.42 (m, 2H), 2.37 – 2.17 (m, 2H), 2.14 – 2.00 (m, 1H), 1.96 – 1.85 (m, 1H), 1.72 – 1.59 (m, 3H), 1.50 – 1.43 (m, 1H), 1.42 – 1.20 (m, 3H), 1.03 – 0.95 (m, 6H), 0.91 – 0.78 (m, 3H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 172.43, 172.25, 172.13, 171.94, 170.40, 170.21, 157.95, 157.73, 149.01, 148.93, 147.50, 147.40, 142.12, 142.07, 137.83, 136.99, 133.61, 133.50, 129.87, 129.85, 129.46, 129.26, 127.08, 126.72, 125.16, 124.89, 120.36, 120.30, 119.98, 119.89, 115.14, 114.69, 112.10, 111.92, 111.82, 111.48, 111.45, 111.27, 76.40, 76.15, 65.31, 65.21, 56.04, 55.99, 52.52, 52.26, 51.24, 51.11, 45.05, 44.60, 38.55, 38.39, 38.22, 38.02, 32.94, 32.86, 31.53, 31.29, 26.89, 25.60, 25.38, 24.64, 24.45, 23.88, 23.85, 21.10, 20.92, 8.55, 8.47.

Abbreviations

Aq.	Aqueous
$BH_3 \cdot SMe_2$	Borane dimethyl sulfide complex
CH	Cyclohexane
COMU	(1-Cyano-2-ethoxy-2-oxoethylidenaminooxy)dimethylamino-morpholino-carbenium hexafluorophosphate
DIC	N,N′-Diisopropylcarbodiimide
DCM	Dichloromethane
DIPEA	N,N-Diisopropylethylamine
DMAP	4-(Dimethylamino)pyridine
eq.	Equivalent (unit)
EtOAc	Ethyl acetate
EDC	N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride
ee	Enantiomeric excess
er	Enantiomeric ratio
FKBP	FK506-binding protein
FP	Fluorescence polarization
GR	Glucocorticoid receptor
HATU	1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid Hexafluorophosphate
HOAt	1-Hydroxy-7-azabenzotriazole
HR	High resolution
Hsp90	Heat shock protein 90
iPrOH	Isopropyl alcohol, Propan-2-ol
LC-MS	Liquid chromatography mass spectrometry
LiHMDS	Lithium-bis(trimethylsilyl)amide
MS	Mass spectrometry
mTOR	Mechanistic target of Rapamycin
µM	micromolar (unit)
n-Hex	Hexane
nM	nanomolar (unit)
NaHMDS	Sodium-bis(trimethylsilyl)amide
NMI	1-Methylimidazole
NMR	Nuclear magnetic resonance
Pd/C	Palladium on carbon
PPIase	peptidyl-prolyl cis/trans isomerase
PR	Progesterone receptor

Pt/C	Platinum on carbon
R_f	Retention factor
rt	room temperature
SAR	Structure-affinity relationship
sat.	saturated
T3P	Propylphosphonic anhydride
TCFH	Chloro-N,N,N′,N′-tetramethylformamidinium hexafluorophosphate
t-BuOH	tert-Butyl alcohol, 2-Methylpropan-2-ol
TFA	Trifluoroacetic acid
TLC	Thin-layer chromatography
TPR	Tetratricopeptide repeat
SPhos	Dicyclohexyl(2′,6′-dimethoxy[1,1′-biphenyl]-2-yl)phosphane

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