The versatility of asymmetric aminoethyl-tetrazines in bioorthogonal chemistry

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Chapter 7: Summary and future prospects

This thesis describes the synthesis, analysis of various tetrazine bearing compounds and use in bioorthogonal chemistry. In **chapter 1** a literature survey of tetrazine chemistry developments throughout history is provided. Additionally an introduction to the emergence of click chemistry, bioorthogonal reactions, and IEDDA chemistry is provided. These developments are the foundations on which the work described in this thesis is built upon.

Chapter 2 reports the synthesis of tetrazine-modified alanine building blocks as functionalized amino acids to substitute natural amino acids in activity-based probes (ABP's) for bioorthogonal activity-based protein profiling (ABPP) of cysteine, serine and threonine peptidases.[1][2] One building block, Cbz-Tzm-OH (compound **2**, **chapter 2**), proved to be readily available following the developed route of synthesis, and could be incorporated in small oligopeptide chloromethylketones (CMK)[3, 4] and benzyloxymethylketones (BOMK)[5, 6] providing structures closely related to known peptidase inhibitors. CMK inhibitor **1** (**Figure 1**) could be readily prepared and was successfully used bioorthogonal ABPP. Unfortunately, the CMK warhead was too reactive and resulted the modification of numerous unspecified proteins when applied to cell lysates. BOMK inhibitor **2** (**Figure 1**) was also readily prepared, however, provided poor protein modification.

As a **future prospect**, it would be interesting to prepare ABPs containing a fluoromethylketone (FMK) warhead[7], such as inhibitor **3** (**Figure 1**). It should be possible to synthesize this inhibitor using intermediates from this thesis as described in literature[8], and may result in an ABP featuring a more reactive warhead compared to compound **2** and a less reactive warhead compared to compound **1**[1, 3].

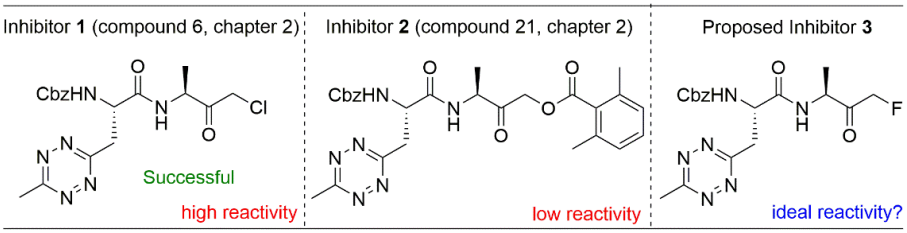
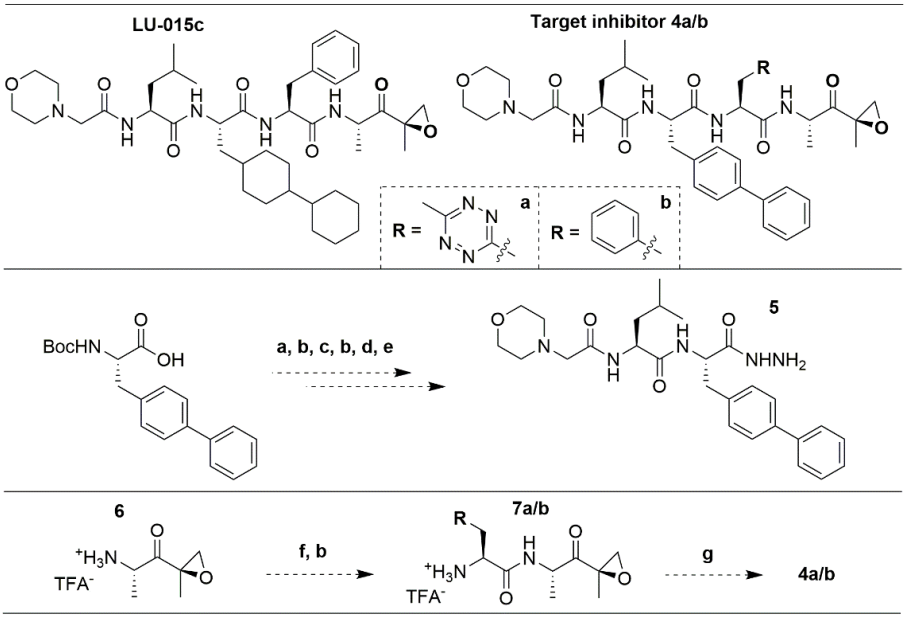


Figure 1: Structures of synthesized CMK inhibitor **1**, synthesized BOMK inhibitor **2**, and proposed FMK inhibitor **3**.

Chapter 2 also presents the synthesis of Boc-Tzm-OH (compound **12**, **chapter 2**) as an building block towards ABPs. However, after attachment of a warhead on its C-terminus, any effort to deprotect the Boc-group was unsuccessful.

As a **future prospect**, Boc-Tzm-OH could be used in the design of alternative ABPs. The non-canonical amino acid may be incorporated through replacement of the phenyl amino acid in the human proteasome and immunoproteasome B5c and B5i selective inhibitor **LU-015c**[9a] (**Figure 2**). Initial target inhibitors can be synthesized using commercially available Boc-Bip-OH instead of Boc-BiCha-OH, because this amino acid change has been shown to have only little influence on similarly used constructs[9b]. Compounds **5** and **6** may be synthesized according to literature procedures [9a, 9c]. Condensation of compound **6** with Boc-Tzm-OH or Boc-Phe-OH using HCTU, following Boc-deprotection should obtain compounds **5a/b**, which should be condensed with compound **5** to obtain target peptide epoxyketones **4a/b**.



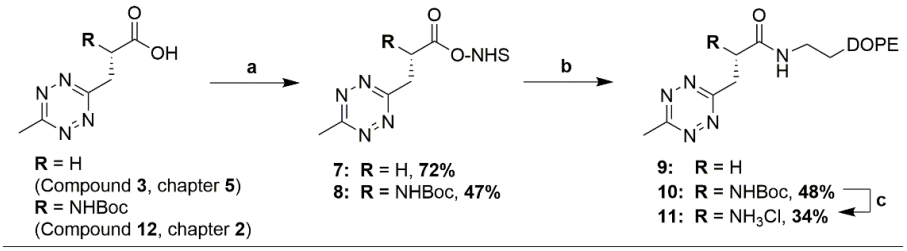
a) MeI, $KHCO_3$, DMF, **b**) TFA, DCM, **c**) Boc-Ile-OH, HCTU, DiPEA, DCM, **d**) 4-morpholineacetic acid, HCTU, DiPEA, DCM, **e**) hydrazine, MeOH, **f**) Boc-Phe-OH or Boc-Tzm-OH, HCTU, DiPEA, DCM, **g**) compound **5**, tBuONO, 4M HCl in dioxane, DMF/DCM.

Figure 2: Proposed 9-step synthesis of peptide epoxyketones **4a** (control) and **4b** (tetrazine).

Chapter 3 describes the synthesis of a library of functionalized tetrazines, as well as the synthesis and optimization of a variety of reactive alkenes. The work includes kinetic studies to determine the reactivity of the library of tetrazines towards the synthesized variety of alkenes. Additionally, these tetrazines were attached to Bodipy-FL to obtain fluorophore-tetrazine tags for bioorthogonal live cell fluorescence microscopy of alkene-functionalized molcecules present in the cell. Functionalized DOPE-lipids[10], mannosamines[11] and the naturally occurring strained

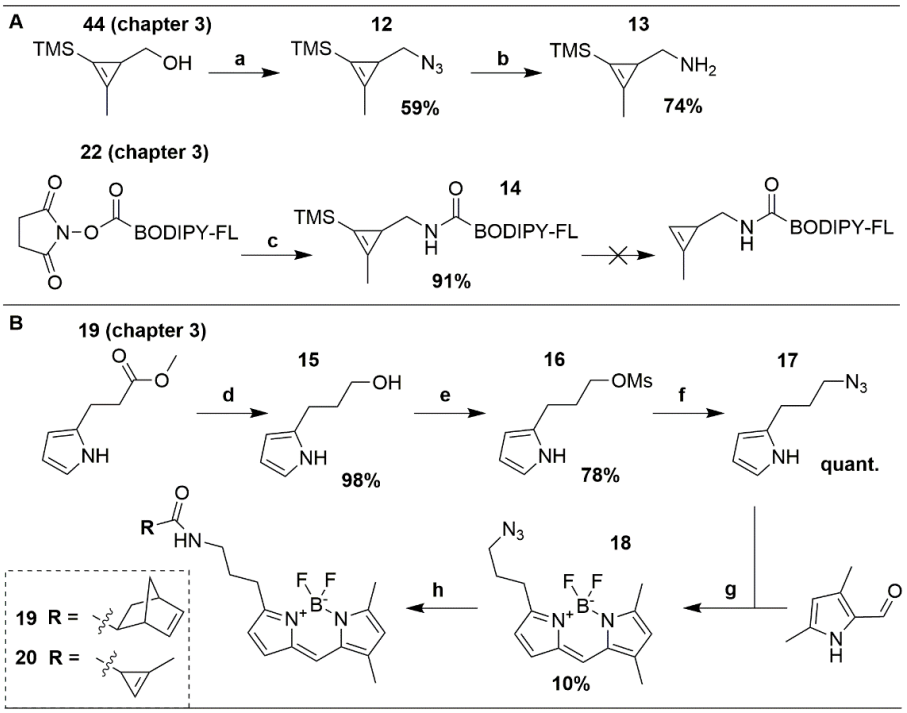
alkene-bearing sterculic acid[12] were incubated in cells and subsequently incubated with the tetrazine fluorophores. This resulted in weak fluorescence for the DOPE-lipids, as well as the mannosamines. Surprisingly, cells incubated with sterculic acid gave a strong fluorescence signal for a specific set of tetrazines. Tetrazines functionalized with the amino-methyl-phenyl linker gave a very strong fluorescence signal, while no fluorescence was observed for tetrazines functionalized with an aminoethyl linker.

As a **future prospect**, for the fluorescent imaging of alkene-bearing DOPE-lipids, tetrazine-functionalized modified DOPE-lipids **8**, **9** and **10** can be synthesized (**Figure 3**) starting from either Boc-Tzm-OH or 4-carboxyethyl-methyltetrazine (compound **3**, **chapter 5**). These functionalized lipids may be used for fluorescent imaging using commercially available or synthesized alkene-modified fluorophores **18** and **19** (**Figure 4**).



a) N-hydroxy succinimide, DCC, THF, **b**) DOPE-C_2H_4-NH_2, TEA, $CHCl_3$, **c**) 2M HCl dioxane/DCM.

Figure 3: Synthesis of tetrazine functionalized DOPE-lipids **8**, **9**, and **10**.



a) DPPA, DBU, THF, **b**) PPh_3, THF/H_2O, **c**) **12**, TEA, DMF, **d**) $LiAlH_4$, Et_2O, **e**) MsCl, TEA, DCM, **f**) NaN_3, DMF, **g**) $POCl_3$, DCM, then BF_3*OEt_2, TEA, **h**) PPh_3, THF/H_2O, then compound **35 (chapter 3)** or compound **42 (chapter 3)**, DMF.

Figure 4: (**A**) Attempted synthesis of alkene-functionalized Bodipy-FL. (**B**) Successful synthesis of alkene-functionalized Bodipy-FL **18** and **19**.

Chapter 4 describes the synthesis of highly water-soluble tetrazine fluorophores. These fluorophores were able to access the dense hydrophilic glycan coating (glycocalyx) around the cell surface and by doing so label the metabolically incorporated alkene-functionalized mannosamine (compound **62**, **Chapter 3**). By using sterculic acid and Bodipy-FL tetrazine (compound **25**, **Chapter 3**) as a second bioorthogonal pair, live cells could be incubated and labeled simultaneously to achieve multicomponent labeling ("dual-labeling") of alkene-bearing biomolecules. This was achieved by using only bioorthogonal IEDDA chemistry without any significant cross reactivity.

Building on the success of these highly water-soluble tetrazine fluorophores, as a **future prospect**, tetrazine-alkyne handles **21-26** (**Figure 5**) were synthesized. These molecules may be used to change the functionality of azide-functionalized biomolecules to alkene-functionalized biomolecules, and used in biological experiments together with the tetrazine fluorophores.

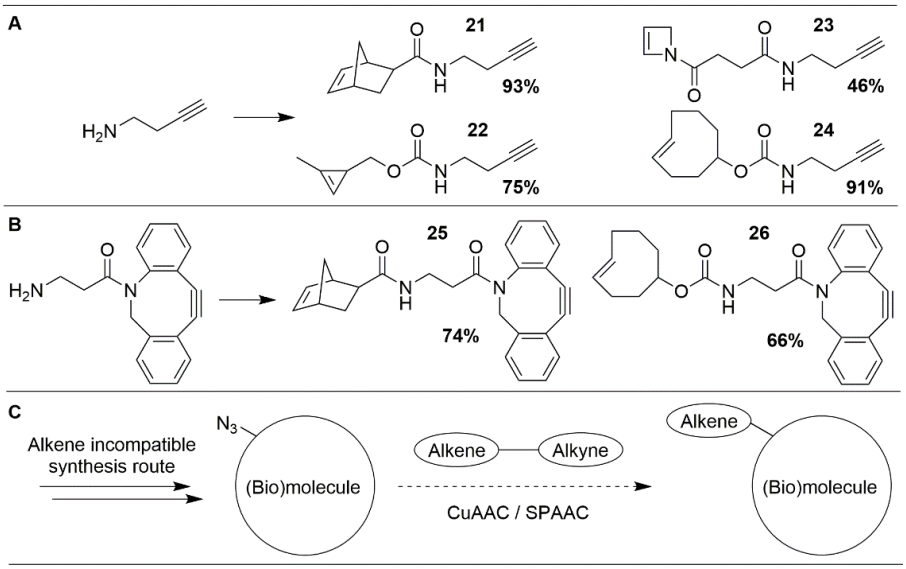
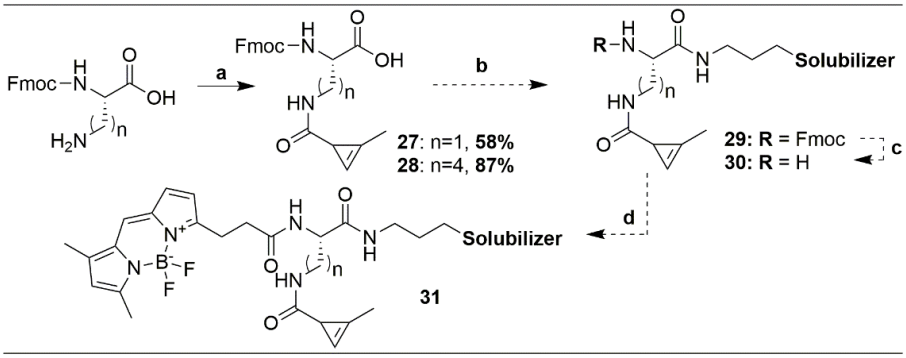


Figure 5: (**A**) Successful synthesis of propargyl-based alkene-alkynes. (**B**) Successful synthesis of DIBAC-based alkene-alkynes. (**C**) Proposed CuAAC or SPAAC mediated conversion of azide-functionalized biomolecules into alkene-functionalized biomolecules.

As another **future prospect**, it may be possible to apply the synthesis route of these fluorophores for the preparation of highly water-soluble alkene-functionalized fluorophores such as Bodipy-FL **31** (**Figure 6**).



a) Compound **42 (chapter 3)**, TEA, DMF, **b**) compound **2 (chapter 4)**, **c**) 20% Piperidine in DMF, **d**) compound **22 (chapter 3)**, TEA, DMF.

Figure 6: Synthesis of acyl-cyclopropene functionalized amino acids **27** and **28**, and proposed synthesis towards highly soluble Bodipy-FL-alkenes **31**.

As yet another **future prospect**, the highly water-soluble fluorophores may be used for the labeling of molecules less-abundant at the cells surface, such as alkene- and tetrazine-functionalized peptide epitopes, which were successfully synthesized (**Figure 7**). Through standard solid phase peptide synthesis Fmoc-SIINFEKL-OH **32** was prepared. **32** was then used to synthesize **33-36**, which may be used for MHC-I mediated presentation at the cell surface of APCs[13, 14]. Additionally using tetrazine-epitopes in combination with highly water-soluble alkene-functionalized fluorophores may also be a viable approach.

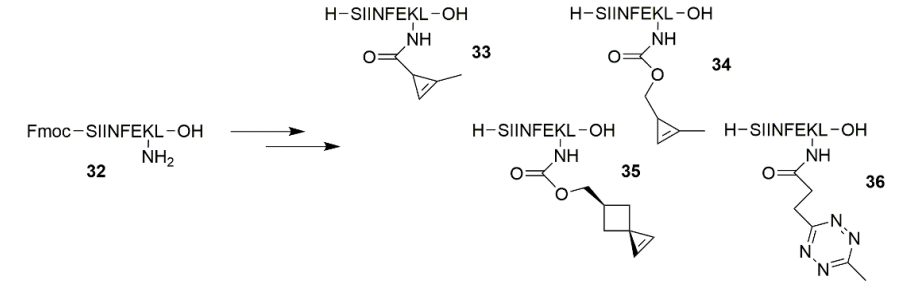
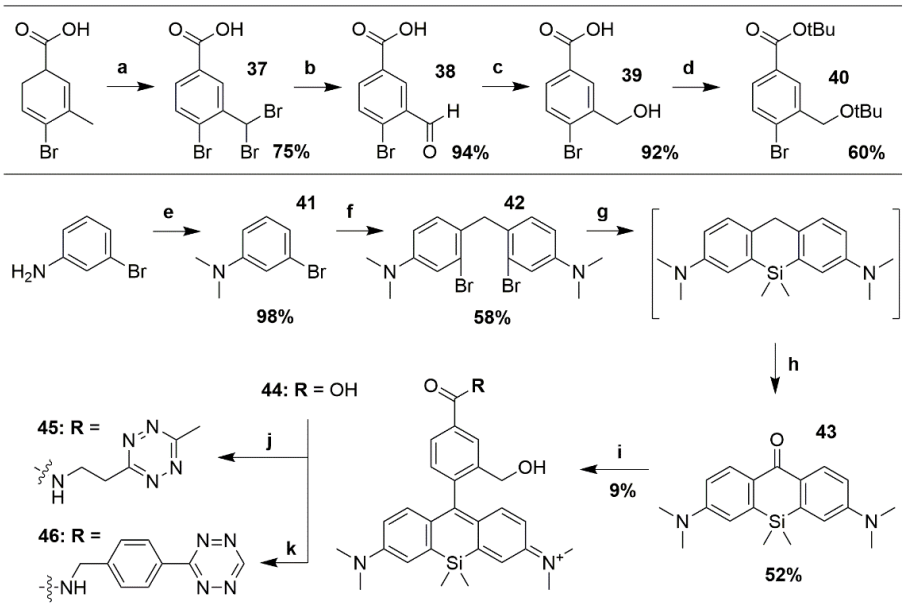


Figure 7: Successful synthesis of alkene-functionalized and tetrazine-functionalized "SIINFEKL" epitopes at the lysine position.

As a final **future prospect** for this chapter, to increase the resolution of fluorescence imaging, super-resolution may be applied by modifying the spontaneously blinking fluorophore "HMSiR" [15] at its carboxyl position, which was successfully synthesized (**Figure 8**). The molecule may then be used to label and quantify cell-surface labeled molecules[16, 17].



a) NBS, AIBN, DCE, **b**) Na_2CO_3(aq.), **c**) $NaBH_4$, THF, **d**) $MgSO_4$, H_2SO_4, tBuOH, DCM, **e**) formamide, H_2SO_4, THF then $NaBH_4$, **f**) formamide, AcOH **g**) Sec-BuLi, $SiMe_2Cl_2$, THF, **h**) $KMnO_4$, Acetone, **i**) **40**, THF then BuLi, then **42**, workup then TFA, **j**) EDC*HCl, N-hydroxy succinimide, DMF, then compound 10 chapter 3 **j**) EDC*HCl, N-hydroxy succinimide, DMF, then compound **10 (chapter 3)**, **k**) EDC*HCl, N-hydroxy succinimide, DMF, then compound **1 (chapter 3)**.

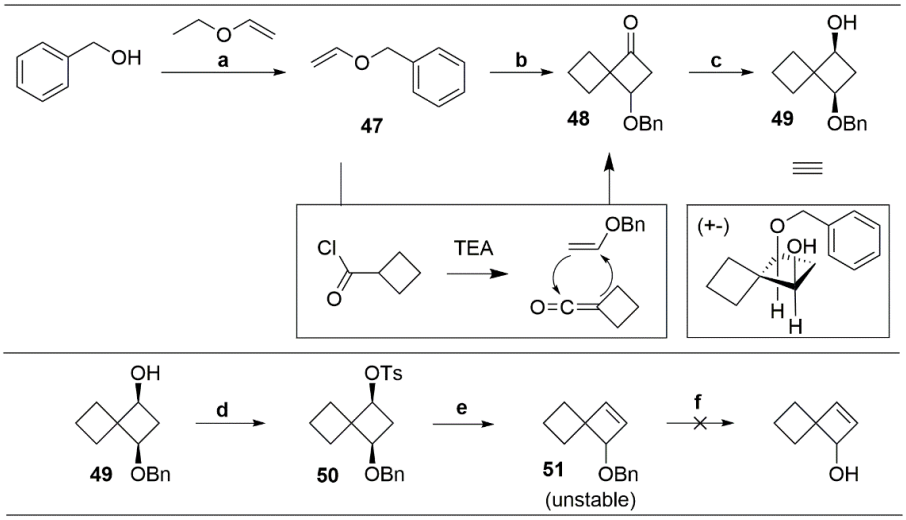
Figure 8: Successful synthesis of tetrazine-functionalized naturally blinking "HMSiR" fluorophores.

In **Chapter 5** the library of tetrazines from chapter 3 was analyzed on their capability (with respect to IEDDA rate, elimination rate and elimination efficiency) to perform a IEDDA-pyridazine elimination tandem reaction[18]. The library, consisting tetrazines functionalized with free amines or N-Boc protected amines with various spacers (methyl, ethyl, or methyl-phenyl) was analyzed on their reactivity and elimination speed with AMC-coumarin, a previously employed method from the literature[19]. The results were compared a set of literature tetrazines[19] including carboxy-functionalized tetrazines[20]. The data showed that amino-ethyl functionalized tetrazines, in particular tetrazines **6** (chapter 5) and **7** (chapter 5), showed unprecedented elimination rates, combined with respectable elimination efficiency, which was only achievable with other tetrazines by lowering the pH to non-physiological levels[20]. Unlike these other tetrazines, due to their amino-ethyl functionality, tetrazines **6** (chapter 5) and **7** (chapter 5) were not negatively affected by pH changes, or the lack of an acidic environment. This makes them highly likely an excellent choice when a high elimination rate is required.

In **Chapter 6** a tool was designed and synthesized from bifunctional trans-cyclooctane and the EDANS/DABCYL quencher pair to be able to correctly determine the reaction and elimination rates of tetrazines when releasing alkylic amines. This tool replaces the previously used AMC-coumarin method as it contained an aniline instead of a primary amine. With the help of computational modelling of the multi-step reaction in *Coach 7* and analysis of the results using *Graphpad PRiSM*, the tool was used to determine specific properties of individual tetrazines on their reaction rate and elimination rate. Additionally multiple simultaneous processes were identified and quantified and with that the rate-limiting steps could be determined.

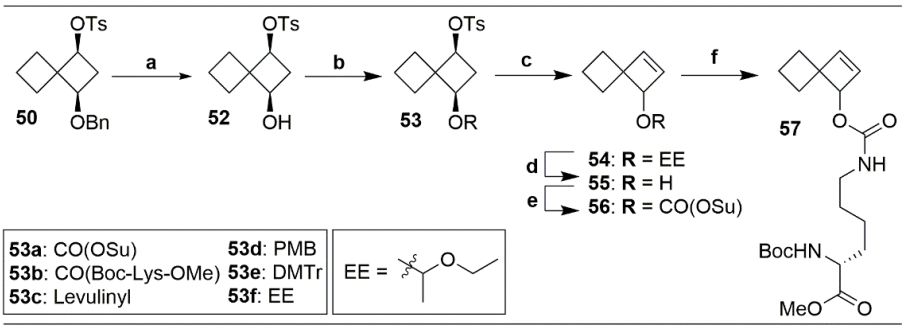
A **future prospect** to the work described here, would be the design, synthesis, and characterization of new strained alkenes that may have desirable properties for future research. One attempt was made here in the synthesis of a strained alkene termed "spiroheptene". First (**Figure 9**), benzyl vinyl ether **47** was prepared via mercury(II) trifluoroacetate catalysed vinylation of benzyl alcohol.[21] Spiroheptane **48** was synthesized via a [2+2]-cycloaddition of the *in situ* generated ketene[22] and vinyl ether **47,** which was reduced to obtain spiroheptane **48** as an enantiomeric mixture of cis-positioned (hydroxy and benzyloxy) functional groups. To form the spiroheptene structure through elimination of the hydroxyl moiety, this moiety was mesylated, tosylated or triflated, to be followed by their elimination though the use of KOtBu. Neither triflate nor mesylate were obtained in useful quantities, while tosylated spiroheptane **50** was obtained in good yield (79%) and was successfully eliminated to form spiroheptene **51**.[23] Storage of spiroheptene **51** at -20°C for several months led to the degradation of the compound, indicating a limited use for these types of molecules. Removal of the benzyl group to obtain 3-hydroxy-spiroheptene by chromium chloride and lithium iodide appeared to be unsuccessful.[24] The approach was discontinued as hydrogenation was not an option in the presence of the already formed spiroheptene functionality. As an alternative approach (**Figure 10**) spiroheptane **50** was hydrogenized to remove the benzyl group, resulting in spiroheptane **52**. The free hydroxyl was then functionalized with a variety of groups, which were successful for some (**53a**, **53b**, **53c**, **53f**) and failed for the others (**53d**, **53e**). Using **53f**, previously shown to be useful in the synthesis of spirohexene, the tosyl group was eliminated to form spiroheptene **54**. Further attempts on the elimination reaction on **53a** and **53b** appeared to be unsuccessful and returned the initial material, increasing the amount of KOtBu equivalents resulted in the degradation of the material. Compound **53c** degraded using the initial elimination procedure. Spiroheptene **54** was deprotected under acidic conditions to obtain spiroheptene **55**, which was directly used to prepare spiroheptene **56**

containing a succinimide ester. Finally spiroheptene **56** was successfully reacted with Boc-Lys-OMe resulting in spiroheptene **57**.



a) $Hg(TFA)_2$, 0°C to r.t., 2h, 75%; **b)** cyclobutanecarbonyl chloride, TEA, ACN, 90°C, 3h, 66%; **c)** $NaBH_4$, MeOH, 0°C to r.t., 3h, 69%. **d)** TsCl, pyridine, r.t., 3h, 79%; **e)** KOtBu, dry DMSO, r.t., 3h, 24% **f)** $CrCl_2$, LiI, H_2O, EtOAc, 65°C, o.n., 0%.

Figure 9: Synthesis of spiroheptene intermediates **50** and **51**.



a) Pd/C, H_2, MeOH, r.t., 3h, 88%; **b)** **53a**: $CO(OSu)_2$, DIPEA, dry ACN, r.t., o.n., 62%; **53b:** Compound **53a**, Boc-Lys-OMe acetate salt, TEA, dry DCM, rt, 4 h, 48%; for **53c**: levulinic acid, DCC, DMAP, DCM, 0° to rt, 3h, 76%; **53d**: NaH, dry DMF, 0 °C, 15 min, then PMB-Cl, 0°C, 15 min, 0%; **53e**: DMTr-Cl, pyridine, rt, o.n., 0%; **53f**: ethyl vinyl ether, PPTS, DCM, 0°C, 1h, 86%; **c)** KOtBu, dry DMSO, rt, 3h, 88%. **d)** 2M HCl (aq.), ACN, 0°, 1h; **e)** $CO(OSu)_2$, DIPEA, dry ACN, rt, overnight, 49%, **f)** Boc-Lys-OMe acetate salt, TEA, dry DCM, rt, 45 min, 32%.

Figure 10: Synthesis of target spiroheptene **56** as activated ester and spiroheptene modified lysine **57**.

Compound Synthesis

Compound 7: 0.125 mmol (21 mg) of compound **3 (chapter 5)** was dissolved in 1.0 mL of THF, 0.150 mmol (31 mg) of DCC and 0.156 mmol (18 mg) of N-hydroxy succinimide were added and the reaction mixture was stirred for 3 hours at room temperature. Reaction completion was checked by TLC (Rf = 0.6, 50% EtOAc in DCM). The reaction mixture was filtered over a pad of celite (in a glass pipette) and concentrated using rotary evaporation. Purification was performed twice by silica column chromatography using an 2.5%-20% EtOAc in DCM eluent followed by silica column chromatography using an EtOAc in pentane eluent resulting in 24 mg (0.091 mmol, 72.4%) of compound **7** as a pink solid**. ^{1}H NMR (400 MHz, $CDCl_3$) δ:** 3.74 (t, *J* = 7.2 Hz, 2H), 3.38 (t, *J* = 7.2 Hz, 2H), 3.05 (s, 3H), 2.81 (s, 4H). **^{13}C NMR (101 MHz, $CDCl_3$) δ**: 168.96, 168.07, 167.57, 167.38, 29.27, 28.06, 25.66, 21.26.

Compound 8: 0.198 mmol (56 mg) of compound **12 (chapter 2)** was dissolved in 2.0 mL of THF, 0.242 mmol (50 mg) of DCC and 0.243 mmol (28 mg) of N-hydroxy succinimide were added and the reaction mixture was stirred for 2 hours at room temperature. Reaction completion was checked by TLC (Rf = 0.6, 50% EtOAc in DCM). The reaction mixture was filtered over a pad of celite (in a glass pipette) and concentrated using rotary evaporation. Purification was performed twice by silica column chromatography using an 20%-60% EtOAc in pentane eluent followed by silica column chromatography using an 5%-20% EtOAc in DCM eluent, but failed to separate the starting material from the product. The fractions containing product were collected and concentrated using rotary evaporation. The residue was redissolved in EtOAc extracted with sat. $NaHCO_3$ (aq.), dried using $MgSO_4$, filtered and concentrated using rotary evaporation resulting in 35 mg (0.092 mmol, 46.5%) of compound **8** as a pink solid**. ^{1}H NMR (400 MHz, $CDCl_3$) δ**: 5.87 (d, *J* = 9.1 Hz, 1H), 5.34 (dt, *J* = 9.2, 5.4 Hz, 1H), 3.95 (d, *J* = 5.2 Hz, 2H), 3.05 (s, 4H), 2.77 (s, 5H), 1.39 (s, 11H). **^{13}C NMR (101 MHz, $CDCl_3$) δ**: 168.45, 168.18, 166.83, 166.06, 154.86, 80.98, 50.17, 37.43, 28.28, 25.60, 21.29.

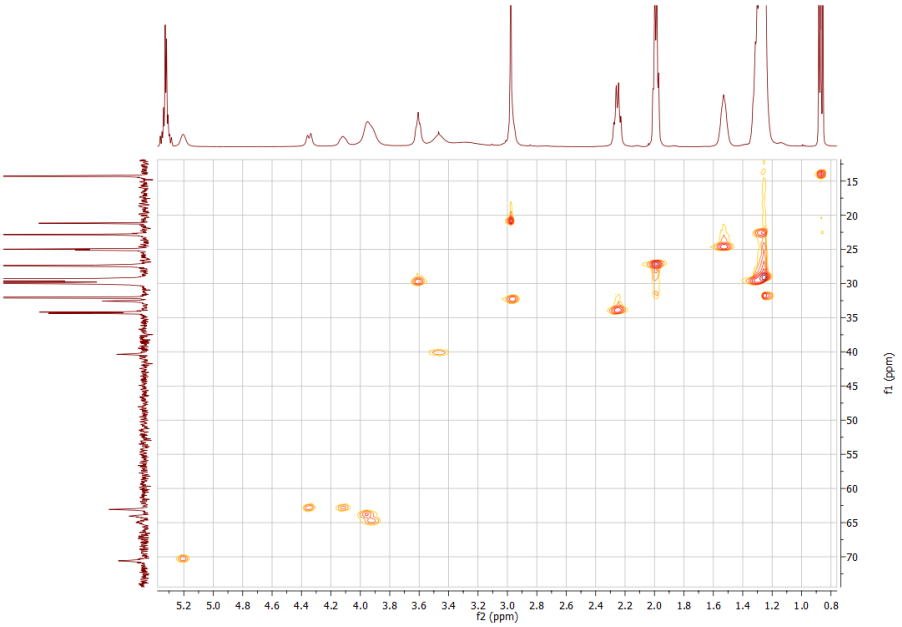
Compound 9: 0.075 mmol (20 mg) of compound **7** was dissolved in 1 mL of $CHCl_3$, 0.050 mmol (0.37 mg) of DOPE and 0072 mmol (10 µL, 7.2 mg) of TEA were added respectively and the reaction mixture was stirred for 30 minutes. 2 mL of $CHCl_3$ was added, the solution was washed with 0.1M HCl (aq.) and the organic layer was directly used for purification. Purification was performed by silica column chromatography using an 2%-5% MeOH in DCM eluent resulting in compound **9**. **^{1}H NMR (500 MHz, $CDCl_3$) δ**: 7.52 (br, 1H), 5.39 – 5.25 (m, 4H), 5.20 (s, 1H), 4.35 (d, *J* = 11.1 Hz, 1H), 4.12 (s, 1H), 3.95 (br, 4H), 3.61 (s, 2H), 3.47 (s, 2H), 3.29 (br, 1H), 2.98 (s, 5H), 2.25 (dd, *J* = 15.6, 7.9 Hz, 4H), 2.04 – 1.92 (m, 8H), 1.53 (s, 4H), 1.38 – 1.15 (m, 36H), 0.87 (t, *J* = 6.9 Hz, 6H). **^{13}C NMR (126 MHz, $CDCl_3$) δ**: 173.87, 173.63, 169.14, 167.47, 130.14, 129.76, 87.18, 70.60, 64.99, 64.04, 63.07, 40.36, 34.35, 34.18, 32.57, 32.05, 29.92, 29.69, 29.48, 29.35, 29.30, 27.38, 25.07, 24.97, 22.83, 21.17, 14.26.

Compound 10: 0.078 mmol (30 mg) of compound **8** was dissolved in 1 mL of $CHCl_3$, 0.050 mmol (0.37 mg) of DOPE and 0072 mmol (10 µL, 7.2 mg) of TEA were added respectively and the reaction mixture was stirred for 30 minutes. 5 mL of $CHCl_3$ was added, the solution was washed with 5 mL 1M HCl (aq.) and the organic layer was directly used for purification. Purification was performed by silica column chromatography using an 2%-5% MeOH in DCM eluent containing 2% AcOH resulting in 32 mg (0.036 mmol, 48%) of compound **10**. **^{1}H NMR**

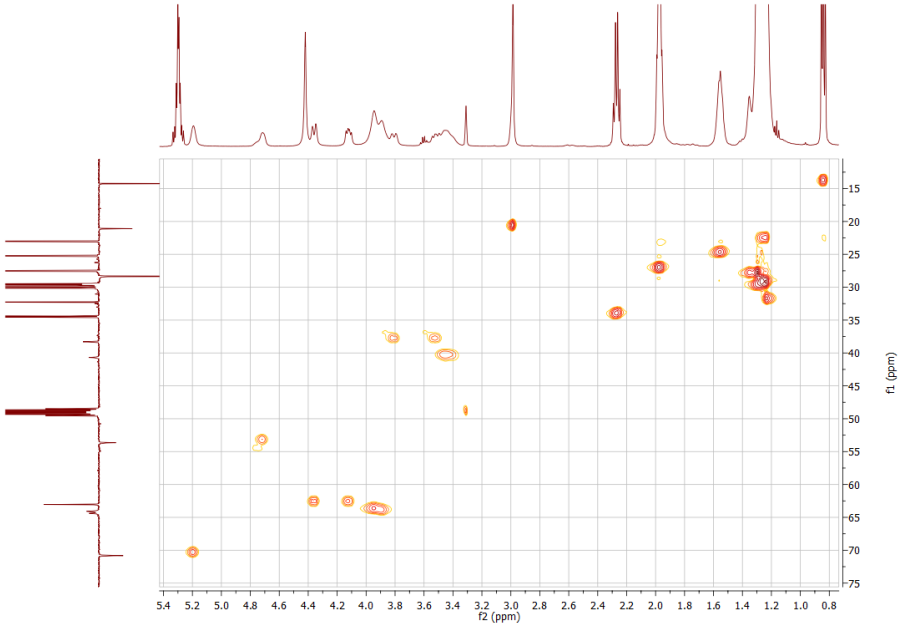
(500 MHz, MeOD) δ: 5.36 – 5.22 (m, 4H), 5.19 (s, 1H), 4.72 (s, 1H), 4.36 (d, *J* = 11.3 Hz, 1H), 4.12 (dd, *J* = 11.5, 7.0 Hz, 1H), 3.94 (s, 2H), 3.89 (s, 2H), 3.81 (d, *J* = 13.0 Hz, 1H), 3.65 – 3.35 (m, 3H), 2.99 (s, 3H), 2.27 (q, *J* = 7.6 Hz, 4H), 1.97 (dd, *J* = 11.5, 6.0 Hz, 8H), 1.56 (d, *J* = 5.1 Hz, 4H), 1.41 – 1.10 (m, 45H), 0.84 (t, *J* = 6.9 Hz, 6H). **^{13}C NMR (126 MHz, MeOD) δ**: 174.24, 173.90, 171.77, 167.89, 167.34, 156.10, 130.33, 129.99, 129.97, 80.60, 70.82, 64.39, 64.08, 63.05, 53.65, 40.69, 38.30, 34.54, 34.39, 32.25, 30.09, 29.85, 29.65, 29.64, 29.62, 29.58, 29.51, 29.49, 29.47, 29.45, 28.34, 27.53, 27.51, 25.25, 25.19, 22.99, 21.09, 14.25.

Compound 11: 10 µmol (10 mg) of compound **10** was dissolved in 1 mL of DCM and 1mL of 4M HCl in dioxane and stirred for 2 hours at room temperature. The reaction mixture was concentrated using rotary evaporation and purified using silica column chromatography using an 1%-20% MeOH in $CHCl_3$ eluent resulting in 3 mg (3,4 µmol, 34%) of compound **11**. **^{1}H NMR (400 MHz, MeOD) δ**: 5.37 – 5.23 (m, 4H), 5.19 (s, 1H), 4.67 (s, 1H), 4.35 (d, *J* = 11.7 Hz, 1H), 4.16 – 4.06 (m, 1H), 3.96 (s, 5H), 3.77 – 3.53 (m, 2H), 3.31 (s, 1H), 3.03 (s, 3H), 2.28 (dd, *J* = 14.2, 6.9 Hz, 4H), 1.97 (d, *J* = 5.2 Hz, 8H), 1.55 (s, 4H), 1.25 (d, *J* = 14.8 Hz, 36H), 0.84 (t, *J* = 6.8 Hz, 6H). **^{13}C NMR (101 MHz, MeOD) δ:** 174.31, 173.98, 168.67, 165.77, 130.35, 129.98, 129.96, 70.66, 64.64, 64.45, 62.92, 51.80, 43.08, 43.00, 40.75, 35.89, 34.55, 34.39, 32.24, 30.08, 29.85, 29.65, 29.63, 29.60, 29.57, 29.50, 29.48, 29.43, 27.52, 27.49, 25.25, 25.19, 22.99, 21.21, 14.25.

HSQC ($CDCl_3$) (compound 9):



HSQC (MeOD) (compound 10):



Compound 12: 0.79 mmol (0.323 g) of compound **44 (Chapter 3)** was dissolved in 2 mL of dry THF and cooled to 0 °C. 1.0 mmol (0.15 mL) of DBU and 1.0 mmol (0.22 mL) of DPPA were added and the reaction mixture was stirred overnight while warming to room temperature. Reaction completion was checked by TLC (Rf = 0.4, 100% pentane). Air was blown through the reaction mixture to evaporate the THF, forming a suspension. Then, 2 mL of a 1:1 (v:v) Pentane:DCM mixture was added to the suspension. The liquid fraction was then separated from the solid and directly purified with silica column chromatography using an 0-2% Et_2O in pentane eluent. Product fractions were collected and carefully concentrated using rotary evaporation (30 °C, 200 mbar) and carefully co-evaporated twice using $CHCl_3$ (30 °C, 100 mbar) resulting in 83 mg (0.47 mmol, 59%) of compound **12** as a yellow oil (unstable at room temperature). **^{1}H NMR (300 MHz, $CDCl_3$) δ**: 3.075 (m, 2H), 2.214 (s, 3H), 1.568 (t, 1H), 0.168 (s, 9H). **^{13}C NMR (75 MHz, $CDCl_3$) δ:** 134.94, 112.53, 59.98, 18.80, 13.85, -1.18.

Compound 13: 1.22 mmol (0.222 g) of compound **12** was dissolved in 2.4 mL of a 5:1 (v:v) THF:H_2O mixture, 1.6 mmol (0.418 g) of PPh_3 was added and the reaction mixture was stirred overnight at room temperature. Reaction completion was checked by TLC (Rf = 0.2, 10% MeOH in DCM). 5 mL of 1M HCl (aq.) was added and the THF was removed using rotary evaporation, and additional 5 mL of 1M HCl (aq.) was added, 10 mL of Et_2O was added and the mixture was stirred vigorously for 10 minutes to dissolve any remaining solids. The layers were separated and the water layer was neutralized to pH = 9 and extracted using DCM. The organic layer was dried using $MgSO_4$, filtered, concentrated using rotary evaporation, co-evaporated twice using $ChCl_3$. The resulting product, 0.14 g (0.90 mmol, 73.6%) of compound **13** as a yellow oil, was used without further purification. **^{1}H NMR (300 MHz, $CDCl_3$) δ**: 2.588 (m, 2H),

2.251 (s, 3H), 1.435 (t, 1H), 1.5-1.4 (br, 1H), 0.145 (s, 9H). **^{13}C NMR (75 MHz, $CDCl_3$) δ:** 136.90, 112.64, 48.81, 23.55, 13.67, -0.87.

Compound 14: 0.11 mmol (0.044 g) of compound **22 (chapter 3)** was dissolved in 1.5 mL dry DMF, 0.32 mmol (0.050 g) of compound 1**2** and 0.43 mmol (60 µL) of TEA were added respectively and the reaction mixture was stirred for 30 minutes at room temperature. Reaction completion was checked by TLC (Rf = 0.4, 10% EtOAc in DCM). 15 mL of EtOAc was added and the reaction mixture was washed with 0.1M HCl (aq.), washed with sat. $NAHCO_3$ (aq.), washed with brine, dried using $MgSO_4$, filtered and concentrated using rotary evaporation. Purification was performed with silica column chromatography using an 10-20% EtOAc in DCM eluent resulting in 43 mg (0.10 mmol, 91%) of compound **14** as a red solid. **^{1}H NMR (400 MHz, $CDCl_3$) δ**: 7.07 (s, 1H), 6.86 (d, *J* = 3.9 Hz, 1H), 6.28 (d, *J* = 4.0 Hz, 1H), 6.10 (s, 1H), 5.55 (s, 1H), 3.26 (t, *J* = 7.6 Hz, 2H), 3.10 (ddt, *J* = 66.2, 13.5, 5.1 Hz, 2H), 2.60 (t, *J* = 7.6 Hz, 2H), 2.55 (s, 3H), 2.24 (s, 3H), 2.13 (s, 3H), 1.37 (t, *J* = 4.7 Hz, 1H), 0.12 (s, 8H). **^{13}C NMR (101 MHz, $CDCl_3$) δ:** 71.39, 160.10, 157.85, 143.82, 135.98, 135.14, 133.51, 128.44, 123.86, 120.42, 117.67, 111.81, 77.48, 77.16, 76.84, 46.43, 36.43, 25.24, 19.24, 15.03, 13.21, 11.42, -1.02.

Compound 15: 2.49 mmol (0.381 g) of compound **19 (Chapter 3)** was dissolved in 20 mL of Et_2O and cooled to 0 °C. 4.0 mmol (2 mL of a 2M solution in THF) of $LiAlH_4$ was added dropwise to the solution and the reaction mixture was stirred overnight while warming to room temperature. Reaction completion was checked by TLC (Rf = 0.15, 10% EtOAc in pentane). The reaction was quenched by dropwise addition of 5 mL of a 1M NaOH (aq.) solution over 5 minutes while stirring vigorously. The organic layer was separated from the water layer, 5 mL of Et_2O was added to the water layer while stirring for 5 minutes, before separating the organic layer. The organic layers were combined, dried using Na_2SO_4, filtered and concentrated using rotary evaporation. The resulting product, 0.306 g (2.44 mmol, 98%) of compound **15** as a pale oil, was used without further purification.

Compound 16: 2.0 mmol (0.252 g) of compound **15** was dissolved in 10 mL of dry DCM and cooled to 0 °C. 3.9 mmol (0.55 mL) of TEA, and 2.6 mmol (0.20 mL) of methanesulfonyl chloride were added and the reaction mixture was stirred for 1 hour. Reaction completion was checked by TLC (Rf = 0.8, 75% EtOAc in pentane). 50 mL of DCM was added to the reaction mixture and the solution was washed with 1M HCl (aq.), washed with sat. $NaHCO_3$ (aq.), dried using $MgSO_4$, filtered and concentrated using rotary evaporation. The resulting product, 1.55 mmol (0.314 g, 77.5%) of compound **16**, was used without further purification.

Compound 17: 0.144 mmol (0.292 g) of compound **16** was dissolved in 8 mL dry DMF, 4.61 mmol (0.30 g) of NaN_3 was added and the reaction mixture was tired overnight at 70 °C. Reaction completion was checked by TLC (Rf = 0.85, 50% EtOAc in pentane). 20 mL of EtOAc was added to the reaction mixture and the solution was washed with 20 mL of water. The water layer was extracted twice using 10 mL EtOAc, all organic layers were combined and the resulting solution was washed multiple times using brine until the organic layer became clear. The organic layer was then dried using $MgSO_4$, filtered and concentrated using rotary evaporation. The resulting product, (quantitative) compound **17**, was used without further purification. **1H NMR (400 MHz, CDCl3) δ:** 6.69 (dd, J = 4.0, 2.5 Hz, 1H), 6.16 (dd, J = 5.7, 2.8

Hz, 1H), 5.96 (s, 1H), 3.34 (t, J = 6.7 Hz, 2H), 2.72 (t, J = 7.4 Hz, 2H), 1.98 – 1.86 (m, 2H). **13C NMR (101 MHz, CDCl3) δ**: 130.77, 116.66, 108.54, 105.58, 50.75, 28.96, 24.73.

Compound 18: 1.9 mmol (0.27 g) of compound **17** was dissolved in dry DCM and cooled to 0 °C, 2.1 mmol (0.260 g) of 3,5-dimethyl-1H-pyrrole-2-carboxaldehyde and 2.1 mmol (0.20 mL) of $POCl_3$ were added respectively and the reaction mixture was stirred for 6 hours while warming to room temperature. The reaction mixture was again cooled to 0 °C and 8.6 mmol (1.2 mL) of TEA was dropwise added to the solution, followed by dropwise addition of 7.6 mmol (0.94 mL) of BF_3 etherate and the reaction mixture was stirred overnight. Reaction completion was checked by TLC (Rf = 0.8, 25% EtOAc in pentane). The reaction mixture was poured into 400 mL EOAc, washed with 1M HCl (aq.), washed with sat. $NAHCO_3$ (Aq.), washed with brine, dried using $MGSO_4$, filtered and concentrated using rotary evaporation. Purification was performed with silica column chromatography using an 5-10% EtOAc in pentane eluent resulting in 55 mg (0.181 mmol, 9.5%) of compound **18** as a black solid. **1H NMR (400 MHz, CDCl3) δ:** 7.07 (s, 1H), 6.89 (d, J = 3.9 Hz, 1H), 6.26 (d, J = 3.9 Hz, 1H), 6.10 (s, 1H), 3.38 (t, J = 7.0 Hz, 2H), 3.04 (t, J = 7.7 Hz, 2H), 2.55 (s, 3H), 2.23 (s, 3H), 2.10 – 1.93 (m, 2H). **13C NMR (101 MHz, CDCl3) δ**: 160.25, 157.78, 143.85, 135.20, 133.33, 128.26, 123.82, 120.47, 116.72, 51.03, 28.22, 25.88, 15.01, 11.36.

Compound 19: 30 µmol (10 mg) of compound **18** was dissolved in 1 mL of THF, 100 µL of H_2O and 60 µmol (38 mg, 1.6 mmol/g) of polymer-bound PPh_3 were added and the reaction mixture was stirred for two days at room temperature. Reaction completion was checked by TLC (Rf = 0.2, 10% TEA in EtOAc). 6 mL of 0.1M HCl (aq.) was added and the water layer was washed three times with Et_2O. To the water layer 2 mL of sat. $NaHCO_3$ (aq.) was added and extracted three times with EtOAc. The organic layers were combined, dried using $MgSO_4$, filtered and concentrated using rotary evaporation. The obtained crude bodipy-amine intermediate was directly used by dissolving it in 1 mL of DMF, 110 µmol (27 mg) of compound **35 (chapter 3)** was added and the reaction mixture was stirred for 1 hour. Reaction completion was checked by TLC (Rf = 0.9, 100% EtOAc). 5 mL of 0.1M HCL (aq.) was added and the watery solution was extracted twice with EtOAc. The organic layers were combined, washed with brine and concentrated using rotary evaporation. Purification was performed with silica column chromatography using an 25-35% EtOAc in pentane eluent resulting in 1 mg (2.5 µmol, 8%) of compound **19**. **^{1}H NMR (400 MHz, $CDCl_3$) δ:** 7.08 (s, 1H), 6.92 (d, *J* = 4.1 Hz, 1H), 6.31 (d, *J* = 4.1 Hz, 1H), 6.12 (t, *J* = 4.2 Hz, 2H), 6.09 – 5.99 (m, 2H), 3.39 – 3.22 (m, 2H), 3.02 (t, *J* = 7.3 Hz, 2H), 2.93 (s, 1H), 2.89 (s, 1H), 2.56 (s, 3H), 2.26 (s, 3H), 1.95 (dd, *J* = 15.5, 9.2 Hz, 3H), 1.91 – 1.85 (m, 1H), 1.68 (d, *J* = 8.3 Hz, 2H), 1.36 – 1.20 (m, 4H).

Compound 20: 30 µmol (10 mg) of compound **18** was dissolved in 1 mL of THF, 200 µL of H_2O and 40 µmol (10 mg) of PPh_3 were added and the reaction mixture was stirred overnight at room temperature. Reaction completion was checked by TLC (Rf = 0.2, 10% TEA in EtOAc). 10 mL of 1.2M HCl (aq.) was added and the water layer was washed two times with Et_2O. To the water layer 50 mL of sat. $NaHCO_3$ (aq.) was added and extracted once with DCM and two times with EtOAc. The organic layers were combined, dried using $MgSO_4$, filtered and concentrated using rotary evaporation. The obtained crude bodipy-amine intermediate was directly used by dissolving it in 0.5 mL of DMF, 60 µmol (15 mg) of compound **42 (chapter 3)** was added and the reaction mixture was stirred overnight at room temperature. Reaction completion

was checked by TLC (Rf = 0.6, 100% EtOAc). 5 mL of 0.1M HCL (aq.) was added and the watery solution was extracted twice with EtOAc. The organic layers were combined, washed with brine and concentrated using rotary evaporation. Purification was performed with silica column chromatography using an 25-50% EtOAc in pentane eluent resulting in 5.0 mg (14 µmol, 47%) of compound **20** as a red solid. The NMR showed a non-removable PPh_3 impurity, and HPLC purification was required to remove this impurity. **^{1}H NMR (400 MHz, $CDCl_3$) δ:** 7.09 (s, 1H), 6.92 (d, *J* = 4.0 Hz, 1H), 6.40 (dt, *J* = 2.6, 1.2 Hz, 1H), 6.30 (d, *J* = 4.1 Hz, 1H), 6.12 (s, 1H), 5.99 (s, 1H), 3.37 – 3.22 (m, 2H), 2.98 (t, *J* = 7.3 Hz, 2H), 2.57 (s, 3H), 2.26 (s, 3H), 2.16 (d, *J* = 1.2 Hz, 3H), 2.00 (d, *J* = 1.6 Hz, 1H), 1.97 – 1.87 (m, 2H).

Compound 21: 0.40 mmol (94 mg) of compound **35 (chapter 3)** was dissolved in 2 mL of DCM, 0.80 mmol (51 µL) of propargylamine and 0.80 mmol (111 µL) of TEA were added respectively and the reaction mixture was stirred for 1 hour at room temperature. Reaction completion was checked by TLC (Rf = 0.75 in 10% EtOAc/DCM). The crude mixture was directly used for purification by silica column chromatography using an EtOAc in DCM eluent, resulting in 65 mg (0.37 mmol, 93%) of compound **21** as a white solid. **^{1}H NMR (400 MHz, $CDCl_3$) δ:** 6.27 (s, 1H), 6.10 (dd, *J* = 5.6, 2.9 Hz, 1H), 6.05 (dd, *J* = 5.6, 3.0 Hz, 1H), 4.01 (dd, *J* = 5.3, 2.6 Hz, 2H), 2.90 (d, *J* = 1.3 Hz, 1H), 2.87 (s, 1H), 2.19 (t, *J* = 2.6 Hz, 1H), 2.06 – 1.98 (m, 1H), 1.92 – 1.81 (m, 1H), 1.67 (d, *J* = 8.2 Hz, 1H), 1.35 – 1.23 (m, 2H). **^{13}C NMR (101 MHz, $CDCl_3$) δ:** 175.58, 138.30, 135.98, 79.94, 77.48, 71.37, 47.12, 46.36, 44.35, 41.58, 30.49, 29.28.

Compound 22: 0.47 mmol (116 mg) of compound **45 (chapter 3)** was dissolved in 2.5 mL of DCM, 1.0 mmol (64 µL) of propargylamine and 1.0 mmol (140 µL) of TEA were added respectively and the reaction mixture was stirred overnight at room temperature. Reaction completion was checked by TLC (Rf = 0.30 in 10% EtOAc/Pentane). The crude mixture was directly used for purification by silica column chromatography using an 5%-10% EtOAc in DCM eluent resulting in 58 mg (0.35 mmol, 75%) of compound **22** as a colorless oil. **^{1}H NMR (400 MHz, $CDCl_3$) δ:** 6.53 (s, 1H), 5.02 (s, 1H), 4.05 – 3.81 (m, 4H), 2.22 (t, *J* = 2.5 Hz, 1H), 2.10 (d, *J* = 0.9 Hz, 3H), 1.61 (t, *J* = 4.5 Hz, 1H). **^{13}C NMR (101 MHz, $CDCl_3$) δ:** 156.49, 120.70, 102.12, 80.09, 72.87, 71.45, 30.82, 17.20, 11.72.

Compound 23: 0.18 mmol (50 mg) of *p*-nitrophenyl 2-azetine-succinate was dissolved in 2 mL of DCM, 0.91 mmol (58 µL) of propargylamine and 0.90 mmol (125 µL) of TEA were added respectively and the reaction mixture was stirred overnight at room temperature. Reaction completion was checked by TLC (Rf = 0.25 in 100% EtOAc). The crude mixture was directly used for purification by silica column chromatography using an 50%-100% EtOAc in pentane eluent, followed by silica column chromatography using an 75%-100% EtOAc in DCM eluent resulting in 16 mg (0.083 mmol, 46%) of compound **23** as a white solid. **^{1}H NMR (400 MHz, $CDCl_3$) δ**: 6.77 (d, *J* = 78.1 Hz, 1H), 6.65 (s, 1H), 5.72 (d, *J* = 12.1 Hz, 1H), 4.51 (d, *J* = 57.1 Hz, 2H), 4.04 – 3.98 (m, 2H), 2.68 – 2.57 (m, 2H), 2.56 (s, 2H), 2.20 (t, *J* = 2.5 Hz, 1H). **^{13}C NMR (101 MHz, $CDCl_3$) δ**: 171.75, 166.12, 137.34, 136.99, 114.15, 114.06, 79.74, 71.44, 58.87, 56.83, 30.91, 30.81, 29.29, 27.41, 26.45.

Compound 24: 0.069 mmol (20 mg) of compound **57a (chapter 3)** was dissolved in 0.5 mL of DCM, 0.34 mmol (22 µL) of propargylamine and 0.34 mmol (48 µL) of TEA were added respectively and the reaction mixture was stirred overnight at room temperature. Reaction

completion was checked by TLC (Rf = 0.60 in 20% EtOAc/Pentane). 20 mL of DCM was added and the reaction mixture was washed twice with sat. $NaHCO_3$ (aq.), washed twice with 10% $KHSO_4$ (aq.), dried with $MgSO_4$, filtered and concentrated using rotary evaporation. Purification was performed by silica column chromatography using an 5%-10% EtOAc in pentane eluent resulting in 13 mg (0.63 mmol, 91%) of compound **24** as a colorless oil. **^{1}H NMR (400 MHz, $CDCl_3$) δ**: 5.63 – 5.47 (m, 2H), 5.01 – 4.86 (m, 2H), 3.99 (dd, *J* = 5.7, 2.4 Hz, 2H), 2.39 – 2.21 (m, 5H), 2.21 – 2.01 (m, 2H), 1.91 – 1.73 (m, 2H), 1.73 – 1.60 (m, 1H), 1.60 – 1.43 (m, 1H), 1.28 – 1.14 (m, 1H). **^{13}C NMR (101 MHz, $CDCl_3$) δ**: 155.75, 135.49, 131.86, 80.00, 71.70, 70.95, 41.16, 34.41, 32.69, 30.93, 30.08, 28.13.

Compound 25: 38 µmol (9 mg) of compound **35 (chapter 3)** was dissolved in 0.2 mL of DCM, 40 µmol (11 mg) of DBCO-amine and 43 µmol (6 µL) of TEA were added respectively and the reaction mixture was stirred for 1 hour at room temperature. Reaction completion was checked by TLC (Rf = 0.4 in 20% EtOAc/DCM). The crude mixture was directly used for purification by silica column chromatography using an 10%-20% EtOAc in DCM eluent, resulting in 11 mg (28 µmol, 74%) of compound **25** as a colorless oil. **^{1}H NMR (400 MHz, $CDCl_3$) δ:** 7.69 (d, *J* = 7.6 Hz, 1H), 7.44 – 7.20 (m, 7H), 6.11 – 6.05 (m, 1H), 6.05 – 5.93 (m, 2H), 5.15 (d, *J* = 13.9 Hz, 1H), 3.70 (d, *J* = 13.9 Hz, 1H), 3.41 – 3.20 (m, 2H), 2.84 (s, 1H), 2.68 (dd, *J* = 88.0, 1.4 Hz, 1H), 2.50 – 2.36 (m, 1H), 2.05 – 1.90 (m, 1H), 1.78 (ddd, *J* = 5.4, 4.6, 2.2 Hz, 1H), 1.73 – 1.61 (m, 1H), 1.52 (dd, *J* = 30.1, 8.4 Hz, 1H), 1.28 – 1.23 (m, 1H), 1.22 – 1.13 (m, 1H). **^{13}C NMR (101 MHz, $CDCl_3$) δ:** 175.45, 175.28, 172.49, 172.43, 151.18, 151.13, 148.11, 138.16, 138.14, 136.19, 136.16, 132.21, 129.15, 128.71, 128.69, 128.55, 128.45, 127.96, 127.40, 125.79, 125.69, 123.10, 123.07, 122.62, 114.85, 107.92, 107.90, 55.63, 53.57, 47.43, 46.88, 46.49, 46.31, 44.74, 44.61, 41.66, 41.65, 35.31, 35.20, 35.10, 30.61, 30.05.

Compound 26: 41 µmol (12 mg) of compound **57a (chapter 3)** was dissolved in 0.2 mL of DCM, 40 µmol (11 mg) of DBCO-amine and 100 µmol (14 µL) of TEA were added respectively and the reaction mixture was stirred overnight at room temperature. Reaction completion was checked by TLC (Rf = 0.55 in 50% EtOAc/pentane). The crude mixture was directly used for purification by silica column chromatography using an 20%-40% EtOAc in pentane eluent, followed by silica column chromatography using an 20%-5% EtOAc in DCM eluent resulting in 12 mg (27 µmol, 66%) of compound **26** as a colorless oil. **^{1}H NMR (400 MHz, $CDCl_3$) δ**: 7.70 (d, *J* = 7.5 Hz, 1H), 7.44 – 7.19 (m, 7H), 5.57 – 5.38 (m, 2H), 5.16 (d, *J* = 14.0 Hz, 1H), 5.12 – 5.08 (m, 1H), 4.85 – 4.76 (m, 1H), 3.71 (d, *J* = 13.9 Hz, 1H), 3.36 – 3.11 (m, 2H), 2.49 (m, 1H), 2.32 – 2.13 (m, 4H), 2.13 – 1.89 (m, 2H), 1.88 – 1.66 (m, 2H), 1.59 (m, 1H), 1.56 – 1.21 (m, 1H), 1.21 – 1.05 (m, 1H). **^{13}C NMR (101 MHz, $CDCl_3$) δ**: 172.23, 156.15, 151.23, 148.12, 135.65, 135.53, 132.12, 132.08, 131.78, 131.62, 129.24, 129.19, 128.64, 128.52, 128.51, 128.00, 127.35, 127.32, 125.85, 125.80, 122.96, 122.79, 115.03, 107.74, 70.09, 69.97, 55.71, 55.68, 41.20, 41.17, 36.95, 36.79, 35.58, 35.38, 34.52, 34.45, 32.78, 30.06, 28.05.

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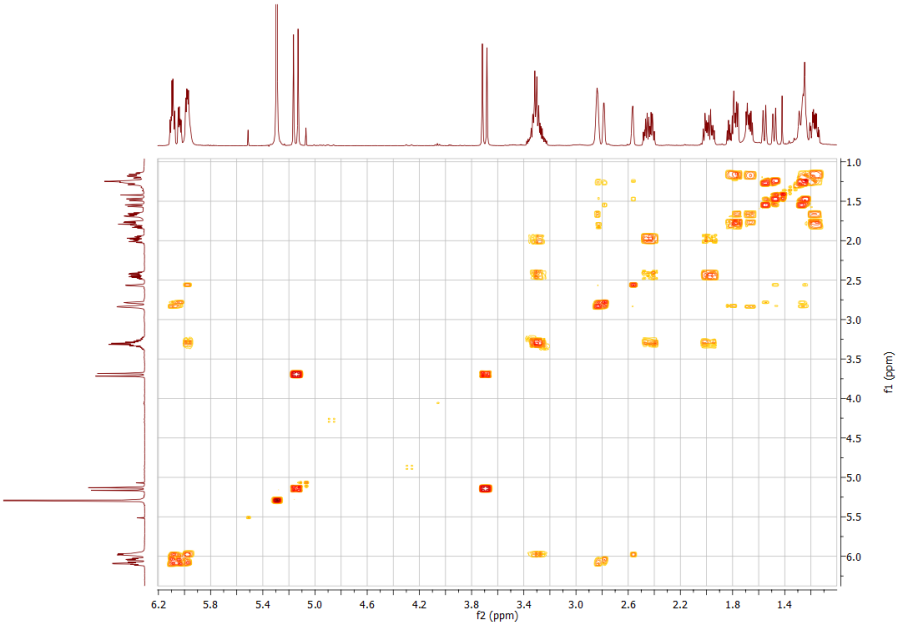

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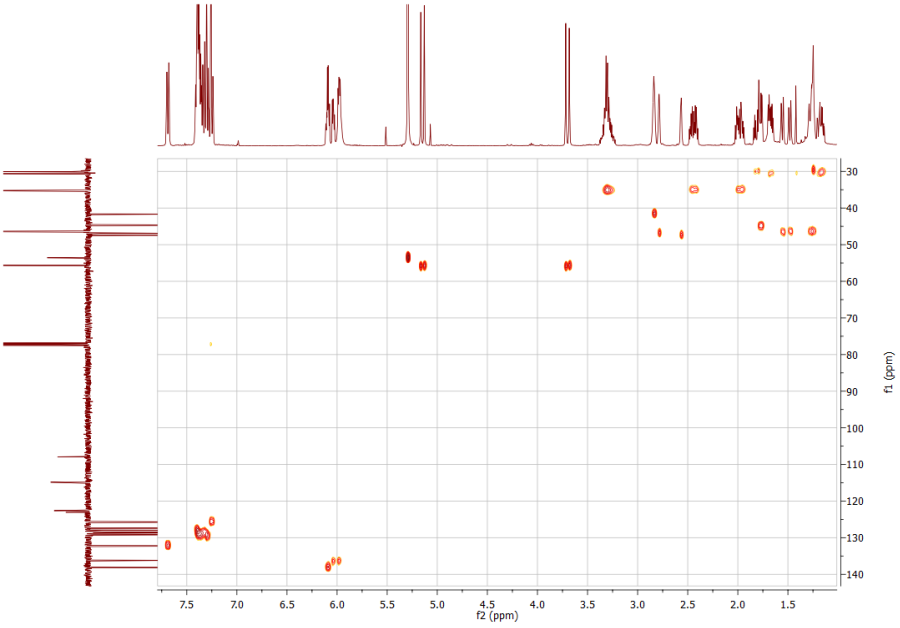

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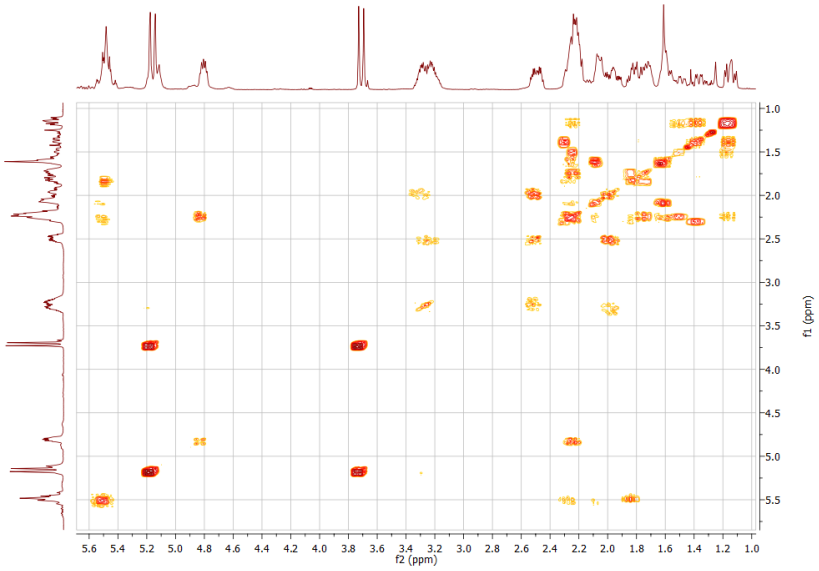

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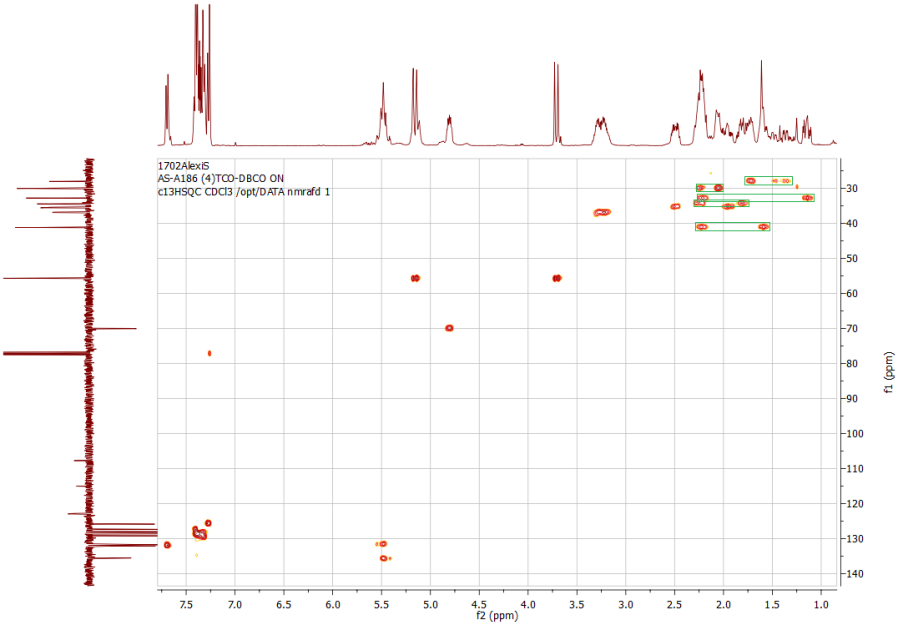


Compound 27: 0.24 mmol (63 mg) of compound **42** was dissolved in 0.5 mL dimethylformamide, 0.36 mmol (130 mg) Fmoc-Dap-OH hydrochloride, 0.36 mmol (63 µL) DiPEA were added and the reaction mixture was stirred overnight at room temperature. Reaction completion was checked by TLC tracking the consumption of starting material (Rf = 0.8, 10% Et_2O in pentane) and the formation of product (Rf = 0.1, 100% EtOAc + 0.5% AcOH). 10 mL of 0.1M HCl (aq.) and 2 mL brine were added to the reaction mixture and the resulting solution was extracted twice with 10 mL EtOAc. The organic layers were combined, dried using $MgSO_4$, filtered and concentrated using rotary evaporation. Purification was performed with silica column chromatography using an 1-5% MeOH in dichloromethane containing 1% AcOH eluent resulting in 0.57 mg (0.14 mmol, 58%) of compound **27** as a colorless oil. **TLC**: Rf = 0.2, 100% EtOAc + 0.5% AcOH). **^{1}H NMR (400 MHz, $CDCl_3$) δ**: 9.42 (s, 1H), 7.73 (d, *J* = 7.4 Hz, 2H), 7.58 (t, *J* = 7.6 Hz, 2H), 7.37 (t, *J* = 7.3 Hz, 2H), 7.31 – 7.21 (m, 2H), 6.57 (d, *J* = 5.1 Hz, 1H), 6.43 – 6.22 (m, 2H), 4.54 – 4.28 (m, 3H), 4.18 (t, *J* = 6.9 Hz, 1H), 3.81 – 3.56 (m, 2H), 2.07 (d, *J* = 5.9 Hz, 3H), 2.01 (s, 1H). **^{13}C NMR (101 MHz, $CDCl_3$) δ:** 178.99, 172.50, 156.66, 143.89, 143.69, 141.34, 141.31, 127.85, 127.23, 125.27, 120.07, 113.23, 67.47, 67.43, 55.05, 54.86, 47.11, 41.89, 22.18, 10.55, 10.52.

Compound 28: 0.20 mmol (52 mg) of compound **42** was dissolved in 1 mL dimethylformamide, 0.30 mmol (110 mg) Fmoc-Lys-OH was added and the reaction mixture was stirred overnight at room temperature. Reaction completion was checked by TLC tracking the consumption of starting material (Rf = 0.8, 10% Et_2O in pentane) and the formation of product (Rf = 0.1, 100% EtOAc + 0.5% AcOH). 10 mL of 0.1M HCl (aq.) and 4 mL brine were added to the reaction mixture and the resulting solution was extracted with 10 mL EtOAc. The organic layer was dried using $MgSO_4$, filtered and concentrated using rotary evaporation. Purification was performed with silica column chromatography using an 1-5% MeOH in dichloromethane containing 1% AcOH eluent resulting in 0.78 mg (0.174 mmol, 87%) of compound **28** as a colorless oil. **TLC**: Rf = 0.2, 100% EtOAc + 0.5% AcOH). **^{1}H NMR (400 MHz, $CDCl_3$) δ:** 9.28 (s, 1H), 7.73 (d, *J* = 7.5 Hz, 2H), 7.62 – 7.55 (m, 2H), 7.37 (t, *J* = 7.4 Hz, 2H), 7.31 – 7.24 (m, 2H), 6.35 (s, 1H), 5.96 (t, *J* = 8.8 Hz, 1H), 5.91 (d, *J* = 2.8 Hz, 1H), 4.39 (dd, *J* = 12.9, 7.3 Hz, 1H), 4.33 (d, *J* = 7.2 Hz, 2H), 4.17 (t, *J* = 7.1 Hz, 1H), 3.24 (pd, *J* = 13.4, 6.7 Hz, 2H), 2.09 (s, 3H), 1.99 (s, 1H), 1.95 – 1.71 (m, 2H), 1.50 (dd, *J* = 13.3, 6.5 Hz, 2H), 1.46 – 1.31 (m, 2H**). ^{13}C NMR (101 MHz, $CDCl_3$) δ:** 177.72, 177.68, 174.72, 156.38, 144.00, 143.81, 141.31, 127.76, 127.16, 125.28, 125.25, 120.01, 113.55, 113.48, 67.08, 53.75, 47.19, 39.32, 39.24, 32.02, 29.19, 22.31, 22.24, 10.65.

Compound 32 (A): 0.25 mmol (1.25 g) of 0.2 mmol/g Fmoc-Leucine preloaded Tentagel S and 1 mmol of Fmoc-Lys(Boc)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Phe-OH, Fmoc-Asn(Trt)-OH, Fmoc-Ile-OH (2x), Fmoc-Ser(tBu)-OH were used in solid phase peptide synthesis to prepare Fmoc-SIINFEKL-OH **32** as a colorless solid. LC-MS analysis: (Alltima C_{18} analytical column, linear gradient in 12.5 minutes; H_2O: 80 → 0% ; ACN: 10 → 90% ; aq. 0.5% TFA: 10%) **LC:** Rt: 6.34 min **MS:** $[C_{60}H_{84}N_{10}O_{15}]^+$: found 1185.6, calculated 1185.6.

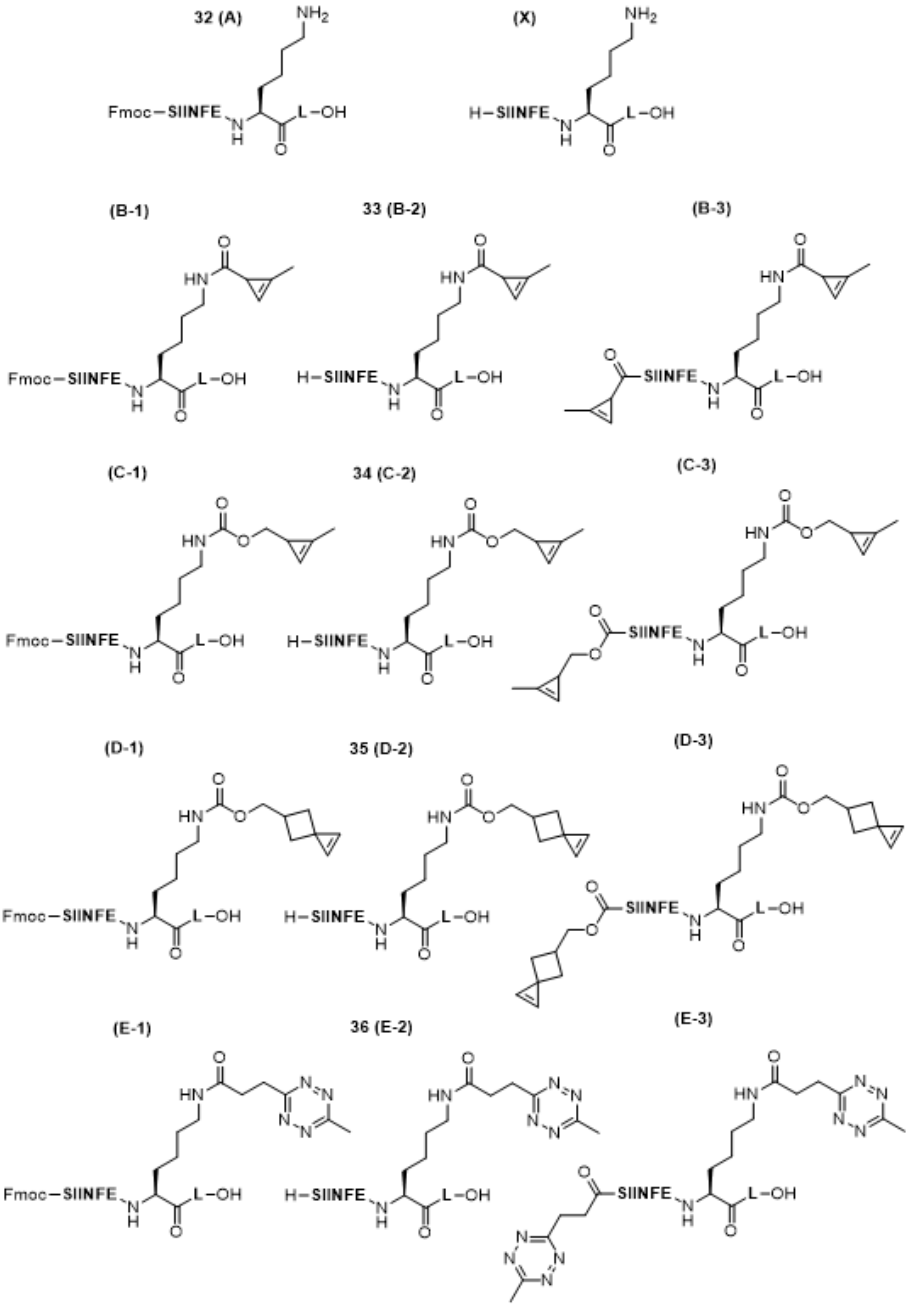
Compound 33 (B-2): **(Step 1)** 23 µmol (27 mg) of compound **32** was added to 3 mL DMF, 0.47 mmol (44 µL) N-methyl morpholine and 98 µmol (26 mg) of compound **42 (chapter 3)** were added to the solution and the reaction mixture was stirred for three days at room temperature. Reaction completion was checked by LC-MS tracking the consumption of starting compound

32. **(Step 2)** 0.75 mL of piperidine was added to form a 20% piperidine in DMF solution and the reaction mixture was stirred for 2 hours at room temperature. Reaction completion was checked by LC-MS tracking the consumption of intermediate **B-1**. The solution was dropwise titrated into an 25 mL of Et_2O, cooled to -20 °C for 30 minutes and centrifuged to form a crude pellet containing the product **33** (**B-2**), as well as double functionalized byproduct **B-3**, and unknown likely-oxidized byproduct **B-1^{ox}**. The crude product was then purified using C_{18}-reverse phase HPLC to obtain compound **B-2** as a white powder. LC-MS analysis: (Alltima C_{18} analytical column, linear gradient in 12.5 minutes; H_2O: 80 → 0% ; ACN: 10 → 90% ; aq. 0.5% TFA: 10%) **LC:** Rt: 5.2 min (**32**), 5.9 min (**B-3**), 6.8 min (**B-1^{ox}**), 7.5 min (**B-1**), **MS:** $[C_{65}H_{89}N_{10}O_{16}]^+$: found 1265.4, calculated 1265.6 (**B-1**), $[C_{50}H_{79}N_{10}O_{14}]^+$: found 1043.4, calculated 1043.6 (**32**), $[C_{55}H_{83}N_{10}O_{15}]^+$: found 1123.4, calculated 1123.6 (**B-3**) , $[C_{65}H_{89}N_{10}O_{17}]^+$: found 1281.3, calculated 1281.6 (**B-1^{ox}**)

Compound 34 (C-2): **(Step 1)** 31 µmol (35 mg) of compound **32** was added to 2 mL DMF, 236 µmol (26 µL) N-methyl morpholine and 120 µmol (30 mg) of compound **45 (chapter 3)** were added to the solution and the reaction mixture was stirred for five days at room temperature. Reaction completion was checked by LC-MS tracking the consumption of starting compound **32**. **(Step 2)** 0.5 mL of piperidine was added to form a 20% piperidine in DMF solution and the reaction mixture was stirred for 2 hours at room temperature. Reaction completion was checked by LC-MS tracking the consumption of intermediate **C-1**. The solution was dropwise titrated into an 25 mL of Et_2O, cooled to -20 °C for 30 minutes and centrifuged to form a crude pellet containing the product **34**, as well as double functionalized byproduct **C-3**, unprotected peptide **X**, and unknown likely-oxidized byproducts **32^{ox}** and **C-1^{ox}**. The crude product was then purified using C_{18}-reverse phase HPLC to obtain compound **34** as a white powder. LC-MS analysis: (Alltima C_{18} analytical column, linear gradient in 12.5 minutes; H_2O: 80 → 0% ; ACN: 10 → 90% ; aq. 0.5% TFA: 10%) **LC:** Rt: 4.7 min (**X**), 5.6 min (**34**), 7.0 min (**C-3**), 7.3 min (**C-1^{ox}**), 7.9 min (**C-1**), **MS:** $[C_{45}H_{75}N_{10}O_{13}]^+$: found 963.5, calculated 963.6 (**X**), $[C_{60}H_{85}N_{10}O_{15}]^+$: found 1185.6, calculated 1185.6 (**32**), $[C_{60}H_{85}N_{10}O_{16}]^+$: found 1201.5, calculated 1201.6 (**32^{ox}**), $[C_{66}H_{91}N_{10}O_{17}]^+$: found 1295.3, calculated 1295.7 (**C-1**), $[C_{66}H_{91}N_{10}O_{18}]^+$: found 1311.3, calculated 1311.7 (**C-1^{ox}**), $[C_{51}H_{81}N_{10}O_{15}]^+$: found 1073.5, calculated 1073.6 (**34**), $[C_{57}H_{87}N_{10}O_{17}]^+$: found 1183.3, calculated 1183.6 (**C-3**).

Compound 35 (D-2): **(Step 1)** 37 µmol (44 mg) of compound **32** was added to 1 mL DMF, 199 µmol (22 µL) N-methyl morpholine and 80 µmol (20 mg) of compound **52 (chapter 3)** were added to the solution and the reaction mixture was stirred for three days at room temperature. Reaction completion was checked by LC-MS tracking the consumption of starting compound **32**. **(Step 2)** 0.25 mL of piperidine was added to form a 20% piperidine in DMF solution and the reaction mixture was stirred for 2 hours at room temperature. Reaction completion was checked by LC-MS tracking the consumption of intermediate **D-1**. The solution was dropwise titrated into 25 mL of Et_2O, cooled to -20 °C for 30 minutes and centrifuged to form a crude pellet containing the product **35**, as well as double functionalized byproduct **D-3**. The crude product was then purified using C_{18}-reverse phase HPLC to obtain compound **35** as a white powder. LC-MS analysis: (Alltima C_{18} analytical column, linear gradient in 12.5 minutes; H_2O: 80 → 0% ; ACN: 10 → 90% ; aq. 0.5% TFA: 10%) **LC:** Rt: 6.0 min (**35**), 7.6 min (**D-3**), 7.4 min (**D-1**), **MS:** $[C_{68}H_{93}N_{10}O_{17}]^+$: found 1322.0, calculated 1321.7 (**D-1**), $[C_{53}H_{83}N_{10}O_{15}]^+$: found 1099.5, calculated 1099.6 (**35**), $[C_{61}H_{91}N_{10}O_{17}]^+$: found 1235.4, calculated 1235.7 (**D-3**).

Compound 36 (E-2): **(Step 1)** 31 µmol (35 mg) of compound **32** was added to 3 mL DMF, 146 µmol (16 µL) N-methyl morpholine and 41 µmol (11 mg) of compound **3 (chapter 5)** were added to the solution and the reaction mixture was stirred for three days at room temperature. Reaction completion was checked by LC-MS tracking the consumption of starting compound **32**. **(Step 2)** 0.75 mL of piperidine was added to form a 20% piperidine in DMF solution and the reaction mixture was stirred overnight at room temperature. Reaction completion was checked by LC-MS tracking the consumption of intermediate **E-1**. The solution was dropwise titrated into an 25 mL of Et_2O, cooled to -20 °C for 30 minutes and centrifuged to form a crude pellet containing the product **36**, as well as double functionalized byproduct **E-3**, and unknown likely-oxidized byproduct **E-1^{ox}**. The crude product was then purified using C_{18}-reverse phase HPLC to obtain compound **36** as a powder. LC-MS analysis: (Alltima C_{18} analytical column, linear gradient in 12.5 minutes; H_2O: 80 → 0% ; ACN: 10 → 90% ; aq. 0.5% TFA: 10%) **LC:** Rt: 4.4 min (**36**), 5.0 min (**E-3**), 5.9 min (**E-1^{ox}**), 6.6 min (**E-1**), **MS:** $[C_{66}H_{91}N_{14}O_{16}]^+$: found 1335.9, calculated 1335.7 (**E-1**), $[C_{66}H_{91}N_{14}O_{17}]^+$: found 1351.8, calculated 1351.6 (**E-1^{ox}**), $[C_{51}H_{81}N_{14}O_{14}]^+$: found 1113.5, calculated 1113.6 (**36**), $[C_{57}H_{87}N_{18}O_{15}]^+$: found 1263.7, calculated 1263.7 (**E-3**).


32 (A)
(X)
(B-1)
33 (B-2)
(B-3)
(C-1)
34 (C-2)
(C-3)
(D-1)
35 (D-2)
(D-3)
(E-1)
36 (E-2)
(E-3)
Fmoc–SIINFE
H–SIINFE
L–OH

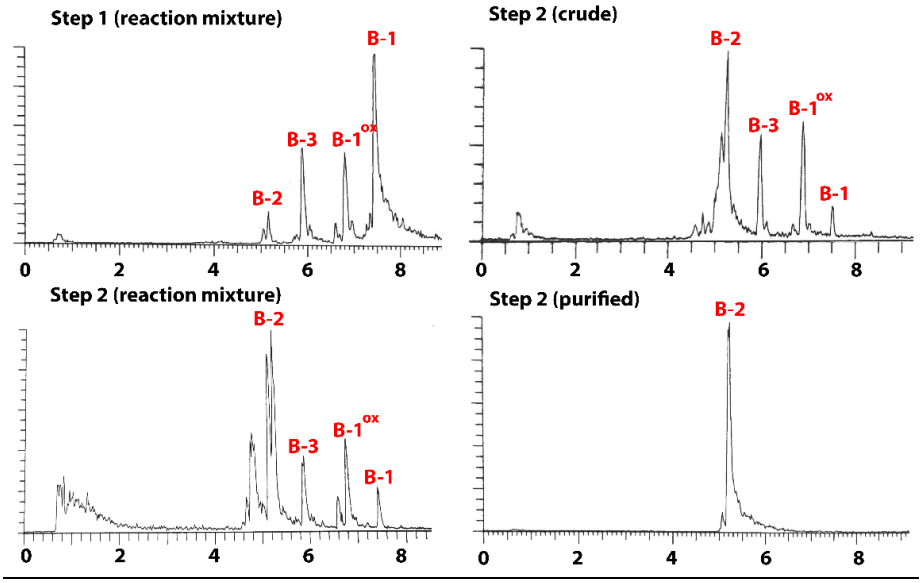


Figure: LCMS trace (m/z) of the two-step reaction and between Fmoc-SIINFEKL-OH and acyl-cyclopropene **42 (chapter 3)**.

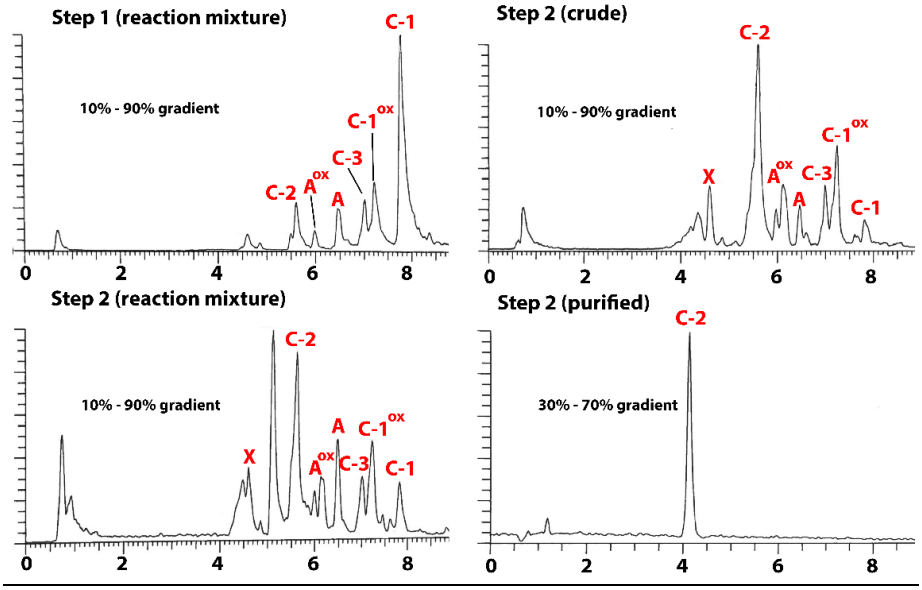


Figure: LCMS trace (m/z) of the two-step reaction between Fmoc-SIINFEKL-OH and alkyl-cyclopropene **45 (chapter 3)**.

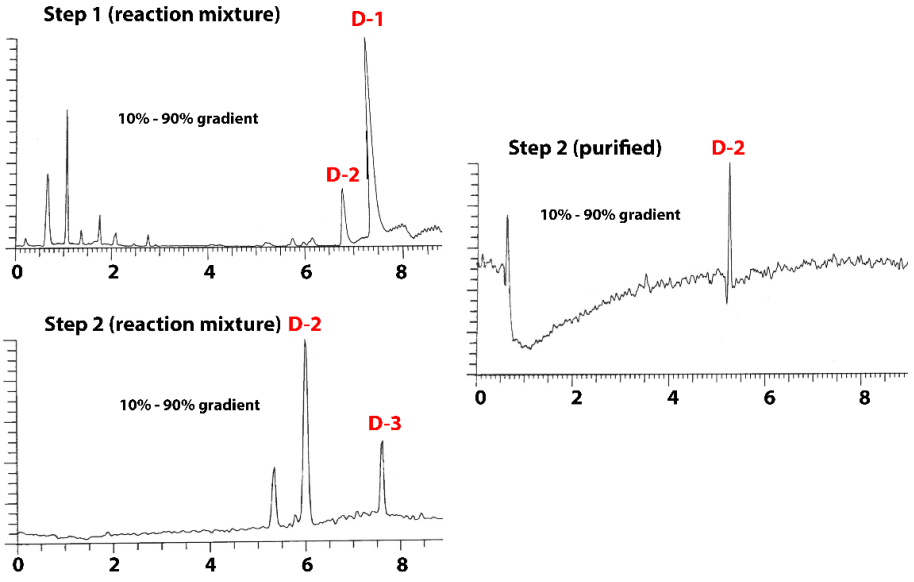


Figure: LCMS trace (m/z) of the two-step reaction between Fmoc-SIINFEKL-OH and spirohexene **52 (chapter 3)**.

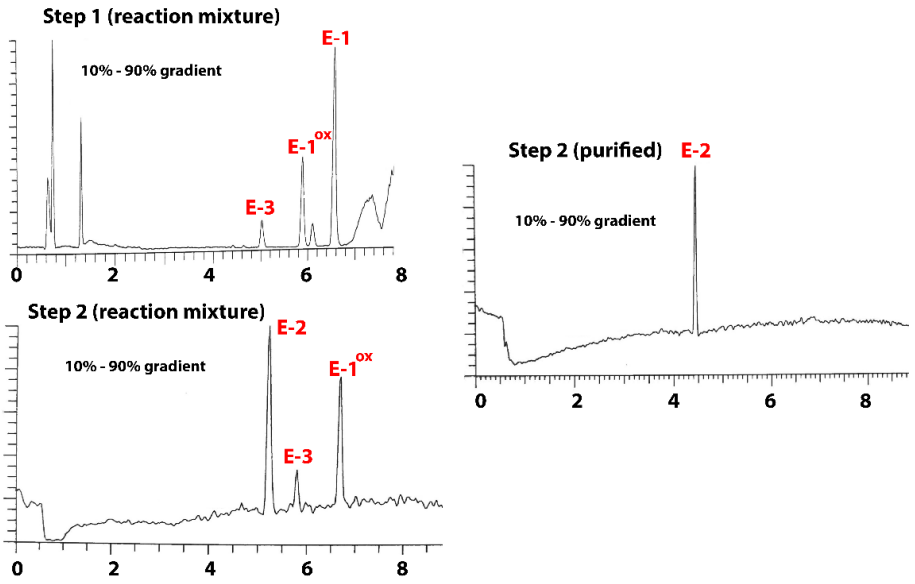


Figure: LCMS trace (m/z) of the two-step reaction between Fmoc-SIINFEKL-OH and carboxyethyl-tetrazine **3 (chapter 5)**.

Compound 37: 5.0 mmol (1.08 g) of 4-bromo-2-methylbenzoic acid was suspended in 20 mL of dry DCE, 11 mmol (2.0 g) of NBS and 0.11 mmol (18 mg) of AIBN were added and the reaction mixture was stirred overnight at 95 °C. The reaction mixture was cooled to room temperature and formed a suspension. Reaction completion was checked by TLC (Rf = 0.5, 2% AcOH in 1:1 EtOAc/DCM). 50 mL of sat. $NaHCO_3$ (aq.) and 50 mL of H_2O were added to the mixture and it was washed using 100 mL of DCM. The water layer was acidified using 75 mL of 1M HCl (aq.) and extracted using 100 mL of EtOAc. The organic layer was then washed multiple times with 0.1M HCl (aq.) until all remaining succinimide was washed away (visible by TLC at RF = 0.3, 2% AcOH in 1:1 EtOAc/DCM). The organic layer was dried using NaSO4, filtered and concentrated using rotary evaporation. Reaction completion was checked by NMR (^{1}H NMR (400 MHz, MeOD)), which could distinguish between mono-brominated intermediate (**δ**: 4.740 (CH_2Br)) and di-brominated product (**δ**: 7.313 ($CHBr_2$)). Because only 62% of the material was di-brominated, the reaction and purification steps were repeated using 5.0 mmol of NBS resulting in 1.40 g (3.76 mmol, 75.2%) of compound **37** as a slightly yellowish solid. **^{1}H NMR (300 MHz, DMSO) δ**: 8.470 (s, 1H), 7.806 (s, 2H), 7.383 (s, 1H). **^{13}C NMR (75 MHz, DMSO) δ:** 165.92, 140.25, 133.87, 131.93, 131.33, 131.10, 40.62.

Compound 38: 3.13 mmol (1.17 g) of compound **37** was suspended in 20 mL of 10% wt. $NaCO_3$ (aq.) and the reaction mixture was warmed to 70 °C for 4 hours. Reaction completion was checked by TLC (Rf = 0.6, 10% MeOH in EtOAc). While warm, the reaction mixture was filtered ,100 mL of H_2O was added, and the watery solution was washed with 100 mL EtOAc. The water layer was acidified to pH = 1 using 50 mL of 1M HCl (aq.) and extracted with 100 mL EtOAc. The organic layer was dried using $MgSO_4$, filtered and concentrated using rotary evaporation resulting in 0.675 g (2.95 mmol, 94.2%) of compound **38** as an off-white solid. **^{1}H NMR (400 MHz, DMSO) δ:** 13.50 (s, 1H), 10.22 (d, J = 0.5 Hz, 1H), 8.29 (d, J = 2.2 Hz, 1H), 8.06 (dd, J = 8.3, 1.8 Hz, 1H), 7.92 (d, J = 8.3 Hz, 1H). **^{13}C NMR (101 MHz, DMSO) δ**: 191.10, 165.89, 135.59, 134.69, 133.36, 130.94, 130.58, 130.14.

Compound 39: 2.69 mmol (0.615 g) of compound **38** was dissolved in 10 mL of THF, cooled to 0 °C, 4.15 mmol (0.157 g) of $NaBH_4$ was added and the reaction mixture was stirred for 2 hours. Reaction completion was checked by TLC (Rf = 0.8, 10% MeOH in EtOAc). The reaction mixture was poured into a mixture of 50 mL of EtOAc and 50 mL 1M HCl (aq.), while vigorously stirring. The organic layer was collected, dried using $MgSO_4$, filtered and concentrated using rotary evaporation resulting in 0.573 g (248 mmol, 92.4%) of compound **39** as a white solid. **^{1}H NMR (300 MHz, DMSO) δ**: 8.109 (s, 1H), 7.8-7.6 (m, 2H), 5.605 (br, 1H), 4.607 (s, 1H). **^{13}C NMR (75 MHz, DMSO) δ:** 166.91, 141.64, 132.39, 130.14, 129.21, 128.65, 125.69, 62.37.

Compound 40: 8.3 mmol (1.0 g) of anhydrous $MgSO_4$ was suspended in 10 mL dry DCM and stirred for 15 minutes. Then 0.10 mL of H_2SO_4 was added and the mixture was stirred for 15 minutes. To the solution 0.498 mmol (0.115 g) of compound **39** and 1.0 mL of tBuOH were added, the vessel was sealed air-tight and the reaction mixture was stirred for 4 days at room temperature. The formed white suspension was quenched by pouring it into 50 mL sat. $NaHCO_3$ (aq.) while vigorously stirring. To obtain the crude product, and tert-butyl ester intermediate, the watery solution was extracted with 100 mL of EtOAc. To recover the remaining starting-material and tert-butyl ether intermediate, the water layer was acidified using 1M HCl (aq.) and extracted with EtOAc. The crude product fraction was dried using

$MgSO_4$, filtered and concentrated using evaporation. Purification was performed by silica column chromatography using an 2%-12% EtOAc in pentane eluent resulting in 0.102 g (0.297 mmol, 59.6%) of compound **40** (TLC Rf = 0.6 in 2% EtOAc in pentane)**.** The tert-butyl ester intermediate (TLC Rf = 0.1 in 2% EtOAc in pentane) was also collected and combined with crude starting material and tert-butyl ether intermediate to be re-used. **^{1}H NMR (400 MHz, $CDCl_3$) δ:** 8.14 (s, 1H), 7.71 (dd, *J* = 8.2, 1.9 Hz, 1H), 7.54 (d, *J* = 8.3 Hz, 1H), 4.50 (s, 3H), 1.58 (s, 9H), 1.32 (s, 9H). **^{13}C NMR (101 MHz, $CDCl_3$) δ**: 165.37, 139.44, 132.31, 131.38, 130.08, 129.30, 127.24, 81.38, 74.10, 63.50, 28.28, 27.75.

Compound 41: 300 mmol (25 mL) formaldehyde was dissolved in 250 mL THF, cooled to 0 °C and 60 mL of H_2SO_4 was added to the solution. Then, dropwise over 10 minutes, 100 mmol (11 mL, 17.2 g) of 3-bromo aniline was added and the reaction mixture was stirred for an additional 10 minutes. Reaction completion was checked by TLC (Intermediate, Rf = 0.8, 25% EtOAc in pentane). 400 mmol (15 g) of $NaBH_4$ was portion wise added over a period of 30 minutes, then the reaction mixture was stirred for 1 hour while warming to room temperature. Reaction completion was checked by TLC (Rf = 0.9, 25% EtOAc in pentane). The reaction was quenched by addition of 400 mL sat. $NaHCO_3$ (aq.), THF present in the solution was removed using rotary evaporation, and the remaining watery solution was extracted twice with 500 mL $CHCl_3$. The organic layers were combined, washed with sat. $NaHCO_3$ (aq.) dried using $MgSO_4$, filtered and concentrated using rotary evaporation. Purification was performed by silica column chromatography using an 0%-4% EtOAc in pentane eluent resulting in 19.52 g (98.42 mmol, 98.4%) of compound **41** as a pale oil**. ^{1}H NMR (500 MHz, $CDCl_3$) δ**: 7.08 (t, *J* = 8.0 Hz, 1H), 6.86 – 6.80 (m, 2H), 6.63 (dd, *J* = 8.6, 2.3 Hz, 1H), 2.95 (s, 6H).

Compound 42: 88.85 mmol (17.77 g) of compound **41** was dissolved in 170 mL AcOH and 44 mmol (1.332 g, 3.6 mL of a 37% wt. solution) formaldehyde was added dropwise over 10 minutes. The reaction mixture was stirred at 80 °C for 1.5 hours, cooled to room temperature, and the reaction mixture was concentrated using rotary evaporation. The residue was suspended in 300 mL of sat. $NaHCO_3$ (aq.) and extracted three times with EtOAc (500, 100, 100 mL). The organic layers were combined, dried using $MgSO_4$ and concentrated using rotary evaporation. Purification was performed by silica column chromatography using an 2%-10% EtOAc in pentane eluent resulting in 12.84 g (31.16 mmol, 70.1%) of compound **42** as a white solid. **^{1}H NMR (500 MHz, $CDCl_3$) δ:** 6.99 (d, *J* = 2.1 Hz, 2H), 6.90 (d, *J* = 8.5 Hz, 2H), 6.62 (dd, *J* = 8.5, 2.1 Hz, 2H), 4.06 (s, 2H), 2.95 (s, 12H). **^{1}H NMR (300 MHz, $CDCl_3$) δ:** 6.97 (d, 2H), 6.88 (d, 2H), 6.61 (dd, 2H), 4.03 (s, 2H), 2.93 (s, 12H). **^{13}C NMR (75 MHz, $CDCl_3$) δ**: 150.09, 130.85, 127.12, 125.69, 116.30, 111.9340.62, 39.96.

Compound 43: 1.45 mmol (0.597 g) of compound **42** was co-evaporated in dry dioxane, dissolved in 58 mL dry THF and cooled to -78 °C. 3.1 mL *sec*-butyl lithium was added dropwise to the solution over 10 minutes and the reaction mixture was stirred for another 30 minutes. 2.6 mmol (0.32 mL) of di-chloro-di-methyl silane was added and the reaction mixture was stirred for 2 hours while warming to room temperature. The resulting dark-green solution was quenched by the addition of 2 mL 1M HCl (aq.), resulting in the formation of a black precipitate, which dissolved after the addition of 50 mL H_2O. THF present in the solution was removed using rotary evaporation, and the remaining watery solution was adjusted to pH = 10 by adding 100 mL of sat. $NaHCO_3$ (aq.). The resulting dark blue solution was extracted twice with

100 mL of EtOAc, obtaining a colorless water layer and yellow organic layers. Formation of the intermediate was checked by TLC (TEA neutralized TLC plate, Rf = 0.8, 10% EtOAc in pentane). The organic layer was dried using Na_2SO_4, filtered and evaporated using rotary evaporation, resulting in a dark green oil containing crude intermediate. The crude intermediate was directly used by dissolving it in 10 mL dry acetone, and cooling to -15°C using an iced salt bath. 600 mg of $KMnO_4$ was added in portions of 100 mg over a time period of 45 minutes to the solution and the reaction mixture was stirred for another 75 minutes. The reaction mixture was then diluted with dry acetone, filtered using filter paper, filtered using a pad of celite and concentrated using rotary evaporation into a black slurry. Purification was performed by silica column chromatography using 10%-40% EtOAc in pentane eluent resulting in 0.247 g (0.762 mmol, 52.6%) of compound **43** as a yellow (slightly green) solid. **^{1}H NMR (400 MHz, $CDCl_3$) δ**: 8.40 (d, *J* = 9.0 Hz, 1H), 6.84 (dd, *J* = 9.0, 2.8 Hz, 1H), 6.79 (d, *J* = 2.8 Hz, 1H), 3.09 (s, 6H), 0.47 (s, 2H). **^{13}C NMR (101 MHz, $CDCl_3$) δ:** 185.39, 151.55, 140.59, 131.70, 129.75, 114.35, 113.24, 40.13, -0.88.

Compound 44: 134 µmol (46 mg) of compound **40** was co-evaporated with dry dioxane, dissolved in 2.0 mL of dry THF and cooled to -78 °C. 170 µmol (0.10 mL of a 1.7M solution in pentane) tert-butyl lithium was cooled to -78 °C and added dropwise over 5 minutes. The reaction mixture was stirred for 15 minutes at -78 °C. Then, 32 µmol (10.5 mg) of compound **43** was co-evaporated with dry dioxane, dissolved in 1.0 mL of dry THF, cooled to -78 °C and dropwise added to the reaction mixture over 1 minute. The reaction mixture was allowed to stir for 2 hours while warming to room temperature. The reaction was quenched by the slow addition of 0.5 mL of 1M HCl (aq.), neutralized using 10 mL of sat. $NaHCO_3$ (aq.), and the watery solution was extracted using 10 mL of EtOAc. The organic layer was concentrated using rotary evaporation resulting a residue containing the tert-butylated crude intermediate. The residue was dissolved in 1 mL of TFA and stirred for 2 days at room temperature. The resulting suspension was filtered using a syringe filter, concentrated using rotary evaporation and purified using C_{18}-reverse phase HPLC to obtain 1.3 mg (2.8 µmol, 8.8%) of compound **44** including a small amount of impurities. **LC-MS analysis:** (Alltima C_{18} analytical column, linear gradient in 12.5 minutes; H_2O: 80 → 0% ; ACN: 10 → 90% ; aq. 0.5% TFA: 10%) **LC:** Rt: 5.18 min **MS:** $[C_{27}H_{31}N_2O_3Si]^+$: found 459.40, calculated 459.21.

Compound 44 (repurification method): Crude and semi-purified material from several reaction were combined to a single flask. Purification was performed by silica column chromatography using an 0.5%-2% H_2O in ACN eluent containing 1% AcOH resulting in 4 mg (8.7 µmol) of compound **44** as a colorless oil/solid**. ^{1}H NMR (600 MHz, MeOD) δ**: 8.02 (s, 1H), 7.85 (d, *J* = 7.8 Hz, 1H), 7.01 (d, *J* = 8.9 Hz, 2H), 6.98 (d, *J* = 2.8 Hz, 2H), 6.88 (d, *J* = 8.0 Hz, 1H), 6.71 (dd, *J* = 8.9, 2.9 Hz, 2H), 5.40 (s, 2H), 2.93 (s, 11H), 0.59 (s, 3H), 0.49 (s, 3H). **^{13}C NMR (151 MHz, MeOD) δ:** 170.24, 153.59, 150.54, 139.70, 139.38, 135.51, 132.12, 130.32, 129.99, 124.60, 124.04, 117.57, 115.70, 94.17, 73.71, 40.82, 0.24, -0.30.

Compound 45: In an Eppendorf tube 8.7 µmol (4 mg) of compound **44** was dissolved in 40 µL of DMF. Then, 16 µmol (2 mg) of N-hydroxy succinimide and 16 µmol (3 mg) of EDC hydrochloride salt were added and the reaction mixture was thoroughly mixed using a micropipette and left to react for one hour at room temperature. No reaction was observed, so over the course of several hours 16 µmol of TEA, 16 µmol of EDC hydrochloride salt and 16

μmol of N-hydroxy succinimide were added and the reaction mixture was left to react overnight at room temperature. Reaction completion was checked by TLC tracking the consumption of starting material (Rf = 0.4, 10% MeOH in DCM) and the formation of succinimide ester intermediate (Rf = 0.9, 10% MeOH in DCM).

To the reaction mixture 29 μmol (4 mg) of compound **10 (chapter 3)** and 25 μmol (3.5 μL of TEA were added and the reaction mixture was thoroughly mixed using a micropipette and left to react for two hours at room temperature. Reaction completion was checked by TLC tracking the consumption of succinimide ester intermediate (Rf = 0.60, 75% EtOAc in pentane) and the formation of product (Rf = 0.40, 75% EtOAc in pentane). 2 ml of EtOAc was added to the solution and the organic mixture was washed twice with 0.5 mL sat. $NaHCO_3$ (aq.) and concentrated using rotary evaporation. Purification was performed by silica column chromatography using an 0.5%-2% H_2O in ACN eluent resulting in compound **45** as a colorless oil/solid**.**

Compound 46: In an Eppendorf tube 2 μmol (1 mg) of compound **44** was dissolved in 10 μL of DMF. Then, 4 μmol (10 μL of a 0.4M solution in DMF) of TEA, 8 μmol (10 μL of a 0.8M solution in DMF) of N-hydroxy succinimide, 1.5 mg of EDC hydrochloride salt and an additional 10 μL of DMF were added and the reaction mixture was thoroughly mixed using a micropipette and left to react for two hours at room temperature. Reaction completion was checked by TLC tracking the consumption of starting material (Rf = 0.4, 10% MeOH in DCM) and the formation of succinimide ester intermediate (Rf = 0.9, 10% MeOH in DCM). LC-MS analysis: (Alltima C_{18} analytical column, linear gradient in 12.5 minutes; H_2O: 80 → 0% ; ACN: 10 → 90% ; aq. 0.5% TFA: 10%) **LC:** Rt: 5.77 **MS:** $[C_{31}H_{34}N_3O_5Si]^+$: found 556.3, calculated 556.2.

To the reaction mixture 4 μmol (1 mg) of compound **1 (chapter 3)** and 8 μmol (20 μL of a 0.4M solution in DMF) of TEA were added and the reaction mixture was thoroughly mixed using a micropipette and left to react for one hour at room temperature. Reaction completion was checked by TLC tracking the consumption of succinimide ester intermediate (Rf = 0.60, 75% EtOAc in pentane) and the formation of product (Rf = 0.65, 75% EtOAc in pentane). 0.5 mL of sat. $NaHCO_3$ (aq.) was added to the solution and it was extracted with 0.5 mL of EtOAc. The organic layer was washed twice with 0.5 mL sat. $NaHCO_3$ (aq.) and concentrated using rotary evaporation. The crude product was then purified using C_{18}-reverse phase HPLC to obtain compound **46.** LC-MS analysis: (Alltima C_{18} analytical column, linear gradient in 12.5 minutes; H_2O: 80 → 0% ; ACN: 10 → 90% ; aq. 0.5% TFA: 10%) **LC:** Rt: 6.01 **MS:** $[C_{36}H_{38}N_7O_2Si]^+$: found 628.2, calculated 628.3.

Benzyl vinyl ether 47: 202 mmol (21 mL) of benzyl alcohol was dissolved in 998 mmol (96 mL) vinyl ethyl ether and cooled down to 0 °C. 20 mmol (9 mg) $Hg(TFA)_2$ was added and the reaction mixture stirred for 2 hours while warming to room temperature. Reaction completion was checked by TLC (Rf = 0.4 in 100% Pentane). Et_2O was added to the reaction mixture and the organic layer was washed four times with $NaHCO_3$, dried with $MgSO_4$, filtered and concentrated using rotary evaporation. Silica chromatography was performed 0%-4% Et_2O in pentane eluent resulting in 150 mmol (20.2 g, 75.2%) of the benzyl vinyl ether **47** as a colorless oil. **^{1}H NMR (400 MHz, $CDCl_3$) δ:** 7.51 – 7.33 (m, 5H), 6.65 (dd, J = 14.3, 6.8 Hz, 1H), 4.82 (s,

2H), 4.38 (dd, J = 14.3, 2.1 Hz, 1H), 4.16 (dd, J = 6.8, 2.2 Hz, 1H). **^{13}C NMR (101 MHz, $CDCl_3$) δ:** 151.73, 136.96, 128.59, 128.00, 127.64, 127.53, 87.41, 77.48, 77.16, 76.84, 70.11.

Spiroheptane 48: 19.5 mmol (2.2 mL, 2.30 g) cyclobutanecarbonyl chloride and 35.3 mmol (4.73 g) benzyl vinyl ether **47** were dissolved in 18 mL dry ACN. 21.58 mmol (3 ml) TEA was added and the mixture was refluxed (90 °C) for 3 hours. Reaction completion was checked by TLC (Rf = 0.35 in 5% EtOAc/Pentane). 32 mL ACN was added and the reaction mixture was filtered, concentrated using rotary evaporation, redissolved in Et_2O, washed with 1M HCl (aq.), washed with brine, dried with $MgSO_4$, filtered, and concentrated using rotary evaporation. Purification was performed by silica column chromatography using an 1%-4% EtOAc in pentane eluent resulting in 11.6 mmol (2.50 g, 65.6%) of the spiroheptane **48** as a colorless oil. **^{1}H NMR (400 MHz, $CDCl_3$) δ:** 7.45 – 7.20 (m, 5H), 4.78 – 4.42 (m, 2H), 4.06 (dd, J = 6.7, 5.5 Hz, 1H), 3.06 (dd, J = 17.6, 6.8 Hz, 1H), 2.92 (dd, J = 17.5, 5.5 Hz, 1H), 2.58 (dtd, J = 11.0, 8.9, 8.4, 1.9 Hz, 1H), 2.43 (t, J = 8.0 Hz, 2H), 2.37 – 2.28 (m, 1H), 2.24 – 2.15 (m, 1H), 2.11 – 1.93 (m, 3H), 1.93 – 1.82 (m, 1H). **^{13}C NMR (101 MHz, $CDCl_3$) δ:** 210.79, 137.87, 128.61, 127.97, 127.72, 77.48, 77.16, 76.84, 71.89, 71.46, 66.64, 50.35, 27.78, 24.45, 16.66.

Spiroheptane 49: 3.72 mmol (805 mg) of spiroheptane **48** was dissolved in 17 mL dry MeOH and cooled to 0°C. 11.3 mmol (429 mg) of $NaBH_4$ was added and the reaction mixture was stirred for 3 hours while warming to room temperature. Reaction completion was checked by TLC (Rf = 0.4 in 25 % EtOAc/Pentane). The reaction was quenched by the slow addition of 85 mL water, and extracted twice with EtOAc. The organic layers were combined, washed with water, washed with brine, dried with $MgSO_4$, filtered and concentrated using rotary evaporation. Purification was performed by silica column chromatography using an 10%-25% EtOAc in pentane eluent resulting in 2.55 mmol (556 mg, 68.9%) of the spiroheptane **49** as a colorless oil. **^{1}H NMR (400 MHz, $CDCl_3$) δ:** 7.44 – 7.11 (m, 5H), 4.63 – 4.43 (m, 2H), 3.60 (t, J = 7.7 Hz, 1H), 3.41 (dd, J = 8.1, 6.6 Hz, 1H), 2.51 (dt, J = 11.3, 6.8 Hz, 1H), 2.39 – 2.23 (m, 1H), 2.17 (ddd, J = 11.6, 8.9, 6.2 Hz, 1H), 2.04 (t, J = 7.5 Hz, 2H), 1.98 – 1.78 (m, 2H), 1.53 (dt, J = 11.1, 8.2 Hz, 1H). **^{13}C NMR (101 MHz, $CDCl_3$) δ:** 138.57, 128.48, 127.68, 77.48, 77.16, 76.84, 72.19, 71.07, 66.74, 54.11, 36.72, 30.50, 19.18, 17.39.

Spiroheptane 50: 3.02 mmol (659 mg) of spiroheptane **49** was co-evaporated with dioxane and dissolved in 3 mL dry pyridine. 6.02 mmol (1.15 g) of *p*-toluenesulfonyl chloride was added and the reaction mixture was stirred for 3 hours at room temperature. Reaction completion was checked by TLC (Rf = 0.75 in 25% EtOAc/Pentane). 80 mL of 0.1M HCl (aq.) was added to the reaction mixture and extracted twice with Et_2O. The organic layers were combined, washed twice with 0.1M HCl (aq.), washed with water, washed with brine, dried with $MgSO_4$, filtered and concentrated using rotary evaporation. Purification was performed by silica column chromatography using an 1%-40% Et_2O in pentane eluent resulting in 2.38 mmol (886 mg, 78.9%) of the spiroheptane **50** as a white crystalline solid. **^{1}H NMR (400 MHz, $CDCl_3$) δ:** 7.80 (d, J = 8.4 Hz, 2H), 7.52 – 6.68 (m, 6H), 4.50 (d, J = 10.2 Hz, 2H), 4.19 (dd, J = 8.4, 7.0 Hz, 1H), 3.38 (dd, J = 8.3, 6.5 Hz, 1H), 2.45 (s, 3H), 2.44 – 2.18 (m, 3H), 2.05 – 1.89 (m, 2H), 1.89 – 1.60 (m, 3H). **^{13}C NMR (101 MHz, $CDCl_3$) δ:** 144.87, 138.13, 134.08, 129.92, 128.52, 127.93, 127.85, 127.68, 74.02, 71.77, 71.31, 53.83, 34.49, 29.95, 21.76, 20.31, 17.02.

Compound 51: 0.752 mmol (280 mg) of spiroheptane **50** was co-evaporated with dioxane and dissolved in 10 mL dry DMSO. 1.13 mmol (127 mg) KOtBu was pre-suspended in 5 mL dry DMSO and added to the solution. The reaction mixture was stirred for 5 hours at room temperature. Reaction completion was checked by TLC (Rf = 0.9 in 5% EtOAc/Pentane). 50 mL 1M HCl and Et_2O were added to the reaction mixture. The organic layer was washed twice with 1M HCl (aq.), washed three times with water, washed once with brine, dried with $MgSO_4$, filtered and concentrated using rotary evaporation. Purification was performed by silica column chromatography using an 0%-5% Et_2O in pentane eluent resulting in 0.18 mmol (37 mg, 24.3%) of the spiroheptene **51** as a colorless oil. **^{1}H NMR (400 MHz, $CDCl_3$) δ:** 7.59 – 7.13 (m, 5H), 6.34 (dd, J = 2.8, 1.2 Hz, 1H), 6.12 (dd, J = 2.8, 0.6 Hz, 1H), 4.88 – 4.46 (m, 2H), 4.22 (t, J = 0.8 Hz, 1H), 2.58 – 2.34 (m, 1H), 2.13 (dddd, J = 12.7, 9.0, 5.1, 2.1 Hz, 3H), 1.97 – 1.79 (m, 2H). **^{13}C NMR (101 MHz, $CDCl_3$) δ:** 145.36, 135.47, 128.50, 127.78, 127.65, 83.91, 77.48, 77.16, 76.84, 71.11, 30.19, 28.03, 16.56.

Compound 52: 0.268 mmol (100 mg) of spiroheptane **50** was dissolved in 8 mL MeOH and degassed using sonication under a nitrogen atmosphere for 30 min. 10 mg Pd/C was added to the solution, the mixture was flushed with hydrogen and stirred overnight at room temperature under a hydrogen atmosphere. Reaction completion was checked by TLC (Rf = 0.8 in 25% EtOAc/Pentane). The reaction mixture was concentrated using rotary evaporation resulting in 0.24 mmol (67 mg, 88%) of the spiroheptane **52** as colorless oil. **^{1}H NMR (400 MHz, $CDCl_3$) δ:** 7.77 (d, J = 8.3 Hz, 2H), 7.38 – 7.29 (m, 2H), 4.15 (dd, J = 8.2, 6.9 Hz, 1H), 3.60 (dd, J = 8.3, 6.8 Hz, 1H), 2.41 (d, J = 14.4 Hz, 4H), 2.31 (s, 1H), 2.19 (dd, J = 8.3, 7.1 Hz, 2H), 1.95 – 1.83 (m, 2H), 1.80 – 1.60 (m, 3H). **^{13}C NMR (101 MHz, $CDCl_3$) δ:** 144.99, 133.80, 129.95, 127.87, 77.48, 77.16, 76.84, 73.80, 66.01, 54.57, 36.72, 29.50, 21.73, 19.82, 16.91.

Compound 53a: 0.12 mmol (34 mg) of spiroheptane **52** was co-evaporated with dioxane and dissolved in 1 mL dry ACN. 0.26 mmol (67 mg) of N-N'-disuccinimidyl carbonate and 0.18 mmol (30 µl, 22 mg) DIPEA were added and the reaction mixture was stirred overnight at room temperature. Reaction completion was checked by TLC (Rf = 0.15 in 25% EtOAc/Pentane). The mixture was concentrated using rotary evaporation, re-dissolved in Et_2O, washed three times with 0.1 M HCl (aq.), washed once with sat. $NaHCO_3$ (aq.), washed once with brine, dried with $MgSO_4$, filtered and concentrated using rotary evaporation. Purification was performed by silica column chromatography using an 25%-60% EtOAc in pentane eluent resulting in 0.073 mmol (31 mg, 61.9%) of the spiroheptane **53a** as a colorless oil. **^{1}H NMR (400 MHz, $CDCl_3$) δ:** 7.80 (d, J = 8.0 Hz, 2H), 7.35 (d, J = 8.0 Hz, 2H), 4.50 (t, J = 7.5 Hz, 1H), 4.29 (t, J = 7.5 Hz, 1H), 2.83 (s, 4H), 2.57 (dt, J = 12.2, 7.0 Hz, 1H), 2.45 (s, 3H), 2.41 – 2.30 (m, 1H), 2.25 (dd, J = 11.4, 5.3 Hz, 1H), 2.16 – 1.93 (m, 3H), 1.79 (td, J = 9.1, 6.5 Hz, 2H). **^{13}C NMR (101 MHz, $CDCl_3$) δ:** 168.64, 151.07, 145.29, 133.61, 130.10, 127.94, 77.48, 77.16, 76.84, 74.11, 72.79, 53.71, 33.67, 29.58, 25.65, 25.56, 21.80, 21.02, 16.69.

Compound 53b: 0.14 mmol (60 mg) of spiroheptane **53a** was dissolved in 4.3 mL dry DCM. 0.17 mmol (45 mg) Boc-Lys-OMe acetate salt and 0.32 mmol (44 µl, 32 mg) TEA were added and the reaction mixture was stirred for 4 hours at room temperature. Reaction completion was checked by TLC (Rf = 0.75 in 50% EtOAc/Pentane). 10 mL of 0.1M HCl was added to the reaction mixture and extracted with DCM. The organic layer was washed twice with 0.1M HCl (aq.), washed once with brine, dried with $MgSO_4$, filtered and concentrated using rotary

evaporation. Purification was performed by silica column chromatography using an 10%-50% EtOAc in pentane eluent resulting in 0.069 mmol (39 mg, 48.2%) of the spiroheptane **53b** as a colorless oil. **^{1}H NMR (500 MHz, $CDCl_3$) δ:** 7.81 – 7.76 (m, 2H), 7.34 (d, J = 8.1 Hz, 2H), 5.07 (d, J = 8.8 Hz, 1H), 4.88 (s, 1H), 4.39 (td, J = 7.7, 4.8 Hz, 1H), 4.32 – 4.13 (m, 2H), 3.71 (s, 3H), 3.21 – 3.08 (m, 2H), 2.44 (s, 4H), 2.25 (ddt, J = 12.4, 9.0, 4.5 Hz, 1H), 2.15 – 2.05 (m, 2H), 1.94 – 1.85 (m, 1H), 1.84 – 1.68 (m, 4H), 1.66 – 1.57 (m, 1H), 1.56 – 1.46 (m, 2H), 1.42 (d, J = 1.4 Hz, 9H), 1.40 – 1.28 (m, 2H). **^{13}C NMR (126 MHz, $CDCl_3$) δ:** 173.32, 155.96, 145.02, 133.84, 129.99, 127.96, 127.87, 80.05, 77.41, 77.16, 76.91, 74.05, 67.47, 53.77, 53.75, 53.21, 52.41, 40.72, 34.09, 32.56, 29.61, 29.35, 28.42, 22.51, 21.77, 20.87, 16.84.

Compound 53c: 0.354 mmol (100 mg) of spiroheptane **52** was dissolved in 1.8 mL DCM and cooled to 0°C. 0.37 mmol (77 mg) of N,N'-Dicyclohexylcarbodiimide (DCC), 0.086 mmol (11 mg) of DMAP and 0.60 mmol (62 µL, 70 mg) levulinic acid were added and the reaction mixture stirred for 3 hours while warming to room temperature. Reaction completion was checked by TLC (Rf = 0.7 in 50% EtOAc/Pentane). The reaction mixtutre was washed with 30 sat. $NaHCO_3$ and the water layer was extracted with DCM and EtOAc. The organic layers were combined, dried with $MgSO_4$, filtered and concentrated using rotary evaporation. Purification was performed by silica column chromatography using an 10%-40% EtOAc in pentane eluent resulting in 0.270 mmol (103 mg, 76.3%) of the spiroheptane **53c** as a colorless oil. **^{1}H NMR (400 MHz, $CDCl_3$) δ:** 7.81 – 7.71 (m, 2H), 7.40 – 7.27 (m, 2H), 4.41 (dd, J = 8.3, 7.0 Hz, 1H), 4.23 (dd, J = 8.2, 7.0 Hz, 1H), 2.72 (td, J = 6.4, 1.6 Hz, 2H), 2.60 – 2.52 (m, 2H), 2.49 – 2.39 (m, 4H), 2.30 – 2.20 (m, 1H), 2.14 (s, 3H), 2.12 – 2.01 (m, 2H), 1.86 (ddt, J = 16.5, 12.2, 8.0 Hz, 2H), 1.70 (q, J = 7.7 Hz, 2H). **^{13}C NMR (101 MHz, $CDCl_3$) δ:** 206.45, 172.31, 145.02, 133.69, 129.95, 127.84, 127.80, 77.48, 77.16, 76.84, 73.85, 67.66, 53.58, 37.80, 33.70, 29.83, 29.57, 27.69, 21.69, 20.89, 16.67.

Compound 53f: 1.12 mmol (316 mg) of spiroheptane **52** was dissolved in DCM and cooled to 0°C. 0.571 mmol (144 mg) pyridinium *p*-toluenesulfonate (PPTS) was added, 6.71 mmol (642 µL, 484 mg) of vinyl ethyl ether was added dropwise over 1 min and the reaction mixture was stirred at 0°C for 1 hour. Reaction completion was checked by TLC (Rf = 0.85 in 25% EtOAc/Pentane). The reaction mixture was washed three times with sat. $NaHCO_3$, washed once with brine, dried with $MgSO_4$, filtered and concentrated using rotary evaporation. This resulted in 0.958 mmol (339 mg, 85.6%) of the spiroheptane **53f** as a colorless oil. **^{1}H NMR (400 MHz, $CDCl_3$) δ:** 8.01 – 7.62 (m, 2H), 7.34 (d, J = 8.0 Hz, 2H), 4.69 (dt, J = 31.8, 5.3 Hz, 1H), 4.19 (ddd, J = 8.6, 6.9, 1.5 Hz, 1H), 3.76 – 3.27 (m, 3H), 2.47 – 2.42 (m, 3H), 2.40 – 2.18 (m, 2H), 1.94 (dtd, J = 15.7, 8.4, 7.9, 4.0 Hz, 2H), 1.85 – 1.68 (m, 3H), 1.27 (dd, J = 5.3, 3.3 Hz, 3H), 1.16 (td, J = 7.0, 2.7 Hz, 3H). **^{13}C NMR (101 MHz, $CDCl_3$) δ:** 144.87, 134.11, 129.94, 127.96, 99.35, 98.77, 77.48, 77.16, 76.84, 74.28, 74.24, 67.21, 66.85, 61.33, 60.94, 54.12, 36.08, 35.23, 29.92, 29.46, 21.78, 20.55, 20.44, 17.03, 16.93, 15.33.

Compound 54: 0.958 mmol (339 mg) spiroheptane **53f** was co-evaporated with dioxane and dissolved in 19 mL dry DMSO. 1.43 mmol (160 mg) KOtBu was added and the reaction mixture was stirred for 3 hours at room temperature. Reaction completion was checked by TLC (Rf =0.8 in 5% EtOAc/Pentane). 100 mL of sat. NH_4Cl (as.) was added to the reaction mixture and extracted twice with Et_2O. The organic layers were combined, washed twice with sat. NH_4Cl (aq.), washed five times with water, washed once with brine, dried with $MgSO_4$, filtered and

concentrated using rotary evaporation. This resulted in 0.844 mmol (154 mg, 88.1%) of spiroheptene **54** as a pale yellow oil. **^{1}H NMR (500 MHz, $CDCl_3$) δ:** 6.31 (ddd, J = 15.2, 2.8, 1.1 Hz, 1H), 6.08 (ddd, J = 12.5, 2.8, 0.6 Hz, 1H), 4.96 – 4.63 (m, 1H), 4.34 (dt, J = 13.5, 0.8 Hz, 1H), 3.94 – 3.02 (m, 2H), 2.41 – 2.24 (m, 1H), 2.17 – 1.96 (m, 3H), 1.88 – 1.71 (m, 2H), 1.37 (dd, J = 9.0, 5.3 Hz, 3H), 1.22 (t, J = 7.1 Hz, 3H). **^{13}C NMR (126 MHz, $CDCl_3$) δ:** 145.26, 145.11, 136.34, 135.83, 99.38, 98.81, 79.83, 78.87, 77.41, 77.16, 76.91, 61.39, 60.51, 56.90, 56.75, 30.36, 30.07, 28.06, 27.98, 20.81, 20.80, 16.41, 16.17, 15.49, 15.48.

Compound 56: 0.685 mmol (125 mg) of spiroheptene **54** was dissolved in 1.4 mL ACN and cooled to 0°C. 1.4 mL 2M HCl (aq.) was added and the reaction mixture stirred for 35 min at 0°C. Reaction completion was checked by TLC (Rf = 0.25 in 5% EtOAc/Pentane). 50 mL of sat. $NaHCO_3$ was added and extracted twice with Et_2O. The organic layers were combined, washed twice with sat. $NaHCO_3$ (aq.), washed once with brine, dried with $MgSO_4$, filtered and concentrated using rotary evaporation. This resulted in 0.65 mmol (72 mg) of the spiroheptene **55** intermediate as a colorless oil. Spiroheptene **55** was dissolved in 5.5 mL dry ACN, 1.37 mmol (239 µl, 177 mg) DIPEA and 1.37 mmol (351 mg) N-N'-disuccinimidyl carbonate were added, and the reaction mixture was stirred overnight at room temperature. Reaction completion was checked by TLC (Rf = 0.25 in 10% EtOAc/Pentane). The mixture was concentrated using rotary evaporation, re-dissolved in Et_2O, washed three times with sat. $NaHCO_3$ (aq.), washed three times with 0.1 M HCl (aq.), washed once with brine, dried with $MgSO_4$, filtered and concentrated using rotary evaporation. Purification was performed by silica column chromatography using an 10%-100% Et_2O in pentane eluent, resulting in 0.34 mmol (85 mg, 49%, over two steps) of spiroheptene **56** as a colorless oil. **^{1}H NMR (400 MHz, $CDCl_3$) δ:** 6.49 (dd, J = 2.8, 1.5 Hz, 1H), 6.06 (d, J = 2.7 Hz, 1H), 5.17 (d, J = 1.5 Hz, 1H), 2.83 (s, 4H), 2.36 – 2.18 (m, 2H), 2.18 – 2.04 (m, 2H), 1.94 – 1.71 (m, 2H). **^{13}C NMR (101 MHz, $CDCl_3$) δ:** 168.85, 151.25, 148.10, 132.24, 83.79, 77.48, 77.16, 76.84, 56.27, 29.78, 28.12, 25.58, 16.03.

Compound 57: 0.080 mmol (20 mg) of spiroheptene **56** was dissolved in 2.5 mL dry DCM, 0.100 mmol (26 mg) Boc-Lys-OMe acetate salt and 0.180 mmol (25 µL) TEA were added and the reaction mixture was stirred for 45 minutes at room temperature. Reaction completion was checked by TLC (Rf = 0.8 in 5% MeOH/DCM). The reaction was quenched with 50 mL sat. NH_4Cl (aq.) and extracted twice with DCM. The organic layers were combined, washed twice with sat. NH_4Cl (aq.), washed three times with sat. $NaHCO_3$ (aq.), washed with brine, dried with $MgSO_4$, filtered and concentrated using rotary evaporation. Silica chromatography was performed using 0.25%-1% MeOH in DCM eluent, resulting in 0.025 mmol (10 mg, 31.5%) of the spiroheptene **57** as a colorless oil. **^{1}H NMR (500 MHz, $CDCl_3$) δ:** 6.38 (dd, J = 2.8, 1.4 Hz, 1H), 6.04 (d, J = 2.7 Hz, 1H), 5.12 (d, J = 4.1 Hz, 1H), 5.06 (d, J = 10.4 Hz, 1H), 4.80 (s, 1H), 4.29 (d, J = 7.7 Hz, 1H), 3.73 (s, 3H), 3.20 (q, J = 6.7 Hz, 2H), 2.33 – 2.22 (m, 1H), 2.18 – 2.09 (m, 1H), 2.11 – 1.98 (m, 2H), 1.93 – 1.72 (m, 3H), 1.65 (s, 1H), 1.53 (dt, J = 13.2, 5.9 Hz, 2H), 1.43 (s, 9H), 1.38 (d, J = 8.9 Hz, 2H). **^{13}C NMR (126 MHz, $CDCl_3$) δ**: 173.41, 156.45, 146.55, 133.97, 80.09, 78.41, 77.42, 77.17, 76.91, 56.53, 53.33, 52.44, 40.69, 40.61, 32.56, 32.48, 30.00, 29.61, 28.47, 28.15, 22.54, 16.19.

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