Self adjuvanting immunopeptides : design and synthesis

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Chapter 3: Design, synthesis and immunological evaluation of simplified self-adjuvanting TLR-2 stimulating peptides

This chapter is a part of a patent application: "Adjuvant compounds", NL2018803, filing date 27-04-**2017**)

Introduction

The human immune system consists of two interdependent parts, namely the adaptive and the innate system. As part of the innate immune system dendritic cells (DCs) express pattern recognition receptors (PRRs) that can bind non-self molecular structures, termed pathogen associated molecular patterns (PAMPs). Upon binding of a PAMP to the corresponding PRR, signal transduction pathways are initiated that ultimately lead to adaptive immune responses. The best known PRRs are the Toll-like receptors and in humans ten distinct TLRs have been identified.[1] Lipopeptides derived from the outer membrane of *Escherichia coli* are well known agonists for the TLR-2.[2-5] Currently, the synthetic Pam_3CysSK_4 lipopeptide, one of the most potent agonist for TLR-2, is applied in the development of new vaccines. For instance, conjugates of immunogenic peptides with Pam_3CysSK_4 resulted in highly potent self-adjuvanting vaccines conjugates.[6-9] However, the widening of the applicability of this class conjugates is hindered by solubility issues. Conjugates of Pam_3Cys ligand and antigenic peptides exhibit regularly unwanted aggregation or insolubility in mixtures of DMSO and/or aqueous buffers, by which the immunological evaluation can be hampered. This is certainly true when these conjugates are combined with labels or other PRR ligands (see Chapter 2). Although several studies towards the optimization of TLR2 ligands have been reported a break-through in terms of solubility came from the group of David[10-12], who developed mono-and bis-palmitoylated cysteines derivatives as potent TLR-2 ligands (**1** and **2**, respectively, Figure 1). Remarkable is the finding that their optimal ligand **1** is only active on human TLR-2 and is completely inactive in a murine model. As part of a new peptide lipidation method the group of Brimble[13] prepared and evaluated a conjugate of TLR-2 ligand **1** and peptide epitope NLVPMVATV derived from the cytomegalovirus. Based on their results, and the efforts of the group of Filippov on the development of new TLR2 ligands and the corresponding conjugates with antigenic peptides[14] five new TLR-2 ligands (**3**, **4**, **5**, **6** and **7**), two new conjugates (**8** and **9**) and conjugates labeled with the fluorophore Cy5 (**10** and **11**) were designed, prepared and evaluated (Figure 1).

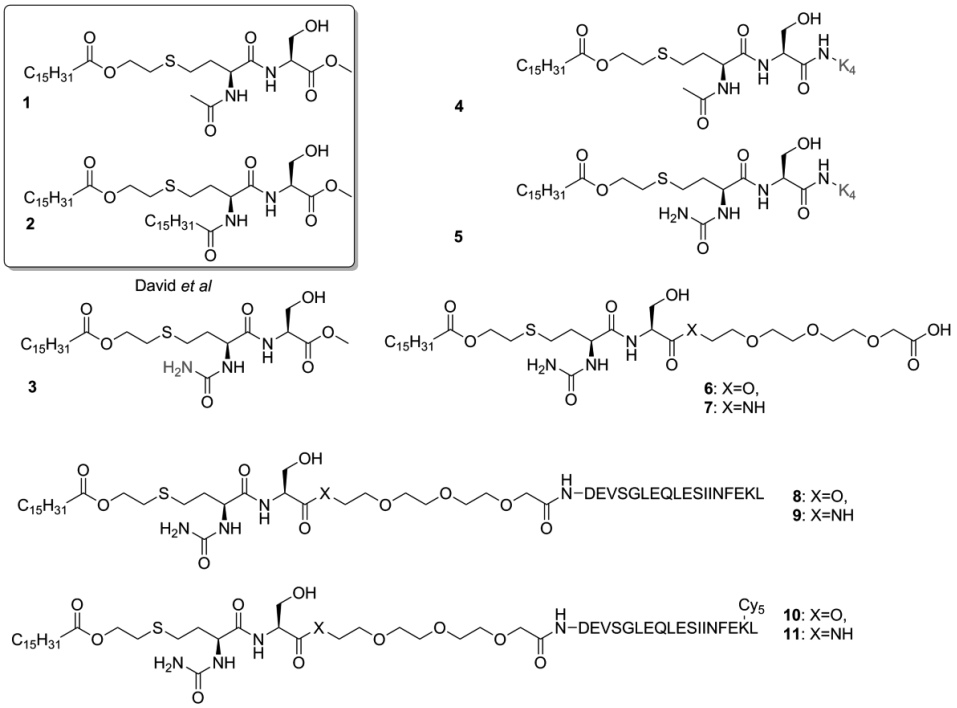
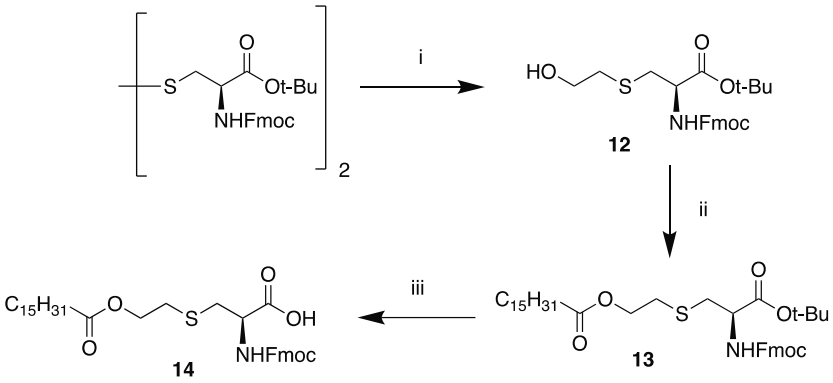


Figure 1. Target self-adjuvanting simplified TLR-2 lipopeptide.

The choice of the urea moiety in ligand **3** is based on the favorable influence of this functionality in the recently developed TLR-2 ligand, termed UPam[14]. In ligands **4** and **5** a tetra-lysine (K_4) segment, a frequently used solubility handle in the field of TLR-2 ligands, was appended. A triethylene glycol (TEG) segment with the same numbers of atoms as tetra-lysine was included as another solubility handle in the ligands **6** and 7[15]. Guided by the outcome of immunological evaluation, the latter ligands were coupled via an ester or amide linkage to MHC 1 epitope DEVSGLEQLESIINFEKL to give conjugates **8** and **9**, respectively. In addition, the corresponding labeled conjugates **10** and **11** were prepared. In this chapter, the synthesis of the prepared ligands and conjugates as well as their preliminary immunological evaluation are described.

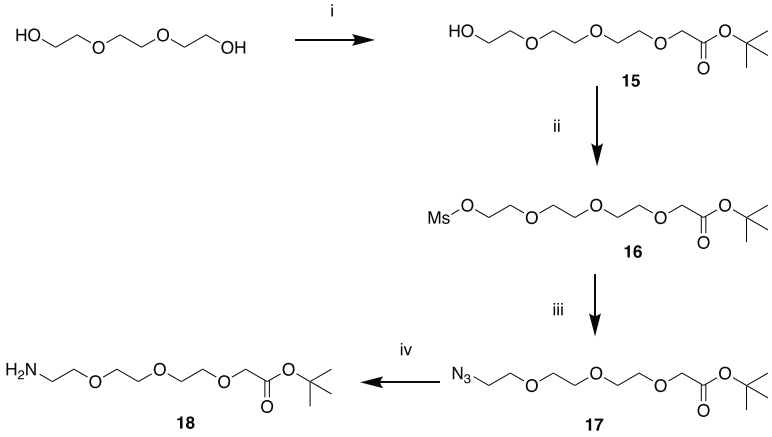
Results and discussion

To arrive at the projected ligands and conjugates (Figure 1), attention was first directed to the synthesis of cysteine derivative **14** (Scheme 1) and TEG spacers **15** and **18** (Scheme 2). Toward cysteine derivative **14** a published procedure was slightly adapted.[16]



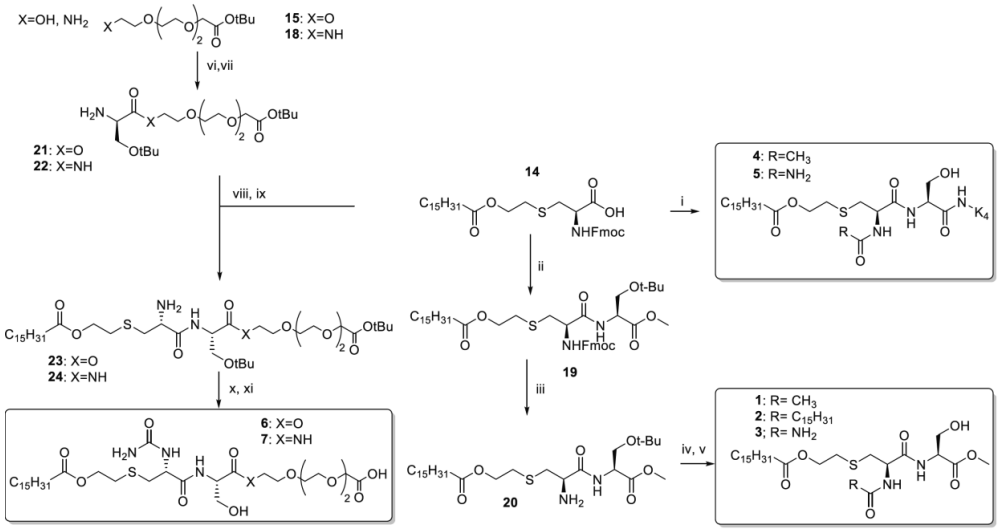
Scheme 1. Synthesis of simplified TLR-2 ligand building block. i) 1) Zn, H_2SO_4, HCl, MeOH 2) Oxirane, RT, 50% ii) Palmitic acid, DIC, DMAP, DCM, 88% iii) TFA, RT, 90%.

In a one pot-procedure commercially available Fmoc-Cysteine-tBu was first reduced using activated zinc in an acidic environment and upon completion of the reduction oxirane was added at 0°C to give alcohol **12** in 50% yield (Scheme 1). Subsequent esterification of **12** with palmitic acid to **13** using diisopropyl carbodiimide (DIC) and DMAP proceeded smoothly. Finally, tBu ester in **13** was cleaved with neat TFA to give target cysteine derivative **14** in 40% overall yield.



Scheme 2. Synthesis of TEG spacer. i) t-Butyl bromoacetate, NaH, TBAI, THF, RT, 40% ii) MsCl, TEA, DCM, RT, qt. iii) NaN_3, DMF, 60°C, 96% iv) 1) PPh_3, THF, RT 2) H_2O, RT, 45%

The preparation of the TEG spacer **18**, as illustrated in Scheme 2, started with a Williamson alkylation of triethylene glycol, with t-butyl bromoacetate in the presence of a catalytic amount of TBAI to yield the alcohol TEG spacer **15**. The alkylation was accompanied by the formation of substantial amounts of bis-substituted product. The synthesis of the amino TEG spacer **18** began with mesylation of **15**, followed by substitution with sodium azide to give compound **17**. Staudinger reduction of the azide in **17** led to isolation of the amine TEG spacer **18** in a moderate yield.



Scheme 3. Synthesis of TLR-2 ligands. i) Fmoc SPPS; TFA/TIS/H_2O (95/2.5/2.5) ii) H-Ser(tBu)-OMe.HCl, DIC, HOBt, Et_3N, DCM, 88% iii) 2% DBU, 2% Piperidine, DMF, RT, 88% iv) R= $C_{15}H_{31}$: Acetic anhydride, TEA, DCM, RT, 94%; R= $C_{15}H_{31}$: Palmitoyl chloride, pyridine, RT, qt.; R = NH_2: TMS-CN, i-PrOH, DCM, RT, qt. v) TFA, RT, 62%-94% vi) X = OH: Fmoc-Ser(tBu)-OH, DIC, DMAP, DCM, RT, qt.; X =NH_2: Fmoc-Ser(tBu)-OH, DIC, HOBt, DCM, RT, 47% vii) 2% DBU, 2% piperidine, DMF, RT, **21**:76%, **22** :qt. viii) DIC, HOBt, DCM, RT, X = O: 71%, X = NH: qt. ix) DBU, octanethiol, DCM, RT, **23**: 90%, **24** :88% x) TMS-CN, *i*-PrOH, DCM, RT, X = O: 90%, X = NH: 82% Xi) TFA/TIS/H_2O (95/2.5/2.5) RT, **6**: 80%, **7**:71%.

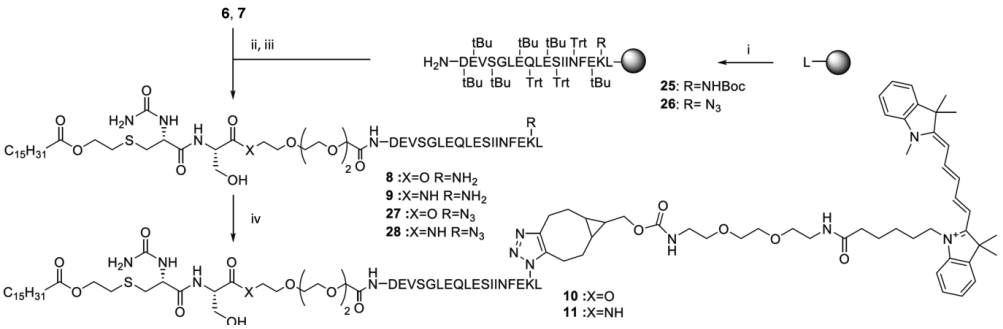
The reference ligands of the group of David (**1** and **2**) and the urea modified version **3** were prepared starting from cysteine derivative **14** (Scheme 3). In a standard procedure **14** was coupled with NH_2-Ser(tBu)-OMe using DIC and HOBt as coupling agents to give **19**. Ensuing Fmoc cleavage with a mixture of piperidine and DBU in dry DMF to prevent cleavage of the methyl ester gave common precursor **20**. Acylation of amine **20** with acetic anhydride or palmitoyl chloride, and subsequent cleavage of the tBu ether with neat TFA provided compounds **1** and **2**, respectively. En route to modified version **3** the urea moiety was installed by treatment of **20** with TMS isocyanate in the presence of isopropanol. Although the role of isopropanol is unclear, it appears to be mandatory for the success of this reaction. Similarly, to the synthesis of **1** and **2**, tBu ester was removed using neat TFA to afford ligand **3**.

Cysteine derivative **14** was also used in the solid phase peptide synthesis (SPPS) to tetra-lysine containing ligands **4** and **5**. With the aid of an automated peptide synthesizer and standard Fmoc chemistry immobilized SK_4 was prepared using Tentagel S RAM resin, Fmoc-Lys(Boc) and Fmoc-Ser(Ot Bu)-OH. Subsequent manual condensation of building block **14** with immobilized SK_4 under influence of PyBop was followed by Fmoc removal to give the immobilized peptide having a free amine. Acetylation of this amine with acetyl chloride and TFA/TIS mediated protecting group removal and cleavage from the solid support led the isolation of ligand **4**. Alternatively, the same immobilized peptide with a free amine was treated with TMS isocyanate as described for the conversion of **20** into **3** and subsequent TFA/TIS treatment gave after purification ligand **5**.

The ligands **6** and **7** were prepared in solution phase. First TEG spacers **15** and **18** were elongated with a serine moiety by a DIC mediated condensation with Fmoc-Ser(tBu)-OH, followed by Fmoc cleavage to afford **21** and **22**. Next cysteine derivative **14** was coupled to the free amine in **21** and **22** by the same sequence of events except that octanethiol was used as a scavenger during Fmoc

cleavage. Installation of the urea moiety at the newly obtained amine was performed as described above and acidic cleavage of the tBu groups yielded ligands **6** and **7**.

Having ligands **6** and **7** in hand the SPPS assembly of conjugates **8-9** and **27-28** was undertaken (Scheme 4). Immobilized peptides **25** and **26**, having a Boc protected lysine or an azidonorleucine incorporated were prepared with standard SPPS. Ligands **6** and **7** were appended manually by pre-activation with HCTU. The progress of the reaction was monitored with the aid of the Kaiser test. Upon completion of the synthesis, removal of the protecting groups and cleavage from the resin with a TFA cocktail led after HPLC purification to the isolation of both the conjugates **8-9** and the conjugates **27-28**, having an azido group. Conjugates **27** and **28** were labelled with Cy5-BCN in DMSO as described Chapter 2.



Scheme 4. Synthesis of labelled and unlabeled TLR2-ligand conjugates. i) Fmoc SPPS ii) HCTU, DIPEA, NMP iii) TFA/TIS/H_2O (95/2.5/2.5) iv) Cy5-BCN, DMSO.

Preliminary immunological evaluation was performed using two different cell types, TLR-2 transfected HEK cells and isolated human DC. Figure 2 shows that the ligands, provided with a TEG spacer (**6** and **7**) show enhanced capacity to activate both TLR2 HEK cells (Figure 2A) and human monocyte-derived dendritic cells (Figure 2B). Their TLR2-mediated IL production (IL-8 and IL-12 respectively) was improved compared to the original ligand of the group of David (compound **1**) and also to a chirally pure version of the classical Pam_3CSK_4 (Pam-R) ligand. The TEG spacer outperforms the usual K4 solubility handle as incorporated in ligand **5**. On the basis of these data it was decided to prepare and evaluate conjugates **8** and **9**, in which ligands **6** and **7** are covalently connected to the N-terminus of MHC 1 epitope DEVSGLEQLESIINFEKL as well as the corresponding Cy_5 labeled conjugates **10** and **11**. As shown in Figure 3A and 3B, using both HEK cells and human DC the IL production (IL-8 and IL-12 respectively) of the unlabeled conjugates remain largely intact while a decrease is seen with the labelled conjugates **10** and **11**. The IL production of the positive controls (Pam-R ligand and LPS) have the same order of magnitude.

Next conjugates **8** and **9** were evaluated for antigen presentation and conjugates **10** and **11** for uptake in dendritic cells. Antigen presentation was determined using SIINFEKL-specific T cells which are activated by antigen presentation by dendritic cells. Murine dendritic cells were first incubated with the conjugates **8** and **9** and subsequently with specific T cells. Figure 4 shows moderate antigen presentation by the DC. This could be expected as maturation of the murine DC does not occur with these human-specific TLR2 ligand. For the same reason the mouse bio-active Pam-R conjugate shows a much stronger antigen presentation in this assay. The uptake by

dendritic cells was analyzed using the Cy5 fluorophore-labeled conjugate compound **10**. Figure 4 shows efficient engulfment of the compounds in both human (Figure 5A) and murine dendritic cells (Figure 5B) in similar endo-lysosomal compartments as shown in earlier studies with Pam_3CSK_4-peptide conjugates[14]. This efficient uptake in mouse dendritic cells is in accordance with the previous findings of Khan *et al.* that uptake of Pam-based lipopeptides by DC is independent of TLR2 triggering.[17]

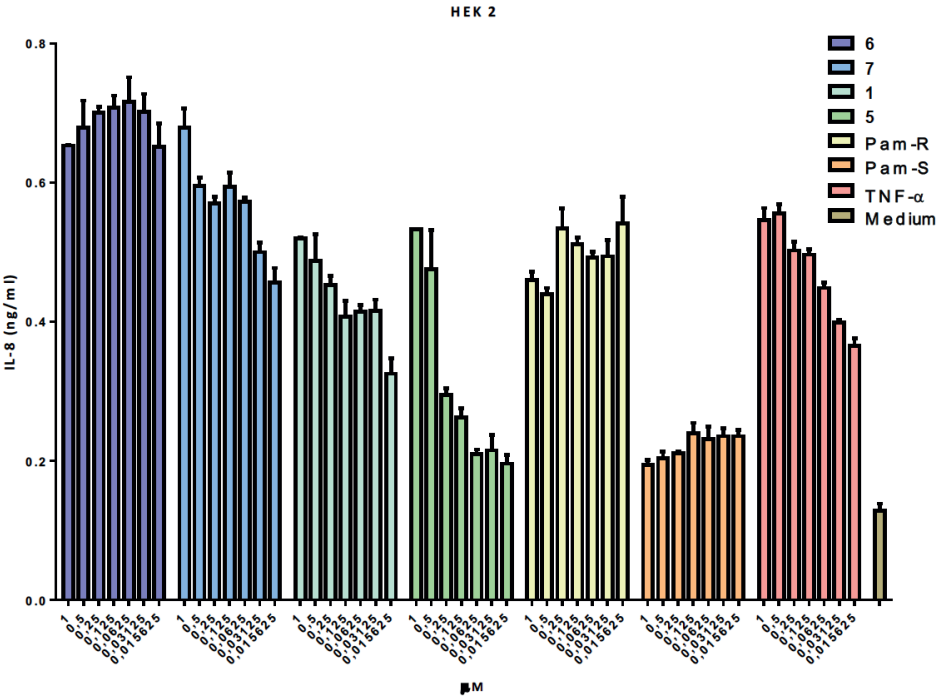


Figure 2A. Ability of the lipophilic ligands in triggering human IL-8 production via TLR-2. (a) HEK TLR-2 cells were incubated with titrated amounts of compounds **1**, **5**, **6**, **7**; R-Pam_3Cys, TNF-alpha (positive controls), S-Pam_3Cys (negative control) for 24 h. Error bars represent SD.

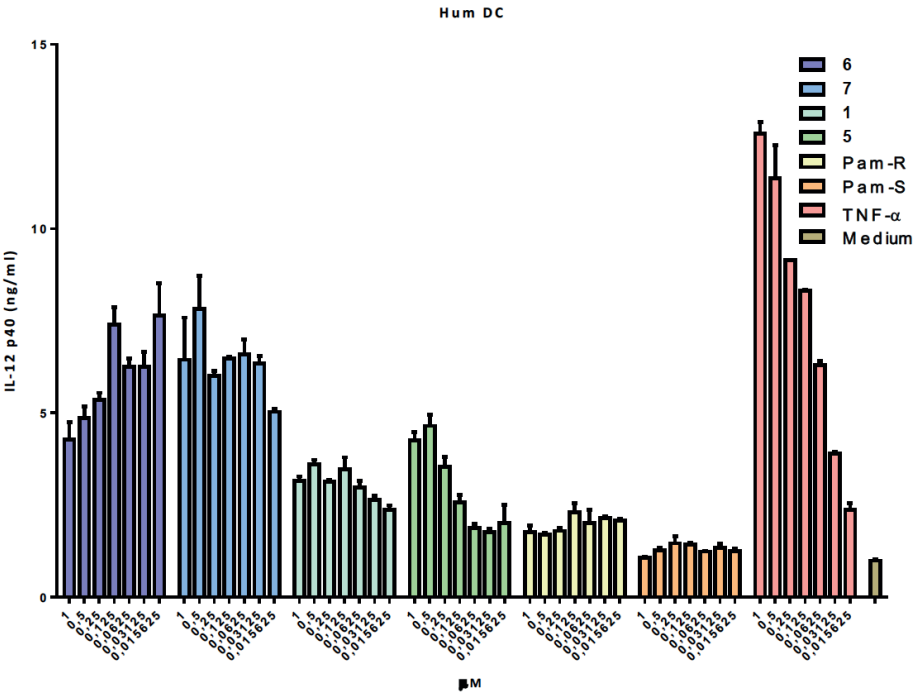


Figure 2B. Activation of human dendritic cells. DCs were stimulated with titrated amounts of compounds **1**, **5**, **6**, **7**; R-Pam$_3$Cys, TNF-alpha (positive controls), S-Pam$_3$Cys (negative control) for 48h. Supernatants were harvested and analyzed for IL-12 cytokine secretion by ELISA. One representative from three independent experiments is shown.

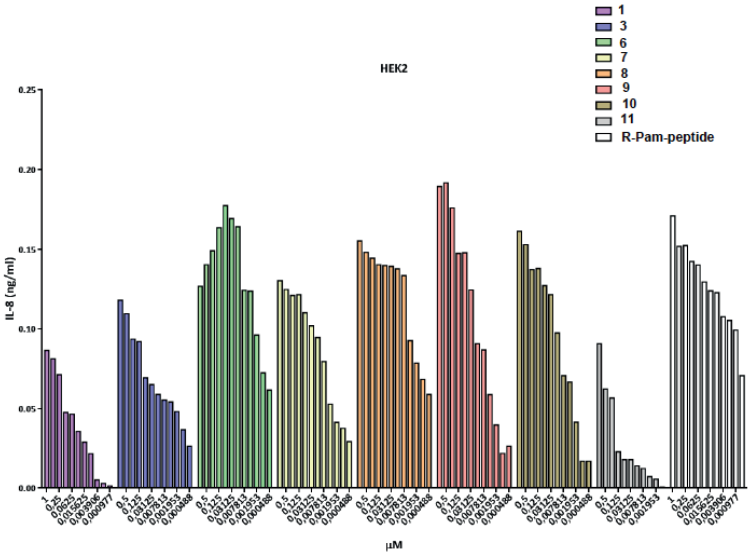


Figure 3A. Ability of the lipophilic ligands and lipopeptides in triggering human IL-8 production via TLR-2. (a) HEK TLR-2 cells were incubated with titrated amounts of compounds **1**, **3**, **6** – **11** or R-Pam3Cys, (positive control) for 24 h.

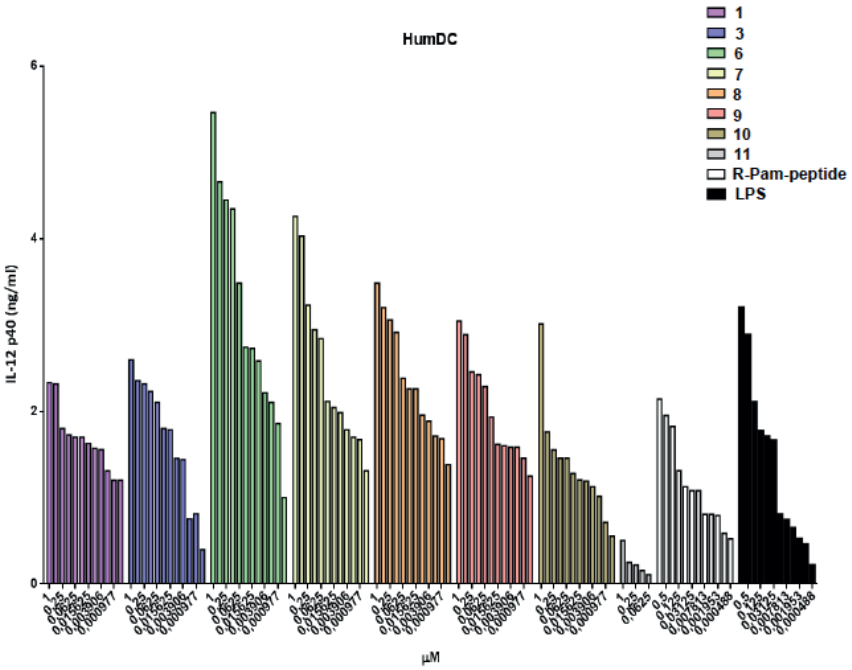


Figure 3B. Activation of human dendritic cells. DCs were stimulated with titrated amounts of compounds **1**, **3**, **6** – **11**; R-Pam$_3$Cys-lipopeptide, LPS (positive controls). Supernatants were harvested and analyzed for IL-12 cytokine secretion by ELISA as in Figure 2B.

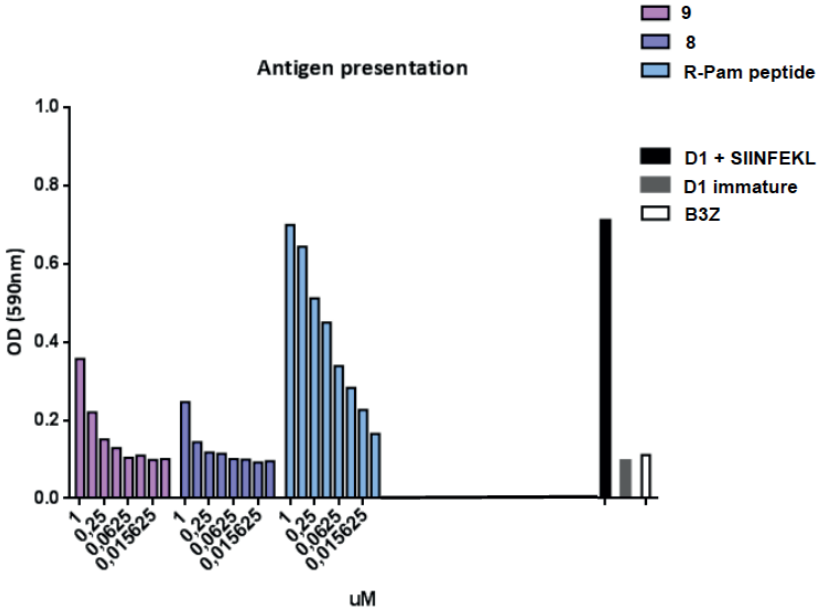


Figure 4. Antigen presentation of SIINFEKL MHC-I epitope by Dendritic cells loaded with lipopeptides **8** and **9.**

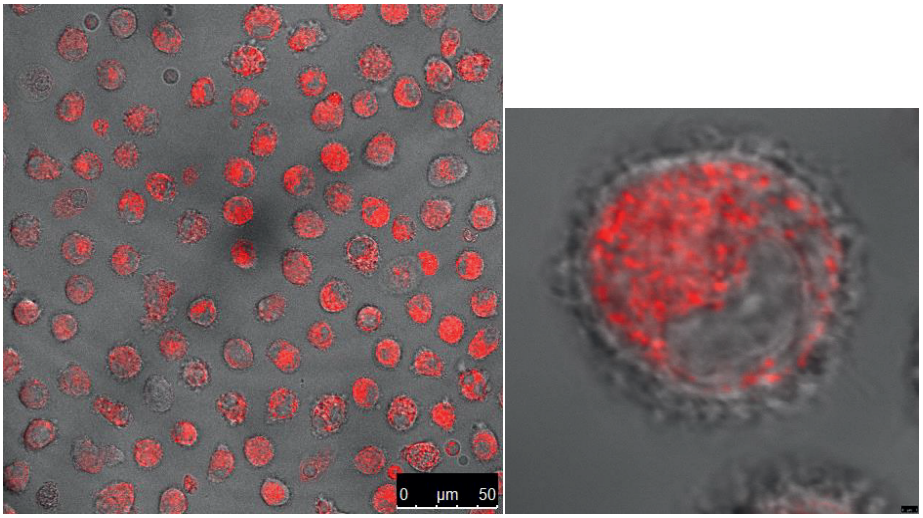


Figure 5A. Uptake of conjugate **10** by human moDC. The cells were incubated for 15 min with compound **10** (1µM). The uptake and localization of the compounds were analyzed with confocal laser scanning microscopy. The images are representative for multiple cells in at least 3 experiments.

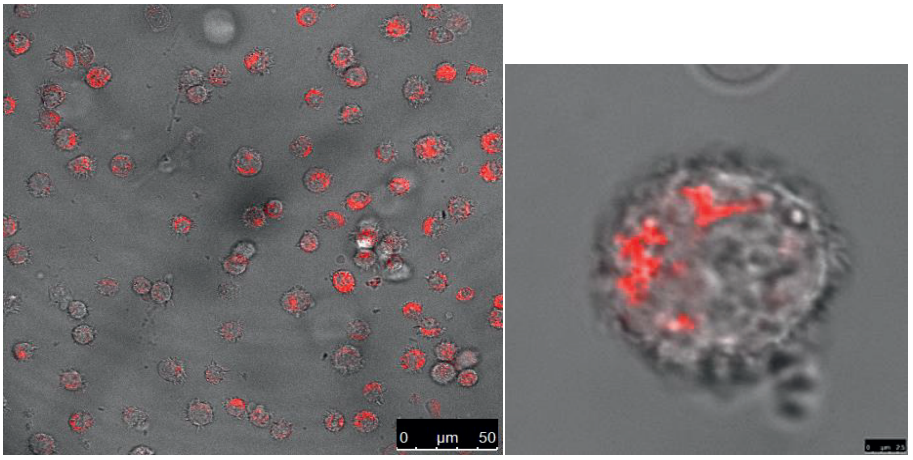


Figure 5B. Uptake of conjugate **10** by mouse D1-cells. The cells were incubated for 15 min with compound **10** (1µM). The uptake and localization of the compounds were analyzed with confocal laser scanning microscopy. The images are representative for multiple cells in at least 3 experiments.

Conclusion

This chapter describes the synthesis of five new TLR-2 ligands (**3**, **4**, **5**, **6** and **7**), two new conjugates (**8** and **9**) and the corresponding conjugates labeled with the fluorophore Cy5 (**10** and **11**). Preliminary immunological evaluation of ligands **5**, **6** and **7** shows that the presence of 4 lysines (K_4) as a solubility handle next to the serine in TLR-2 ligand **5** does not contribute favorably to the IL production. It can be hypothesized that steric hindrance contributes to this loss of activity. Ligands **6** and **7** which are provided with a less sterically demanding triethylene glycol spacer retain the water solubility and are much more potent than their lysine alternative (**5**). Attachment

of Ligands **6** and **7** to the N terminus of the DEVSGLEQLESIINFEKL model epitope furnished conjugates **8** and **9.** The agonistic activity of **8** and **9** remains mainly intact with conjugate **8** being slightly more active than amide analogue **9**. Internalization of the labeled conjugate **10** could be properly visualized on both human monocyte derived DC and mouse dendritic cells. These results are an incentive to synthesize and evaluate conjugates similar to **8** provided with a human specific epitope to allow the assessment of antigen presentation by DCs

Experimental

All solvents used under anhydrous conditions were stored over 4Å molecular sieves, except for methanol, which was stored over 3Å molecular sieves. Solvents used for workup and column chromatography were of technical grade from Sigma Aldrich and used directly. Unless stated otherwise, solvents were removed by rotary evaporation under reduced pressure at 40°C. Reactions were monitored by TLC-analysis using Merck 25 DC plastikfolien 60 F254 with detection by spraying with 1% $KMnO_4$, 10% Na_2CO_3 (aq) (unless stated otherwise) followed by charring at approx. 150°C. Column chromatography was performed on Fluka silicagel (0.04 – 0.063 mm). Analytical LC/MS was conducted on a JASCO system using an Alltima C_{18} analytical column (5µ particle size, flow: 1.0 ml/min), on which the absorbance was measured at 214 and 254 nm. Solvent system for LC/MS: A: 100% water, B: 100% acetonitrile, C: 1% TFA. High resolution mass spectra were recorded by direct injection (2 µL of a 2 µM solution in water/acetonitrile; 50/50; v/v and 0.1% formic acid) on a mass spectrometer (Thermo Finnigan LTQ Orbitrap) equipped with an electrospray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10, capillary temperature 250 °C) with resolution R = 60000 at m/z 400 (mass range m/z = 150-2000) and dioctylpthalate (m/z = 391.2842) as a "lock mass". The high-resolution mass spectrometer was calibrated prior to measurements with a calibration mixture (Thermo Finnigan). ^{1}H and ^{13}C NMR spectra were recorded with a Brüker AV 400 (400/100 MHz) and all individual signal were assigned using 2D-NMR spectroscopy. Chemical shifts are given in ppm (δ) relative to TMS (0 ppm) and coupling constants are given in Hz. Optical rotations were measured in $CHCl_3$. IR spectra were recorded on a Shimadzu FTIR-8300 and are reported in cm^{-1}. Specific rotation was measured in chloroform at 10 mg/mL concentration.

N-Fluorenylmethoxycarbonyl-S-[2 hydroxy ethyl]-(R)-cysteine tert-Butyl ester (12) To a solution of protected cysteinedisulfide (1.04 mmol, 835 mg) in THF (10 mL) was added zinc powder (7 mmol, 455 mg, <10 µm) and a 100:7:1 solution of MeOH:37% HCl:98% H_2SO_4 (5 mL). The mixture was stirred for 15 min at RT. Oxirane (10 mmol, 0.51 mL) was added at 0°C and the mixture was stirred overnight at RT. TLC analysis (2:8 EA:pentane, R_f=0,7) showed complete conversion and the reaction mixture was filtrated and concentrated *in vacuo*. The crude was dissolved in EtOAc and washed with 10% $KHSO_4$ (aq). The solution was dried ($MgSO_4$), filtrated and concentrated *in vacuo*. Purification by silica gel column chromatography (4:6 EA:pentane, R_f=0,4) yielded compound **12** (1.07 mmol, 477 mg) in a 50% yield.
IR(cm^{-1}): 2917,2850, 1742, 1660, 1463.
HRMS $[M+H]^+$: 444.18392 (calculated), 444.18436 (measured).
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (d, *J* = 7.5 Hz, 2H), 7.60 (d, *J* = 7.3 Hz, 2H), 7.38 (t, *J* = 7.4 Hz, 2H), 7.30 (t, *J* = 7.4 Hz, 2H), 5.90 (d, *J* = 7.9 Hz, 1H), 4.55 – 4.45 (m, 1H), 4.39 (m, 2H), 4.22 (t, *J* = 7.0 Hz, 1H), 3.77 – 3.66 (m, 2H), 3.06 – 2.87 (m, 2H), 2.80 – 2.65 (m, 2H), 1.47 (s, 9H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 169.75, 156.00, 143.85, 141.31, 127.75, 127.11, 125.15, 120., 83.04, 67.15, 60.90, 54.62, 47.14, 36.31, 35.04, 28.02,
N-Fluorenylmethoxycarbonyl-S-[2 palmitoyloxy ethyl]-(R)-cysteine tert-Butyl ester (13)
Compound **8** (2.23 mmol, 987 mg) was dissolved in dry DCM (30 mL). Palmitic acid (6.69 mmol, 1.72 g), DIC (8.92 mmol, 1.41 mL) and DMAP (1.1 mmol, 0.14 g) were added. The mixture was stirred overnight at RT under argon atmosphere. TLC analysis (4:6 EA:pentane, R_f=0.4) showed complete conversion. Glacial acetic acid (1 mL) was added and the mixture was stirred for 15 min at RT. The reaction mixture was filtrated and concentrated *in vacuo*. The crude was dissolved in PE and filtrated. The filtrate was purified

by silica gel column chromatography (5:95 EA:pentane, R_f=0.15) and compound **9** (1.97 mmol, 1.34 g) was obtained with a 88% yield.

$[\alpha]_D$: +2°

IR(cm^{-1})= 3333, 2916, 2850, 1733, 1699, 1532.

HRMS $[M+H]^+$: 682.41354 (measured), 682.41359 (calculated).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, *J* = 7.5 Hz, 2H), 7.61 (d, *J* = 7.3 Hz, 2H), 7.39 (t, *J* = 7.4 Hz, 2H), 7.30 (t, *J* = 7.4 Hz, 2H), 5.73 (d, *J* = 7.6 Hz, 1H), 4.52 (dt, *J* = 7.5, 4.8 Hz, 1H), 4.46 – 4.28 (m, 2H), 4.22 (m, 3H), 3.04 (m, 2H), 2.77 (t, *J* = 6.6 Hz, 2H), 2.32 – 2.20 (m, 2H), 1.58 (d, *J* = 6.8 Hz, 2H), 1.45 (d, *J* = 26.7 Hz, 9H), 1.21 (m, 24H), 0.94 – 0.78 (t, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 173.61, 169.61, 155.75, 143.83, 141.33, 127.76, 127., 125.17, 120.02, 82.99, 67.18, 63.13, 54.38, 47.16, 34.92, 34.20, 31.99, 31.43, 29.76, 29.73, 29.68, 29.54, 29.44, 29.34, 29.20, 28.03, 24.94, 22.77, 14.22

N-Fluorenylmethoxycarbonyl-S-[2 palmitoyloxy ethyl]-(R)-cysteine (14)

Compound **9** (0.47 mmol, 0.32 g) was dissolved in neat TFA (5 mL) and stirred for 1 hour at RT. TLC analysis (10% EtOAc in PE, R_f= 0.3) showed complete conversion. The reaction mixture was concentrated and co-evaporated with toluene *in vacuo*. The crude was adsorbed on Celite and purified by silica gel column chromatography (15:85 EA:pentane + 1% acetic acid, R_f=0.15). Compound **10** (0.42 mmol, 0.26 g) was obtained with a 90% yield.

$[\alpha]_D$: +12.4°

IR(cm^{-1})= 3317, 2500-3200, 2916,2848, 1732, 1691, 1537

HRMS $[M+H]^+$: 626.36067 (measured), 626.35099 (calculated).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (s, 1H, COOH), 7.75 (d, *J* = 7.5 Hz, 2H, C-H Fmoc), 7.60 (d, *J* = 6.2 Hz, 2H C-H Fmoc), 7.39 (t, *J* = 7.4 Hz, 2H C-H Fmoc), 7.30 (t, *J* = 7.4 Hz, 2H C-H Fmoc), 5.79 (d, *J* = 7.8 Hz, 1H, N_{25}), 4.66 (m, 1H, C_2), 4.50 – 4.33 (m, 2H, C_{23}), 4.31 – 4.08 (m, 3H, C_{5+24}), 3.09 (m, 2H, C_3), 2.77 (t, *J* = 6.4 Hz, 2H, C_4), 2.28 (t, *J* = 7.6 Hz, 2H, C_7), 1.57 (m, 2H, C_8), 1.24 (m, 24H, $C_{9\text{-}20}$), 0.88 (t, *J* = 6.8 Hz, 3H, C_{21}).

^{13}C NMR (101 MHz, $CDCl_3$) δ 174.61 ($C_{1,6}$), 174.11 ($C_{1,6}$), 156.11 (C_{22}), 143.72 (C_q Fmoc), 141.40 (C_q Fmoc), 127.87 (C-H Fmoc), 127.20 (C-H Fmoc), 125.21 (C-H Fmoc), 120.12 (C-H Fmoc), 67.51 (C_{23}), 63.23 (C_5), 53.73 (C_2), 47.17 (C_{24}), 34.48 (C_3), 34.29 (C_7), 32.05, 31.35 (C_4), 29.83 ($C_{9\text{-}19}$), 29.79 ($C_{9\text{-}19}$), 29.75 ($C_{9\text{-}19}$), 29.61 ($C_{9\text{-}19}$), 29.49 ($C_{9\text{-}19}$), 29.40 ($C_{9\text{-}19}$), 29.26 ($C_{9\text{-}19}$), 24.99 (C_8), 22.82 (C_{20}), 14.27 (C_{21}).

$HO\text{-}(CH_2CH_2O)_3CH_2C(O)OtBu$ (15)

Triethyleneglycol (40 mmol, 5.3 mL) was dissolved in dry THF (200 mL) under argon atmoshphere. Sodium hydride (0.84 g, 21 mmol) was added at 0°C. Tetrabutylammonium iodide (2.0 mmol, 0.37 g) was added. tert-Butyl bromoacetate (20 mmol, 3.0 mL) was added and the reaction was stirred overnight at RT under argon atmosphere. The reaction mixture was filtrated and the THF was evaporated. The crude was adsorbed on Celite and purified by silica gel column chromatography (8:2 EA:pentane). Compound **15** (7.7 mmol, 2.0 g) was obtained with a 40% yield.

IR(cm^{-1})=2873(C-H, stretch), 1742(C=O, stretch).

HRMS $[M+H]^+$: 265.16298 (measured), 265.16456 (calculated).

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.02 (s, 2H), 3.78 – 3.66 (m, 10H), 3.61 (dd, *J* = 5.3, 3.8 Hz, 2H), 1.48 (s, 9H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 169.67, 81.72(C_{10}), 72.66, 70.62, 70.60, 70.49, 70.23, 68.97, 61.65, 28.10.

$MsO\text{-}(CH_2CH_2O)_3CH_2C(O)OtBu$ (16)

Compound **15** (4.73 mmol, 1.25 g) was dissolved in DCM (50 mL). TEA (9.46 mmol, 1.30 mL) was added and the mixture was cooled to 0°C. MsCl (5.31 mmol, 0.41 mL) was slowly added. The mixture was heated to RT and stirred for 3 hours. TLC analysis (EA) indicated complete conversion. The reaction mixture was diluted with DCM and washed with 10% $KHSO_4$ (aq) (3x), 10% $NaHCO_3$ (aq) (3x) and brine (1x). The solution was dried (Na_2SO_4), filtrated and evaporated *in vacuo*. The crude was purified by silica gel column chromatography (5:5 EA:pentane → 8:2 EA:pentane, Δ=10%). Compound **16** (4.25 mmol, 1.45 g) was obtained with a 90% yield.

IR(cm^{-1})=2872(C-H, stretch), 1742(C=O, stretch).

HRMS $[M+H]^+$: 343.14186 (measured), 343.14211 (calculated).

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.01 (s, 2H), 3.80 – 3.76 (m, 2H), 3.73 – 3.65 (m, 8H), 3.09 (s, 3H), 1.48 (s, 9H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 169.58, 81.61, 70.68, 70.60, 70.53, 70.52, 69.37, 69.01, 68.96, 37.70, 28.11.

N_3-$(CH_2CH_2O)_3CH_2C(O)OtBu$ (17)

Compound **16** (2.96 mmol, 1.01 g) was dissolved in DMF (30 mL). Sodium azide (9 mmol, 585 mg) was added and the mixture was heated to 60°C. After 4 hours of stirring TLC analysis (8:2 EA:pentane) showed complete conversion. The mixture was diluted with EA and washed with 5% $NaHCO_3$ (aq) (4x). The solution was dried (Na_2SO_4), filtrated and evaporated *in vacuo*. The crude was purified by silica gel column chromatography (4:6 EA:pentane → 8:2 EA:pentane, Δ=10%). Compound **17** (2.65 mmol, 766 mg) was obtained with a 90% yield.

IR(cm^{-1})=2869(C-H, stretch), 2099(N=N=N, stretch), 1745(C=O, stretch).

HRMS $[M+Na]^+$: 312.15314 (measured), 312.15299 (calculated).

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.03 (s, 2H), 3.76 – 3.65 (m, 10H), 3.44 – 3.35 (m, 2H), 1.48 (s, 9H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 169.56, 81.42, 70.61, 70.58, 70.56, 70.54, 69.94, 68.92, 50.58, 28.01 .

NH_2-$(CH_2CH_2O)_3CH_2C(O)OtBu$ (18)

Compound **17** (2.22 mmol, 642 mg) was dissolved in dry THF (25 mL) under argon atmosphere. Triphenylphosphine (2.7 mmol, 707 mg) was added and the mixture was stirred for 20 hours. TLC analysis (6:4 pentane:EA) indicated complete conversion. Water was added until white crystals were formed in the solution. The mixture was diluted with DCM and washed with 10% $NaHCO_3$ (aq) (3x). The solution was dried (Na_2SO_4), filtrated and evaporated *in vacuo*. The crude was absorbed on Celite and purified by silica gel column chromatography (1% MeOH in DCM + 1% TEA → 10% MeOH in DCM + 1 % TEA, Δ=2.5%). Compound **18** (1 mmol, 264 mg) was obtained with a 45% yield.

IR(cm^{-1})= 3400(1° N-H, stretch), 2874(C-H, stretch), 1742(C=O, stretch).

HRMS $[M+H]^+$: 264.18073 (measured), 264.18055 (calculated).

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.40 (t, *J* = 5.5 Hz, 1H), 4.04 (s, 2H), 3.71 (m, 4H), 3.63 (s, 4H), 3.55 (t, *J* = 5.2 Hz, 2H), 3.37 (m, 2H), 1.48 (s, 9H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 169.79, 81.84, 70.80, 70.76, 70.51, 70.48, 70.17,69.06,40.19, 28.22

FmocNH-Cys($EtOC(O)C_{15}H_{31}$)-Ser(tBu)-OMe (19)

Compound **14** (1.27 mmol, 794 mg) was dissolved in dry DMF (10 mL). H-Ser(OtBu)-OMe.HCl (1.52 mmol, 321 mg) was added. A solution of TEA (1.91 mmol, 0.26 mL) in DMF (10 mL) was slowly added to the reaction mixture. HOBt (1.91 mmol, 258 mg) and DIC (1.9 mmol, 0.30 mL) were added and the mixture was stirred at RT for 2 hours under argon atmosphere. TLC analysis (3:7 EA:pentane, R_f=0.2, ninhydrine) showed complete conversion. The crude was dissolved in DCM and washed with H_2O. The solution was dried ($MgSO_4$), filtrated, concentrated and co-evaporated with toluene *in vacuo*. The crude was adsorbed on Celite and purified by silica gel column chromatography (2:8 EA:pentane, R_f=0.15). **19** (1.12 mmol, 873 mg) was obtained with a 88% yield.

$[\alpha]_D$: +8°

IR(cm^{-1})= 3298, 2918,2848, 1734, 1660, 1531

HRMS $[M+H]^+$: 783.46038 (measured), 783.46126 (calculated).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (d, *J* = 7.5 Hz, 2H), 7.59 (d, *J* = 7.4 Hz, 2H), 7.38 (t, *J* = 7.5 Hz, 2H), 7.29 (t, *J* = 7.4 Hz, 3H), 5.99 (d, *J* = 7.1 Hz, 1H), 4.69 (dd, *J* = 5.1, 3.1 Hz, 1H), 4.54 – 4.30 (m, 3H), 4.23 (m, 3H), 3.82 (dd, *J* = 9.1, 2.7 Hz, 1H), 3.72 (s, 3H), 3.57 (dd, *J* = 9.1, 3.0 Hz, 1H), 2.98 (d, *J* = 3.9 Hz), 2.84 (s, 2H), 2.29 (t, *J* = 7.6 Hz, 2H), 1.59 (dd, *J* = 14.0, 7.0 Hz, 2H), 1.26 (m, 24H), 1.12 (s, 9H), 0.88 (t, *J* = 6.8 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 173.66,170.43, 170.14, 155.91, 143.76, 141.26, 127.72, 127.07, 125.15, 119.97, 73.56, 67.27, 62.92, 61.62, 54.14, 53.21, 52.43, 47.08, 34.91, 34.16, 31.93, 30.99, 29.70, 29.66, 29.62,29.48,29.37, 29.29, 29.15, 27.26, 24.88, 22.70, 14.15.

NH_2-Cys($EtOC(O)C_{15}H_{31}$)-Ser(tBu)-OMe (20)

19 (0.50 mmol, 0.39 g) was dissolved in dry DMF (10 mL). A solution of 2% piperidine and 2%DBU in DMF (10 mL) was slowly added. The mixture was stirred for 15 min at RT) under argon atmosphere. TLC analysis (95:5 DCM:MeOH, R_f=0.8, ninhydrine) showed complete conversion. The mixture was taken up in EA and washed with 10% $KHSO_4$(aq)(2x) and H_2O(2x). The solution was dried ($MgSO_4$), filtrated, concentrated and co-evaporated with toluene *in vacuo*. The crude was adsorbed on Celite and purified by silica gel column

chromatography (99% DCM, 1% MeOH + 0.1% TEA, R_f=0.1). Compound **20** (0.44 mmol, 0.25 g) was obtained with an 88% yield.
$[\alpha]_D$: -49.2°
IR(cm^{-1})= 3178,3082, 2917, 2851, 1744, 1677, 1660, 1464
HRMS $[M+H]^+$: 561.39046 (measured), 561.39318 (calculated).
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (d, *J* = 8.5 Hz, 1H), 4.67 (dt, *J* = 8.6, 3.2 Hz, 1H), 4.22 (t, *J* = 6.7 Hz, 2H), 3.83 (dd, *J* = 9.1, 3.2 Hz, 1H), 3.75 (s, 3H), 3.56 (m, 2H), 3.09 (dd, *J* = 13.6, 3.8 Hz, 1H), 2.83 – 2.74 (m, 3H), 2.32 (t, *J* = 7.6 Hz, 2H), 1.62 (m, 2H), 1.27 (s, 24H), 1.15 (s, 9H), 0.88 (t, *J* = 6.8 Hz, 3H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 173.68,173.26, 170.92, 73.47, 63.12, 62.06, 54.19, 52.76, 52.41, 37.93, 34.25, 32.00, 30.68, 29.76, 29.73, 29.68, 29.54, 29.43, 29.35, 29.21, 27.39, 24.98, 22.77, 14.20

AcNH-Cys(EtOC(O)$C_{15}H_{31}$)-Ser(tBu)-OMe
Compound **20** (0.17 mmol, 95 mg) was dissolved in dry DCM (2 mL). A solution of TEA (0.25 mmol, 36 µL) in dry DCM (1 mL) was slowly added. Acetic anhydride (0.34 mmol, 32 µL) was added and the mixture was stirred overnight at RTunder argon atmosphere. TLC analysis (93:7 DCM:MeOH, R_f=0,3, ninhydrine) showed complete conversion. The mixture was taken up in DCM and washed with a saturated solution of NH_4Cl (aq). The solution was dried ($MgSO_4$), filtrated and concentrated *in vacuo*. The crude was dissolved in DCM and purified by silica gel column chromatography (99:1 DCM:MeOH, R_f=0.15, ninhydrine). **AcNH-Cys(EtOC(O)$C_{15}H_{31}$)-Ser(tBu)-OMe** (0.16 mmol, 96 mg) was obtained with a 94% yield.
$[\alpha]_D$: +17.8°
IR(cm^{-1})= 3285, 2916, 2849, 1737, 1660
HRMS $[M+H]^+$: 603.40167 (measured), 603.40375 (calculated).
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29 (d, *J* = 8.2 Hz, 1H), 6.77 (d, *J* = 7.4 Hz, 1H), 4.72 – 4.60 (m, 2H), 4.26 (t, *J* = 6.6 Hz, 2H), 3.83 (dd, *J* = 9.1, 3.0 Hz, 1H), 3.75 (s, 3H), 3.57 (dd, *J* = 9.1, 3.3 Hz, 1H), 3.03 – 2.80 (m, 4H), 2.32 (t, *J* = 7.6 Hz, 2H), 2.04 (s, 3H), 1.61 (m, 2H), 1.35 – 1.20 (m, 24H), 1.14 (s, 9H), 0.88 (t, *J* = 6.8 Hz, 3H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 173.71, 170.44, 170.34, 170.02, 73.57, 62.83, 61.54, 53.24, 52.46, 52.44, 34.59, 34.22, 31.95, 30.97, 29.72, 29.68, 29.64, 29.50, 29.39, 29.31, 29.18, 27.29, 24.91, 23.11, 22.72, 14.16 .
$C_{15}H_{31}$C(O)NH-Cys(EtOC(O)$C_{15}H_{31}$)-Ser(tBu)-OMe
Compound **20** (0.17 mmol, 95 mg) was dissolved in dry pyridine (2 mL). Palmitoyl chloride (0.21 mmol, 63 µL) was added and the mixture was stirred at RT under argon atmosphere for 45 min. TLC analysis (93:7 DCM:MeOH, R_f=0.3, ninhydrine) showed complete conversion. The reaction mixture was taken up in DCM and washed with 10% $KHSO_4$ (aq). The solution was dried ($MgSO_4$), filtrated and concentrated *in vacuo*. The crude was dissolved in DCM and purified by silica gel column chromatography (99.5:0.5 DCM:MeOH → 99:1 DCM:MeOH, R_f=0.1). **$C_{15}H_{31}$C(O)NH-Cys(EtOC(O)$C_{15}H_{31}$)-Ser(tBu)-OMe** (0.17 mmol, 0.13 g) was obtained with a quantitative yield.
$[\alpha]_D$: +10.6°
IR(cm^{-1})= 3283, 2915, 2848, 1737, 1639
HRMS $[M+H]^+$: 799.62276 (measured), 799.62285 (calculated).
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (d, *J* = 8.2 Hz, 1H), 6.74 (d, *J* = 7.4 Hz, 1H), 4.71 – 4.62 (m, 2H), 4.26 (t, *J* = 6.6 Hz, 2H), 3.83 (dd, *J* = 9.1, 3.0 Hz, 1H), 3.74 (s, 3H), 3.57 (dd, *J* = 9.1, 3.3 Hz, 1H), 3.00 – 2.82 (m, 4H), 2.32 (m, 2H), 2.28 – 2.20 (m, 2H), 1.69 – 1.56 (m, 4H), 1.37 – 1.20 (m, 48H), 1.14 (s, 9H), 0.88 (t, *J* = 6.8 Hz, 6H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 173.64, 173.20, 170.51, 170.34, 73.54, 62.84, 61.47, 53.23, 52.40, 52.22, 36.46, 34.57, 34.17, 33.97, 31.91, 30.90, 29.69,29.65, 29.63, 29.49, 29.47, 29.35, 29.28, 29.26, 29.15, 27.23, 25.57, 24.87, 22.68, 14.10
NH_2C(O)NH-Cys(EtOC(O)$C_{15}H_{31}$)-Ser(tBu)-OMe
Compound **20** (0.39 mmol, 0.22 g) was dissolved in dry DCM (10 mL). Isopropanol (8.0 mmol, 0.61 mL) was added. 85% (Trimethylsilyl)isocyanate (4.0 mmol, 0.65 mL) was added and the mixture was stirred overnight at RT under argon atmosphere. TLC analysis (1:1 EA:pentane + 1% TEA) indicated completion of

reaction. Celite was added to the reaction mixture and the DCM was evaporated *in vacuo*. The adsorbed crude was purified by silica gel column chromatography (1:1 EA:pentane +1% TEA → 95:5 EA:MeOH + 1% TEA). **$NH_2C(O)NH$-Cys($EtOC(O)C_{15}H_{31}$)-Ser(tBu)-OMe** (0.40 mmol, 0.24 g) was obtained with a quantitative yield.

$[\alpha]_D$: +10.3°

IR(cm^{-1})=2916, 2849, 1733, 1645,

HRMS $[M+H]^+$: 604.39816 (measured), 604.39900 (calculated).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (d, *J* = 8.1 Hz, 1H), 6.46 (d, *J* = 7.9 Hz, 1H), 5.15 (m, 1H), 4.69 – 4.54 (m, 2H), 4.31 – 4.18 (m, 2H), 3.80 (dd, *J* = 9.1, 3.3 Hz, 1H), 3.74 (s, 3H), 3.58 (dd, *J* = 9.2, 3.5 Hz, 1H,), 2.94 (m, 2H), 2.83 (m, 2H), 2.31 (t, *J* = 7.6 Hz, 2H), 1.60 (m, 2H), 1.35 – 1.20 (m, 24H), 1.14 (s, 9H), 0.88 (t, *J* = 6.8 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 173.99,171.66, 170.62, 158.57, 73.70, 63.01, 61.68, 53.44, 53.33, 52.51, 35.35, 34.29, 32.00, 31.13, 29.77, 29.74, 29.57, 29.44, 29.38, 29.25, 27.34, 24.97, 22.76, 14.20.

AcNH-Cys($EtOC(O)C_{15}H_{31}$)-Ser(OH)-OMe (1)

AcNH-Cys($EtOC(O)C_{15}H_{31}$)-Ser(tBu)-OMe (0.24 mmol, 145 mg) was dissolved in TFA (5 mL) and was stirred at RT. After 30 min the mixture was dropped in Et_2O and precipitated overnight at -20° C. Compound **1** (0.15 mmol, 82 mg) was obtained with a 62% yield.

$[\alpha]_D$: +7.0°

IR(cm^{-1})= 3281, 2914, 2849, 1737, 1630.

HRMS $[M+H]^+$: 547,33966 (measured), 547.34115 (calculated).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (d, *J* = 7.9 Hz, 1H), 6.91 (d, *J* = 7.5 Hz, 1H), 4.71 (dd, *J* = 13.7, 6.8 Hz, 1H), 4.67 – 4.61 (m, 1H), 4.33 – 4.16 (m, 2H), 3.94 (ddd, *J* = 25.2, 11.6, 3.5 Hz, 2H), 3.78 (s, 3H), 2.96 (qd, *J* = 13.9, 6.5 Hz, 2H), 2.81 (t, *J* = 6.7 Hz, 2H), 2.32 (t, *J* = 7.6 Hz, 2H), 2.05 (s, 3H), 1.68 – 1.54 (m, 2H), 1.36 – 1.19 (m, 24H), 0.88 (t, *J* = 6.8 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 174.17, 171.31, 170.71, 170.64, 62.88, 62.61, 55.12, 52.85, 34.56, 34.34, 32.04, 31.14, 29.81, 29.78, 29.74, 29.61, 29.48, 29.40, 29.27, 25.00, 23.10, 22.81, 14.24

$C_{15}H_{31}$C(O)NH-Cys($EtOC(O)C_{15}H_{31}$)-Ser(OH)-OMe (2)

$C_{15}H_{31}$C(O)NH-Cys($EtOC(O)C_{15}H_{31}$)-Ser(tBu)-OMe (0.31 mmol, 245 mg) was dissolved in TFA (5 mL) and was stirred at RT. After 30 min the mixture was dropped in Et_2O and precipitated over weekend at -20°C. Compound **2** (0.29 mmol, 217 mg) was obtained with a 94% yield.

$[\alpha]_D$: +2.9°

IR(cm^{-1})= 3313, 2910, 2848, 1739, 1639

HRMS $[M+H]^+$: 743.56003 (measured), 743.56025 (calculated).

^{1}H NMR (400 MHz, $CDCl_3$) δ7.61 (d, *J* = 7.7 Hz, 1H), 6.87 (d, *J* = 7.3 Hz, 1H), 4.77 – 4.58 (m, 2H), 4.36 – 4.15 (m, 2H), 3.96 (m, 2H), 3.80 (d, *J* = 10.2 Hz, 3H), 3.05 – 2.89 (m, 2H), 2.82 (t, *J* = 6.6 Hz, 2H), 2.29 (m, 4H), 1.61 (m, 4H), 1.37 – 1.16 (m, 48H), 0.88 (t, *J* = 6.6 Hz, 6H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 174.95, 174.34, 170.83, 170.50, 62.88, 62.61, 55.14, 52.91, 52.77, 36.47, 34.43, 34.36, 32.05, 31.15, 29.83, 29.79, 29.63, 29.49, 29.42, 29.36, 29.28, 25.75, 25.00, 22.82, 14.24 .

$NH_2C(O)NH$-Cys($EtOC(O)C_{15}H_{31}$)-Ser(OH)-OMe (3)

$NH_2C(O)NH$-Cys($EtOC(O)C_{15}H_{31}$)-Ser(tBu)-OMe (0.37 mmol, 225 mg) was dissolved in TFA (5 mL) and was stirred at RT. After 30 min the mixture was co-evaporated with toluene *in vacuo*. After silica gel column chromatography purification (1% MeOH in EA → 4% MeOH in EA) compound **3** (0.33 mmol, 180 mg) was obtained with a 89% yield.

$[\alpha]_D$: +3.3°

IR(cm^{-1})= 3286, 2916, 2849, 1742, 1639

HRMS $[M+H]^+$: 548.33519 (measured), 548.33640 (calculated).

^{1}H NMR (400 MHz, DMSO) δ 8.37 (d, *J* = 7.8 Hz, 1H), 6.25 (d, *J* = 8.7 Hz, 1H), 5.68 (s, 2H), 5.08 (t, *J* = 5.6 Hz,

1H), 4.49 – 4.39 (m, 1H), 4.36 (m, 1H), 4.21 – 4.06 (m, 2H), 3.71 (m, 1H), 3.67 – 3.56 (m, 4H,), 2.83 (dd, *J* = 13.7, 5.2 Hz, 1H), 2.75 (m, 2H), 2.65 (dd, *J* = 13.7, 7.7 Hz, 1H), 2.28 (t, *J* = 7.4 Hz, 2H), 1.59 – 1.45 (m, 2H), 1.33 – 1.15 (m, 24H), 0.85 (t, *J* = 6.8 Hz, 3H).

^{13}C NMR (101 MHz, DMSO) δ 172.76, 171.34, 170.74, 157.95, 62.88, 61.17, 54.61, 52.25, 51.84, 34.89, 33.40, 31.29, 29.99, 29.04, 28.88, 28.70, 28.44, 24.42, 22.09, 13.96

AcNH-Cys(EtOC(O)$C_{15}H_{31}$)-SerLysLysLysLys-C(O)NH_2 (4)

The SK_4 peptide was prepared by applying Fmoc based protocol starting from Tentagel S RAM resin (4.35 g; loading 0.23 mmol/g) on a AB peptide synthesizer. The amino acids used were Fmoc-Lys(Boc) and Fmoc-Ser(Ot Bu)-OH. To a mixture of resin bound peptide (SK_4) (0.05 mmol; 0.228 g) in NMP/DCM (1:1) was added **14**(0.125 mmol; 0.78 g), PyBop (0.175 mmol; 0.091 g) and DiPEA 1M (0.25 mmol; 0.032 g; 250 µL). The DiPEA was added in two times, first an amount of 125 µL, after 10 min another amount of 125 µL. The reaction mixture was then stirred overnight on the orbital shaker at rt. The resin was washed three times with DCM and the Fmoc was cleaved with 20% piperidine/DMF (3 times 5 min). The now free amine acetylated by adding acetyl chloride (0.5 mmol; 0.039 g; 35 µL) in pyridine/DCM (1:1), the mixture was stirred for 2.5 h. The solution was washed with DCM three times and the resulting conjugate was cleaved off the resin by adding TFA/TiS/H2O (95/2.5/2.5) (2h). Purification of the conjugate was done by adding the crude to cold diethylether/n-pentane (1:1) (14 mL) and centrifugation (4000 rpm, 5min) was performed. The precipitate was dissolved in magic (MeCN/t BuOH/H2O) (1:3:1). This solution was then subjected to semi-prep HPLC, pure lipopeptide fractions were collected and concentrated by freeze-drying. This yielded conjugate **4**(5.6 µmol; 5.8 mg; 7.7%). LCMS: 10-90% C18, Rt = 6.4 min.

NH_2C(O)NH-Cys(EtOC(O)$C_{15}H_{31}$)-SerLysLysLysLys-C(O)NH_2 (5)

To synthesize **5**, the coupling of building block **14** to the pentapeptide SK_4 was done under the same conditions described by the coupling procedure for compound **4**. After the coupling, DCM wash and the cleavage of the Fmoc, the free amine was treated with TMS-isocyanate (0.5 mmol; 0.058 gr; 68 µL) and isopropanol (1 mmol; 0.06 g; 76 µL) in DCM. Further procedure and purification was done according to the same method explained for compound **4**. This provided compound **5** (2.9 µmol; 3.0 mg; 4%). LCMS: 10-90% C18, Rt = 6.3 min.

FmocNH-Ser(OtBu)-O($CH_2CH_2O)_3CH_2$C(O)OtBu

Compound **15** (2.8 mmol, 1.6 g) was dissolved in dry DCM (30 mL). Fmoc-Ser(OtBu)-OH (3.38 mmol, 1.29 g), DIC (3.36 mmol, 0.52 mL) and DMAP (0.3 mmol, 38 mg) were added and the mixture was stirred for 4 hours at RT under argon atmosphere. TLC-MS indicated complete conversion. The reaction mixture was diluted with DCM and washed with 10% $KHSO_4$(aq)(3x), 10% $NaHCO_3$(aq)(3x) and brine (1x). The solution was dried (Na_2SO_4), filtrated and evaporated *in vacuo*. The crude was dissolved in THF and the urea byproduct was removed by crystallization at -20°C. The crude was further purified by silica gel column chromatography (8:2 pentane:EA→ 7:3 pentane:EA, R_f=0.3). Product (1.74 g, 2.77 mmol) was obtained with a quantitative yield.

$[\alpha]_D$: +6.33°

IR(cm^{-1})=2974, 2873, 1738, 1722, 1717

HRMS [M+H]$^+$: 630.32709 (measured), 630.32727 (calculated).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.5 Hz, 2H), 7.63 (t, *J* = 7.1 Hz, 2H), 7.40 (t, 2H), 7.31 (t, 2H), 5.75 (d, *J* = 8.9 Hz, 1H), 4.56 – 4.49 (m, 1H), 4.48 – 4.07 (m, 5H), 4.01 (s, 2H), 3.86 (dd, *J* = 9.0, 2.8 Hz, 1H), 3.76 – 3.54 (m, 11H), 1.47 (s, 9H), 1.16 (s, 9H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 170.62, 169.64, 156.11, 143.99, 143.81, 141.27, 127.70, 127.08,125.21, 125.17, 119.97, 81.55, 73.47, 70.69, 70.62, 70.57, 69.00, 68.98, 67.16, 64.54, 62.11, 54.67, 47.14, 28.12, 27.35

NH_2-Ser(OtBu)-O($CH_2CH_2O)_3CH_2$C(O)OtBu (21)

FmocNH-Ser(OtBu)-O($CH_2CH_2O)_3CH_2$C(O)OtBu (1.629 g, 2.59 mmol) was dissolved in dry DMF (10 mL). A mixture of 2:2 piperidine and DBU (v:v) in dry DMF (30 mL) was added. The mixture was stirred for 15 minutes at RT under argon atmosphere. TLC (6:4 pentane:EA) indicated complete conversion. The reaction mixture was diluted with EA and washed with 0.25% $KHSO_4$(aq) (3x), 10% $NaHCO_3$(aq) (3x) and brine (1x). The solution was dried (Na_2SO_4), filtrated and evaporated *in vacuo*. The crude was adsorbed

on Celite and purified with silica gel column chromatography (DCM →95:5 DCM:MeOH, Δ= 0.5%). Compound **21** (1.97 mmol, 802 mg) was obtained with a 76% yield.
$[\alpha]_D$: -8.95°
IR(cm^{-1})= 2974,2872, 1742
HRMS $[M+H]^+$: 408.25702 (measured), 408.25919 (calculated).
^{1}H NMR (400 MHz, $CDCl_3$) δ 4.29 (m, 2H), 4.02 (s, 2H), 3.76 – 3.51 (m, 13H), 1.48 (s, 9H), 1.09 (s, 9H,).
^{13}C NMR (101 MHz, $CDCl_3$) δ 174.22, 169.74, 81.72, 73.23, 70.81, 70.72, 70.65, 70.33, 69.12, 69.09, 64.12, 63.83, 55.26, 28.22, 27.57

FmocNH-Cys(EtOC(O)$C_{15}H_{31}$)-Ser(OtBu)-O($CH_2CH_2O)_3CH_2C$(O)OtBu
Compound **21** (0.70 mmol, 0.29 g) was dissolved in dry DMF (5 mL). Compound **14** (0.50 mmol, 0.31 g), HOBt (0.70 mmol, 94 mg) and DIC (0.70 mmol, 0.11 mL) was added. The mixture was stirred overnight at RT under argon atmosphere. TLC-MS indicated complete conversion. The mixture was diluted with EA and washed with 10% $NaHCO_3$(aq) (2x), 10% $KHSO_4$(aq) (2x) and brine (1x). The solution was dried (Na_2SO_4), filtrated and evaporated *in vacuo*. The crude was dissolved in THF and the urea byproduct was removed by crystallization at -20°C. The crude was adsorbed on Celite and purified by silica gel column chromatography (8:2 pentane:EA→ 5:5 pentane:EA, Δ=10%). Product (0.35 mmol, 0.36 g) was obtained with a 71% yield.
$[\alpha]_D$: +4.83°
IR(cm^{-1})= 2923, 2852, 1733, 1739
HRMS $[M+H]^+$: 1015.59320 (measured), 1015.59234 (calculated).
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.5 Hz, 2H), 7.60 (d, *J* = 7.4 Hz, 2H), 7.40 (t, *J* = 7.4 Hz, 2H), 7.30 (m, 3H), 5.88 (d, *J* = 6.5 Hz, 1H), 4.70 (dt, *J* = 8.2, 3.0 Hz, 1H), 4.48 – 4.34 (m, 3H), 4.32 – 4.17 (m, 5H), 4.01 (s, 2H), 3.86 (dd, *J* = 9.1, 2.9 Hz, 1H), 3.75 – 3.65 (m, 10H), 3.59 (dd, *J* = 9.1, 3.1 Hz, 1H), 2.98 (s, 2H), 2.86 (s, 2H), 2.30 (t, *J* = 7.6 Hz, 2H), 1.59 (m, 2H), 1.47 (s, 9H), 1.27 (d, *J* = 22.4 Hz, 24H), 1.13 (s, 9H), 0.88 (t, *J* = 6.8 Hz, 3H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 173.79, 170.01, 169.75, 143.85, 141.41, 127.85, 127.21, 125.27, 125.22, 81.71, 73.69, 70.83, 70.73, 70.69, 70.66, 69.13, 69.06, 67.38, 64.70, 63.02, 61.72, 54.25, 53.37, 47.23, 35.06, 34.31, 32.05, 31.13, 29.82, 29.78, 29.75, 29.60, 29.48, 29.42, 29.29, 28.24, 27.44, 25.01, 22.82, 14.25 .

NH_2-Cys(EtOC(O)$C_{15}H_{31}$)-Ser(OtBu)-O($CH_2CH_2O)_3CH_2C$(O)OtBu (23)
FmocNH-Cys(EtOC(O)$C_{15}H_{31}$)-Ser(OtBu)-O($CH_2CH_2O)_3CH_2C$(O)OtBu (0.29 mmol, 0.29 g) was dissolved in dry DCM (5 mL). Octanethiol (1.5 mmol, 0.25 mL), then DBU (0.03 mmol, 5 µL) was added and the mixture was stirred overnight at RT. TLC (8:2 EA:pentane) indicated complete conversion. Celite was added to the reaction mixture and the DCM was evaporated *in vacuo*. The adsorbed crude was purified by silica gel column chromatography (EA + 1% TEA → 9:1 EA:MeOH + 1% TEA). Compound **23**(0.26 mmol, 203 mg) was obtained with a 90% yield.
$[\alpha]_D$: -41.42°
IR(cm^{-1})=2918, 2850, 1742, 1677, 1662.
HRMS $[M+H]^+$: 793.52330 (measured), 793.52426 (calculated).
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (d, *J* = 8.6 Hz, 1H), 4.69 (dt, *J* = 8.6, 3.2 Hz, 1H), 4.30 (t, 2H), 4.22 (t, *J* = 6.7 Hz, 2H), 4.02 (d, *J* = 2.5 Hz, 2H), 3.85 (dd, *J* = 9.0, 3.1 Hz, 1H), 3.75 – 3.66 (m, 10H), 3.63 – 3.52 (m, 2H), 3.09 (dd, *J* = 13.6, 3.8 Hz, 1H), 2.82 – 2.70 (m, 3H), 2.31 (t, *J* = 7.5 Hz, 2H), 1.67 – 1.56 (m, 2H), 1.48 (s, 9H), 1.36 – 1.21 (m, 23H), 1.13 (d, *J* = 9.3 Hz, 9H), 0.88 (t, *J* = 6.8 Hz, 3H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 173.61, 173.24, 170.35, 169.65, 81.55, 73.37, 72.58, 70.74, 70.66, 70.62, 70.35, 69.03, 64.51, 63.06, 62.02, 54.16, 52.72, 37.88, 34.21, 31.95, 30.63, 29.72, 29.68, 29.64, 29.50, 29.39, 29.31, 29.17, 28.14, 27.38, 24.94, 22.72, 14.17

NH_2C(O)NH-Cys(EtOC(O)$C_{15}H_{31}$)-Ser(OtBu)-O($CH_2CH_2O)_3CH_2C$(O)OtBu
Compound **23** (0.23 mmol, 0.18 g) was dissolved in dry DCM (20 mL). Isopropanol (4.6 mmol, 0.35 mL) was added. 85% (Trimethylsilyl)isocyanate (2.3 mmol, 0.37 mL) was added and the mixture was stirred

overnight at RT under argon atmosphere. TLC (EA, R_f=0.5, ninhydrine) indicated complete conversion. The mixture was diluted with DCM and washed with 10% $NaHCO_3$ (aq) (2x), 10% $KHSO_4$ (aq) (2x) and brine (1x). The solution was dried (Na_2SO_4), filtrated and evaporated *in vacuo*. The crude was adsorbed on Celite and purified by silica gel column chromatography (8:2 EA:pentane + 1% TEA). Product (0.21 mmol, 0.18 g) was obtained with a 90% yield.

$[\alpha]_D$: +3°

IR(cm^{-1})=2917, 2849, 1739, 1733, 1641.

HRMS $[M+H]^+$: 836.52912 (measured), 836.53007 (calculated).

^{1}H NMR (400 MHz, $CDCl_3$) δ7.41 (d, *J* = 8.2 Hz, 1H), 6.15 (d, *J* = 7.6 Hz, 1H), 4.98 (s, 2H), 4.64 (dt, *J* = 8.1, 3.3 Hz, 1H), 4.51 (dd, *J* = 13.5, 6.4 Hz, 1H), 4.37 – 4.19 (m, 4H), 4.05 – 4.00 (m, 2H), 3.84 (dd, *J* = 9.1, 3.3 Hz, 1H), 3.75 – 3.62 (m, 10H), 3.59 (dd, *J* = 9.1, 3.4 Hz, 1H), 3.08 – 2.89 (m, 2H), 2.87 – 2.81 (m, 2H), 2.32 (t, 2H), 1.65 – 1.56 (m, 3H), 1.47 (s, 9H), 1.35 – 1.21 (m, 24H), 1.15 (s, 9H), 0.88 (t, *J* = 6.9 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 174.03, 171.33, 170.11, 158.33, 73.66, 70.83, 70.68, 70.65, 70.60, 69.06,64.71, 63.04, 61.71, 53.62, 53.45, 35.11, 34.35, 32.04, 31.14, 29.82, 29.78, 29.61, 29.48, 29.42, 29.29, 28.24, 27.45, 25.02, 22.81, 14.25

$NH_2C(O)NH$-Cys($EtOC(O)C_{15}H_{31}$)-Ser(OH)-$O(CH_2CH_2O)_3CH_2C(O)OH$ (6)

$NH_2C(O)NH$-Cys($EtOC(O)C_{15}H_{31}$)-Ser(OtBu)-$O(CH_2CH_2O)_3CH_2C(O)OtBu$ (50 µmol, 42 mg) was dissolved in dry DCM (2 mL). TFA (2 mL) was added and the mixture was stirred for 1 hour at RT under argon atmosphere. LC-MS indicated complete conversion. The reaction mixture was dropped in a tube with Et_2O (9 mL) and left at -20°C overnight. The tube was centrifuged and the precipitate was collected. Compound **6** (39 µmol, 28 mg) was obtained with an 80% yield.

$[\alpha]_D$: -6.3°

IR(cm^{-1})= 3291, 2916(C-H, stretch), 2849, 1739, 1641.

HRMS $[M+H]^+$: 724.40325 (measured), 724.40487 (calculated).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (s, 1H), 6.53 (s, 1H), 4.67 (s, 2H), 4.43 (s, 1H), 4.37 – 4.07 (m, 5H,), 3.95 (m, 2H), 3.69 (d, *J* = 26.1 Hz, 10H), 2.97 (s, 2H), 2.79 (s, 2H), 2.31 (t, *J* = 7.5 Hz, 2H), 1.59 (m, 2H,), 1.25 (m, 24H), 0.88 (t, *J* = 6.7 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 174.07, 70.49, 68.98, 63.17, 34.34, 32.06, 31.04, 29.85, 29.80, 29.66, 29.50, 29.33, 25.04, 22.83, 14.27.

FmocNH-Ser(OtBu)-$NH(CH_2CH_2O)_3CH_2C(O)OtBu$

Compound **18** (0.79 mmol, 0.17 g) was dissolved in dry DCM (30 mL). Fmoc-Ser(OtBu)-OH (1 mmol, 383 mg), DIC (1 mmol, 0.16 mL) and HOBt (1 mmol, 134 mg) were added and the mixture was stirred for 4 hours at RT under argon atmosphere. TLC-MS indicated complete conversion. The reaction mixture was diluted with DCM and washed with 10% $KHSO_4$(aq)(3x), 10% $NaHCO_3$(aq)(3x) and brine (1x). The solution was dried (Na_2SO_4), filtrated and evaporated *in vacuo*. The crude was adsorbed on Celite and purified by silica gel column chromatography (8:2 pentane:EA→ 5:5 pentane:EA, Δ=10%). Product (0.37 mmol, 235 mg) was obtained with a 47% yield. R_f= 0.5 at 8:2 EA:pentane.

$[\alpha]_D$: +15.8°

IR(cm^{-1})=2974, 2868, 1723, 1717, 1668.

HRMS $[M+H]^+$: 629.34200 (measured), 629.34326 (calculated).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.4 Hz, 2H), 7.61 (d, *J* = 6.8 Hz, 2H), 7.40 (t, *J* = 7.4 Hz, 2H), 7.31 (t, *J* = 7.3 Hz, 2H), 7.09 (s, 1H), 5.85 (s, 1H), 4.39 (d, *J* = 4.6 Hz, 2H), 4.23 (t, *J* = 6.9 Hz, 2H), 4.00 (s, 2H), 3.84 – 3.73 (m, 1H), 3.73 – 3.53 (m, 10H), 3.53 – 3.44 (m, 2H), 3.40 (t, *J* = 8.1 Hz, 1H), 1.46 (s, 9H,), 1.20 (s, 9H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 170.38, 141.40, 127.83, 127.19, 125.26, 120.10, 70.77, 70.66, 70.62, 70.43,69.93, 69.08, 62.07, 54.68, 47.28, 39.54, 28.23, 27.53

NH_2-Ser(OtBu)-$NH(CH_2CH_2O)_3CH_2C(O)OtBu$

FmocNH-Ser(OtBu)-$NH(CH_2CH_2O)_3CH_2C(O)OtBu$ (0.16 mmol, 0.10 g) was dissolved in dry DCM (5 mL). Octanethiol (0,80 mmol. 0,14 mL) was added. DBU (0,02 mmol, 3 µL) was added and the mixture was stirred at RT overnight under argon atmosphere. TLC analysis (8:2 EA:pentane) indicated completion of reaction. The DCM was evaporated *in vacuo* and the mixture was diluted with EA. The crude was purified by silica gel column chromatography (EA + 1% TEA → 9:1 EA:MeOH + 1% TEA). Compound **22** (0.16 mmol, 65 mg) was obtained with a quantitative yield.

$[\alpha]_D$: -9.1°
IR(cm^{-1})=2973, 2930, 2872, 1746, 1661.
HRMS $[M+H]^+$: 407.27390 (measured), 407.27518 (calculated).
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (s, 1H), 4.02 (s, 2H), 3.74 – 3.54 (m, 11H), 3.47 (m, 4H), 1.48 (s, 9H), 1.19 (s, 9H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 173.25,169.68, 81.69, 73.40, 70.70, 70.59, 70.54, 70.29, 69.96, 69.01, 64.07, 55.52, 38.91, 28.14, 27.55.

FmocNH-Cys(EtOC(O)$C_{15}H_{31}$)-Ser(OtBu)-NH($CH_2CH_2O)_3CH_2C$(O)OtBu
Compound **22** (0.28 mmol, 0.11 g) was dissolved in dry DCM (10 mL). Compound **14** (0,25 mmol, 156 mg), HOBt (0.30 mmol, 40 mg) and DIC (0.30 mmol, 50 µL) were added and the mixture was stirred overnight at RT under argon atmosphere. TLC analysis (EA) indicated completion of reaction. The reaction mixture was diluted with DCM and washed with 10% $KHSO_4$ (aq) (3x), 10% $NaHCO_3$ (aq) (3x) and brine (1x). The solution was dried (Na_2SO_4), filtrated and evaporated *in vacuo*. The crude was adsorbed on Celite and purified by silica gel column chromatography (8:2 pentane:EA→EA). product (0.25 mmol, 0.25 g) was obtained with a quantitative yield. R_f= 0.6 EA.
$[\alpha]_D$: +7.3°
IR(cm^{-1})=3300, 2920, 2820, 1736, 1733, 1641
HRMS $[M+H]^+$: 1014.60823 (measured), 1014.60832 (calculated).
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.5 Hz, 2H), 7.60 (d, *J* = 7.4 Hz, 2H), 7.43 – 7.26 (m, 5H), 7.12 (t, *J* = 5.2 Hz, 1H), 5.96 (d, *J* = 7.0 Hz, 1H), 4.51 – 4.31 (m, 4H), 4.30 – 4.18 (m, 3H), 4.01 (s, 2H), 3.81 (dd, *J* = 8.5, 3.3 Hz, 1H), 3.73 – 3.54 (m, 10H), 3.50 – 3.42 (m, 2H), 3.42 – 3.35 (m, 1H), 3.06 – 2.90 (m, 2H), 2.82 (s, 2H), 2.30 (t, *J* = 7.6 Hz, 2H), 1.60 (m, 2H), 1.47 (s, 9H), 1.21-1.35 (m, 24H), 1.17 (s, 9H), 0.88 (t, 3H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 173.60, 171.07, 169.95, 169.69, 143.72, 143.69, 141.26, 127.72, 127.07, 125.12, 119.97, 81.55, 73.97, 70.65, 70.53, 70.49, 70.27, 69.69, 68.94, 67.26, 62.83, 61.31, 54.44, 53.45, 47.08, 39.42, 34.68, 34.13, 31.91, 31.00, 29.67, 29.64, 29.60, 29.46, 29.34 , 29.28, 29.14, 28.08, 27.36, 24.86, 22.67, 14.18 .

NH_2-Cys(EtOC(O)$C_{15}H_{31}$)-Ser(OtBu)-NH($CH_2CH_2O)_3CH_2C$(O)OtBu (24)
FmocNH-Cys(EtOC(O)$C_{15}H_{31}$)-Ser(OtBu)-NH($CH_2CH_2O)_3CH_2C$(O)OtBu (0.22 mmol, 0.23 g) was dissolved in dry DCM (1 mL) . Octanethiol (1.1 mmol, 0.19 mL) was added. DBU (22 µmol, 3.3 µL) was added and the mixture was stirred for 3 hours at RT under argon atmosphere. TLC analysis (EA) indicated completion of reaction. Celite was added and the DCM was evaporated *in vacuo*. The crude was purified by silica gel column chromatography (1:1 EA:pentane→ 9:1 EA:MeOH +1% TEA). Compound **24** (0.20 mmol, 154 mg) was obtained with an 88% yield.
$[\alpha]_D$: -1.1°
IR(cm^{-1})=2923, 2852, 1734, 1647.
HRMS $[M+H]^+$: 792.53907 (measured), 792.54024 (calculated).
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (d, *J* = 7.3 Hz, 1H), 7.09 (t, *J* = 5.3 Hz, 1H), 4.42 (td, *J* = 7.3, 4.2 Hz, 1H), 4.22 (t, *J* = 6.8 Hz, 2H) 4.02 (s, 2H), 3.76 (dd, *J* = 8.7, 4.2 Hz, 1H), 3.67-3.56 (dd, *J* = 23.1, 3.7 Hz, 11H), 3.48 (t, *J* = 4.8 Hz, 2H), 3.39 (t, *J* = 8.1 Hz, 1H), 3.06 (dd, *J* = 13.5, 4.0 Hz, 1H), 2.77 (dt, *J* = 10.3, 4.1 Hz, 3H), 2.31 (t, *J* = 7.5 Hz, 2H), 1.60 (m, *J* = 14.4, 7.3 Hz, 3H), 1.48 (s, 9H), 1.26 (s, 24H), 1.20 (s, 9H), 0.88 (t, *J* = 6.8 Hz, 3H)
^{13}C NMR (101 MHz, $CDCl_3$) δ 173.56, 170.18, 169.61, 81.60, 73.88, 70.66, 70.53, 70.29, 69.83, 68.95, 62.98,61.57, 54.30, 52.93, 39.36, 37.86, 31.91, 30.64, 29.68, 29.64, 29.60, 29.46, 29.35, 29.27, 29.13, 28.11, 27.40, 24.89, 22.68, 14.13.

NH_2C(O)NH-Cys(EtOC(O)$C_{15}H_{31}$)-Ser(OtBu)-NH($CH_2CH_2O)_3CH_2C$(O)OtBu
Compound **24** (0.18 mmol, 142 mg) was dissolved in dry DCM (10 mL). Isopropanol (3.5 mmol, 0.27 mL) was added. 85% (Trimethylsilyl)isocyanate (1.8 mmol, 0.28 mL) was added and the mixture was stirred overnight at RT under argon atmosphere. Celite was added and the DCM was evaporated *in vacuo*. The adsorbed crude was purified by silica gel column chromatography (8:2 EA:pentane + 1% TEA → 95:5 EA:MeOH + 1% TEA). Product (0.14 mmol, 0.12 g) was obtained with a 82% yield. R_f=0.5 EA.

$[\alpha]_D$: +10
IR(cm^{-1})=2917, 2849, 1733, 1634
HRMS $[M+H]^+$: 835.54504 (measured), 835.54606 (calculated).
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56 (t, *J* = 6.1 Hz, 2H), 6.35 (d, *J* = 6.6 Hz, 1H), 5.54 (s, 2H), 4.51 (m, 2H), 4.22 (t, *J* = 6.7 Hz, 2H), 4.02 (s, 2H), 3.82 (dd, *J* = 8.9, 3.7 Hz, 1H), 3.73 – 3.55 (m, 11H), 3.45 (dd, *J* = 8.9, 5.7 Hz, 1H), 3.36 (dt, *J* = 12.7, 3.7 Hz, 1H), 2.95 (m, 2H), 2.79 (t, *J* = 6.7 Hz, 2H), 2.31 (m, *J* = 7.6 Hz, 2H), 1.65 – 1.55 (m, 2H), 1.48 (s, 9H), 1.35 – 1.21 (m, 24H), 1.16 (s, 9H), 0.88 (t, *J* = 6.9 Hz, 3H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 173.80, 171.34, 170.34, 169.86, 159.15, 82.09, 73.62, 70.69, 70.67, 70.37, 70.28, 69.89, 69.00, 62.91, 61.62, 54.07, 53.52, 39.62, 34.63, 34.21, 31.95, 30.85, 29.73, 29.70, 29.53, 29.39, 29.34, 29.21, 28.16, 27.43, 24.93, 22.72, 14.17

$NH_2C(O)NH$-Cys($EtOC(O)C_{15}H_{31}$)-Ser(OH)-$NH(CH_2CH_2O)_3CH_2C(O)OH$ (7)

$NH_2C(O)NH$-Cys($EtOC(O)C_{15}H_{31}$)-Ser(OtBu)-$NH(CH_2CH_2O)_3CH_2C(O)OtBu$ (0.14 mmol, 0.11 g) was dissolved in dry DCM (2mL). TFA (2 mL) was added and the mixture was stirred for 1 hour. The TFA was evaporated *in vacuo*. The crude was dissolved in DCM and dropped in a tube with Et_2O (35 mL). Compound **7** (0.10 mmol, 74 mg) was obtained with a 71% yield.
$[\alpha]_D$: -2°
IR(cm^{-1})=3282, 2917, 2849, 1729, 1638
HRMS $[M+H]^+$: 723.42011 (measured), 723.42086 (calculated).
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (d, *J* = 5.7 Hz, 1H), 7.89 (s, 1H), 6.66 (s, 1H), 4.58 (s, 2H), 4.21 (m, 4H), 3.86 (d, *J* = 29.0 Hz, 2H), 3.65 (dd, *J* = 38.0, 20.2 Hz, 10H), 3.48 (m, 2H), 2.93 (s, 2H), 2.79 (t, *J* = 6.2 Hz, 2H), 2.30 (t, *J* = 7.6 Hz, 2H), 1.58 (m, 2H), 1.23 (m, 24H), 0.88 (t, *J* = 6.7 Hz, 3H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 173.99, 173.77, 172.33, 170.67, 160.19, 70.68, 70.45, 70.14, 69.61, 68.44, 63.02, 62.57, 55.65, 53.85, 39.66, 34.70, 34.28, 32.04, 30.83, 29.82, 29.78, 29.65, 29.48, 29.32, 25.00, 22.80, 14.24 .

NH_2-DEVSGLEQLESIINFEKL-resin bound (25)

Preloaded leucine resin (0.05 mmol) was subjected to solid phase Fmoc peptide synthesis using standard Fmoc protected amino acid building block (NovaBiochem, 0.25 mmol, 5eq), HCTU as an activating agent, and Fmoc cleavage as the final step.

NH_2-DEVSGLEQLESIINFEK(^{N3})L-resin bound (26)

Peptide-resin **26** was synthesized using the same procedure than **25** using Fmoc-azidonorleucine instead of Fmoc-Lys(Boc)-OH for the first coupling.

$NH_2C(O)NH$-Cys($EtOC(O)C_{15}H_{31}$)-Ser(OH)-$O(CH_2CH_2O)_3CH_2C(O)NH$-DEVSGLEQLESIINFEKL-OH (8)

Resin **25** (12 µmol) was put in a syringe and suspended in NMP until resin appeared sufficiently swollen. **6**(12 µmol, 10 mg) was preactivated with HCTU (12 µmol, 5 mg) to form a 0,2M solution, which was added to **25**. A solution of 1M DIPEA in NMP (12 µL) was added and the syringe was shaken for 15 min, after which 1M DIPEA in NMP (12 µL) was added again. The syringe was shaken overnight, when a Kaiser test indicated completion of reaction. The resin was washed with DCM (3x) after which a solution of TFA/TIS/H_2O (95/2.5/2.5) was added. The syringe was shaken for 75 min and the solution was dropped in Et_2O. After overnight precipitation and HPLC purification compound **8**(0.6 µmol, 1.7 mg) was obtained.
HRMS [(M+2H)/2]: 1385.23537 (measured),1385.22598(calculated)

$NH_2C(O)NH$-Cys($EtOC(O)C_{15}H_{31}$)-Ser(OH)-$NH(CH_2CH_2O)_3CH_2C(O)NH$-DEVSGLEQLESIINFEKL-OH (9)

The procedure used to synthesize **8** was also used to obtain **9**. **7**(48 µmol, 35 mg) was used instead of **6**, thus **9** (1.5 µmol, 4.3 mg) was obtained.
HRMS [(M+2H)/2]:1384,74236 (measured). 1384,73397(calculated).

$NH_2C(O)NH$-Cys($EtOC(O)C_{15}H_{31}$)-Ser(OH)-$O(CH_2CH_2O)_3CH_2C(O)NH$-DEVSGLEQLESIINFEK(^{N3})L-OH (27)

26 bound (20 µmol) was put in a syringe and suspended in NMP until resin appeared sufficiently swollen. **6** (20 µmol, 14 mg) was preactivated with HCTU (20 µmol, 8.3 mg) to form a 0.2M solution, which was added to **26**. A solution of 1M DIPEA in NMP (20 µL) was added and the syringe was shaken for 15 min, after which 1M DIPEA in NMP (20 µL) was added again. The syringe was shaken overnight, when a Kaiser test indicated completion of reaction. The resin was washed with DCM (3x) after which a solution of

TFA/TIS/H_2O (95/2.5/2.5) was added. The syringe was shaken for 75 min and the solution was dropped in Et_2O. After overnight precipitation and HPLC purification compound **27** (1.4 µmol, 4.0 mg) was obtained.

$NH_2C(O)NH$-Cys(EtOC(O)$C_{15}H_{31}$)-Ser(OH)-NH($CH_2CH_2O)_3CH_2C(O)$NH-DEVSGLEQLESIINFEK(^{N3})L-OH (28)

The procedure used to synthesize **27** was also used to obtain **28**. **7**(20 µmol, 14 mg) was used instead of **6**, thus **28** (0.75 µmol, 2.09 mg) was obtained.

$NH_2C(O)NH$-Cys(EtOC(O)$C_{15}H_{31}$)-Ser(OH)-O($CH_2CH_2O)_3CH_2C(O)$NH-DEVSGLEQLESIINFEK(^{Cy5})L-OH (10)

Compound **27** (0.3 µmol, 0.8 mg) was dissolved in dry DMSO (100 µL). Cy5-BCN (1.08 µmol, 0.9 mg) in dry DMSO (100 µL) was added to the solution. The reaction mixture was stirred at RT for 1 week. After HPLC purification, **10** (30 nmol, 1 mg) was obtained.

HRMS [(M+1H)/2]: 1792.97317 (measured),1792.96897 (calculated).

$NH_2C(O)NH$-Cys(EtOC(O)$C_{15}H_{31}$)-Ser(OH)-NH($CH_2CH_2O)_3CH_2C(O)$NH-DEVSGLEQLESIINFEK(^{Cy5})L-OH (11)

Compound **28** (0.3 µmol, 0.8 mg) was dissolved in dry DMSO (100 µL). Cy5-BCN (0.36 µmol, 0.3 mg) in dry DMSO (100 µL) was added to the solution. The reaction mixture was stirred at RT for 1 week. After HPLC purification, **11** (0.1 µmol, 3.5 mg) was obtained.

HRMS [(M+1H)/2]: 1791.47912(measured),1791.471375 (calculated).

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