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Cross-metathesis and ring-closing metathesis reactions of amino acid-based substrates

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Abstract

Olefin tethers of variable length, introduced into a natural amino acid (side-chain of Ser, Cys; N-terminus of Arg; C-terminus of Phe and Tic; and in both the side-chain and either the N- or C-terminus of Ser, Cys and Tyr), undergo metathesis on treatment with Grubbs' second generation catalyst. Side-chain linked dimers of Ser, Cys and Tyr were obtained by cross-metathesis, while olefin installation at the N- and C-terminus led to dimers of Arg and Phe (or Tic), respectively. Ring-closing metathesis of the doubly alkenylated derivatives of Ser, Cys and Tyr gave 12-, 20- and 24-membered macrocycles.

Keywords: Cross-metathesis (CM); Ring-closing metathesis (RCM); Amino acid dimerization; Cyclic amino acids

1. Introduction

Inter- and intramolecular cross-linking of peptides and proteins is central to a number of important biological processes.[1] Cross-linking of peptides (via biaryl dityrosine[2] and cysteine disulfide bond formation) helps to define protein structure and hence function, while cross-linking of peptides to carbohydrates is involved in immune recognition, cell-adhesion, protease protection and inflammation.[3–5] In addition, intramolecular effects such as peptide backbone and/or side-chain amide bond cross-linking[6] and dimerization or oligomerization of a peptide can result in an improved pharmacological profile relative to the monomer.[7–13] For example, N-terminal linked multimers of neurotensin exhibit improved binding affinities[9] and the 'tail-to-tail' linkage of opioid pharmacophores allows selective interaction with the different opioid subtype receptors.[8,11,14–16]

Ring-closing metathesis (RCM) and cross-metathesis (CM) provide convenient and versatile methods by which to mimic these important natural processes. Recent examples of the use of CM include tethering of amino acids to sugars and fatty acids,[17] while RCM has been used to prepare low molecular weight bioactive cyclic peptidomimetics[18–22] and cyclic amino acid-based building blocks.[23] Secondary structures such as α-helical structures,[24] β-turn motifs[25,26] and β-strands[27,28] can be stabilized by RCM. Olefin metathesis also provides significant potential for the post-translational modification of peptides and proteins by modification of a residue in an existing peptide or protein, or by incorporating a pre-derivatized residue into a solid phase synthesis protocol. However, it is important to note that existing work in these areas is, with few exceptions,[17] limited to the use of non-natural amino acids (e.g., allylglycine).[29–33]

Abbreviations: Boc_2O, di-*tert*-butyl dicarbonate; BOP-Cl, bis(2-oxo-3-oxazolidinyl)phosphinic chloride; CM, cross-metathesis; DIPEA, *N*,*N*-diisopropylethylamine; DMAP, 4-dimethylaminopyridine; EDCI, 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide hydrochloride; HATU, *N*,*N*,*N*′,*N*′-tetramethyl-*O*-(7-azabenzotriazol-1-yl)uronium hexafluorophosphate; HOBt, 1-hydroxybenzotriazole; TBTU, *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate; TEA, triethylamine; TFA, trifluoroacetic acid; Tic, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid; Pbf, 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl; RCM, ring-closing metathesis.

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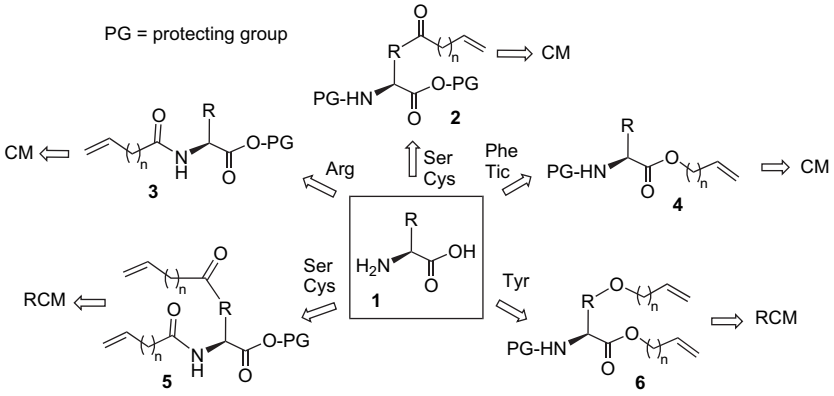


Figure 1. Metathesis precursors **2**–**6**.

Herein we report the synthesis of olefin-based derivatives of natural amino acids (Fig. 1, **2**–**6**) and their application in metathesis-induced dimerization, conjugation and cyclization studies. An olefin tether of controllable length is attached via the side-chain of either serine or cysteine (see structure **2**), the N-terminus (structure **3**), the C-terminus (structure **4**) or a combination (**5** and **6**). Cysteine and serine were chosen because they contain an appropriate side-chain functionality (R) for modification and they provide an opportunity to prepare stable mimics of natural disulfide bonds. Arginine was selected as a type **3** olefin-containing derivative because it represents an important branching point in multivalent variants of biologically active peptides.[9,10,34] Type **4** derivatives provide precursors for the preparation of novel 'tail-to-tail' linked opioid ligands containing a C-terminal Phe or Tic,[†] which are known to be critical for interaction with target receptor subtypes.[11,8] Dienes **5** and **6** provide an opportunity for the preparation of cyclic structures via RCM of the side-chain to either the N- or C-terminus, respectively.

It is important to note that the alkene tether length of **2**–**6** can be tailored to a particular biological situation, for example to study ligand interactions with receptors or enzymes by (i) identifying the optimal olefin spacer length for potency, (ii) providing long tethers to avoid steric problems known to be a problem in bivalent opioid research,[14,35] (iii) providing dimeric structures capable of binding two active sites in receptor complexes simultaneously.[7,36]

2. Results and discussion

2.1. Preparation of the metathesis precursors (Table 1)

The side-chains of serine and cysteine were acylated with 4-pentenoic acid and 10-undecenoic acid, in the presence of bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOP-Cl), to give the alkene tether derivatives **8**–**12** suitable for metathesis (Table 1, entries 1–4). The N-terminal acylated arginines **14** (88%) and **15** (83%) were prepared by coupling the hydrochloride salt of H–Arg(Pbf)–OMe **13** to 4-pentenoic and 10-undecenoic acid in the presence of the uronium salt TBTU (Table 1, entries 5 and 6). C2 and C8 olefin tethers were introduced at the C-terminus of phenylalanine and the conformationally constrained Phe analogue 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (Tic), by separate reactions of **16** and **19** with 3-butenol or with 9-decenol (Table 1, entries 7–10). Precursors for RCM were prepared from cysteine, serine and tyrosine (Table 1, entries 11–16). In particular, cysteine methyl ester **22** was acylated with either 4-pentenoic acid (entry 11) or 10-undecenoic acid (entry 12), in the presence of BOP-Cl, to give the *N*,*S*-diacylated products **23** and **24** in 85 and 44% yield, respectively. Serine methyl ester **25** was acylated with 4-pentenoic acid (entry 13) or 10-undecenoic acid (entry 14), in the presence of HATU, to give the *N*,*O*-diacylated products **26** (59%) and **27** (54%). The tyrosine-based RCM precursors **29** (82%) and **30** (69%) were prepared similarly from commercially available Boc–Tyr(OAllyl)–OH **28** on treatment with 3-butenol and 9-decenol, respectively.

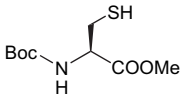
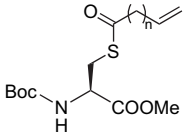
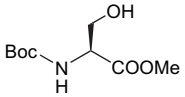
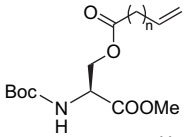
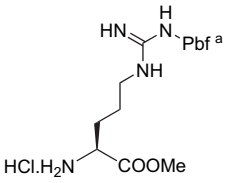
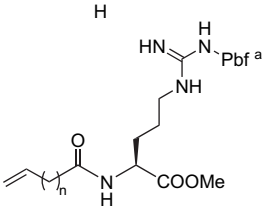
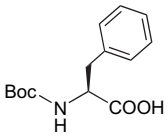
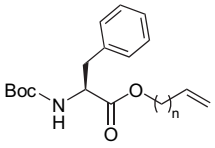
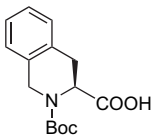
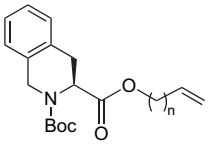
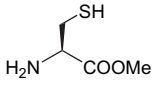
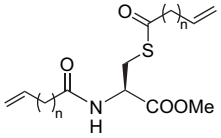
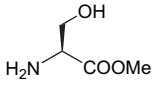
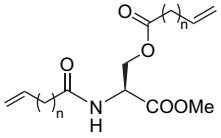
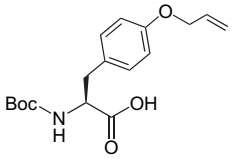
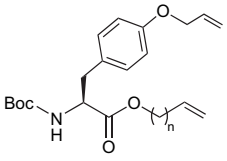
2.2. Metathesis experiments (Tables 2–4)

The olefin tethered amino acid-based substrates (shown in Table 1) were subjected to CM and RCM conditions and the results are summarized in Tables 2–4. Cross-metathesis was performed at a concentration of 0.05 M in the presence of 10 mol % Grubbs' second generation catalyst **31** (Fig. 2). A reaction time of 6 h (at reflux under a gentle flow of inert gas) was sufficient to drive the reaction to completion. DMSO was added at the completion of the reaction to facilitate removal of generated ruthenium byproducts.[52] A higher catalyst loading of 20 mol % was used in the ring-closing experiments, which were otherwise identical.

N-Boc-*S*-pentenoyl cysteine methyl ester **8** was treated with Grubbs' second generation catalyst **31** under the above conditions to give olefin homodimer **33** in 45% yield (Table 2, entry 1). Similar treatment of *N*-Boc-*S*-decenoyl cysteine methyl ester **9** gave the chain extended analogue **34** in 73% yield (Table 2, entry 2), while the two serine-based dimers (**35** and **36**)

[†] Dmt-Tic is a potent δ-opioid receptor antagonist, the potency of which is enhanced upon dimerization (see Ref. 11). These dimers are believed to be useful for the treatment of drug addiction or alcohol dependence, but the optimum tether length to bind two active sites simultaneously remains to be identified.

Table 1
Synthesis of metathesis precursors

Entry	Amino acid substrate		Conditions	Reaction product	*n*	Product (yield, %)
1		**7**	a_1		2	**8** (93)
2					8	**9** (82)
3		**10**	b		2	**11** (89)
4					8	**12** (84)
5		**13**	c_1		2	**14** (88)
6					8	**15** (83)
7		**16**	c_2		2	**17** (87)
8					8	**18** (86)
9		**19**	c_2		2	**20** (80)
10					8	**21** (60)
11		**22**	a_2		2	**23** (85)
12					8	**24** (44)
13		**25**	d		2	**26** (59)
14					8	**27** (54)
15		**28**	c_2		2	**29** (82)
16					8	**30** (69)

Reagents and conditions: (a_1) 4-pentenoic acid or 10-undecenoic acid (1.1 equiv), BOP-Cl (1.2 equiv), Et_3N (2.4 equiv), CH_2Cl_2, 0 °C then rt, 4 h; (a_2) 4-pentenoic acid or 10-undecenoic acid (1.1 equiv), BOP-Cl (1.2 equiv), Et_3N (2.4 equiv), CH_2Cl_2, 0 °C then rt, 18 h; (b) 4-pentenoic acid or 10-undecenoic acid (1.1 equiv), EDCI (1.1 equiv), DMAP (0.01 equiv), CH_2Cl_2, rt, 16 h; (c_1) 4-pentenoic acid or 10-undecenoic acid (1 equiv), TBTU (1.1 equiv), Et_3N (3 equiv), CH_2Cl_2, rt, 2 h; (c_2) 3-butenol or 9-decenol (1 equiv), TBTU (1.1 equiv), Et_3N (3 equiv), CH_2Cl_2, rt, overnight; (d) 4-pentenoic acid or 10-undecenoic acid (2.2 equiv), HATU (2.4 equiv), DIPEA (4.8 equiv), DMF, rt, 16 h.

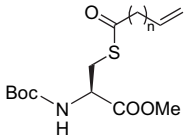
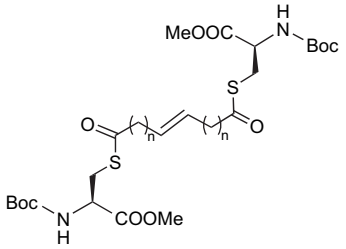
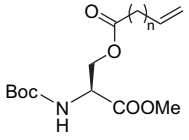
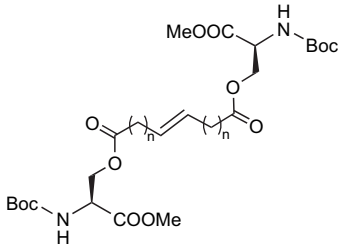
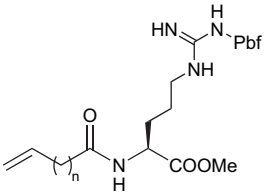
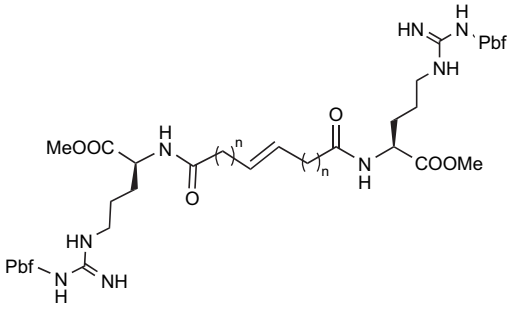
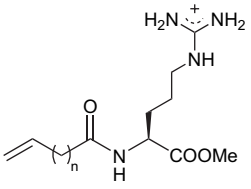
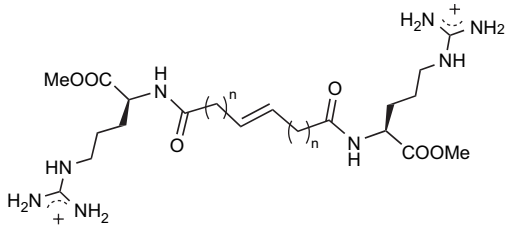
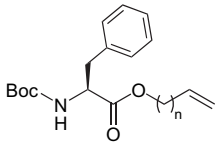
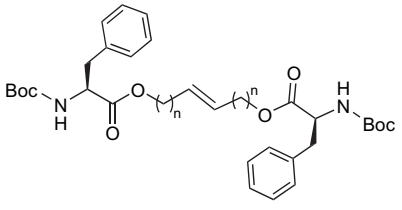
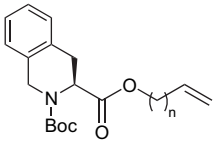
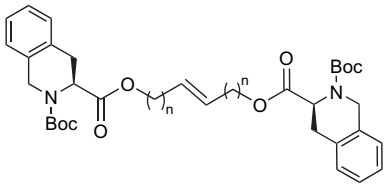
[a] Protective group: Pbf, 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl.

were prepared from *N*-Boc-*O*-pentenoyl serine methyl ester **11** and **12** in isolated yields of 86 and 62%, respectively (Table 2, entries 3 and 4). Catalyst **31** also induced dimerization of α-amine acylated arginines **14** and **15** to give the desired self-metathesis products **37** and **38** in respective yields of 74 and 66% (Table 2, entries 5 and 6). These results demonstrate that the Pbf-protected guanidine functionality of the arginine side-chain is compatible with catalyst **31**, an important result for the future preparation of multivalent variants of biologically active peptides.[9,10,32] Removal of the sulfonyl-type protecting group of **14**, on treatment with trifluoroacetic acid,[38] gave the water soluble guanidinium salt **39** as a possible substrate for CM reactions under polar solvent conditions, as an initial step towards extending the chemistry to an aqueous environment. Second generation Hoveyda–Grubbs catalyst **32** (0.05 equiv) was added to a solution of **39** in MeOH-d_4 and the reaction was monitored by ^{1}H NMR. However, an overnight reaction at 45 °C returned only starting material **39**, with

longer reaction times resulting in some double bond isomerization.[37] Catalyst **32** was employed in this study since it is reported to promote CM in methanol, although Blechert and Connon[37] have reported that ammonium salts are often unreactive to CM and give rise to isomerization of the double bond under the reaction conditions.

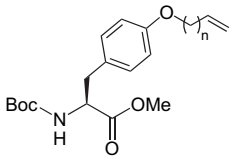
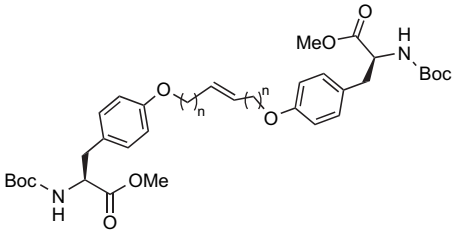
Dimers **41** through **44** were prepared as potential precursors of bivalent opioid ligands, as discussed earlier (and in footnote[†]). Thus, dimerization of Phe-derived **17** and the longer tethered analogue **18** gave **41** and **42** in yields of 68 and 76% (Table 2, entries 8 and 9). The analogous Tic-based substrates **20** and **21** underwent CM to give the bivalent structures

Table 2
Self-metathesis compounds **33**–**46**

Entry	Amino acid substrate	*n*	Substrate	Reaction product	*n*	Product (yield, %)
1		2	**8**		2	**33** (45)
2		8	**9**		8	**34** (73)
3		2	**11**		2	**35** (86)
4		8	**12**		8	**36** (62)
5		2	**14**		2	**37** (74)
6		8	**15**		8	**38** (66)
7		2	**39**		2	**40** (0)
8		2	**17**		2	**41** (68)
9		8	**18**		8	**42** (76)
10		2	**20**		2	**43** (89)
11		8	**21**		8	**44** (56)

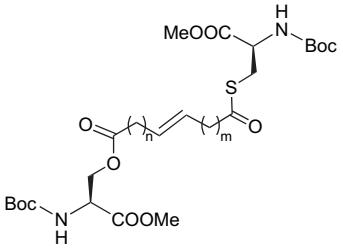
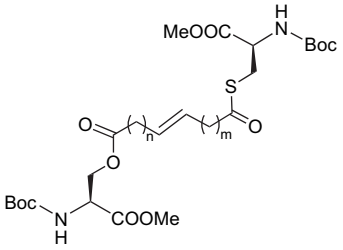
(*continued*)

Table 2 (*continued*)

Entry	Amino acid substrate	*n*	Substrate	Reaction product	*n*	Product (yield, %)
12		1	**45**		1	**46** (67)

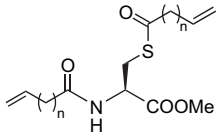
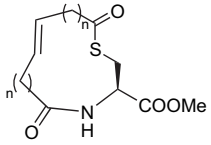
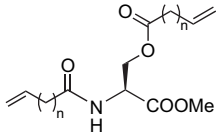
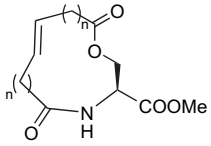
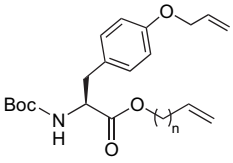
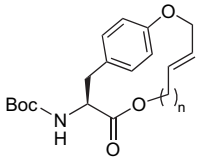
Reagents and conditions: Grubbs' second generation catalyst **31** (0.2 equiv), CH_2Cl_2, argon flow, reflux 6 h, then DMSO (50 equiv relative to **31**), rt, 12 h. Entry 7: Hoveyda–Grubbs catalyst **32** (0.05 equiv), MeOH-d_4, nitrogen flow, 45 °C, 12 h to 3 days.[37]

Table 3
Cross-coupled metathesis products **47** and **48**

Entry	CM partner 1	CM partner 2	Reaction product	*n*	*m*	Product (yield, %)
1	**12**	**8**		8	2	**47** (51)
2	**12**	**9**		8	8	**48** (66)

Reagents and conditions: 1 equiv of CM partner 1, 2 equiv of CM partner 2, Grubbs' second generation catalyst **31** (0.2 equiv), CH_2Cl_2, argon flow, reflux 6 h, then DMSO (50 equiv relative to **31**), rt, 12 h.

Table 4
Ring-closing metathesis products **49–54**

Entry	Amino acid substrate	*n*	Diene	Reaction product	*n*	Product (yield, %)
1		2	**23**		2	**49** (74)
2		8	**24**		8	**50** (43)
3		2	**26**		2	**51** (45)
4		8	**27**		8	**52** (15)
5		2	**29**		2	**53** (0)
6		8	**30**		8	**54** (62)

Reagents and conditions: Grubbs' second generation catalyst **31** (0.2 equiv), CH_2Cl_2, argon flow, reflux 6 h, then DMSO (50 equiv relative to **31**), rt, 12 h.

43 (89%) and **44** (56%) (Table 2, entries 10 and 11). This CM strategy provides potential to access chimeric structures by linking selective, olefin containing, pharmacophores. The last example of self-metathesis dimerization involved cross-linking commercially available *N*-Boc-*O*-allyl tyrosine methyl ester **45** (Table 2, entry 12) to give **46** in a yield of 67%.

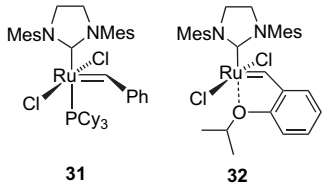


Figure 2. Grubbs' second generation catalyst **31** and Hoveyda–Grubbs second generation catalyst **32**.

Structure **46** represents an analogue of a naturally occurring tyrosine cross-linked dimer.[2]

Table 3 summarizes the results of cross-linking two different amino acids using a CM strategy. The modified serine derivative **12** was coupled to cysteine-based **8** upon treatment with catalyst **31** to give the cross-linked product **47** in 51% yield, along with the homodimer **33** as a result of the use of a two-fold excess of **8** relative to **12**. The serine derivative **12**, containing the C8 alkene tether, was coupled to cysteine-based **9** to give the mixed and extended dimer **48** in 66% yield. Again, a self-metathesis dimer (**34**) was observed since **9** was used in two-fold excess relative to **12**. A statistical mixture of the cross-metathesis product and the homodimers is expected, as reactions between these types of olefins are considered to be nonselective. Terminal olefins **8**, **9** and **12** are prone to homodimerization, whereas the homodimers themselves (disubstituted alkenes) are substrates for secondary metathesis pathways.[39]

All self-metathesis and cross-metathesis products (Tables 2 and 3) were isolated as a single alkene isomer as evidenced by ^{1}H and ^{13}C NMR data. In each case this isomer was assigned the thermodynamically more stable *E* configuration since olefin metathesis using catalyst **31** is believed to occur under thermodynamic control.[40] This assignment is supported by an observed vicinal olefinic coupling constant of 15.2 Hz for **47**.

Next a series of dienes (Table 4, entries 1–6) were reacted with catalyst **31** in order to investigate RCM strategies. Diene **23** cyclized to give the 12-membered cysteine-based cyclic amino acid **49** as a single isomer in 74% yield, the X-ray crystal structure of which revealed an *E* geometry for the double bond (see Fig. 3). Diene **24** similarly underwent RCM to give the larger, 24-membered cysteine-based cyclic amino acid **50** (43%), the sample of which contained trace amounts of a ring-contracted product presumably resulting from double bond migration preceding RCM. The occurrence of double bond migration in olefin metathesis is well known.[41–43]

The *N*,*O*-dipentenoyl serine analogues **26** and **27** were also treated with catalyst **31** to give the 12-membered and 24-membered cyclic amino acids **51** (45%) and **52** (15%), respectively. Traces of ring-contracted products were also present in the isolated samples of **51** and **52**, however, these were not purified or characterized. Lactone **51** has been previously reported by Schreiber.[44] An attempted RCM with **29** gave only complex mixtures, with none of the desired lactone **53** being observed by NMR. This result is in accord with related work by Bressy and Piva,[45] which showed that an olefin tether containing a minimum of four methylene units (i.e., $n=4$) is needed to obtain cyclic lactones of this type, albeit in low yield. Interestingly, the longer tethered precursor **30** did give the desired lactone **54** on treatment with **31** and in a respectable yield of 62%. The configuration of the constituent double bonds of **49** and **51** was assigned *E* on the basis of X-ray crystal structures. As would be expected the cycles in the X-ray structures of **49** and **51** are almost identical (Fig. 3). The alkenes of the larger cycles **50** and **52** were assigned *E* by analogy, the thermodynamically more stable alkene isomer on the basis of molecular modelling.

2.3. Extension/application: dipeptidic moieties/PDF inhibitors

Finally, we report studies on the synthesis of **56** as an initial step towards the preparation of analogues of **55** (Fig. 4), a known peptide deformylase (PDF) inhibitor that possesses

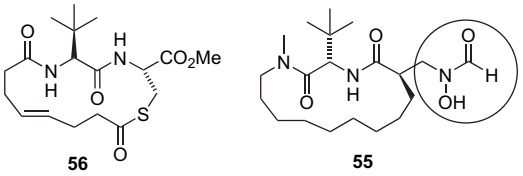


Figure 4. Comparison of **56** to PDF inhibitor **55**[47] (metal-chelating warhead circled).

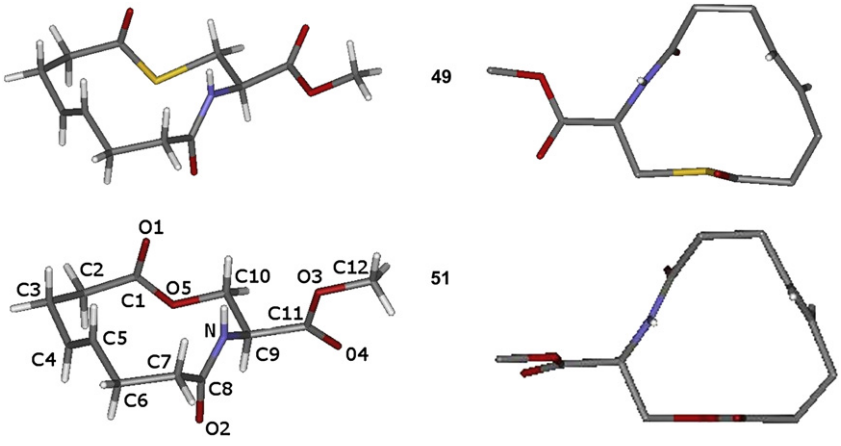
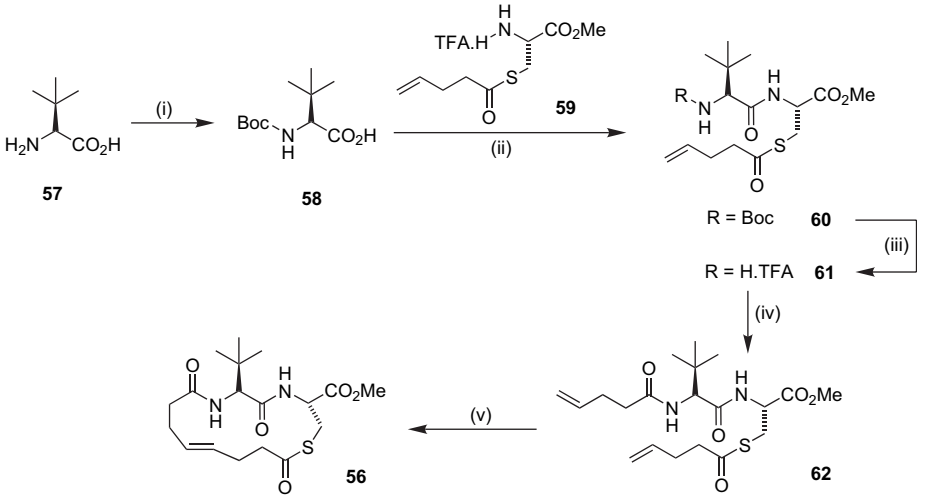


Figure 3. X-ray crystal structures of **49** and **51**.



Scheme 1. Synthesis of cyclic dipeptide **56**. Reagents and conditions: (i) Boc_2O (1.1 equiv), NaOH (1.1 equiv), water/*tert*-butyl alcohol (1:1 v/v), rt, 18 h (99%); (ii) EDCI (1.3 equiv), HOBt (1.5 equiv), DIPEA (1.1 equiv), CH_2Cl_2, rt, 16 h (46%); (iii) TFA, CH_2Cl_2, rt, 18 h; (iv) 4-pentenoic acid (1.1 equiv), EDCI (1.3 equiv), HOBt (1.5 equiv), DIPEA (1.1 equiv), CH_2Cl_2, rt, 16 h (72%, over two steps); (v) Grubbs' second generation catalyst **31** (0.2 equiv), CH_2Cl_2, argon flow, reflux 6 h, then DMSO (50 equiv relative to **31**), rt, 12 h (65%).

antibacterial properties.[46,47] Commercially available *tert*-L-leucine **57** was treated with Boc_2O in the presence of sodium hydroxide to give Boc-protected **58** in quantitative yield (Scheme 1). Boc-L-*tert*-leucine **58** and *S*-pentenoyl cysteine methyl ester **59** were coupled on treatment with EDCI/HOBt in CH_2Cl_2 to give **60** in 46%. The Boc group of dipeptide **60** was then removed using TFA in CH_2Cl_2 to give **61**, which was acylated with 4-pentenoic using EDCI/HOBt to give the dipeptide diene **62** in 72% yield over two steps. This RCM precursor was then cyclized on treatment with Grubbs' second generation catalyst **31** to give the 15-membered compound *E*-**56** in 65% yield. The double bond of **56** was again assigned the thermodynamically more stable *E* configuration, as molecular modelling suggests *E*-**56** to be significantly more thermodynamically stable than *Z*-**56** (molecular models shown in Fig. 5). It is interesting to note that a hydrogen bond is apparent in both structures, between the *N*-pentenoyl carbonyl and cysteine amide for the *E* isomer and between the thio ester carbonyl and the *tert*-leucine amide for the *Z*. Ongoing work is centred on converting the methyl ester of **56** into a metal-chelating warhead as is found in **55** and studying biological properties of this derivative.[48]

3. Conclusion

An olefin tether of variable length was introduced into natural amino acids via the side-chain (Ser, Cys), N-terminus (Arg), C-terminus (Phe, Tic) or a combination of both (Ser, Cys, Tyr) to provide substrates for metathesis (see Tables 1–4). Cross-metathesis of the side-chain derivatized Cys, Ser and Tyr derivatives (**8** and **9**, **11** and **12** and **45**) gave **33**–**36**, **46** and **47** and **48**, while dimerization of the N_α-acyl arginines **14** and **15** gave **37** and **38**, respectively. The Phe and Tic alkenyl esters **17** and **18** and **20** and **21** were cross-linked to give dimers **41**–**44**. Ring-closing metathesis of the doubly alkenylated Cys, Ser and Tyr derivatives (**23** and **24**, **26** and **27** and **30**) gave 12-, 20- and 24-membered macrocycles (**49**–**52** and **54**), with the alkene configuration of **49** and **51** being assigned the *E* configuration by X-ray crystallography. The methodology was also applied to the synthesis of a novel peptide deformylase inhibitor template **56**.

The approach presented is general (using the side-chain of serine and cysteine, and potentially aspartic/glutamic acid, lysine and threonine) and versatile (length of olefin tether), enabling access to analogues of natural peptide cross-links

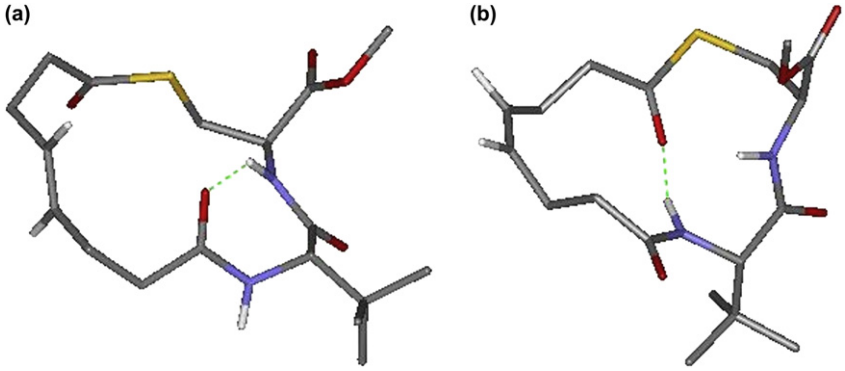


Figure 5. Minimized energy structures of *E*-**56** (a) and *Z*-**56** (b).

and bioconjugates. Bioactive (peptidic) monomers can be linked at various positions, using the olefin tethered side-chain and/or main-chain functionalities. The double bond in the metathesis product also provides a point for further elaboration and modification (e.g., via dihydroxylation or amino hydroxylation). RCM provides access to novel cyclic peptidomimetics in two steps from the natural amino acids, where the length of the olefin tether determines the ring size.

4. Experimental

4.1. General

Thin Layer Chromatography (TLC) was performed on a plastic sheet precoated with silica gel $60F_{254}$ (Merck) and visualized using ultraviolet light, vanillin and/or potassium permanganate dip. Column chromatography was performed using 230–400 mesh Merck Silica Gel 60. CH_2Cl_2 was freshly distilled from calcium hydride under an inert atmosphere and stored over 4 Å molecular sieves. HPLC grade DMF was purchased from Aldrich and stored over 4 Å molecular sieves. Melting points (mp) were determined on an Electrothermal apparatus and are uncorrected. Electrospray ionization mass spectra were detected on a Micromass LCT TOF mass spectrometer, with a probe voltage of 3200 V, temperature of 150 °C and a source temperature of 80 °C. ^{1}H NMR spectra were obtained on a Varian Inova spectrometer, operating at 500 MHz. ^{13}C NMR spectra were recorded on a Varian Inova Unity 300 spectrometer, operating at 75 MHz. Solvents and chemical shifts (δ), using TMS (δ_H 0.00 ppm), $CDCl_3$ ($CHCl_3$ at δ_H 7.26 ppm, $CDCl_3$ at δ_C 77.23 ppm) or CD_3OD (CHD_2OD at δ_H 3.30 ppm, CD_3OD at δ_C 49.3 ppm) as internal standards, are reported for each compound.

4.2. X-ray crystallography

All measurements were made with a Seimens CCD area detector using graphite monochromized Mo Kα (λ=0.71073 Å) radiation at 92F. The data reduction was performed using SAINT.[49] Intensities were corrected for Lorentz and polarization effects and for absorption using SADABS. Space groups were determined from systematic absences and checked for higher symmetry. The structures were solved by direct methods using SHELXS,[50] and refined on *F* with all data using full-matrix least squares procedures with SHELXL-97.[51] All non-hydrogen atoms were refined with anisotropic displacement parameters. All hydrogen atoms were fixed in idealized positions. Absolute structural determinations were based on the Flack parameter. In all cases, final Fourier syntheses showed no significant residual electron density in chemical sensible positions.

The crystallographic data have been submitted with the Cambridge Crystallographic Data Centre. CCDC-676321 (compound **49**) and CCDC-676322 (compound **51**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

4.3. Molecular modelling

E- and *Z*-Configurations of cyclic compounds were constructed in silico using Schrödinger's Maestro build function and minimized using the MMFFs forcefield in vacuo, which is reputed to be best for comparing with crystal structures.[53,54] The same forcefield was then used to perform a conformational search on each structure. Conformations within 12 kJ mol^{-1} of the lowest energy conformation were saved to generate a low energy ensemble for each structure. Each ensemble was then used to calculate the Boltzmann weighted average energy of each compound allowing the most thermodynamically stable double bond configuration of each compound to be identified.

4.4. Preparation of the metathesis substrates (Table 1)

4.4.1. Synthesis of (2R)-2-tert-butoxycarbonylamino-3-(pent-4-enoylsulfanyl)-propionic acid methyl ester **8**

Et_3N (2.4 equiv) was added dropwise to a stirred solution of 4-pentenoic acid (267 mg, 2.67 mmol, 1.1 equiv) in dry CH_2Cl_2 (15 mL) under an inert atmosphere. After stirring for 20 min, this solution was added dropwise to a solution of BOP-Cl (741 mg, 2.92 mmol, 1.2 equiv) in dry CH_2Cl_2 (15 mL) at 0 °C. A solution of Boc–L-Cys–OMe (572 mg, 2.43 mmol, 1 equiv) in dry CH_2Cl_2 (15 mL) was then added dropwise at 0 °C. After 15 min the reaction was allowed to warm to rt and stirring continued overnight. The solvent was removed by evaporation under reduced pressure and the residue dissolved in ethyl acetate. The solution was washed twice with water (10 mL), and the combined organic layers were washed with aqueous saturated sodium bicarbonate (10 mL), water (10 mL) and brine (10 mL) sequentially. The combined organic layers were dried ($MgSO_4$), and the solvent removed by evaporation under reduced pressure. The crude product was purified by flash chromatography on silica (ethyl acetate/petroleum ether, 1:4) to give **8** (717 mg, 93%) as a white solid.

HRMS (M+H) found 318.1375 (calcd for $C_{14}H_{24}NO_5S$ 318.1375); R_f (20% ethyl acetate/petroleum ether) 0.48; mp: 33–35 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.42 (s, 9H, C(*Me*)$_3$), 2.38 (m, 2H, $COCH_2CH_2$), 2.64 (t, 2H, ^3J 7.5 Hz, $COCH_2$), 3.33 (m, 2H, CH_2S), 3.73 (s, 3H, OCH_3), 4.51 (br m, 1H, NHC*H*(α)), 5.00 (dd, 1H, ^2J 1.3 Hz, ^3J 10.3 Hz, CH=CH*H*), 5.04 (m, 1H, CH=C*H*H), 5.21 (br d, 1H, ^3J 7.5 Hz, N*H*(α)), 5.76 (m, 1H, C*H*=CH_2); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 27.5, 28.6, 30.1, 42.3, 51.8, 52.4, 79.0, 115.3, 135.3, 154.4, 170.3, 196.6.

4.4.2. Synthesis of (2R)-2-tert-butoxycarbonylamino-3-(undec-10-enoylsulfanyl)-propionic acid methyl ester **9**

TEA (2.4 equiv) was added dropwise to a stirred solution of 10-decenoic acid (516 mg, 2.80 mmol, 1.1 equiv) in dry CH_2Cl_2 (15 mL) under an inert atmosphere. After stirring for 20 min, this solution was added dropwise to a solution of

BOP-Cl (774 mg, 3.05 mmol, 1.2 equiv) in dry CH_2Cl_2 (15 mL) at 0 °C. A solution of Boc–L-Cys–OMe (598 mg, 2.54 mmol, 1 equiv) in dry CH_2Cl_2 (15 mL) was then added dropwise at 0 °C. After 15 min the reaction was allowed to warm to rt and stirring continued overnight. The solvent was removed by evaporation under reduced pressure and the residue dissolved in ethyl acetate. The solution was washed twice with water (10 mL), and the combined organic layers were washed with aqueous saturated sodium bicarbonate (10 mL), water and brine (10 mL) sequentially. The combined organic layers were dried ($MgSO_4$), and the solvent removed by evaporation under reduced pressure. The crude product was purified by flash chromatography on silica (ethyl acetate/petroleum ether, 1:4) to give **9** (840 mg, 82%) as a colourless oil.

HRMS (M+Na) found 424.2119 (calcd for $C_{20}H_{35}NO_5SNa$ 424.2134); R_f (20% ethyl acetate/petroleum ether) 0.73; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.26–1.47 (m, 10H, CH_2), 1.42 (s, 9H, C(*Me*)$_3$), 1.63 (m, 2H, COCH$_2$CH_2), 2.01 (m, 2H, CO(CH$_2$)$_7$CH_2), 2.53 (t, 2H, ^3J 7.6 Hz, COCH_2), 3.32 (m, 2H, CH_2S), 3.73 (s, 3H, O*Me*), 4.51 (br m, 1H, NHC*H*(α)), 4.91 (m, 1H, CH=CH*H*), 4.97 (m, 1H, CH=C*H*H), 5.22 (br d, 1H, ^3J 6.7 Hz, N*H*(α)), 5.79 (m, 1H, C*H*=CH$_2$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 25.0, 27.7, 28.3, 28.4, 28.5, 28.6, 28.7, 30.3, 33.2, 43.3, 51.9, 52.6, 79.1, 113.7, 138.3, 154.5, 170.4, 197.4.

4.4.3. Synthesis of (2S)-pent-4-enoic acid-(2-tert-butoxy-carbonylamino-2-methoxycarbonyl)-ethyl ester *11*

To a stirred solution of Boc–L-Ser–OMe (2 g, 9.10 mmol, 1 equiv) and 4-pentenoic acid (1.04 g, 10.4 mmol, 1.14 equiv) in dry CH_2Cl_2 (80 mL) was added DMAP (11 mg, 0.09 mmol, 0.01 equiv) and the solution was cooled to 0 °C. EDCI (1.89 g, 9.92 mmol, 1.09 equiv) was added, the solution was stirred at 0 °C for 2 h and was then allowed to warm to rt with stirring overnight. The solvent was removed by evaporation under reduced pressure and the residue dissolved in ethyl acetate and water (80 mL, 1:1 v/v). The organic layer was dried ($MgSO_4$), and the solvent removed by evaporation under reduced pressure. The crude product was purified by flash chromatography on silica (ethyl acetate/petroleum ether, 3:7) to give **11** (2.51 g, 89%) as a white solid.

HRMS (M+H) found 302.1601 (calcd for $C_{14}H_{24}NO_6$ 302.1604); R_f (30% ethyl acetate/petroleum ether) 0.67; mp: 35–37 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.42 (s, 9H, C(*Me*)$_3$), 2.32 (m, 2H, COCH$_2$CH_2), 2.39 (m, 2H, COCH_2), 3.73 (s, 3H, O*Me*), 4.31 (dd, 1H, ^3J 3.6 Hz, ^2J 11.3 Hz, OC*H*H), 4.41 (dd, 1H, ^3J 3.6 Hz, ^2J 11.3 Hz, OCH*H*), 4.54 (m, 1H, NHC*H*(α)), 4.98 (dd, 1H, ^2J 1.4 Hz, ^3J 10.3 Hz, CH=CH_{cis}H$_{trans}$), 5.02 (dd, 1H, ^2J 1.4 Hz, ^3J 17.2 Hz, CH=CH$_{cis}$$H_{trans}$), 5.27 (br d, 1H, ^3J 8.0 Hz, N*H*(α)), 5.77 (m, 1H, C*H*=CH$_2$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 27.7, 28.1, 32.6, 52.0, 52.4, 63.5, 79.3, 115.0, 136.0, 154.7, 169.7, 171.8.

4.4.4. Synthesis of (2S)-undec-10-enoic acid-(2-tert-butoxy-carbonylamino-2-methoxycarbonyl)-ethyl ester *12*

To a stirred solution of Boc–L-Ser–OMe (300 mg, 1.37 mmol, 1 equiv) and 10-decenoic acid (287 mg, 1.56 mmol, 1.14 equiv) in dry CH_2Cl_2 (10 mL) was added DMAP (2 mg, 0.01 mmol, 0.01 equiv) and the solution was cooled to 0 °C. EDCI (285 mg, 1.49 mmol, 1.09 equiv) was added, the solution was stirred at 0 °C for 2 h and was then allowed to warm to rt with stirring overnight. The solvent was removed by evaporation under reduced pressure and the residue dissolved in ethyl acetate and water (30 mL, 1:1 v/v). The organic layer was dried ($MgSO_4$), and the solvent removed by evaporation under reduced pressure. The crude product was purified by flash chromatography on silica (ethyl acetate/petroleum ether, 1:4) to give **12** (444 mg, 84%) as a colourless oil.

HRMS (M+Na) found 408.2361 (calcd for $C_{20}H_{35}NO_6Na$ 408.2362); R_f (20% ethyl acetate/petroleum ether) 0.52; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.26–1.45 (m, 10H, CH_2), 1.43 (s, 9H, C(*Me*)$_3$), 1.57 (m, 2H, COCH$_2$CH_2), 2.01 (m, 2H, CO(CH$_2$)$_7$CH_2), 2.27 (t, 2H, ^3J 7.6 Hz, COCH_2), 3.74 (s, 3H, OCH_3), 4.29 (dd, 1H, ^3J 3.5 Hz, ^2J 11.2 Hz, OC*H*H), 4.43 (dd, 1H, ^3J 3.5 Hz, ^2J 11.2 Hz, OCH*H*), 4.54 (m, 1H, NHC*H*), 4.90 (m, 1H, CH=C*H*H), 4.96 (m, 1H, CH=CH*H*), 5.27 (br d, 1H, ^3J 8.0 Hz, N*H*), 5.78 (m, 1H, C*H*=CH$_2$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 24.5, 27.9, 28.5, 28.7, 28.9, 29.0, 33.5, 33.6, 52.3, 52.7, 63.6, 79.7, 113.9, 138.7, 154.9, 170.0, 172.9.

4.4.5. Synthesis of N^{α}-(2S)-pent-4-enoyl-N^{ω}-(2,2,4,6,7-pentamethyldihydro-benzofuran-5-sulfonyl)-arginine methyl ester *14*

To a stirred solution of 4-pentenoic acid (0.053 mL, 0.76 mmol, 1 equiv) in dry CH_2Cl_2 (10 mL) were added Et_3N (0.32 mL, 2.26 mmol, 3 equiv) and TBTU (0.269 g, 0.84 mmol, 1.1 equiv). The solution was stirred at rt for 5 min and N^{ω}-(2,2,4,6,7-pentamethyldihydro-benzofuran-5-sulfonyl)-L-arginine methyl ester hydrochloride **13** (400 mg, 0.84 mmol, 1.1 equiv) was subsequently added. The reaction was stirred for 2 h at rt and the reaction mixture was then diluted with CH_2Cl_2 (10 mL) and extracted with 1 N aqueous HCl (10 mL), aqueous saturated sodium bicarbonate (10 mL) and brine (10 mL). The organic layer was dried (Na_2SO_4), filtered and the solvent removed by evaporation under reduced pressure. No further purification was needed (purity >95%). Compound **14** was isolated as a white foam (450 mg, 88%).

MS (M+H) found 523 (calcd for $C_{25}H_{39}N_4O_6S$ 523.2512); R_f (5% MeOH/CH_2Cl_2) 0.41; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.46 (s, 6H, C$_{quat}$(*Me*)$_2$ (Pbf)), 1.55 (m, 2H, CH_2(γ)), 1.85 (m, 2H, CH_2(β)), 2.09 (s, 3H, C$_7$*Me* (Pbf)), 2.32 (m, 4H, COCH_2CH_2), 2.51 (s, 3H, C$_4$*Me* (Pbf)), 2.57 (s, 3H, C$_6$*Me* (Pbf)), 2.96 (s, 2H, CH_2 (Pbf)), 3.24 (m, 2H, CH_2(δ)), 3.72 (s, 3H, COO*Me*), 4.54 (m, 1H, C*H*(α)), 4.99 (m, 2H, CH=C*H*H and CH=CH*H*), 5.78 (m, 1H, C*H*=CH$_2$), 6.27 (br s, 3H, C(δ)N*H*C(=N*H*)N*H*), 6.62 (d, 1H, ^3J 6.6 Hz, N*H*(α)); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 12.7, 18.1, 19.5, 25.5, 28.8, 29.6, 30.1, 35.5, 38.8, 43.4, 49.6, 52.7, 86.6, 118.9, 117.7, 124.8, 132.3, 133.1, 136.8, 138.5, 156.5, 158.9, 172.9, 173.8.

4.4.6. Synthesis of N^{α}-(2S)-undec-10-enoyl-N^{ω}-(2,2,4,6,7-pentamethyldihydro-benzofuran-5-sulfonyl)-arginine methyl ester *15*

To a stirred solution of 10-undecenoic acid (0.15 mL, 0.76 mmol, 1 equiv) in dry CH_2Cl_2 (10 mL) were added

Et_3N (0.32 mL, 2.26 mmol, 3 equiv) and TBTU (0.269 g, 0.84 mmol, 1.1 equiv). The solution was stirred at rt for 5 min and N^{ω}-(2,2,4,6,7-pentamethyldihydro-benzofuran-5-sulfonyl)-L-arginine methyl ester hydrochloride **13** (400 mg, 0.84 mmol, 1.1 equiv) was subsequently added. The reaction was stirred for 2 h at rt and the reaction mixture was then diluted with CH_2Cl_2 (10 mL) and extracted with 1 N aqueous HCl (10 mL), aqueous saturated sodium bicarbonate (10 mL) and brine (10 mL). The organic layer was dried (Na_2SO_4), filtered and the solvent removed by evaporation under reduced pressure. No further purification was needed (purity >95%). Compound **15** was isolated as a pale yellow oil (381 mg, 83%).

MS (M+H) found 607 (calcd for $C_{31}H_{51}N_4O_6S$ 607.3451); R_f (5% MeOH/CH_2Cl_2) 0.35; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.27 (m, 10H, C_4–C_8 CH_2 undecenoyl), 1.46 (s, 6H, $C_{quat}(Me)_2$ (Pbf)), 1.58 (m, 4H, C_3 CH_2 undecenoyl and $CH_2(\gamma)$), 1.65 and 1.85 (2m, 2H, C_9 CH_2 undecenoyl), 2.03 (m, 2H, $CH_2(\beta)$), 2.09 (s, 3H, C_7Me (Pbf)), 2.20 (t, 2H, ^3J 7.6 Hz, C_2 CH_2 undecenoyl), 2.51 (s, 3H, C_4Me (Pbf)), 2.57 (s, 3H, C_6Me (Pbf)), 2.96 (s, 2H, CH_2 (Pbf)), 3.25 (m, 2H, $CH_2(\delta)$), 3.73 (s, 3H, COO*Me*), 4.54 (m, 1H, C*H*(α)), 4.98 (m, 2H, CH=C*H*H and CH=CH*H*), 5.81 (m, 1H, C*H*=CH_2), 6.24 (br s, 2H, C(δ)N*H*C(=NH)N*H*), 6.35 (br s, 1H, C(δ)NHC(=N*H*)NH), 6.46 (d, 1H, ^3J 6.6 Hz, N*H*(α)); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 12.6, 18.1, 19.4, 25.5, 25.8, 28.8, 29.1, 29.3, 29.4, 29.5, 30.3, 36.5, 40.8, 43.5, 51.8, 52.7, 61.4, 86.6, 114.4, 117.7, 124.8, 132.5, 133.3, 138.5, 139.3, 156.5, 158.9, 172.9, 174.2.

*4.4.7. Synthesis of (2S)-2-tert-butoxycarbonylamino-3-phenyl-propionic acid but-3-enyl ester **17***

To a stirred solution of Boc–L-Phe–OH (2 g, 7.5 mmol, 1 equiv) in dry CH_2Cl_2 (40 mL) were added Et_3N (3.1 mL, 22.5 mmol, 3 equiv) and TBTU (2.65 g, 8.25 mmol, 1.1 equiv). The solution was stirred at rt for 5 min and 3-buten-1-ol (0. 71 mL, 8.25 mmol, 1.1 equiv) was subsequently added. The reaction was stirred overnight at rt and the reaction mixture was then diluted with CH_2Cl_2 (40 mL) and extracted with 1 N aqueous HCl (20 mL), aqueous saturated sodium bicarbonate (20 mL) and brine (20 mL). The organic layer was dried (Na_2SO_4), filtered and the solvent removed by evaporation under reduced pressure. The crude product was purified by flash chromatography on silica (CH_2Cl_2/MeOH, 99:1) to give **17** (2.09 g, 87%) as a white solid.

MS (M+Na) found 342 (calcd for $C_{18}H_{26}NO_4Na$ 342.1681); R_f (1% MeOH/CH_2Cl_2) 0.38; mp: 76–79 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.41 (s, 9H, C(*Me*)$_3$), 2.34 (m, 2H, $COCH_2CH_2$), 3.07 (m, 2H, CH_2Ar), 4.15 (t, 2H, ^3J 6.7 Hz, OCH_2CH_2), 4.57 (dd, 1H, ^3J 15.4, 6.2 Hz, C*H*(α)), 4.96 (br d, 1H, ^3J 7.0 Hz, N*H*(α)), 5.07 (m, 1H, CH=CH_2), 5.70 (m, 1H, C*H*=CH_2), 7.13 (apparent d, 2H, ^3J 7.8 Hz, C*H* arom), 7.25 (m, 3H, C*H* arom); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 28.5, 33.1, 38.9, 54.6, 64.6, 80.3, 117.7, 127.2, 128.7, 129.5, 133.8, 136.3, 155.9, 172.1.

*4.4.8. Synthesis of (2S)-2-tert-butoxycarbonylamino-3-phenyl-propionic acid dec-9-enyl ester **18***

To a stirred solution of Boc–L-Phe–OH (1 g, 3.75 mmol, 1 equiv) in dry CH_2Cl_2 (20 mL) were added Et_3N (1.55 mL, 11.25 mmol, 3 equiv) and TBTU (1.325 g, 4.125 mmol, 1.1 equiv). The solution was stirred at rt for 5 min and 9-decen-1-ol (0.735 mL, 4.13 mmol, 1.1 equiv) was subsequently added. The reaction was stirred overnight at rt and the reaction mixture was then diluted with CH_2Cl_2 (20 mL) and extracted with 1 N aqueous HCl (20 mL), aqueous saturated sodium bicarbonate (20 mL) and brine (20 mL). The organic layer was dried (Na_2SO_4), filtered and the solvent removed by evaporation under reduced pressure. The crude product was purified by flash chromatography on silica (CH_2Cl_2/MeOH, 99:1) to give **18** (1.30 g, 86%) as a glassy white solid.

MS (M+Na) found 426 (calcd for $C_{24}H_{37}NO_4Na$ 426.2723); R_f (1% MeOH/CH_2Cl_2) 0.36; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.28 (br m, 10H, CH_2 C_3–C_7 dec-9-enyl ester), 1.42 (s, 9H, C(*Me*)$_3$), 1.61 (m, 2H, CH_2 C_2 dec-9-enyl ester), 2.05 (m, 2H, CH_2 C_8 dec-9-enyl ester), 3.07 (m, 2H, CH_2Ar), 4.08 (t, 2H, ^3J 7.0 Hz, CH_2 C_1 dec-9-enyl ester), 4.59 (dd, 1H, ^3J 15.3, 6.0 Hz, C*H*(α)), 5.02 (m, 2H, CH=CH_2), 5.05 (br s, 1H, N*H*(α)), 5.81 (m, 1H, C*H*=CH_2), 7.15 (m, 2H, C*H* arom), 7.25 (m, 3H, C*H* arom); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 25.9, 28.4, 28.5, 29.0, 29.1, 29.2, 29.4, 33.9, 38.5, 54.6, 65.6, 79.8, 114.3, 127.0, 128.6, 129.4, 136.2, 139.2, 155.2, 172.0.

*4.4.9. Synthesis of (2S)-2-tert-butoxycarbonylamino-1,2,3,4-tetrahydro-isoquinoline-3-carboxylic acid but-3-enyl ester **20***

To a stirred solution of Boc–L-Tic–OH **19** (300 mg, 1.08 mmol, 1 equiv) in dry CH_2Cl_2 (10 mL) were added Et_3N (0.450 mL, 3.26 mmol, 3 equiv) and TBTU (382 mg, 1.19 mmol, 1.1 equiv). The solution was stirred at rt for 5 min and 3-buten-1-ol (0.102 mL, 1.19 mmol, 1.1 equiv) was subsequently added. The reaction was stirred overnight at rt and the reaction mixture was then diluted with CH_2Cl_2 (10 mL) and extracted with 1 N aqueous HCl (10 mL), aqueous saturated sodium bicarbonate (10 mL) and brine (10 mL). The organic layer was dried (Na_2SO_4), filtered and the solvent removed by evaporation under reduced pressure. The crude product was purified by flash chromatography on silica (CH_2Cl_2/MeOH, 99:1) to give **20** (286 mg, 80%) as a yellow oil.

MS (M+Na) found 354 (calcd for $C_{19}H_{25}NO_4Na$ 354.1784); R_f (1% MeOH/CH_2Cl_2) 0.40; ^{1}H NMR ($CDCl_3$, 500 MHz) δ (cis/trans rotamer mixture) 1.46 and 1.53 (2s, 9H, cis/trans C(*Me*)$_3$), 2.24 (m, 2H, $COCH_2CH_2$), 3.20 (m, 2H, CH_2Ar), 4.07 (t, 2H, ^3J 6.8 Hz, OCH_2CH_2), 4.48 and 4.52 (2d, 1H, ^2J 7.0 Hz, NC*H*HAr), 4.67 and 4.72 (2d, 1H, ^2J 7.0 Hz, NCH*H*Ar), 4.80 (apparent t, 0.5H, J 5.2 Hz, C*H*(α) cis or trans), 5.01 (m, 2H, CH=CH_2), 5.13 (dd, 0.5H, ^3J 5.8, 3.3 Hz, C*H*(α) cis or trans), 5.62 (m, 1H, C*H*=CH_2), 7.16 (m, 4H, C*H* arom); ^{13}C NMR ($CDCl_3$, 75 MHz) δ (cis/trans rotamer mixture) 28.5 and 28.6, 31.4 and 31.9, 33.1, 44.3 and 44.8, 52.7, 53.6, 54.5, 64.3, 80.7, 117.4 and 117.5, 126.4, 126.5, 126.7, 126.9, 127.0, 127.1, 128.0, 128.7, 132.0, 132.3, 133.8, 133.9, 134.7, 155.6 and 156.2, 171.6 and 172.9.

*4.4.10. Synthesis of (2S)-2-tert-butoxycarbonylamino-1,2,3,4-tetrahydro-isoquinoline-3-carboxylic acid dec-9-enyl ester **21***

To a stirred solution of Boc–L-Tic–OH **19** (300 mg, 1.08 mmol, 1 equiv) in dry CH_2Cl_2 (10 mL) were added Et_3N (0.450 mL, 3.26 mmol, 3 equiv) and TBTU (382 mg, 1.19 mmol, 1.1 equiv). The solution was stirred at rt for 5 min and 9-decen-1-ol (0.212 mL, 1.19 mmol, 1.1 equiv) was subsequently added. The reaction was stirred overnight at rt and the reaction mixture was then diluted with CH_2Cl_2 (10 mL) and extracted with 1 N aqueous HCl (10 mL), aqueous saturated sodium bicarbonate (10 mL) and brine (10 mL). The organic layer was dried (Na_2SO_4), filtered and the solvent removed by evaporation under reduced pressure. The crude product was purified by flash chromatography on silica (CH_2Cl_2/MeOH, 99:1) to give **21** (270 mg, 60%) as a colourless oil.

MS (M+Na) found 438 (calcd for $C_{25}H_{37}NO_4Na$ 438.2723); R_f (1% MeOH/CH_2Cl_2) 0.31; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.25 (m, 10H, C_3–C_7 CH_2 dec-9-enyl ester), 1.46 and 1.53 (2s, 9H, C(*Me*)$_3$), 1.58 (br s, 2H, CH_2 C_2 dec-9-enyl ester), 2.04 (m, 2H, CH_2 C_8 dec-9-enyl ester), 3.15 (m, 2H, CH_2Ar), 4.00 (t, 2H, ^3J 5.8 Hz, CH_2 C_1 dec-9-enyl ester), 4.46 and 4.54 (2br s, 1H, NC*H*HAr), 4.65 and 4.74 (2br s, 1H, NCH*H*Ar), 4.79 (apparent t, 1H, *J* 5.0 Hz, C*H*(α) cis or trans), 4.99 (m, 2H, CH=CH_2), 5.14 (dd, ^3J 5.9, 3.0 Hz, C*H*(α) cis or trans), 5.81 (m, 1H, C*H*=CH$_2$), 7.17 (m, 4H, C*H* arom); ^{13}C NMR ($CDCl_3$, 75 MHz) δ (cis/trans mixture) 25.4, 28.1, 28.3, 28.6, 28.7, 28.8, 29.0, 31.0, 31.5, 33.4, 43.8 and 44.4, 52.3 and 54.1, 64.8, 80.2, 113.9, 126.0, 126.5, 127.5, 128.2, 130.9, 131.9, 132.7, 133.6, 138.8, 154.6 and 155.2, 171.1 and 171.7.

*4.4.11. Synthesis of (2R)-2-(pent-4-enoylamino)-3-(pent-4-enoylsulfanyl)-propionic acid methyl ester **23***

To a stirred solution of 4-pentenoic acid (321 mg, 3.2 mmol, 2.2 equiv) in dry CH_2Cl_2 (10 mL) under an inert atmosphere was added dropwise Et_3N (876 mg, 8.6 mmol, 5.9 equiv) at 0 °C. After stirring for 20 min, this solution was added dropwise to a solution of BOP-Cl (890 mg, 3.5 mmol, 2.4 equiv) in dry CH_2Cl_2 (20 mL) at 0 °C. A solution of L-Cys–OMe·HCl **22** (250 mg, 1.5 mmol, 1 equiv) in dry CH_2Cl_2 (10 mL) was then added dropwise at 0 °C. After 15 min the reaction was allowed to warm to rt with stirring overnight. The solvent was removed by evaporation under reduced pressure and the residue dissolved in ethyl acetate (50 mL). The solution was washed twice with water (25 mL) and the organic layer washed twice with saturated aqueous sodium bicarbonate (25 mL), water (25 mL) and brine solution (25 mL) sequentially. The resulting organic layer was dried ($MgSO_4$), and the solvent was removed by evaporation under reduced pressure. The crude product was purified by flash chromatography on silica (ethyl acetate/petroleum ether, 1:4) to give **23** (372 mg, 85%) as a colourless oil.

HRMS (M+H) found 300.1273 (calcd for $C_{14}H_{22}NO_4S$ 300.1270); R_f (40% ethyl acetate/CH_2Cl_2) 0.61; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.27 (m, 2H, COCH_2), 2.36 (m, 4H, =CHCH_2), 2.63 (t, 2H, ^3J 7.3 Hz, COCH_2), 3.35 (m, 2H, CH_2S), 3.72 (s, 3H, O*Me*), 4.77 (m, 1H, C*H*(α)), 4.97–5.06 (m, 4H, CH=CH_2), 5.77 (m, 2H, C*H*=CH$_2$), 6.28 (br d, 1H, ^3J 7.1 Hz, N*H*(α)); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 28.5, 28.7, 29.7, 34.4, 42.19, 51.3, 51.8, 114.6, 115.2, 135.3, 136.3, 167.0, 171.8, 197.0.

*4.4.12. Synthesis of (2R)-2-(undec-10-enoylamino)-3-(undec-10-enoylsulfanyl)-propanoic acid methyl ester **24***

To a stirred solution of 10-undecenoic acid (590 mg, 3.2 mmol, 2.2 equiv) in dry CH_2Cl_2 (10 mL) under an inert atmosphere was added dropwise Et_3N (876 mg, 8.6 mmol, 5.9 equiv) at 0 °C. After stirring for 20 min, this solution was added dropwise to a solution of BOP-Cl (890 mg, 3.5 mmol, 2.4 equiv) in dry CH_2Cl_2 (20 mL) at 0 °C. A solution of L-Cys–OMe·HCl **22** (250 mg, 1.5 mmol, 1 equiv) in dry CH_2Cl_2 (10 mL) was then added at 0 °C. After 15 min the reaction was allowed to warm to rt with stirring overnight. The solvent was removed by evaporation under reduced pressure and the residue dissolved in ethyl acetate (50 mL). The solution was washed twice with water (25 mL) and the organic layer washed twice with saturated aqueous sodium bicarbonate (25 mL), water (25 mL) and brine solution (25 mL) sequentially. The resulting organic layer was dried ($MgSO_4$), and the solvent was removed by evaporation under reduced pressure. The crude product was purified by flash chromatography on silica (ethyl acetate/petroleum ether, 1:1) to give **24** (300 mg, 44%) as a white solid.

HRMS (M+H) found 468.3166 (calcd for $C_{26}H_{46}NO_4S$ 468.3148); R_f (50% ethyl acetate/CH_2Cl_2) 0.96; mp: 40–41 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.30 (m, 20H, CH_2), 1.59 (m, 4H, CH_2), 2.00 (m, 4H, CH_2CH=CH$_2$), 2.16 (t, 2H, ^3J 7.6 Hz, NHCOCH_2), 2.52 (t, 2H, ^3J 7.5 Hz, SCOCH_2), 3.32 (pseudo d, 2H, *J* 5.5 Hz, CH_2S), 3.71 (s, 3H, O*Me*), 4.76 (m, 1H, NHC*H*), 4.89 (m, 2H, CH=CH_2), 4.95 (ddd, 2H, ^2J 1.6 Hz, ^4J 3.4 Hz, ^3J 17.1 Hz, CH=CH_2), 5.77 (m, 2H, C*H*=CH$_2$), 6.24 (d, 1H, ^3J 7.5 Hz, N*H*(α)); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 25.2, 25.3, 28.5, 28.6, 28.7, 28.8, 28.9, 28.9, 29.0, 29.0, 30.2, 33.4, 33.5, 36.0, 43.6, 51.7, 52.3, 113.9, 113.9, 138.6, 138.7, 170.5, 172.8, 198.5.

*4.4.13. Synthesis of (2S)-pent-4-enoic acid-[2-methoxycarbonyl-2-(pent-4-enoylamino)]-methyl ester **26***

To a stirred solution of L-Ser–OMe·HCl **25** (200 mg, 1.7 mmol, 1 equiv) and 4-pentenoic acid (370 mg, 3.74 mmol, 2.2 equiv) in DMF (50 mL) were added DIPEA (1.05 g, 8.16 mmol, 4.8 equiv) and HATU (1.53 g, 4.08 mmol, 2.4 equiv) and the solution was stirred overnight. Water (100 mL) was added and the resulting solution extracted with ethyl acetate (50 mL). The organic layer was then washed with aqueous saturated sodium bicarbonate (25 mL), saturated ammonium chloride (25 mL) and brine (25 mL). The organic layer was dried ($MgSO_4$), and the solvent was removed by evaporation under reduced pressure. The crude product was purified by flash chromatography on silica (ethyl acetate/petroleum ether, 2:3) to give **26** (280 mg, 59%) as a pale yellow oil.

HRMS (M+H) found 284.1494 (calcd for $C_{14}H_{22}NO_5$ 284.1498); R_f (40% ethyl acetate/CH_2Cl_2) 0.53; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.35 (m, 8H, CH_2CH_2), 3.73 (s, 3H, O*Me*), 4.33 (dd, 1H, ^3J 3.7 Hz, ^2J 11.5 Hz, OC*H*H), 4.43 (dd, 1H, ^3J 3.7 Hz, ^2J 11.5 Hz, OCH*H*), 4.83 (m, 1H, NHC*H*(α)), 5.01 (m, 4H, CH=CH_2), 5.78 (m, 2H, C*H*=CH_2), 6.32 (d, 1H, ^3J 7.6 Hz, N*H*(α)); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 28.3, 29.0, 32.8, 35.0, 51.3, 52.4, 63.4, 115.2, 115.3, 136.1, 136.5, 169.7, 172.1, 172.2.

4.4.14. Synthesis of (2S)-undec-10-enoic acid-[2-methoxycarbonyl-2-(undec-10-enoylamino)]-ethyl ester **27**

To a stirred solution of L-Ser−OMe·HCl **25** (200 mg, 1.7 mmol, 1 equiv) and 10-undecenoic acid (680 mg, 3.74 mmol, 2.2 equiv) in DMF (50 mL) were added DIPEA (1.05 g, 8.16 mmol, 4.8 equiv) and HATU (1.53 g, 4.08 mmol, 2.4 equiv), and the solution stirred overnight. Water (100 mL) was added and the resulting solution extracted with ethyl acetate (50 mL). The organic layer was washed with aqueous saturated sodium bicarbonate (25 mL), saturated ammonium chloride (25 mL) and brine (25 mL). The resulting organic phase was dried ($MgSO_4$) and the solvent removed by evaporation under reduced pressure. The crude product was purified by flash chromatography on silica (ethyl acetate/petroleum ether, 2:3) to give **27** (409 mg, 54%) as a waxy oil.

HRMS (M+H) found 452.3378 (calcd for $C_{26}H_{46}NO_5$ 452.3376); R_f (30% ethyl acetate/CH_2Cl_2) 0.67; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.31 (m, 20H, CH_2), 1.58 (m, 4H, CH_2), 2.01 (m, 4H, CH_2CH=CH_2), 2.26 (m, 4H, COCH_2), 3.74 (s, 3H, O*Me*), 4.32 (dd, 1H, ^3J 3.5 Hz, ^2J 11.4 Hz, OC*H*H), 4.45 (dd, 1H, ^3J 4.0 Hz, ^2J 11.4 Hz, OCH*H*), 4.85 (m, 1H, NHC*H*(α)), 4.90 (m, 2H, HC=C*H*H), 4.96 (dd, 2H, ^2J 1.3 Hz, ^3J 17.3 Hz, HC=CH*H*), 5.78 (m, 2H, C*H*=CH_2), 6.26 (d, 1H, ^3J 7.8 Hz, N*H*(α)); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 24.5, 25.2, 28.5, 28.6, 28.7, 28.7, 28.8, 28.9, 29.0, 29.1, 33.4, 33.5, 35.6, 35.9, 51.4, 52.4, 63.3, 113.8, 113.9, 138.6, 138.6, 169.9, 173.0, 173.1.

4.4.15. Synthesis of 3-(4-allyloxy-phenyl)-(2S)-2-tert-butoxycarbonylamino-propionic acid but-3-enyl ester **29**

To a stirred solution of Boc−L-Tyr(OAllyl)−OH **28** (1 g, 3.1 mmol, 1 equiv) in dry CH_2Cl_2 (20 mL) were added Et_3N (1.28 mL, 9.3 mmol, 3 equiv) and TBTU (1.095 g, 3.41 mmol, 1.1 equiv). The solution was stirred at rt for 5 min and 3-buten-1-ol (0.293 mL, 3.41 mmol, 1.1 equiv) was subsequently added. The reaction was stirred overnight at rt and the reaction mixture was then diluted with CH_2Cl_2 (20 mL) and extracted with 1 N aqueous HCl (20 mL), aqueous saturated sodium bicarbonate (20 mL) and brine (20 mL). The organic layer was dried (Na_2SO_4), filtered and the solvent removed by evaporation under reduced pressure. The crude product was purified by flash chromatography on silica (CH_2Cl_2/MeOH, 99:1) to give **29** (927 mg, 82%) as a glassy, colourless solid.

MS (M+Na) found 398 (calcd for $C_{21}H_{29}NO_5Na$ 398.2046); R_f (1% MeOH/CH_2Cl_2) 0.36; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.42 (s, 9H, C(*Me*)$_3$), 2.35 (m, 2H, COCH$_2$CH_2), 3.01 (m, 2H, CH_2Ar), 4.14 (t, 2H, ^3J 6.7 Hz, OCH_2CH$_2$), 4.50 and 4.51 (2apparent t, 3H, J 1.7 Hz, ArOCH_2 and NHC*H*(α)), 4.95 (br d, 1H, ^3J 7.6 Hz, N*H*(α)), 5.07 and 5.28 (2m, 2H, ^3J 10.3 Hz, ^4J 3.3 Hz, ^2J 1.4 Hz, CH=CH_2 ester), 5.09 and 5.40 (2m, 2H, ^2J 1.8 Hz, ^4J 3.5 Hz, ^3J 17.3 Hz, CH=CH_2 ether), 5.73 (m, 1H, C*H*=CH$_2$ ester), 6.05 (m, 1H, C*H*=CH$_2$ ether), 6.84 (d, 2H, ^3J 8.8 Hz, C*H* arom), 7.04 (d, 2H, ^3J 8.5 Hz, C*H* arom); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 28.0, 32.6, 37.8, 54.6, 64.0, 68.5, 79.8, 114.6, 117.1, 117.2, 130.0, 133.1, 133.3, 155.8, 157.7, 172.0.

4.4.16. Synthesis of 3-(4-allyloxy-phenyl)-(2S)-2-tert-butoxycarbonylamino-propionic acid dec-9-enyl ester **30**

To a stirred solution of Boc−L-Tyr(OAllyl)−OH **28** (1 g, 3.1 mmol, 1 equiv) in dry CH_2Cl_2 (20 mL) were added Et_3N (1.28 mL, 9.3 mmol, 3 equiv) and TBTU (1.095 g, 3.41 mmol, 1.1 equiv). The solution was stirred at rt for 5 min and 9-decen-1-ol (0. 608 mL, 3.41 mmol, 1.1 equiv) was subsequently added. The reaction was stirred overnight at rt and the reaction mixture was then diluted with CH_2Cl_2 (20 mL) and extracted with 1 N aqueous HCl (20 mL), aqueous saturated sodium bicarbonate (20 mL) and brine (20 mL). The organic layer was dried (Na_2SO_4), filtered and the solvent removed by evaporation under reduced pressure. The crude product was purified by flash chromatography on silica (CH_2Cl_2/MeOH, 99:1) to give **30** (977 mg, 69%) as a pale yellow oil.

MS (M+Na) found 482 (calcd for $C_{27}H_{40}NO_5Na$ 482.2985); R_f (1% MeOH/CH_2Cl_2) 0.38; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.29 (m, 10H, C_3−C_7 CH_2 decenyl ester), 1.42 (s, 9H, C(*Me*)$_3$), 1.59 (m, 2H, CH_2 C_2 decenyl ester), 2.05 (m, 2H, CH_2 C_8 decenyl ester), 3.02 (2br s, 2H, CH_2Ar), 4.08 (t, 2H, ^3J 6.7 Hz, CH_2 C_1 decenyl ester), 4.49 and 4.51 (2apparent t, 3H, J 1.8 Hz, ArOCH_2 and C*H*(α)), 4.97 (br s, 1H, N*H*(α)), 4.93 and 5.27 (2m, 2H, ^3J 10.1 Hz, ^4J 3.6 Hz, ^2J 1.3 Hz, CH=CH_2 ester), 4.99 and 5.39 (2m, 2H, ^2J 1.4, 17.4 Hz, ^4J 3.4 Hz, CH=CH_2 ether), 5.81 (m, 1H, C*H*=CH$_2$ ester), 6.05 (m, 1H, C*H*=CH$_2$ ether), 6.83 (d, 2H, ^3J 8.6 Hz, C*H* arom), 7.04 (d, 2H, ^3J 8.6 Hz, C*H* arom); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 26.0, 28.5, 28.7, 29.1, 29.2, 29.3, 29.5, 33.9, 38.1, 55.4, 65.6, 69.0, 80.1, 114.4, 115.0, 117.7, 128.5, 130.5, 133.6, 139.3, 155.5, 157.9, 172.2.

4.5. Cross-metathesis experiments (Tables 2 and 3)

4.5.1. General procedure

To a solution of alkene (2 equiv) in dry CH_2Cl_2 ($\sim$0.05 M) was added Grubbs' second generation catalyst **31** (0.2 equiv). The solution was refluxed under a flow of inert gas for 6 h, and DMSO added (50 equiv relative to catalyst **31**) and stirred at rt overnight. The solvent was removed by evaporation under reduced pressure to give the crude product.

4.5.2. Synthesis of (2R,2R)-2-tert-butoxycarbonylamino-3-[7-(2-tert-butoxycarbonylamino-2-methoxycarbonyl-ethylsulfanylcarbonyl)-hept-4-enoylsulfanyl]-propionic acid methyl ester **33**

Alkene **8** (30 mg, 0.09 mmol, 2 equiv) in dry CH_2Cl_2 (2 mL) was treated with Grubbs' second generation catalyst

31 (8 mg, 0.009 mmol, 0.2 equiv) according to the general procedure. The crude product was purified by flash chromatography on silica (petroleum ether/ethyl acetate, 7:3) to give **33** (13 mg, 45%) as a brown oil.

HRMS (M+H) found 607.2354 (calcd for $C_{26}H_{43}N_2O_{10}S_2$ 607.2359); R_f (20% ethyl acetate/petroleum ether) 0.48; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.42 (s, 18H, C(*Me*)$_3$), 2.31 (m, 4H, =CHC*H*$_2$), 2.59 (t, 4H, ^3J 7.4 Hz, COC*H*$_2$), 3.32 (m, 4H, SC*H*$_2$), 3.72 (s, 6H, O*Me*), 4.50 (m, 2H, NHC*H*(α)), 5.23 (br d, 2H, ^3J 7.4 Hz, N*H*(α)), 5.41 (m, 2H, C*H*=C*H*); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 24.6, 28.2, 31.0, 43.5, 53.0, 52.6, 80.1, 129.1, 155.0, 170.9, 197.6.

4.5.3. Synthesis of (2R,2R)-2-tert-butoxycarbonylamino-3-[19-(2-tert-butoxycarbonylamino-2-methoxycarbonyl-ethylsulfanylcarbonyl)-nonadec-10-enoylsulfanyl]-propionic acid methyl ester ***34***

Alkene **9** (50 mg, 0.12 mmol, 2 equiv) in dry CH_2Cl_2 (5 mL) was treated with Grubbs' second generation catalyst **31** (10 mg, 0.012 mmol, 0.2 equiv) according to the general procedure. The crude product was purified by flash chromatography on silica (petroleum ether/ethyl acetate, 7:3) to give **34** (35 mg, 73%) as a brown oil.

HRMS (M+H) found 775.4224 (calcd for $C_{38}H_{67}N_2O_{10}S_2$ 775.4237); R_f (30% ethyl acetate/petroleum ether) 0.56; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.24 (m, 20H, C*H*$_2$), 1.41 (s, 18H, C(*Me*)$_3$), 1.60 (m, 4H, COCH$_2$C*H*$_2$), 1.92 (m, 4H, =CHC*H*$_2$), 2.52 (m, 4H, COC*H*$_2$), 3.30 (br m, 4H, SC*H*$_2$), 3.71 (s, 6H, O*Me*), 4.49 (br m, 2H, NHC*H*(α)), 5.22 (br d, 2H, ^3J 7.9 Hz, N*H*(α)), 5.34 (m, 2H, C*H*=C*H*); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 25.5, 28.2, 28.7, 28.9, 29.1, 29.1, 29.5, 30.8, 32.4, 43.9, 52.5, 53.0, 80.0, 130.2, 154.9, 170.9, 198.4.

4.5.4. Synthesis of (2S)-oct-4-enedioic acid bis-(2-tert-butoxycarbonylamino-2-methoxycarbonyl-ethyl) ester ***35***

Alkene **11** (50 mg, 0.17 mmol, 2 equiv) in dry CH_2Cl_2 (5 mL) was treated with Grubbs' second generation catalyst **31** (14 mg, 0.017 mmol, 0.2 equiv) according to the general procedure. The crude product was purified by flash chromatography on silica (CH_2Cl_2/ethyl acetate, 9:1) to give **35** (42 mg, 86%) as a brown oil.

HRMS (M+H) found 575.2818 (calcd for $C_{26}H_{43}N_2O_{12}$ 575.2816); R_f (10% ethyl acetate/CH_2Cl_2) 0.44; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.39 (s, 18H, C(*Me*)$_3$), 2.23 (m, 4H, =CHC*H*$_2$), 2.31 (m, 4H, COC*H*$_2$), 3.70 (s, 6H, O*Me*), 4.27 (m, 2H, OC*H*$_2$), 4.38 (m, 2H, OC*H*$_2$), 4.51 (m, 2H, NHC*H*(α)), 5.32 (br m, 2H, N*H*(α)), 5.38 (m, 2H, C*H*=C*H*); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 27.4, 28.1, 33.6, 52.6, 52.7, 64.0, 80.2, 129.2, 155.0, 170.2, 172.4.

4.5.5. Synthesis of (2S)-icos-10-enedioic acid bis-(2-tert-butoxycarbonylamino-2-methoxycarbonyl-ethyl) ester ***36***

Alkene **12** (60 mg, 0.13 mmol, 2 equiv) in dry CH_2Cl_2 (5 mL) was treated with Grubbs' second generation catalyst **31** (14 mg, 0.017 mmol, 0.2 equiv) according to the general procedure. The crude product was purified by flash chromatography on silica (CH_2Cl_2/ethyl acetate, 9:1) to give **36** (30 mg, 62%) as a brown oil.

HRMS (M+H) found 743.4714 (calcd for $C_{38}H_{67}N_2O_{12}$ 743.4694); R_f (5% ethyl acetate/CH_2Cl_2) 0.45; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.28 (m, 18H, C*H*$_2$), 1.44 (s, 18H, C(*Me*)$_3$), 1.58 (m, 6H, C*H*$_2$), 1.96 (m, 4H, =CHC*H*$_2$), 2.28 (t, 4H, ^3J 7.5 Hz, COC*H*$_2$), 3.75 (s, 6H, O*Me*), 4.30 (m, 2H, OC*H*$_2$), 4.44 (m, 2H, OC*H*$_2$), 4.55 (m, 2H, NHC*H*(α)), 5.27 (br d, 2H, ^3J 8.0 Hz, N*H*(α)), 5.36 (m, 2H, C*H*=CH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 24.7, 28.2, 28.9, 29.1, 29.2, 29.4, 29.5, 32.4, 33.8, 52.6, 52.8, 63.9, 80.1, 130.2, 155.0, 170.2, 173.2.

4.5.6. Synthesis of (2S,2S)-2-{7-[1-methoxycarbonyl-4-(N′-methyl-guanidino)-butylcarbamoyl]-hept-4-enoylamino}-5-(N′-2,2,4,6,7-pentamethyldihydro-benzofuran-5-sulfonyl-guanidino)-pentanoic acid methyl ester ***37***

Alkene **14** (100 mg, 0.19 mmol, 2 equiv) in dry CH_2Cl_2 (5 mL) was treated with Grubbs' second generation catalyst **31** (16 mg, 0.019 mmol, 0.2 equiv) according to the general procedure. The crude product was purified by flash chromatography on silica (CH_2Cl_2/methanol, 9:1) to give **37** (75 mg, 74%) as a light brown oil.

HRMS (M+H) found 1017.4802 (calcd for $C_{48}H_{73}N_8O_{12}S_2$ 1017.4711); R_f (10% MeOH/CH_2Cl_2) 0.25; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.46 (s, 12H, C(*Me*)$_2$ (Pbf)), 1.57 (m, 4H, C*H*$_2$(γ)), 1.80 (m, 4H, C*H*$_2$(β)), 2.09 (s, 6H, C$_7$*Me* (Pbf)), 2.32 (m, 8H, COC*H*$_2$C*H*$_2$), 2.50 (s, 6H, C$_4$*Me* (Pbf)), 2.56 (s, 6H, C$_6$*Me* (Pbf)), 2.96 (s, 4H, C*H*$_2$ (Pbf)), 3.19 (m, 4H, C*H*$_2$(δ)), 3.72 (s, 6H, COO*Me*), 4.53 (m, 2H, C*H*(α)), 5.46 (m, 2H, C*H*=C*H*), 6.34 (br s, 6H, C(δ)N*H*C(=N*H*)N*H*), 6.97 (br s, 2H, N*H*(α)); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 12.6, 18.1, 19.4, 25.9, 26.5, 27.1, 28.5, 28.8, 29.6, 36.0, 40.8, 43.4, 52.1, 52.6, 86.6, 117.7, 124.8, 129.7, 132.3, 133.1, 138.4, 156.6, 158.9, 172.8, 173.6.

4.5.7. Synthesis of (2S,2S)-2-{19-[1-methoxycarbonyl-4-(N′-methyl-guanidino)-butylcarbamoyl]-nonadec-10-enoylamino}-5-(N′-2,2,4,6,7-pentamethyldihydro-benzofuran-5-sulfonyl-guanidino)-pentanoic acid methyl ester ***38***

Alkene **15** (100 mg, 0.17 mmol, 2 equiv) in dry CH_2Cl_2 (5 mL) was treated with Grubbs' second generation catalyst **31** (14 mg, 0.017 mmol, 0.2 equiv) according to the general procedure. The crude product was purified by flash chromatography on silica (CH_2Cl_2/methanol, 95:5) to give **38** (64 mg, 66%) as a light brown foam.

HRMS (M+H) found 1185.6671 (calcd for $C_{60}H_{97}N_8O_{12}S_2$ 1185.6589); R_f (5% MeOH/CH_2Cl_2) 0.20; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.25 (m, 20H, C*H*$_2$ C$_3$–C$_7$), 1.46 (s, 12H, C(*Me*)$_2$ (Pbf)), 1.57 (m, 8H, C*H*$_2$(γ) and C*H*$_2$ C$_2$), 1.71 and 1.83 (2m, 4H, C*H*$_2$(β)), 1.97 (m, 4H, C*H*$_2$ C$_8$), 2.09 (s, 6H, C$_7$Me (Pbf)), 2.22 (t, 4H, ^3J 7.3 Hz, C*H*$_2$ C$_1$), 2.50 (s, 6H, C$_4$*Me* (Pbf)), 2.56 (s, 6H, C$_6$*Me* (Pbf)), 2.96 (s, 4H, C*H*$_2$ (Pbf)), 3.22 (m, 4H, C*H*$_2$(δ)), 3.71 (s, 6H, COO*Me*), 4.51 (m, 2H, C*H*(α)), 5.35 (m, 2H, C*H*=C*H*), 6.30 (br s, 6H, C(δ)N*H*C(=N*H*)N*H*), 6.68 (br s, 2H, N*H*(α)); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 12.1, 17.6, 18.9, 25.1, 25.3, 28.3, 28.5, 28.9, 29.0, 29.1, 29.4, 32.1, 35.6, 40.3, 43.0, 51.5, 52.1, 86.1, 117.2, 124.3, 130.1, 131.9, 132.7, 138.0, 156.1, 158.5, 172.4, 173.7.

*4.5.8. Synthesis of (2S,2S)-2-tert-butoxycarbonylamino-3-phenyl-propionic acid 6-(2-tert-butoxycarbonylamino-3-phenyl-propionyloxy)-hex-3-enyl ester **41***

Alkene **17** (110 mg, 0.34 mmol, 2 equiv) in dry CH_2Cl_2 (7 mL) was treated with Grubbs' second generation catalyst **31** (28 mg, 0.034 mmol, 0.2 equiv) according to the general procedure. The crude product was purified by flash chromatography on silica (CH_2Cl_2/methanol, 99:1) to give **41** (70 mg, 68%) as a white solid.

HRMS (M+H) found 611.3331 (calcd for $C_{34}H_{47}N_2O_8$ 611.3254); R_f (1% MeOH/CH_2Cl_2) 0.27; mp: 50–52 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.41 (s, 18H, C(*Me*)$_3$), 2.32 (m, 4H, OCH$_2$C*H*$_2$), 3.12 (m, 2H, C*H*$_2$(β)), 4.14 (t, 4H, ^3J 6.8 Hz, OC*H*$_2$), 4.60 (dd, 2H, ^3J 15.0, 5.2 Hz, C*H*(α)), 5.04 (br s, 2H, N*H*(α)), 5.45 (m, 2H, C*H*=C*H*), 7.18 (apparent d, 4H, *J* 7.3 Hz, C*H* arom), 7.30 (m, 6H, C*H* arom); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 28.5, 32.0, 38.6, 54.5, 64.8, 80.1, 127.2, 128.4, 128.7, 129.5, 136.3, 155.3, 172.1.

*4.5.9. Synthesis of (2S,2S)-2-tert-butoxycarbonylamino-3-phenyl-propionic acid 18-(2-tert-butoxycarbonylamino-3-phenyl-propionyloxy)-octadec-9-enyl ester **42***

Alkene **18** (210 mg, 0.52 mmol, 2 equiv) in dry CH_2Cl_2 (11 mL) was treated with Grubbs' second generation catalyst **31** (42 mg, 0.052 mmol, 0.2 equiv) according to the general procedure. The crude product was purified by flash chromatography on silica (CH_2Cl_2/methanol, 99:1) to give **42** (119 mg, 76%) as a brown oil.

HRMS (M+H) found 779.5411 (calcd for $C_{46}H_{71}N_2O_8$ 779.5132); R_f (1% MeOH/CH_2Cl_2) 0.24; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.28 (m, 20H, C*H*$_2$), 1.42 (s, 18H, C(*Me*)$_3$), 1.57 (m, 4H, C*H*$_2$), 1.97 (m, 4H, =CHC*H*$_2$), 3.07 (m, 4H, C*H*$_2$(β)), 4.07 (t, 4H, ^3J 6.7 Hz, OC*H*$_2$), 4.56 (dd, 2H, ^3J 16.2, 6.7 Hz, C*H*(α)), 4.99 (br d, 2H, ^3J 8.1 Hz, N*H*(α)), 5.39 (m, 2H, C*H*=C*H*), 7.13 (apparent d, 4H, *J* 7.6 Hz, C*H* arom), 7.26 (m, 6H, C*H* arom); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 26.1, 28.6, 28.7, 29.4, 29.5, 29.6, 29.9, 32.9, 38.8, 54.7, 65.8, 80.1, 127.2, 128.8, 129.6, 130.6, 136.4, 155.3, 172.2.

*4.5.10. Synthesis of (2S,2S)-2-tert-butoxycarbonyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid 6-(2-tert-butoxycarbonyl-1,2,3,4-tetrahydroisoquinolyloxy)-hex-3-enyl ester **43***

Alkene **20** (60 mg, 0.18 mmol, 2 equiv) in dry CH_2Cl_2 (5 mL) was treated with Grubbs' second generation catalyst **31** (15 mg, 0.018 mmol, 0.2 equiv) according to the general procedure. The crude product was purified by flash chromatography on silica (CH_2Cl_2/MeOH, 99:1) to give **43** (51 mg, 89%) as a brown oil.

HRMS (M+H) found 632.3176 (calcd for $C_{36}H_{47}N_2O_8$ 632.3254); R_f (1% MeOH/CH_2Cl_2) 0.25; ^{1}H NMR ($CDCl_3$, 500 MHz) δ (cis/trans mixture) 1.46 and 1.52 (2s, 18H, C(*Me*)$_3$), 2.13 (m, 4H, OCH$_2$C*H*$_2$), 3.15 (m, 4H, C*H*$_2$(β)), 3.97 (t, 4H, ^3J 6.0 Hz, OC*H*$_2$), 4.47 and 4.53 (2d, 2H, ^2J 7.2 Hz, NC*H*$_2$Ar cis or trans), 4.67 and 4.72 (2d, 2H, ^2J 3.3 Hz, NC*H*$_2$Ar cis or trans), 4.79 (apparent t, 1H, *J* 5.7 Hz, C*H*(α) cis or trans), 4.79 (dd, 1H, ^3J 6.0, 3.3 Hz, C*H*(α) cis or trans), 5.22 (m, 2H, C*H*=C*H*), 7.13 (m, 8H, C*H* arom); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 28.6 and 28.7, 31.5 and 32.0, 44.3 and 44.9, 52.8 and 54.6, 64.7, 80.8, 126.5 and 126.6, 126.9 and 127.0, 127.1 and 127.4, 128.1, 128.3, 128.8, 132.1, 133.6, 133.8, 133.9, 134.7, 155.5 and 156.1, 171.4 and 172.8.

*4.5.11. Synthesis of (2S,2S)-2-tert-butoxycarbonyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid 18-(2-tert-butoxycarbonyl-1,2,3,4-tetrahydroisoquinolyloxy)-octadec-9-enyl ester **44***

Alkene **21** (80 mg, 0.2 mmol, 2 equiv) in dry CH_2Cl_2 (5 mL) was treated with Grubbs' second generation catalyst **31** (16 mg, 0.02 mmol, 0.2 equiv) according to the general procedure. The crude product was purified by flash chromatography on silica (CH_2Cl_2/methanol, 99:1) to give **44** (45 mg, 56%) as a brown oil.

HRMS [M(−Boc)+H] found 703.4692 (calcd for $C_{43}H_{63}N_2O_6$ 703.4608); R_f (1% MeOH/CH_2Cl_2) 0.29; ^{1}H NMR ($CDCl_3$, 500 MHz) δ (cis/trans mixture) 1.21 (m, 20H, CH_2), 1.46 and 1.52 (2s, 22H, C(*Me*)$_3$ and CH_2 C$_2$), 1.97 (m, 4H, CH_2 C$_8$), 3.20 and 3.30 (m, 4H, CH_2(β)), 4.04 (t, 4H, ^3J 7.1 Hz, OCH_2 C$_1$), 4.52 and 4.58 (2d, 2H, ^2J 3.6 Hz, NCH_2Ar cis or trans), 4.70 and 4.76 (2br s, 2H, NCH_2Ar cis or trans), 4.83 (apparent t, 1H, *J* 5.4 Hz, C*H*(α) cis or trans), 4.79 (dd, 1H, ^3J 6.8, 3.0 Hz, C*H*(α) cis or trans), 5.43 (m, 2H, C*H*=C*H*), 7.16 (m, 8H, C*H* arom); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 25.9, 28.5 and 28.6, 28.7 and 28.8, 29.4, 29.5, 29.7, 31.5 and 32.0, 32.8, 44.3 and 44.8, 52.8 and 54.5, 65.4, 80.7, 126.4 and 126.5, 126.8 and 126.9, 127.0 and 127.1, 128.0 and 128.7, 130.5, 132.1 and 132.4, 133.1 and 134.1, 155.0 and 155.7, 171.7 and 172.2.

*4.5.12. Synthesis of (2S,2S)-2-tert-butoxycarbonylamino-3-(4-{4-[4-(2-tert-butoxy-carbonyl amino-2-methoxycarbonyl-ethyl)-phenoxy]-but-2-enyloxy}-phenyl)-propionic acid methyl ester **46***

Alkene **45** (100 mg, 0.3 mmol, 2 equiv) in dry CH_2Cl_2 (5 mL) was treated with Grubbs' second generation catalyst **31** (24 mg, 0.03 mmol, 0.2 equiv) according to the general procedure. The crude product was purified by flash chromatography on silica (CH_2Cl_2/MeOH, 99:1) to give **46** (66 mg, 67%) as a brown oil.

HRMS (M+H) found 643.3242 (calcd for $C_{34}H_{47}N_2O_{10}$ 643.3152); R_f (1% MeOH/CH_2Cl_2) 0.31; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.44 (s, 18H, C(*Me*)$_3$), 3.02 (m, 4H, ArCH_2), 3.70 (s, 6H, O*Me*), 4.55 (m, 6H, ArOCH_2 and C*H*(α)), 4.98 (d, 2H, ^3J 8.0 Hz, N*H*(α)), 6.07 (m, 2H, C*H*=C*H*), 6.83 (d, 4H, ^3J 8.0 Hz, C*H* arom), 7.03 (d, 4H, ^3J 8.0 Hz, C*H* arom); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 28.5, 37.8, 52.3, 54.8, 67.9, 80.1, 115.0, 128.5, 128.6, 130.5, 152.5, 157.8, 172.6.

*4.5.13. Synthesis of (2R,2S)-13-(2-tert-butoxy-carbonylamino-2-methoxycarbonyl-ethylsulfanylcarbonyl)-tridec-10-enoic acid 2-tert-butoxycarbonylamino-2-methoxycarbonyl-ethyl ester **47***

To a solution of *O*-decenoyl serine **12** (20 mg, 0.05 mmol, 1 equiv) and *S*-pentenoyl cysteine **8** (33 mg, 0.1 mmol, 2 equiv) in dry CH_2Cl_2 (2 mL) was added Grubbs' second generation catalyst **31** (9 mg, 0.01 mmol, 0.2 equiv). The solution

was stirred at reflux under a flow of inert gas for 6 h, and DMSO added (50 equiv relative to **31**) and stirred at rt overnight. The solvent was removed by evaporation under reduced pressure to give the crude product, which was purified by flash chromatography on silica (petroleum ether/ethyl acetate, 3:2) to give **47** (18 mg, 51%) as a brown oil.

HRMS (M+H) found 675.3527 (calcd for $C_{32}H_{55}N_2O_{11}S$ 675.3527); R_f (40% ethyl acetate/petroleum ether) 0.76; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.24 (m, 10H, CH_2), 1.42 (s, 9H, C(Me)$_3$), 1.43 (s, 9H, C(Me)$_3$), 1.56 (m, 2H, CH_2), 1.94 (m, 2H, =CHCH_2(CH$_2$)$_7$CO), 2.29 (m, 4H, OCOCH_2, =CHCH_2CH$_2$CO), 2.59 (t, 2H, ^3J 7.5 Hz, SCOCH_2), 3.32 (br m, 2H, CH_2S), 3.72 (s, 3H, OMe), 3.74 (s, 3H, OMe), 4.29 (m, 1H, OCHH), 4.43 (m, 1H, OCHH), 4.50 (m, 1H, NHCH(α)), 4.54 (m, 1H, NHCH(α)), 5.22 (br d, 1H, ^3J 7.4 Hz, NH(α)), 5.27 (br d, 1H, ^3J 8.5 Hz, NH(α)), 5.33 (dt, 1H, J 6.7, 15.2 Hz, CH=CH), 5.42 (dt, 1H, J 6.6, 15.2 Hz, CH=CH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 24.7, 27.1, 28.2, 28.4, 28.9, 29.1, 29.2, 29.3, 30.9, 32.4, 33.9, 43.8, 52.6, 52.6, 52.9, 53.0, 63.9, 80.1, 80.2, 127.1, 132.3, 154.9, 155.1, 170.3, 170.9, 173.2, 197.8.

*4.5.14. Synthesis of (2R,2S)-19-(2-tert-butoxy-carbonylamino-2-methoxycarbonyl-ethylsulfanylcarbonyl)-nonadec-10-enoic acid 2-tert-butoxycarbonylamino-2-methoxycarbonyl-ethyl ester **48***

To a solution of *O*-decenoyl serine **12** (20 mg, 0.05 mmol, 1 equiv) and *S*-decenoyl cysteine **9** (42 mg, 0.10 mmol, 2 equiv) in dry CH_2Cl_2 (2 mL) was added Grubbs' second generation catalyst **31** (9 mg, 0.01 mmol, 0.2 equiv). The solution was stirred at reflux under a flow of inert gas for 6 h, and DMSO added (50 equiv relative to **31**) and stirred overnight at rt. The solvent was removed by evaporation under reduced pressure to give the crude product, which was purified by flash chromatography on silica (petroleum ether/ethyl acetate, 7:3) to give **48** (26 mg, 66%) as a brown oil.

HRMS (M+H) found 759.4453 (calcd for $C_{38}H_{67}N_2O_{11}S$ 759.4466); R_f (30% ethyl acetate/petroleum ether) 0.74; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.26 (m, 20H, CH_2), 1.41 (s, 9H, C(Me)$_3$), 1.43 (s, 9H, C(Me)$_3$), 1.59 (m, 4H, COCH$_2$CH_2), 1.93 (m, 4H, =CHCH_2), 2.27 (t, 2H, ^3J 7.6 Hz, OCOCH_2), 2.53 (t, 2H, ^3J 7.5 Hz, SCOCH_2), 3.31 (br m, 2H, SCH_2), 3.72 (s, 3H, OMe), 3.74 (s, 3H, OMe), 4.29 (m, 1H, OCHH), 4.42 (m, 1H, OCHH), 4.50 (m, 1H, NHCH(α)), 4.54 (m, 1H, NHCH(α)), 5.23 (br d, 1H, ^3J 7.7 Hz, NH(α)), 5.27 (br d, 1H, ^3J 8.3 Hz, NH(α)), 5.35 (m, 2H, CH=CH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 24.7, 25.5, 28.21, 28.8, 28.8, 28.9, 29.0, 29.0, 29.1, 29.1, 29.2, 29.2, 29.5, 30.9, 32.5, 33.9, 40.3, 43.9, 52.6, 52.6, 52.9, 53.0, 64.0, 80.1, 80.3, 130.2, 130.3, 155.0, 155.1, 170.3, 170.9, 173.3, 198.5.

4.6. Ring-closing metathesis experiments (Table 4)

*4.6.1. Synthesis of (3R)-5,12-dioxo-1-thia-4-aza-cyclododec-8-ene-3-carboxylic acid methyl ester **49***

To a solution of alkene **23** (34 mg, 0.11 mmol, 1 equiv) in dry CH_2Cl_2 (2 mL) was added Grubbs' second generation catalyst **31** (19 mg, 0.02 mmol, 0.2 equiv). The solution was stirred at reflux under a flow of inert gas for 6 h, and DMSO added (50 equiv relative to **31**) and stirred overnight at rt. The solvent was removed by evaporation under reduced pressure to give the crude product, which was purified by flash chromatography on silica (petroleum ether/ethyl acetate, 1:4) to give **49** (23 mg, 74%) as a white solid. Recrystallization by slow evaporation from CH_2Cl_2/ethyl acetate gave crystals suitable for X-ray crystallography.

HRMS (M+H) found 272.0960 (calcd for $C_{12}H_{18}NO_4S$ 272.0957); R_f (20% ethyl acetate/petroleum ether) 0.48; mp: 146–147 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.02 (m, 1H, COCHHCH$_2$), 2.26–2.43 (m, 5H, =CHCH_2, COCHHCH$_2$), 2.62 (m, 1H, COCHHCH$_2$), 2.69 (m, 1H, COCHHCH$_2$), 3.45 (m, 2H, CH_2S), 3.74 (s, 3H, OMe), 4.80 (m, 1H, NHCH(α)), 5.33 (m, 1H, CH=CH), 5.41 (m, 1H, CH=CH), 5.85 (br s, 1H, NH(α)); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 29.0, 29.4, 29.5, 37.5, 42.7, 51.9, 52.6, 128.2, 131.8, 170.8, 172.4, 199.1.

*4.6.2. Synthesis of (3R)-5,24-dioxo-1-thia-4-aza-cyclotetracos-14-ene-3-carboxylic acid methyl ester **50***

To a solution of alkene **24** (50 mg, 0.11 mmol, 1 equiv) in dry CH_2Cl_2 (5 mL) was added Grubbs' second generation catalyst **31** (18 mg, 0.02 mmol, 0.2 equiv). The solution was stirred at reflux under a flow of inert gas for 6 h, and DMSO added (50 equiv relative to **31**) and stirred overnight at rt. The solvent was removed by evaporation under reduced pressure to give the crude product, which was purified by flash chromatography on silica (ethyl acetate/petroleum ether, 3:7) to give **50** (20 mg, 43%) as a white solid.

HRMS (M+H) found 440.2843 (calcd for $C_{24}H_{42}NO_4S$ 440.2835); R_f (30% ethyl acetate/petroleum ether) 0.58; mp: 94–96 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.30 (m, 16H, CH_2), 1.60 (m, 8H, CH_2), 2.00 (m, 4H, CH_2CH=CH$_2$), 2.16 (m, 2H, NHCOCH_2), 2.55 (m, 2H, SCOCH_2), 3.33 (m, 2H, SCH_2), 3.75 (s, 3H, OCH_3), 4.77 (m, 1H, NHCH(α)), 5.31 (m, 2H, CH=CH), 6.16 (d, 1H, ^3J 8.0 Hz, NH(α)); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 25.9, 25.2, 27.9, 28.2, 28.7, 28.7, 28.8, 28.9, 29.2, 29.4, 29.4, 29.6, 30.0, 32.0, 32.1, 36.8, 43.9, 52.3, 52.7, 130.8, 130.9, 170.8, 173.1, 199.9.

*4.6.3. Synthesis of (3S)-5,12-dioxo-1-oxa-4-aza-cyclododec-8-ene-3-carboxylic acid methyl ester **51***

To a solution of alkene **26** (110 mg, 0.39 mmol, 1 equiv) in dry CH_2Cl_2 (10 mL) was added Grubbs' second generation catalyst **31** (66 mg, 0.08 mmol, 0.2 equiv). The solution was stirred at reflux under a flow of inert gas for 6 h, and DMSO added (50 equiv relative to **31**) and stirred overnight at rt. The solvent was removed by evaporation under reduced pressure to give the crude product, which was purified by flash chromatography on silica (CH_2Cl_2/ethyl acetate, 1:1) to give **51** (45 mg, 45%) as brown crystals. Recrystallization by slow evaporation from CH_2Cl_2/ethyl acetate gave colourless crystals suitable for X-ray crystallography.

HRMS (M+H) found 256.1183 (calcd for $C_{12}H_{18}NO_5$ 256.1185); R_f (30% ethyl acetate/petroleum ether) 0.47; mp: 151–153 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.09–2.44 (m, 8H, CH$_2$CH_2), 3.76 (s, 3H, OMe), 4.24 (dd, 1H, ^3J 3.8 Hz, ^2J

11.5 Hz, OCH*H*), 4.63 (m, 1H, OC*H*H), 4.98 (m, 1H, NHC*H*(α)), 5.39 (ddt, 2H, ^3J 6.3, 11.5, 15.1 Hz, C*H*=C*H*), 5.92 (d, 1H, ^3J 8.6 Hz, N*H*(α)); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 28.8, 28.9, 34.3, 36.4, 51.0, 52.5, 61.1, 130.0, 130.3, 169.6, 171.9, 172.9.

4.6.4. Synthesis of (2S)-5,24-dioxo-1-oxa-4-aza-cyclotetracos-14-ene-3-carboxylic acid methyl ester ***52***

To a solution of alkene **27** (119 mg, 0.26 mmol, 1 equiv) in dry CH_2Cl_2 (10 mL) was added Grubbs' second generation catalyst **31** (45 mg, 0.05 mmol, 0.2 equiv). The solution was stirred at reflux under a flow of inert gas for 6 h, and DMSO added (50 equiv relative to **31**) and stirred overnight at rt. The solvent was removed by evaporation under reduced pressure to give the crude product, which was purified by flash chromatography on silica (ethyl acetate/petroleum ether, 3:7) to give **52** (17 mg, 15%) as a brown solid.

HRMS (M+H) found 424.3068 (calcd for $C_{24}H_{42}NO_5$ 424.3063); R_f (30% ethyl acetate/petroleum ether) 0.30; mp: 88–90 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.28 (m, 20H, CH_2), 1.59 (m, 4H, CH_2), 1.99 (m, 4H, CH_2CH=CH_2), 2.24 (m, 4H, COCH_2), 3.76 (s, 3H, O*Me*), 4.41 (m, 2H, OCH_2), 4.83 (m, 1H, NHC*H*(α)), 5.30 (m, 2H, C*H*=C*H*), 6.17 (d, 1H, ^3J 7.7 Hz, N*H*(α)); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 25.2, 25.9, 28.1, 28.8, 28.8, 28.9, 29.0, 29.1, 29.1, 29.3, 32.1, 33.9, 34.2, 36.6, 51.6, 52.8, 63.1, 130.8, 130.8, 170.0, 172.9, 173.3.

4.6.5. (15-Oxo-2,14-dioxa-bicyclo[16.2.2]docosa-1(21),4,18(22),19-tetraen-16-yl)-carbamic acid tert-butyl ester ***54***

To a solution of alkene **30** (100 mg, 0.22 mmol, 1 equiv) in dry CH_2Cl_2 (100 mL) was added Grubbs' second generation catalyst **31** (30 mg, 0.033 mmol, 0.15 equiv). The solution was stirred at reflux under a flow of inert gas for 6 h, and DMSO added (50 equiv relative to **31**) and stirred overnight at rt. The solvent was removed by evaporation under reduced pressure to give the crude product, which was purified by flash chromatography on silica (CH_2Cl_2/methanol, 99:1) to give **54** (59 mg, 62%) as a light brown foam.

HRMS (M+H) found 432.2748 (calcd for $C_{25}H_{38}NO_5$ 432.2672); R_f (1% MeOH/CH_2Cl_2) 0.24; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.80–1.40 (m, 10H, CH_2), 1.47 (s, 9H, C(*Me*)$_3$), 1.82–2.16 (m, 2H, CH_2 C_2), 2.24 and 2.40 (2m, 2H, CH_2CH=CH_2), 2.76 and 3.15 (2m, 2H, ArCH_2), 3.81–4.01 (m, 4H, ArOCH_2 and OCH_2 C_1), 4.49 (m, 1H, C*H*(α)), 5.13 (br s, 1H, N*H*(α)), 5.32 (m, 1H, OCH$_2$C*H*=CH), 5.47 (m, 1H, OCH$_2$CH=C*H*), 6.79 (m, 2H, C*H* arom), 7.04 (m, 2H, C*H* arom); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 26.0, 28.6, 27.8, 29.0, 29.8, 30.5, 32.1, 32.4, 39.1, 55.4, 65.2, 68.1, 77.5, 114.2, 114.6, 127.9, 129.3, 130.4, 132.5, 133.1, 156.2, 158.6, 172.4.

4.7. Application: preparation of PDF inhibitor analogue ***56*** *(Scheme 1)*

4.7.1. Synthesis of (2S)-2-tert-butoxycarbonylamino-3,3-dimethyl-butyric acid ***58***

Boc_2O (182 mg, 0.84 mmol, 1.1 equiv) was added to a solution of L-*tert*-leucine **57** (100 mg, 0.76 mmol, 1 equiv) and NaOH (34 mg, 0.84 mmol, 1.1 equiv) in water/*tert*-butyl alcohol (1 mL/1 mL). The reaction mixture was stirred overnight at rt and was extracted three times with ethyl acetate (5 mL). The combined organic layers were washed with saturated aqueous sodium bicarbonate solution (5 mL), and the combined water phases were acidified to pH 1.5–2.0. The water phase was extracted four times with ethyl acetate (5 mL) and the combined organic phases were washed with brine solution, dried ($MgSO_4$), and the solvent removed by evaporation under reduced pressure to give **58** (175 mg, 99%) as a colourless solid.

HRMS (M+H) found 232.1485 (calcd for $C_{11}H_{22}NO_4$ 232.1471); mp: 122–123 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.01 (s, 9H, C(*Me*)$_{3Leu}$), 1.43 (s, 9H, C(*Me*)$_{3Boc}$), 4.11 (d, 1H, ^3J 7.2 Hz, C*H*(α)), 5.08 (d, 1H, ^3J 8.3 Hz, N*H*); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 26.4, 28.2, 34.4, 61.6, 79.8, 155.6, 176.2.

4.7.2. Synthesis of (2S,2R)-2-(2-tert-butoxycarbonylamino-3,3-dimethyl-butyrylamino)-3-(pent-4-enoylsulfanyl)-propionic acid methyl ester ***60***

To N_ε-pentenoyl cysteine **8** (1 g, 3.15 mmol, 1 equiv) in CH_2Cl_2 (50 mL) was added TFA (9.4 mL, 40 equiv) and the solution was stirred overnight. Removal of the solvent by evaporation under reduced pressure yielded TFA salt **59** with a quantitative yield. Salt **59** (3.15 mmol) was added to a mixture of Boc–L-Tle–OH **58** (831 mg, 3.6 mmol, 1.14 equiv), DIPEA (0.6 mL, 1.1 equiv), EDCI (782 mg, 4.1 mmol, 1.3 equiv) and HOBt (713 mg, 4.73 mmol, 1.5 equiv) and stirred at rt overnight. The reaction mixture was then diluted with CH_2Cl_2 (50 mL) and extracted with 1 N aqueous HCl (50 mL), aqueous saturated sodium bicarbonate (50 mL) and brine (50 mL). The organic layer was dried (Na_2SO_4), filtered and the solvent removed by evaporation under reduced pressure. The crude product was purified by flash chromatography on silica (ethyl acetate/CH_2Cl_2, 1:4) to give **60** (620 mg, 46%) as a pale yellow oil.

HRMS (M+H) found 431.2214 (calcd for $C_{20}H_{35}N_2O_6S$ 431.2216); R_f (40% ethyl acetate/petroleum ether) 0.75; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.99 (s, 9H, C(*Me*)$_{3Leu}$), 1.43 (s, 9H, C(*Me*)$_{3Boc}$), 2.29 (m, 2H, COCH$_2$CH_2), 2.36 (t, 2H, ^3J 7.0 Hz, COCH_2CH$_2$), 3.30 (dd, 1H, ^3J 5.0 Hz, ^2J 14.2 Hz, SC*H*H), 3.46 (dd, 1H, ^3J 4.6 Hz, ^2J 14.2 Hz, SCH*H*), 3.70 (s, 3H, O*Me*), 4.02 (d, 1H, ^3J 8.3 Hz, C*H*(α)$_{Leu}$), 4.86 (m, 1H, C*H*(α)$_{Cys}$), 4.98 (d, 1H, ^3J 10.1 Hz, N*H*(α)$_{Leu}$), 5.04 (m, 2H, CH=CH_2), 5.81 (m, 1H, C*H*=CH$_2$), 6.38 (d, 1H, ^3J 7.7 Hz, N*H*(α)$_{Cys}$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 26.7, 28.2, 29.2, 30.1, 33.8, 35.2, 51.5, 52.5, 68.6, 80.4, 115.3, 136.8, 155.5, 170.6, 172.3, 199.5.

4.7.3. Synthesis of (2S,2R)-2-[3,3-dimethyl-2-(pent-4-enoylamino)-butyrylamino]-3-(pent-4-enoylsulfanyl)-propionic acid methyl ester ***62***

Dipeptide **60** (620 mg, 1.44 mmol, 1 equiv) was treated with TFA (analogous to Section 4.7.2). TFA salt **61** (1.44 mmol) and 4-pentenoic acid (164 mg, 1.6 mmol, 1.14 equiv) were treated with EDCI (358 mg, 1.87 mmol,

1.3 equiv) and HOBt (326 mg, 2.16 mmol, 1.5 equiv) analogous to procedure given in Section 4.7.2. The crude product was purified by flash chromatography on silica (ethyl acetate/CH_2Cl_2, 1:4) to give **62** (430 mg, 72%) as a colourless oil.

HRMS (M+H) found 413.2115 (calcd for $C_{20}H_{32}N_2O_5S$ 413.2110); R_f (20% ethyl acetate/CH_2Cl_2) 0.20; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.99 (s, 9H, C(*Me*)$_3$), 2.35 (m, 8H, COC*H*$_2$C*H*$_2$), 3.28 (dd, 1H, ^3J 5.4 Hz, ^2J 14.2 Hz, SC*H*H), 3.46 (dd, 1H, ^3J 4.6 Hz, ^2J 14.2 Hz, SCH*H*), 3.72 (s, 3H, O*Me*), 4.42 (d, 1H, ^3J 8.6 Hz, C*H*(α)$_{Leu}$), 4.83 (m, 1H, C*H*(α)$_{Cys}$), 4.98–5.11 (m, 4H, CH=C*H*$_2$), 5.82 (m, 2H, C*H*=CH$_2$), 6.05 (d, 1H, ^3J 8.6 Hz, N*H*(α)$_{Leu}$), 6.35 (d, 1H, ^3J 7.7 Hz, N*H*(α)$_{Cys}$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 26.4, 28.4, 28.9, 29.8, 32.9, 33.5, 34.7, 51.5, 52.2, 67.0, 115.0, 115.1, 136.4, 136.6, 170.2, 172.3, 173.1, 198.6.

*4.7.4. Synthesis of (3S,6R)-6-tert-butyl-5,8,15-trioxo-1-thia-4,7-diaza-cyclopentadec-11-ene-3-carboxylic acid methyl ester **56***

To a solution of diene **57** (230 mg, 0.60 mmol, 1 equiv) in CH_2Cl_2 (10 mL) was added Grubbs' second generation catalyst **31** (102 mg, 0.12 mmol, 0.2 equiv). The solution was stirred at reflux under a flow of inert gas for 6 h, and DMSO (50 equiv relative to catalyst **31**) added and stirred overnight at rt. The solvent was removed by evaporation under reduced pressure to give the crude product, which was purified by flash chromatography on silica (100% ethyl acetate) to give **56** (140 mg, 65%) as white solid.

HRMS (M+H) found 385.1796 (calcd for $C_{18}H_{29}N_2O_5S$ 385.1797); R_f (ethyl acetate) 0.51; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.99 (s, 9H, C(*Me*)$_3$), 2.20 (m, 4H, =CHC*H*$_2$ (3H), COC*H*$_2$ (1H)), 2.44 (m, 3H, =CHC*H*$_2$ (1H), COC*H*$_2$ (2H)), 2.57 (m, 1H, COC*H*$_2$), 3.11 (dd, 1H, ^3J 3.9 Hz, ^2J 14.5 Hz, SC*H*H), 3.46 (dd, 1H, ^3J 9.5 Hz, ^2J 14.5 Hz, SCH*H*), 3.73 (s, 3H, O*Me*), 4.59 (m, 2H, C*H*(α)), 5.52 (m, 2H, *H*C=C*H*), 5.86 (d, 1H, ^3J 9.7 Hz, N*H*), 6.41 (d, 1H, ^3J 7.4 Hz, N*H*); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 26.5, 27.2, 27.6, 29.6, 34.1, 35.4, 35.7, 52.7, 52.9, 65.5, 129.7, 130.2, 170.8, 172.3, 172.5, 200.2.

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