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Design, Synthesis, and Evaluation of a Novel Series of Macrocyclic Inhibitors of Norovirus 3CL Protease

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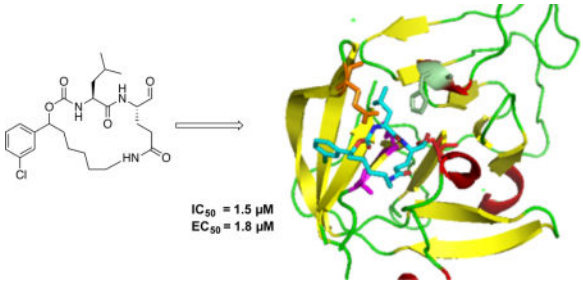
Abstract

Norovirus infections have a major impact on public health worldwide, yet there is a current dearth of norovirus-specific therapeutics and prophylactics. This report describes the discovery of a novel class of macrocyclic inhibitors of norovirus 3C-like protease, a cysteine protease that is essential for virus replication. SAR, structural, and biochemical studies were carried out to ascertain the effect of structure on pharmacological activity and permeability. Insights gained from these studies have laid a solid foundation for capitalizing on the therapeutic potential of the series of inhibitors described herein.

Graphical abstract



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1. Introduction

Noroviruses belong to the family *Caliciviridae* and are classified into at least six genogroups (GI-GVI) [1–3]. Human noroviruses are the primary cause of non-bacterial acute gastroenteritis worldwide [4–5], and are associated with high morbidity and a heavy economic burden [6–8]. In the U.S. alone noroviruses account for >20 million cases annually and impact most severely the young and elderly, as well as immunocompromised individuals [9–12]. Combating norovirus infections presents a formidable challenge because of their robustness, high infectivity, ease of transmission via multiple routes [13–14], the current dearth of norovirus-specific therapeutics/prophylactics and vaccines [15–21], as well as a sub-optimal understanding of norovirus biology and pathophysiology [22–26].

Noroviruses are small, single-stranded, positive sense RNA viruses whose genome (7–8 kb) is covalently linked to a viral protein (VPg, virion protein, genome-linked) at the 5′ end and polyadenylated at the 3′ end [1,24]. The genome consists of three open reading frames (ORFs) that encode a 200 kDa polyprotein (ORF1), a major capsid protein VP1 (ORF2) and a small basic protein VP2 (ORF3) [1,24]. The polyprotein is processed by a virus-encoded protease to generate six mature non-structural proteins, including the viral protease (3C-like protease, 3CLpro or $NS6^{pro}$) and the RNA dependent RNA polymerase ($NS7^{pol}$). Co- and post-translational processing of the polyprotein by 3CLpro is essential for virus replication, consequently, 3CLpro is an attractive target for the discovery of anti-norovirus small molecule therapeutics and prophylactics [15–20].

Norovirus 3CLpro, including Norwalk virus (NV) 3CLpro, is a cysteine protease with a Cys-His-Glu catalytic triad, an extended binding site, and a chymotrypsin-like fold [27–31]. The protease displays a primary substrate specificity requirement for a P_1 glutamine or glutamic acid residue, or equivalent [32]. We have recently described an array of transition state inhibitors and transition state mimics of NV 3CLpro [15]. In continuing our studies in this area, we describe herein the structure-guided design of a novel series of macrocyclic inhibitors of the protease, as well as pertinent structural, biochemical, and cell-based studies.

2. Results and Discussion

2.1. Inhibitor design rationale

Cocrystal structures of NV 3CLpro with peptidyl inhibitors reveal a network of backbone hydrogen bonds involving Ala158, Ala160, Gln110 and His30, as well as two critical hydrogen bonds between His157 and Thr134 and the carbonyl oxygen of the P_1 Gln side chain [27,29,33]. The backbone hydrogen bonds mimic an antiparallel β-sheet and serve to correctly orient and position the inhibitor to the active site [34–35]. It was envisaged that the construction of a macrocyclic structure (Figure 1, structure (I)) capable of (a) maintaining the aforementioned favorable binding interactions and, (b) having a flexible diversity site that is well-suited to exploiting H-bonding and hydrophobic interactions with the S_3-S_4 subsites and modulating physicochemical properties, could potentially result in the identification of a molecule that displays high potency and drug-like characteristics [36–37]. Additional advantages frequently accrued by macrocyclization include higher pharmacological activity and selectivity, enhanced permeability, and improved stability [38–

40]. The interaction of inhibitor (I) with the protease was further probed using different warheads, ring sizes, and P_2 residues, a P_1 alkoxyamide side chain replacement for the P_1 Gln side chain that could potentially engage in intramolecular H-bonding, thereby attenuating cellular permeability [41–46] and, finally, structural variants focused on R_1 which projects toward the S_3-S_4 subsites and affords opportunities for favorable binding interactions.

2.2. Chemistry

The synthesis of inhibitors ***11–21*** by coupling compounds ***3a–k*** (Scheme 1) with compound ***6a*** (Scheme 2B) to yield acyclic compounds ***7a–k*** (Scheme 3). The reaction sequence outlined in Scheme 1 is flexible and permits ready manipulation of the ring size using appropriate alkenyl Grignard reagents. Furthermore, the nature of the P_2 residue (R_2) could be readily manipulated by reacting intermediates ***1a–f*** (Scheme 1) with an appropriate amino acid ester isocyanate ***4a–d*** (Scheme 2A). Ring closure using a metathesis reaction furnished compounds ***8a–k*** which, upon sequential catalytic hydrogenation, reduction with lithium borohydride, and Dess-Martin periodinane oxidation yielded aldehydes ***11–21.*** With the exception of compounds ***14*** and ***36***, these were obtained as diastereomeric mixtures. Aldehydes ***23–24*** having an unsaturated linker were synthesized in an analogous manner (Scheme 4). Aldehyde bisulfite adducts ***25–29*** and α-ketoamide ***31*** were synthesized as shown in Scheme 5. Finally, coupling of intermediate ***3c*** with ***6b*** (Scheme 2C) followed by further elaboration of the product yielded aldehyde ***36*** (Scheme 6)**.**

2.3. Biochemical Studies

The inhibitory activity of the synthesized compounds against NV 3CLpro and their anti-norovirus activity in a cell-based replicon system, were evaluated as described in the experimental section [33,47]. The determined IC_{50} in enzyme assay, EC_{50} against NV in the replicon harboring cells (HG23 cells) or murine norovirus (MNV) in RAW264.7 cells, and CC_{50} values in HG23 cells are listed in Tables 1 and they are the average of at least two determinations.

The low cellular permeability and susceptibility to proteolytic degradation of peptide-based inhibitors provided the impetus behind the design of macrocyclic inhibitor (I). It is evident from the results shown in Table 1 that, with the exception of compounds ***18, 26, 19***, and ***27*** (Table 1) that were inactive, the rest of the compounds inhibited NV 3CLpro and displayed antiviral activity in cell based assays in the replicon harboring cells (NV) as well as RAW264.7 cells (MNV) with IC_{50} and EC_{50} values in the low micromolar range. The antiviral activities against NV and MNV in cell based assays demonstrate that the compounds have good permeability in different cell types (hepatoma cells [HG23] and macrophage-like cells [RAW264.7]). The aldehyde and aldehyde bisulfite adducts had comparable potency, however, replacement of the warhead with an α-ketoamide (Table 1, compounds ***13*** and ***25*** versus α-ketoamide ***31***) diminished activity. These observations are congruent with the results of previous studies with peptidyl and macrocyclic inhibitors of NV 3CLpro [48–49]. Replacement of the P_2 Leu (R_2) residue with cyclohexylalanine (Cha) had a minor effect on potency. This is contrast to the significant boost in potency observed in the dipeptidyl series of acyclic inhibitors having a P_2 Cha [33]. As expected, a P_2 Ala (R_2)

residue resulted in greatly diminished potency, a reflection of the strong preference of NV 3CLpro for a Cha or Leu residue at the P_2 position [32]. Surprisingly, substitution of a spirocyclohexylglycine (1-aminocyclohexaneglycine) at P_2 (R_2) resulted in a dramatic loss of activity (Table 1, compounds ***18, 26, 19***, and ***27***). In those instances, where the diastereomers were separable, these displayed comparable potencies (Table 1, compounds ***14A–B*** and ***36A–B***).

Delineation of the structural determinants impacting pharmacological activity, as well as demonstration of the mechanism of action, was made possible by determining a high resolution cocrystal structure of inhibitor ***13*** with NV 3CLpro. Examination of the active site revealed the presence of prominent difference electron density with inhibitor ***13*** covalently bound to Cys 139 and the entire inhibitor could be traced (Figure 2). The interactions between NV 3CLpro and ***13*** are shown in Figure 3 and electrostatic surface representations of the NV 3CLpro with the inhibitor nestled in the active site are shown in Figure 4. A network of H-bonds involving the backbone of inhibitor ***13*** and residues Gln110, Ala158, and Ala160 that serve to position the inhibitor correctly at the active site are clearly evident. Surprisingly, the His157 and Thr134 residues that are present in the vicinity of the Gln side chain and ordinarily engage in H-bonding interactions with the Gln side chain oxygen [33] are absent and seemingly displaced by Pro136, which forms a H-bond with the oxygen of the tetrahedral adduct (Figure 3). Thus, the loss of the pair of H-bonds between the Gln side chain oxygen and the His157 and Thr134 residues may account for the observed potency (*vide infra*). The inhibitor is covalently bonded to the active site Cys139 residue providing unequivocal demonstration of the mechanism of action of (I). The *m*-chlorophenyl group of inhibitor ***13*** is positioned within a hydrophobic cleft, as shown in Figure 5. Extending the position of the phenyl ring (Table 1, compounds ***11–12, 20*** and ***28***) resulted in a two to four-fold decrease in potency. Furthermore, one of the macrocycles having an unsaturated linker (compound ***24***, Table 1) was more potent than the corresponding macrocycle with a saturated linker (Table 1/compounds ***14A–B***) while the second one (Table 1/***23***) was ~2-fold more potent than compound ***12*** (Table 1). In order to enhance binding and permeability, the P_1 Gln side chain was modified by introducing an additional H-bonding site. The structural modification did not have a major effect on potency (Table 1, compounds ***36A–B***).

The *m*-chlorophenyl moiety occupies a predominantly hydrophobic pocket (Figure 5), consequently, this site was probed by synthesizing macrocycles having an n-pentyl chain. The resulting compounds were found to be ~ 2-fold less active (Table 1, compounds ***21/29*** and compounds ***13/25***). Inhibitor ***21*** is bound to the active site similarly to inhibitor ***13*** (Figures 6 and 7) and engages in similar H-bonding interactions (Figure 8), however, the Pro136 H-bond is missing. The latter, along with the higher entropic penalty associated with the alkyl chain, partially accounts for the lower affinity. The n-pentyl chain is clearly shown to engage in hydrophobic interactions (Figure 9). Modification of this portion of the inhibitor to facilitate the formation of new hydrogen bonds with the side chains of Thr161 or Thr168 could potentially enhance affinity (Figures 5 and 9).

3. Conclusions

Noroviruses are a leading cause of acute gastroenteritis in all age groups worldwide and have a significant impact on public health. There are currently no norovirus-specific therapeutics or prophylactics. In this report we describe the design, synthesis, and anti-norovirus activity of a novel class of macrocyclic inhibitors of NV 3CLpro. These studies provide new insights into the interaction of macrocyclic inhibitors with NV 3CLpro and demonstrate the nuanced interplay of structure, pharmacological activity, and cellular permeability. They also lay the ground work for conducting further studies related to the development of anti-norovirus therapeutics.

4. Experimental section

4.1. General

Reagents and dry solvents were purchased from various chemical suppliers (Aldrich, Acros Organics, ChemImpex, TCI America, Oakwood chemicals, Bachem, and Fisher) and were used as obtained. Silica gel (230–450 mesh) used for flash chromatography was purchased from Sorbent Technologies (Atlanta, GA). Thin layer chromatography was performed using Analtech silica gel plates. The ^{1}H spectra were recorded in $CDCl_3$ or DMSO-d_6 on a Varian XL-400 NMR spectrometer and are reported relative to TMS (δH= 0.00 ppm). High resolution mass spectra (HRMS) were performed at the University of Kansas Mass Spectrometry lab using an LCT Premier mass spectrometer (Waters, Milford, MA) equipped with a time of flight mass analyzer and an electrospray ion source or a G6230B TOF MS (Agilent Technologies, Santa Clara, CA). Visualization was accomplished using UV light and/or iodine.

4.1.1. Synthesis of compounds 1a – f. General procedure—To a 250 mL round bottom flask kept under nitrogen atmosphere was added a 0.5 M solution (35 mmol) of the appropriate Grignard reagent (3-butenyl magnesium bromide or 4-pentenyl magnesium bromide) in THF and the solution was cooled to 0 – 5 °C. A solution of the appropriate aldehyde (35 mmol) in dry THF (20 mL) was added dropwise to the cooled Grignard solution over ~ 1 h. The reaction mixture was allowed to warm up to room temperature and stirred for 5 h under nitrogen. The disappearance of the aldehyde was monitored by TLC. The reaction mixture was cooled to 0 – 5°C and acidified to pH ~3.0 using 5% aqueous hydrochloric acid. The organic solvent was evaporated off and the residue was extracted with ethyl acetate (2 × 150 mL). The combined organic extracts were washed with brine (50 mL) dried over anhydrous Na_2SO_4, filtered, and concentrated. The crude product was purified by column chromatography to yield alcohols ***1a–1f*** as oils.

4.1.1.1. 1-phenylpent-4-en-1-ol 1a: Oil (yield 80%); ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 1.68 – 1.99 (m, 2 H), 1.99 – 2.33 (m, 2 H), 4.58 – 4.74 (m, 1 H), 4.90 – 5.11 (m, 2 H), 5.73 – 5.94 (m, 1 H), 7.20 – 7.50 (m, 5 H). HRMS (ESI) calcd for $C_{11}H_{15}O$: $[M+H]^+$: 163.1123 Found: 163.1126.

4.1.1.2. 1-phenylhex-5-en-2-ol 1b: Oil (yield 80%); ^{1}H NMR (400 MHz, $CDCl_3$-*d*) 1.53 – 1.69 (m, 2 H), 1,91 – 2.02 (m, 2 H), 2.70 – 2.93 (m, 2 H), 3.95 – 4.08 (m, 1 H), 4.88 – 5.09

(m, 3 H), 5.67 – 5.91 (m, 1 H), 7.23 – 7.34 (m, 5 H). HRMS (ESI) calcd for $C_{12}H_{17}O$: [M +H] $^{+}$: 177.1279 Found: 177.1285.

4.1.1.3. 1-(3-chlorophenyl) pent-4-en-1-ol 1c: Oil, yield (80%), ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.71 – 1.96 (m, 2 H), 2.04 – 2.24 (m, 2 H), 4.69 (s, 1 H), 4.97 – 5.13 (m, 2 H), 5.77 – 5.93 (m, 1 H), 7.17 – 7.50 (m, 4 H). HRMS (ESI) calcd for $C_{11}H_{14}ClO$: $[M+H]^{+}$: 197.0733 Found: 197.0735.

4.1.1.4. 1-(3-chlorophenyl) hex-5-en-1-ol 1d: Oil (yield 75%); ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 1.26 – 1.62 (m, 1 H), 1.59 – 1.85 (m, 1 H), 1.83 – 1.89 (m, 2 H), 2.02 – 2.13 (m, 2 H), 4.61 – 4.71 (m, 1 H), 4.95 – 5.08 (m, 3 H), 5.69 – 5.86 (m, 1 H), 7.16 – 7.30 (m, 3 H), 7.35 (s, 1 H). HRMS (ESI) calcd for $C_{12}H_{16}ClO$: $[M+H]^{+}$: 211.0890 Found: 211.0894.

4.11.5. 1-phenyloct-7-en-4-ol 1e: Oil (yield 55%);^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 1.41 – 1.59 (m, 4 H), 1.61 – 1.86 (m, 2 H), 2.04 – 2.26 (m, 2 H), 2.57 – 2.71 (t, 2 H), 3.59 – 3.70 (m, 1 H), 4.91 – 5.10 (m, 3 H), 5.76 – 5.89 (m, 1 H), 7.14 – 7.20 (d, 2 H), 7.23 – 7.30 (t, 3 H). HRMS (ESI) calcd for $C_{14}H_{21}O$: $[M+H]^{+}$: 205.1592 Found: 205.1598.

4.1.1.6. Dec-1-en-5-ol 1f: Oil **(**yield 93%); ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 0.82 – 0.91(t, 2 H), 1.21 – 1.33 (m, 4 H), 1.35 – 1.44 (m, 2 H), 1.47 – 1.61 (m, 2 H), 2.05 – 2.25 (m, 2 H),3.55 – 3.63 (m, 1 H), 4.90 – 5.07 (m, 3 H), 5.76 – 5.89 (m, 1 H). HRMS (ESI) calcd for $C_{10}H_{21}O$: $[M+H]^{+}$: 157.1592 Found: 157.1954.

4.1.2. Synthesis of carbamates 2a – k. General procedure—A solution of compound ***1*** (35 mmol) in dry acetonitrile (60 mL) was treated with trimethylamine (7.1 g; 70 mmol) followed by an appropriate amino acid methyl ester isocyanate ***4*** (35 mmol). The resulting reaction mixture was refluxed for 3 h and then allowed to cool to room temperature. The disappearance of the alcohol was monitored by TLC. The solvent was evaporated and the residue was taken up in ethyl acetate (250 mL) and the organic layer was washed with 5% aqueous HCl (2 × 50 mL) and saturated NaCl (50 mL). The organic layer was dried over anhydrous sulfate, filtered, and concentrated to yield an oily product. Purification by flash chromatography yielded esters ***2a-k*** as colorless oils.

4.1.2.1. Methyl (((1-phenylpent-4-en-1-yl) oxy) carbonyl)-L-leucinate 2a: Oil (yield 80%); ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 0.68 – 1.05 (d, 6 H), 1.40 – 1.73 (m, 1 H), 1.73 – 1.95 (m, 2 H), 1.95 – 2.21 (m, 4 H), 3.64 – 3.86 (s,3 H), 4.45 – 4.53 (m, 1 H), 4.86 – 5.20 (m, 2 H), 5.54 – 5.69 (m, 1 H), 5.69 – 5.92 (m, 1 H), 7.17 – 7.35 (m, 5 H), 7.40 – 7.50 (d, 1 H). HRMS (ESI) calcd for $C_{19}H_{28}NO_4$:$[M+H]^{+}$: 334.2018 Found: 334.2021.

4.1.2.2. Methyl (((1-phenylhex-5-en-2-yl) oxy) carbonyl)-L-leucinate 2b: Oil (yield 80%); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.81 – 0.97 (d, 6 H), 1.53 – 1.69 (m, 5 H), 1,91 – 2.02 (m, 2 H), 2.70 – 2.93 (m, 2 H), 3.71 – 3.78 (s, 3 H), 4.18 – 4.28 (m, 1 H), 4.88 – 5.09 (m, 3 H), 5.67 – 5.91 (m, 1 H), 6.20 – 6 60 (br, 1 H), 7.13 – 7.43 (m, 5 H), 7.71 – 7.80 (d, 1 H). HRMS (ESI) calcd for $C_{20}H_{30}NO_4$: $[M+H]^{+}$: 348.2175 Found: 348.2180.

4.1.2.3. Methyl (((1-(3-chlorophenyl) pent-4-en-1-yl) oxy) carbonyl)-L-leucinate 2c: Oil (yield 80%); ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 0.84 – 1.06 (d, 6 H), 1.41 – 1.72 (m, 1 H), 1.73 – 2.24 (m, 5 H), 2.42 – 2.57 (m, 2 H),3.62 – 3.77 (d, 3 H), 4.23 – 4.42 (m, 1 H), 4.60 – 4.74 (m, 1 H), 4.92 – 5.24 (m, 1 H), 5.54 – 5.68 (m, 1 H), 5.72 – 5.98 (m, 1 H), 7.15 – 7.32 (m, 3 H), 7.41 (s, 1 H). HRMS (ESI) calcd for $C_{19}H_{27}ClNO_4$: [M+H] $^+$: 368.1629 Found: 368.1633.

4.1.2.4. Methyl (2S)-2-((((1-(3-chlorophenyl) pent-4-en-1-yl) oxy) carbonyl) amino)-3-cyclohexylpropanoate 2d: Oil (yield 75%); ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 0.73 – 0.93 (m, 4 H), 1.03 – 1.31 (m, 2H), 1.31 – 1.55 (m, 2 H),1.55 – 1.75 (m, 2 H), 1.74 – 1.89 (m, 4 H), 2.00 – 2.21 (m, 2 H), 3.52 – 3.61 (s, 3H), 4.44 – 4.50 (m, 1 H), 4.95 – 5.18 (m, 3 H), 5.67 – 5.89 (m, 2 H), 7.14 – 7.35 (m, 4 H), 7.44 – 7.49 (s, 1 H). HRMS (ESI) calcd for $C_{22}H_{31}ClNO_4$: [M+H] $^+$: 408.1942 Found: 408.1947.

4.1.2.5. Methyl (((1-(3-chlorophenyl) hex-5-en-1-yl) oxy) carbonyl)-L-leucinate 2e: Oil (yield 80%); ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 0.87 – 0.92 (d, 6 H), 1.30 – 1.62 (m, 3 H), 1.61 – 1.84 (m, 2 H), 1.97 – 2.16 (m, 4 H), 3.61 – 3.70 (s, 3 H), 4.60 – 4.70 (m, 1 H), 4.89 – 5.06 (m, 2 H), 5.66 – 5.87 (m, 2 H), 7.13 – 7.38 (m, 4 H), 7.40 – 7.45 (s, 1 H). HRMS (ESI) calcd for $C_{20}H_{29}ClNO_4$: [M+H] $^+$: 382.1785 Found: 382.1789.

4.1.2.6. Methyl (((1-(3-chlorophenyl) pent-4-en-1-yl) oxy) carbonyl)-L-alaninate 2f: Oil (yield 80%); ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 1.42 (d, *J*=7.03 Hz, 3 H), 1.75 – 1.92 (m, 2 H), 1.90 – 2.14 (m, 2 H), 3.74 (br d, *J*=16.01 Hz, 3 H), 4.27 – 4.39 (m, 1 H), 4.94 – 5.09 (m, 2 H), 5.24 – 5.44 (d, 1 H), 5.55 – 5.68 (m, 1 H), 5.72 – 5.87 (m, 1 H), 7.26 (dd, *J*=4.10, 0.98 Hz, 4 H). HRMS (ESI) calcd for $C_{16}H_{21}ClNO_4$: [M+H] $^+$: 326.1159 Found: 326.1162.

4.1.2.7. Methyl (((1-(3-chlorophenyl) hex-5-en-1-yl) oxy) carbonyl)-L-alaninate 2g: Oil (yield 80%); ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 1.27 – 1.56 (m, 5 H), 1.66 – 2.14 (m, 4 H), 3.71 (br d, *J*=16.01 Hz, 3 H), 4.24 – 4.39 (m, 1 H), 4.89 – 5.06 (m, 2 H), 5.29 – 5.49 (m, 1 H), 5.54 – 5.83 (m, 2 H), 7.10 – 7.36 (m, 4 H). 8.10 – 8.15 (br s, 1 H). HRMS (ESI) calcd for $C_{17}H_{23}ClNO_4$: [M+H] $^+$: 340.1316 Found: 340.1322.

4.1.2.8. Methyl 1-((((1-(3-chlorophenyl) pent-4-en-1-yl) oxy) carbonyl) amino) cyclohexane-1-carboxylate 2h: Oil (yield 70%); ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 1.18 – 1.66 (m, 6H), 1.67 – 2.21 (m, 6 H), 2.23 – 2.40 (m, 2 H), 3.59 (s, 3 H), 4.53 – 4.73 (m, 1 H), 4.93 – 5.13 (m, 2 H), 5.48 – 5.64 (m, 1 H), 5.71 – 5.93 (m, 1 H), 7.24 (m, 4 H). HRMS (ESI) calcd for $C_{20}H_{27}ClNO_4$: [M+H] $^+$: 380.1629 Found: 380.1635.

4.1.2.9. Methyl 1-((((1-(3-chlorophenyl) hex-5-en-1-yl) oxy) carbonyl) amino) cyclohexane-1-carboxylate 2i: Oil, yield (65%); ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 1.30 – 2.16 (m, 16 H), 3.57 – 3.65 (s, 3 H), 4.62 – 4.71 (m, 1 H), 4.88 – 5.08 (m, 2 H), 5.48 – 5.63 (m, 1 H), 5.67 – 5.87 (m, 1 H), 7.11 – 7.30 (m, 3 H), 7.32 – 7.39 (s, 1 H). HRMS (ESI) calcd for $C_{21}H_{29}ClNO_4$: [M+H] $^+$: 394.1785 Found: 394.1790.

4.1.2.10. Methyl (((1-phenyloct-7-en-4-yl) oxy) carbonyl)-L-leucinate 2j: Oil (yield 88%); ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 0.83 – 0.96 (d, 6 H), 1.37 – 1.92 (m, 7 H), 1.95

– 2.07 (m, 3 H), 2.49 – 2.65 (t, 2 H), 3.63 – 3.73 (s, 3 H), 4.28 – 4.48 (m, 1 H), 4.71 – 4.81 (m, 1 H), 4.88 – 5.08 (m, 2 H), 5.48 – 5.63 (m, 1 H), 5.67 – 5.87 (m, 1 H), 7.08 – 7.18 (m, 2 H), 7.18 – 7.28 (m, 3 H). HRMS (ESI) calcd for $C_{22}H_{34}NO_4$: $[M+H]^+$: 376.2488 Found: 376.2452.

4.1.2.11. Methyl ((dec-1-en-5-yloxy) carbonyl)-L-leucinate 2k: Oil (yield 91%); ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 0.83 – 0.90 (t, 2 H),0.90 – 0.98 (d, 6 H), 1.20 – 1.35 (m, 6 H), 1.45 – 1.70 (m, 4 H), 1.98 – 2.15 (m, 4 H), 3.69 – 3.76 (s, 3 H), 4.30 – 4.43 (m, 1 H), 4.70 – 4.80 (m, 1 H), 4.90 – 5.05 (m, 2 H), 5.70 – 5.80 (m, 1 H), 7.80 – 7.89 (d, 1 H). HRMS (ESI) calcd for $C_{18}H_{34}NO_4$: $[M+H]^+$: 328.2488 Found: 328.2454.

4.1.3. Synthesis of acids (3a–g and 3j–k). General procedure A—A solution of ester ***2*** (10 mmol) in tetrahydrofuran (15 mL) was treated with 1M aqueous LiOH (20 mL). The reaction mixture was stirred for 3 h at room temperature while monitoring the disappearance of the ester by TLC. Most of the solvent was evaporated off and the solution was acidified to pH ~3 using 5% hydrochloric acid (10 mL). The aqueous layer was extracted with ethyl acetate (2 × 100 mL) and the combined organic layer was washed with brine (50 mL). The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated to yield compounds ***3a–g*** and ***3j–k*** as colorless oils.

4.1.3.1. (((1-phenylpent-4-en-1-yl) oxy) carbonyl)-L-leucine 3a: Oil (yield 92%); ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 0.68 – 1.05 (d, 6 H), 1.40 – 1.73 (m, 1 H), 1.73 – 1.95 (m, 2 H), 1.95 – 2.21 (m, 4 H), 4.45 – 4.53 (m, 1 H), 4.86 – 5.20 (m, 2 H), 5.54 – 5.69 (m, 1 H), 5.69 – 5.92 (m, 1 H), 7.17 – 7.35 (m, 5 H), 7.40 – 7.50 (d, 1 H). HRMS (ESI) calcd for $C_{18}H_{26}NO_4$: $[M+H]^+$: 320.1862 Found: 320.1865.

4.1.3.2. (((1-phenylhex-5-en-2-yl) oxy) carbonyl)-L-leucine 3b: Oil (yield 91%); 1H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 0.81 – 0.97 (d, 6 H), 1.53 – 1.69 (m, 5 H), 1,91 – 2.02 (m, 2 H), 2.70 – 2.93 (m, 2 H), 4.18 – 4.28 (m, 1 H), 4.88 – 5.09 (m, 3 H), 5.67 – 5.91 (m, 1 H), 6.20 – 6 60 (br., 1 H), 7.13 – 7.43 (m, 5 H), 7.71 – 7.80 (d, 1 H). HRMS (ESI) calcd for $C_{19}H_{28}NO_4$: $[M+H]^+$: 334.2018 Found: 334.2022.

4.1.3.3. (((1-(3-chlorophenyl) pent-4-en-1-yl) oxy) carbonyl)-L-leucine 3c: Oil (yield 92%); ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 0.84 – 1.06 (d, 6 H), 1.41 – 1.72 (m, 1 H), 1.73 – 2.24 (m, 5 H), 2.42 – 2.57 (m, 2 H), 4.23 – 4.42 (m, 1 H), 4.60 – 4.74 (m, 1 H), 4.92 – 5.24 (m, 1 H), 5.54 – 5.68 (m, 1 H), 5.72 – 5.98 (m, 1 H), 7.15 – 7.32 (m, 3 H), 7.41 (s, 1 H). HRMS (ESI) calcd for $C_{18}H_{25}ClNO_4$: $[M+H]^+$: 354.1472 Found: 354.1475.

4.1.3.4. (2S)-2-((((1-(3-chlorophenyl) pent-4-en-1-yl) oxy) carbonyl) amino)-3-cyclohexylpropanoic acid 3d: Oil (yield 90%); ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 0.77 – 1.30 (m, 7 H), 1.58 – 2.21 (m, 8 H), 4.25 – 4.44 (m, 2 H), 4.65 – 4.73 (m, 1 H), 4.95 – 5.22 (m, 2 H), 5.53 – 5.71 (m, 1 H), 5.71 – 5.88 (m, 1 H), 7.14 – 7.41 (m, 4 H), 7.44 – 7.49 (s, 1 H). HRMS (ESI) calcd for $C_{21}H_{29}ClNO_4$: $[M+H]^+$: 394.1785 Found: 394.1791.

4.1.3.5. (((1-(3-chlorophenyl) hex-5-en-1-yl) oxy) carbonyl)-L-leucine 3e: Oil (yield 95%); ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 0.85 – 1.00 (d, 6 H), 1.41 – 1.78 (m, 5 H), 1.95

– 2.17 (m, 4 H), 4.29 – 4.48 (m, 1 H), 4.63 – 4.73 (m, 1 H), 4.84 – 5.01 (m, 1 H), 5.18 – 5.30 (m, 2 H), 5.72 – 5.85 (m, 2 H), 7.14 – 7.40 (m, 4 H). HRMS (ESI) calcd for $C_{19}H27ClNO_4$: $[M+H]^+$: 368.1629 Found: 368.1637.

4.1.3.6. (((1-(3-chlorophenyl) pent-4-en-1-yl) oxy) carbonyl)-L-alanine 3f: Oil (yield 92%); ^1H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 1.34 – 1.47 (d, 3 H), 1.75 – 1.91 (m, 2 H), 1.91 – 2.11 (m, 2 H), 4.28 – 4.46 (m, 1 H), 4.97 – 5.13 (m, 2 H), 5.35 – 5.46 (m, 1 H), 5.57 – 5.66 (m, 1 H), 5.73 – 5.87 (m, 1 H), 7.28 (br d, *J*=5.47 Hz, 3 H), 7.31 – 7.42 (s, 1 H). HRMS (ESI) calcd for $C_{15}H_{19}ClNO_4$: $[M+H]^+$: 312.1003 Found: 312.1010.

4.1.3.7. (((1-(3-chlorophenyl) hex-5-en-1-yl) oxy) carbonyl)-L-alanine 3g: Oil (yield 92%); ^1H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 1.27 – 1.56 (m, 5 H), 1.66 – 2.14 (m, 4 H), 4.24 – 4.39 (m, 1 H), 4.89 – 5.06 (m, 2 H), 5.29 – 5.49 (m, 1 H), 5.54 – 5.83 (m, 2 H), 7.10 – 7.36 (m, 4 H). 8.10 – 8.15 (br s, 1 H). HRMS (ESI) calcd for $C_{16}H_{21}ClNO_4$: $[M+H]^+$: 326.1159 Found: 326.1168.

4.1.3.8. (((1-phenyloct-7-en-4-yl) oxy) carbonyl)-L-leucine 3j: Oil (yield 94%); ^1H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 0.83 – 0.96 (d, 6 H), 1.37 – 1.92 (m, 7 H), 1.95 – 2.07 (m, 3 H), 2.49 – 2.65 (t, 2 H), 4.28 – 4.48 (m, 1 H), 4.71 – 4.81 (m, 1 H), 4.88 – 5.08 (m, 2 H), 5.48 – 5.63 (m, 1 H), 5.67 – 5.87 (m, 1 H), 7.08 – 7.18 (m, 2 H), 7.18 – 7.28 (m, 3 H), 8.10 – 8.16 (d, 1 H). HRMS (ESI) calcd for $C_{21}H_{32}NO_4$: $[M+H]^+$: 362.2331 Found 362.2333.

4.1.3.9. ((Dec-1-en-5-yloxy) carbonyl)-L-leucine 3k: Oil (yield 91%); ^1H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 0.83 – 0.90 (t, 2 H),0.90 – 0.98 (d, 6 H), 1.20 – 1.35 (m, 6 H), 1.45 – 1.70 (m, 4 H), 1.98 – 2.15 (m, 4 H), 4.30 – 4.43 (m, 1 H), 4.70 – 4.80 (m, 1 H), 4.90 – 5.05 (m, 2 H), 5.70 – 5.80 (m, 1 H), 7.80 – 7.89 (d, 1 H). HRMS (ESI) calcd for $C_{17}H_{32}NO_4$: $[M+H]^+$: 314.2331 Found 314.2339.

4.1.3. Synthesis of acids 3h–i. General procedure B—A solution of ester ***2a*** *or* ***2i*** (10 mmol) in tetrahydrofuran (10 mL) and methanol (10 mL) was treated with 2M aqueous LiOH (20 mL) and the reaction mixture was heated to 60°C for 12 h with stirring. The disappearance of the ester was monitored by TLC. The reaction mixture was cooled to room temperature, the solvent was removed under reduced pressure, and the solution was acidified to pH ~3 using 5% hydrochloric acid (~20 mL). The aqueous layer was extracted with ethyl acetate (2 × 100 mL) and the combined extracts were washed with brine (50 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated to yield compounds ***3h–i*** as oils.

4.1.3.10. 1-((((1-(3-chlorophenyl) pent-4-en-1-yl) oxy) carbonyl) amino) cyclohexane-1-carboxylic acid 3h: Oil (yield 90%); ^1H NMR (400 MHz, $CDCl_3$) δ ppm 1.18 – 1.66 (m, 6H), 1.67 – 2.21 (m, 6 H), 2.23 – 2.40 (m, 2 H), 4.69 (dd, *J*=7.62, 5.27 Hz, 1 H), 4.94 – 5.09 (m, 2 H), 5.57 – 5.62 (m, 1 H), 5.72 – 5.90 (m, 1 H), 7.14 – 7.17 (m, 1 H), 7.15 – 7.31 (m, 4 H). HRMS (ESI) calcd for $C_{19}H_{25}ClNO_4$: $[M+H]^+$: 366.1472 Found 366.1478.

4.1.3.11. 1-((((1-(3-chlorophenyl) hex-5-en-1-yl) oxy) carbonyl) amino) cyclohexane-1-carboxylic acid 3i: Oil (yield 95%); ^1H NMR (400 MHz, $CDCl_3$) δ ppm 1.30 – 2.16 (m, 16

H), 4.62 – 4.71 (m, 1 H), 4.88 – 5.08 (m, 2 H), 5.48 – 5.63 (m, 1 H), 5.67 – 5.87 (m, 1 H), 7.11 – 7.30 (m, 3 H), 7.32 – 7.39 (s, 1 H). HRMS (ESI) calcd for $C_{20}H_{27}ClNO_4$: $[M+H]^+$: 380.1629 Found 380.1637.

4.1.4. Synthesis of amino acid methyl ester isocyanates 4a–d. General procedure—The appropriate amino acid methyl ester hydrochloride (55 mmol) was placed in a dry 500-mL RB flask and then dried overnight on the vacuum pump. The flask was flushed with nitrogen and dry dioxane (150 mL) was added followed by trichloromethyl chloroformate (16.26 g, 82.5 mmol). After refluxing for 12 h, the solvent was removed on the rotary evaporator and the residue was vacuum distilled to yield pure isocyanates ***4a–d*** as colorless oils.

4.1.5. Synthesis of compounds 5a–b. General procedure—To a solution of Boc-L-glutamic acid α-methyl ester (80 mmol) in dry DMF (200 mL) were added EDCI (19.93 g; 104 mmol), HOBt (15.92 g; 104 mmol) and the reaction mixture was stirred for 30 min at room temperature. Following the sequential addition of allylamine/*O*-allylhydroxylamine hydrochloride (120 mmol) and DIEA (20.68 g; 160 mmol), the reaction mixture was stirred for 16 h at room temperature. Completion of the reaction was monitored by TLC. The solvent was removed and the residue was partitioned between ethyl acetate (400 mL) and 5% aqueous HCl (100 mL). The layers were separated and the organic layer was further washed with saturated aqueous $NaHCO_3$ (2 × 100 mL), followed by saturated NaCl (100 mL). The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated to yield a yellow-colored oily product. Purification by flash chromatography yielded a white solid.

4.1.5.1. Methyl N^5-allyl-N^2-(tert-butoxycarbonyl)-L-glutaminate 5a: White solid (yield 85%); m.p. 69–70°C; ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 1.42 – 1.50 (s, 9 H), 1.90 – 2.00 (m, 1 H), 2.16 – 2.28 (m. 1 H), 3.74 – 3.80 (s, 3 H), 3.91 – 4.00 (t, 2 H), 4.34 – 4.41 (m, 1 H), 5.09 – 5.22 (m, 2 H), 5.30 – 5.40 (br. s, 1 H), 5.78 – 5.94 (m, 1 H), 6.10 – 6.20 (br. s, 1 H). HRMS (ESI) calcd for $C_{14}H_{25}N_2O_5$: $[M+H]^+$: 301.1763 Found 301.1771.

4.1.5.2. Methyl N^5-(allyloxy)-N^2-(tert-butoxycarbonyl)-L-glutaminate 5b: Sticky oil (yield 54%); ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 1.41 – 1.50 (s, 9 H), 1.78 – 1.96 (br. s, 1 H), 2.12 – 2.28 (m. 4 H), 3.72 – 3.80 (s, 3 H), 4.24 – 4.37 (m, 2 H), 4.44 – 4.51 (m, 1 H), 5.29 – 5.50 (m, 2 H), 5.92 – 6.07 (m, 1 H), 9.29 – 9.42 (br. s, 1 H). HRMS (ESI) calcd for $C_{14}H_{25}N_2O_6$: $[M+H]^+$: 317.1713 Found 317.1718.

4.1.6. Synthesis of compounds 6a–b. General procedure—To a solution of compound ***5*** (43 mmol) in dry DCM (30 mL) was added a solution of 4 M HCl in dioxane (130 mL) with stirring. The reaction mixture was stirred for 3 h at room temperature. The disappearance of the starting material was monitored by TLC. The solvent was evaporated under reduced pressure and compound ***6*** was used in the next step without further purification.

4.1.7. Synthesis of acyclic compounds 7a–k and 32

General procedure: To a solution of **c**ompound ***3*** (15 mmol) in dry DMF (40 mL) was added EDCI (3.74 g, 19.5 mmol, 1.30 eq), HOBt (2.97 g, 19.5 mmol, 1.30 eq) and the mixture was stirred for 30 min at room temperature. In a separate flask, a solution of compound ***6a*** (3.55 g, 15 mmol) in DMF (20 mL) cooled to 0–5°C was treated with diisopropyl ethylamine (DIEA) (7.75 g, 60 mmol, 4 eq), stirred for 30 min, and then added to the reaction mixture containing acid ***3***. The reaction mixture was stirred for 16 h while monitoring the reaction by TLC. The solvent was removed and the residue was partitioned between ethyl acetate (300 mL) and 10% citric acid (50 mL). The layers were separated and the organic layer was further washed with saturated aqueous $NaHCO_3$ (2 × 50 mL), followed by brine (50 mL). The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated to yield a yellow-colored oily product. Purification by flash chromatography yielded esters ***7a–k***.

4.1.7.1. Methyl N^5-allyl-N^2-((((1-phenylpent-4-en-1-yl) oxy) carbonyl)-L-leucyl)-L-glutaminate 7a: Oil (yield 62%); ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 0.68 – 1.05 (d, 6 H), 1.40 – 1.73 (m, 3 H), 1.73 – 1.95 (m, 2 H), 1.95 – 2.21 (m, 6 H), 3.64 – 3.76 (s,3 H), 4.45 – 4.53 (m, 4 H), 4.86 – 5.20 (m, 4 H), 5.54 – 5.69 (m, 2 H), 5.69 – 5.92 (m, 2 H), 7.17 – 7.35 (m, 5 H), 7.40 – 7.50 (d, 1 H), 7.90 – 7. 80 (d, 1 H). HRMS (ESI) calcd for $C_{27}H_{40}N_3O_6$: [M+H] $^+$: 502.2917 Found 502.2921.

4.1.7.2. Methyl N^5-allyl-N^2-((((1-phenylhex-5-en-2-yl) oxy) carbonyl)-L-leucyl)-L-glutaminate 7b: Oil (yield 60%); ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 0.81 – 0.97 (d, 6 H), 1.53 – 1.69 (m, 5 H), 1, 91 – 2.02 (m, 6 H), 2.70 – 2.93 (m, 2 H), 3.73 (s, 3 H), 3.80 – 3.94 (t, 2 H), 4.40 – 4.62 (m, 2 H), 4.88 – 5.04 (m, 2 H), 5.06 – 5.26 (m, 2 H), 5.68 – 5.87 (m, 3 H), 6.20 – 6.41 (br. d, 1 H), 6.91 – 7.10 (d, 1 H), 7.14 – 7.36 (m, 5 H), 7.85 – 7.92 (d, 1 H). HRMS (ESI) calcd for $C_{28}H_{42}N_3O_6$: [M+H] $^+$: 516.3074 Found 516.3080.

4.1.7.3. Methyl N^5-allyl-N^2-((((1-(3-chlorophenyl) pent-4-en-1-yl) oxy) carbonyl)-L-leucyl)-L-glutaminate 7c: Oil (yield 65%); ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 0.74 – 1.02 (d, 6 H), 1.43 – 2.13 (m, 4 H), 2.13 – 2.38 (m, 6 H), 3.60 – 3.71 (s, 3 H), 4.07 – 4.24 (m, 1 H), 4.41 – 4.61 (m, 2 H), 4.91 – 5.25 (m, 1 H), 5.25 – 5.42 (m, 2 H), 5.50 – 5.68 (m, 2 H), 5.70 – 5.92 (m, 2 H), 6.07 – 6.26 (m, 2 H), 6.30 – 6.39 (m, 1 H), 6.96 – 7.34 (m, 4 H), 7.7 – 7.8 (d, 1 H), 8.1 – 8.2 (d, 1 H). HRMS (ESI) calcd for $C_{27}H_{39}ClN_3O_6$: [M+H] +: 536.2527 Found 536.2534.

4.1.7.4. Methyl N^5-allyl-N^2-((2S)-2-((((1-(3-chlorophenyl) pent-4-en-1-yl) oxy) carbonyl) amino)-3-cyclohexylpropanoyl) glutaminate 7d: Oil (yield70%); ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 0.75 – 1.59 (m, 4 H), 1.55 – 1.73 (m, 4 H), 1.74 – 1.89 (m, 4 H), 1.89 – 2.14 (m, 5 H), 2.14 – 2.31 (m, 4 H), 3.65 – 3.76 (d, 3 H), 3.76 – 4.02 (m, 1 H), 4.07 – 4.29 (m, 1 H), 4.40 – 4.61 (m, 2 H), 4.93 – 5.38 (m, 2 H), 5.43 – 5.64 (m, 2 H), 5.69 – 5.90 (m, 3 H), 6.10 – 6.20 (d, 1 H), 6.35 – 6.44 (d, 1 H), 6.86 – 7.05 (d, 1 H), 7.12 – 7.37 (m, 4 H). HRMS (ESI) calcd for $C_{30}H_{43}ClN_3O_6$: [M+H] $^+$: 576.2840 Found 576.2848.

4.1.7.5. Methyl N^5-allyl-N^2-((((1-(3-chlorophenyl) hex-5-en-1-yl) oxy) carbonyl)-L-leucyl)-L-glutaminate 7e: Oil (yield 75%); ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 0.82 – 1.03 (d, 6 H), 1.20 – 2.32 (m, 13 H), 3.73 (br d, *J*=12.89 Hz, 3 H), 4.03 – 4.20 (m, 2 H), 4.38 – 4.59 (m, 2 H), 4.89 – 5.03 (m, 2 H), 5.08 – 5.32 (m, 2 H), 5.48 – 5.67 (m, 1 H), 5.68 – 5.94 (m, 1 H), 5.98 – 6.12 (m, 1 H), 6.21 – 6.34 (br s, 1 H), 6.86 – 7.03 (br s, 2 H), 7.13 – 7.37 (m, 4 H). HRMS (ESI) calcd for $C_{28}H_{41}ClN_3O_6$: $[M+H]^+$: 550.2684 Found 550.2691.

4.1.7.6. Methyl N^5-allyl-N^2-((((1-(3-chlorophenyl) pent-4-en-1-yl) oxy) carbonyl)-L-alanyl)-L-glutaminate 7f: Oil (yield 80%); ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 1.39 (d, *J*=7.03 Hz, 3 H), 1.75 – 1.91 (m, 4 H), 1.91 – 2.11 (m, 4 H), 3.66 – 3.71 (s, 3 H), 4.17 – 4.27 (d, 2 H), 4.41 – 4.61 (m, 2 H), 4.92 – 5.25 (m, 4 H), 5.47 – 5.65 (m, 1 H), 5.71 – 5.88 (m, 2 H), 6.15 – 6.20 (br s, 1 H), 6.40 – 6.45 (br s, 1 H), 7.12 – 7.32 (m, 4 H), 7.50 – 7.60 (d, 1 H). HRMS (ESI) calcd for $C_{24}H_{33}ClN_3O_6$: $[M+H]^+$: 494.2058 Found 494.2067.

4.1.7.7. Methyl N^5-allyl-N^2-((((1-(3-chlorophenyl) hex-5-en-1-yl) oxy) carbonyl)-L-alanyl)-L-glutaminate 7g: Oil (yield 80%); ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 1.30 – 1.39 (m, 2 H), 1.39 – 1.42 (d, *J*=7.03 Hz, 3 H), 1.75 – 1.91 (m, 4 H), 1.91 – 2.11 (m, 4 H), 3.66 – 3.71 (s, 3 H), 4.17 – 4.27 (d, 2 H), 4.41 – 4.61 (m, 2 H), 4.92 – 5.25 (m, 4 H), 5.47 – 5.65 (m, 1 H), 5.71 – 5.88 (m, 2 H), 6.15 – 6.20 (br s, 1 H), 6.40 – 6.45 (br s, 1 H), 7.12 – 7.32 (m, 4 H), 7.50 – 7.60 (d, 1 H). HRMS (ESI) calcd for $C_{25}H_{35}ClN_3O_6$: $[M+H]^+$: 508.2214 Found 508.2218.

4.1.7.8. Methyl N^5-allyl-N^2-(1-((((1-(3-chlorophenyl) pent-4-en-1-yl) oxy) carbonyl) amino) cyclohexane-1-carbonyl)-L-glutaminate 7h: Oil (yield 55%); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.20 – 1.48 (m, 5 H), 1.49 – 1.74 (m, 5 H), 1.75 – 1.90 (m, 4 H), 1.90 – 2.35 (m, 4 H), 3.71 (s, 3 H), 3.76 – 3.91 (m, 2 H), 4.41 – 4.61 (m, 1 H), 4.95 – 5.26 (m, 4 H), 5.72 – 5.91 (m, 3 H), 6.29 – 6.45 (br. d, 1 H), 6.57 – 6.71 (br. s, 1 H), 6.95 – 7.06 (d, 1 H), 7.15 – 7.35 (m, 4 H). HRMS (ESI) calcd for $C_{28}H_{39}ClN_3O_6$: $[M+H]^+$: 548.2527 Found 548.2531.

4.1.7.9. Methyl N^5-allyl-N^2-(1-((((1-(3-chlorophenyl) hex-5-en-1-yl) oxy) carbonyl) amino) cyclohexane-1-carbonyl)-L-glutaminate 7i: Oil (yield 57%); ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 1.16 – 1.53 (m, 7 H), 1.54 – 1.74 (m, 7 H), 1.75 – 2.34 (m, 6 H), 3.71 (s, 3 H), 3.73 – 3.90 (m, 2 H), 4.39 – 4.63 (m, 1 H), 4.90 – 5.27 (m, 4 H), 5.41 – 5.61 (m, 1 H), 5.65 – 5.91 (m, 2 H), 6.29 – 6.44 (br. s, 1 H), 6.60 – 6.66 (d, 1 H), 6.89 – 7.03 (d, 1 H), 7.13 – 7.18 (m, 3 H), 7.21 – 7.31 (s, 1 H). HRMS (ESI) calcd for $C_{29}H_{41}ClN_3O_6$: $[M+H]^+$: 562.2684 Found 562.2685.

4.1.7.10. Methyl N^5-allyl-N^2-((((1-phenyloct-7-en-4-yl) oxy) carbonyl)-L-leucyl)-L-glutaminate 7j: White solid (yield 62%); m.p 99 – 100° C. ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 0.90 – 0.99 (d, 6 H), 1.46 – 1.74 (m, 7 H), 1.75 – 1.82 (t, 2 H),1.90 – 2.12 (m, 4 H), 2.14 – 2.35 (m, 2 H), 2.52 – 2.60 (t, 2 H), 3.70 – 3.75 (s, 3 H), 3.80 – 3.90 (d, 2 H), 4.10 – 4.20 (m, 1 H), 4.48 – 4.59 (m, 1 H), 4.71 – 4.84 (m, 1 H), 4.90 – 5.23 (m, 4 H), 5.70 – 5.90 (m, 2 H), 6.20 – 6.30 (br. s, 1 H), 6.30 – 6.40 (br. s, 1 H), 6.93 – 6.70 (d, 1 H), 7.11 – 7.20 (d, 2 H), 7.22 – 7.30 (m, 3 H). HRMS (ESI) calcd for $C_{30}H_{46}N_3O_6$: $[M+H]^+$: 544.3387 Found 544.3390.

4.1.7.11. Methyl N^5-allyl-N^2-(((dec-1-en-5-yloxy) carbonyl)-L-leucyl)-L-glutaminate 7k: White solid (yield 70%); m.p 103 – 104°C. ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 0.83 – 0.91 (t, 2 H),0.91 – 0.99 (d, 6 H), 1.20 – 1.35 (m, 6 H), 1.45 – 1.70 (m, 4 H), 1.98 – 2.12 (m, 4 H), 2.14 – 2.35 (m, 2 H), 2.52 – 2.60 (t, 2 H), 3.72 – 3.76 (s, 3 H), 3.80 – 3.90 (d, 2 H), 4.10 – 4.20 (m, 1 H), 4.50 – 4.60 (m, 1 H), 4.66 – 4.78 (m, 1 H), 4.91 – 5.24 (m, 4 H), 5.71 – 5.90 (m, 2 H), 6.35 – 6.50 (br. s, 2 H), 6.91 – 7.00 (d, 1 H). HRMS (ESI) calcd for $C_{26}H_{46}N_3O_6$: [M+H] $^+$: 496.3387 Found 496.3388.

4.1.7.12. Methyl N^5-(allyloxy)-N^2-((((1-(3-chlorophenyl) pent-4-en-1-yl)oxy)carbonyl)-L-leucyl)-L-glutaminate 32: Oil (yield 52%); ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 0.83 – 1.00 (dd, 6 H), 1.23 – 1.29 (t, 2 H), 1.43 – 2.15 (m, 5 H), 2.14 – 2.35 (m, 4 H), 3.69 – 3.80 (d, 3 H), 4.03 – 4. 22 (m, 2 H), 4.32 – 4.48 (m, 2 H), 4.93 – 5.06 (m, 2 H), 5.23 – 5.44 (m, 2 H), 5.50 – 5.61 (m, 1 H), 5.73 – 5.84 (m, 1 H), 5.87 – 6.05 (m, 1 H), 6.76 – 6. 89 (br. s, 1 H), 7.13 – 7.20 (s, 1 H), 7.23 – 7.33 (m, 3 H), 9.22 – 9.29 (d, 1 H), 9.43 – 9.51 (d, 1 H). HRMS (ESI) calcd for $C_{27}H_{39}ClN_3O_7$: [M+H] $^+$: 552.2477 Found 552.2479.

4.1.8. Synthesis of compounds 8a–k and 33. General procedure for ring closing metathesis.—A solution of acyclic diene ***7*** (1.21 mmol) in dry DCM (1.5 L) was degassed for 30 min using nitrogen. Chloro dicyclohexyl borane (1 M in hexane) (1.21 mL; 1.21 mmol) and Grubb's 2nd generation catalyst (104 mg; 10 mol%) were added and degassing was continued for 10 min. The reaction mixture was heated to 45 °C and stirred for 30 min under a nitrogen atmosphere. An additional portion of Grubb's 2nd generation catalyst (52 mg; 5 mol%) was added and the solution stirred for 16 h at 45 °C under a nitrogen atmosphere. The reaction was quenched by adding activated charcoal (600 mg) and stirring the reaction for 18 h at room temperature. The reaction mixture was filtered through a Celite bed and the solvent was removed on the rotary evaporator. The crude residue was purified by flash chromatography to give macrocyclic esters ***8a–k*** as off-white solids.

4.1.8.1. Methyl (4S,7S, E)-4-isobutyl-2,5,10-trioxo-17-phenyl-1-oxa-3,6,11-triazacycloheptadec-13-ene-7-carboxylate 8a: Off-white solid (yield 65%); m.p 79 – 80°C. ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 0.68 – 1.05 (d, 6 H), 1.40 – 1.73 (m, 3 H), 1.73 – 1.95 (m, 2 H), 1.95 – 2.21 (m, 6 H), 3.64 – 3.76 (s, 3 H), 3.80 – 3.89 (m, 2 H), 4.45 – 4.53 (m, 2 H), 5.51 – 5.74 (m, 2 H), 5.76 – 5.90 (m, 1 H), 7.17 – 7.35 (m, 5 H), 7.40 – 7.50 (d, 1 H), 7.90 – 7. 80 (d, 1 H), 8.25 – 8.35 (d, 1 H). HRMS (ESI) calcd for $C_{25}H_{36}N_3O_6$: [M +H] $^+$: 474.2604 Found: 474.2608.

4.1.8.2. Methyl (4S,7S, E)-17-benzyl-4-isobutyl-2,5,10-trioxo-1-oxa-3,6,11-triazacycloheptadec-13-ene-7-carboxylate 8b: Off-white solid (yield 68%); m.p 70 – 71°C. ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 0.81 – 0.97 (d, 6 H), 1.53 – 1.69 (m, 4 H), 1,91 – 2.02 (m, 5 H), 2.70 – 2.93 (m, 2 H), 3.73 – 3.80 (s, 3 H), 3.80 – 3.94 (m, 2 H), 4.40 – 4.62 (m, 2 H), 4.88 – 5.04 (m, 1 H), 5.06 – 5.26 (m, 2 H), 5.68 – 5.87 (m, 2 H), 6.20 – 6.41 (br. d, 1 H), 6.91 – 7.10 (d, 1 H), 7.14 – 7.36 (m, 5 H), 7.85 – 7.92 (d, 1 H). HRMS (ESI) calcd for $C_{26}H_{38}N_3O_6$: [M+H] $^+$: 488.2761 Found: 488.2767.

4.1.8.3. Methyl (4S,7S, E)-17-(3-chlorophenyl)-4-isobutyl-2,5,10-trioxo-1-oxa-3,6,11-triazacycloheptadec-13-ene-7-carboxylate 8c: Off-white solid (yield 70%); m.p 84 –

85 °C. ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 0.74 – 0.99 (d, 6 H), 1.42 – 1.83 (m, 3 H), 2.11 – 2.43 (m, 6 H),2.47 – 2.64 (m, 2 H), 3.28 – 3.40 (m, 2 H), 3.69 – 3.81 (s, 3 H), 3.81 – 3.96 (t, 2 H), 4.62 – 4.77 (m, 1 H), 5.24 – 5.35 (m, 1 H), 5.54 – 5.75 (m, 1 H), 5.91 – 6.03 (m, 1 H), 6.14 – 6.24 (d, 1 H), 6.96 – 7.34 (m, 4 H), 8.02 – 8.11 (d, 1 H), 8.60 – 8.65 (d, 1 H). HRMS (ESI) calcd for $C_{25}H_{35}ClN_3O_6$: $[M+H]^+$: 508.2214 Found: 508.2219.

4.1.8.4. Methyl (4S,7S, E)-17-(3-chlorophenyl)-4-(cyclohexylmethyl)-2,5,10-trioxo-1-oxa-3,6,11-triazacycloheptadec-13-ene-7-carboxylate 8d: Off-white solid (yield 69%); m.p (A: 74 – 75°C), (B: 177 – 178°C).^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 0.66 – 0.93 (m, 11 H), 0.95 – 2.32 (m, 10 H), 3.61 – 3.74 (s, 3 H), 3.74 – 3.86 (d, 2 H), 4.00 – 4.20 (m, 1 H), 4.29 – 4.51 (m, 1 H), 4.84 – 5.01 (m, 1 H), 5.42 – 5.83 (m, 2 H), 5.94 – 6.11 (m, 1 H), 6.22 – 6.31 (d, 1 H), 6.75 – 6.92 (d, 1 H), 7.04 – 7.27 (m, 4 H). HRMS (ESI) calcd for $C_{28}H_{38}ClN_3NaO_6$: $[M+Na]^+$: 570.2347 Found: 570.2346.

4.1.8.5. Methyl (4S,7S, E)-18-(3-chlorophenyl)-4-isobutyl-2,5,10-trioxo-1-oxa-3,6,11-triazacyclooctadec-13-ene-7-carboxylate 8e: Off-white solid (yield 70%); m.p 63 – 64°C. ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 0.82 – 1.03 (d, 6 H), 1.20 – 2.32 (m, 7 H), 2.35 – 2.49 (m, 6 H), 3.76 (s, 3 H), 4.01 – 4.25 (m, 2 H), 4.49 – 4.58 (m, 2 H), 5.48 – 5.86 (m, 3 H),6.32 – 6.38 (d, 1 H), 6.89 – 6.93 (d, 1 H), 7.13 – 7.44 (m, 4 H), 8.05 – 8.11 (br s, 1 H). HRMS (ESI) calcd for $C_{26}H_{37}ClN_3O_6$: $[M+H]^+$: 522.2371 Found: 522.2378.

4.1.8.6. Methyl (4S,7S, E)-17-(3-chlorophenyl)-4-methyl-2,5,10-trioxo-1-oxa-3,6,11-triazacycloheptadec-13-ene-7-carboxylate 8f: Off-white solid (yield 70%); m.p 105 – 106°C. ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 1.32 – 1.50 (d, 3 H), 1.55 – 2.44 (m, 4 H), 2.44 – 2.75 (m, 4 H), 3.66 – 3.70 (s, 3 H), 4.05 – 4.10 (d, 2 H), 4.50 – 4.61 (m, 1 H), 4.66 – 4.82 (m, 1 H), 5.46 – 5.73 (m, 2 H), 5.89 – 6.01 (m, 1 H), 6.20 – 6.25 (d, 1 H),6.91 – 6.95 (br s, 1 H), 7.07 – 7.42 (m, 4 H), 8.50 – 8.54 (br s, 1 H). HRMS (ESI) calcd for $C_{22}H_{29}ClN_3O_6$: $[M+H]^+$: 466.1745 Found: 466.1751.

4.1.8.7. Methyl (4S,7S, E)-18-(3-chlorophenyl)-4-methyl-2,5,10-trioxo-1-oxa-3,6,11-triazacyclooctadec-13-ene-7-carboxylate 8g: Off-white solid (yield 70%); m.p 74 – 75°C. ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 1.20 – 1.30 (m, 2 H), 1.37 (br d, *J*=7.03 Hz, 3 H), 1.56 – 2.49 (m, 8 H), 3.76 (s, 3 H), 3.81 – 3.90 (m, 2 H), 4.54 – 4.86 (m, 2 H), 5.48 – 5.80 (m, 3 H), 6.14 – 6.32 (br d, 1 H), 6.90 – 7.02 (d, 1 H), 7.06 – 7.38 (m, 4 H), 7.70 – 7.75 (br s, 1 H). HRMS (ESI) calcd for $C_{23}H_{31}ClN_3O_6$: $[M+H]^+$: 480.1901 Found: 480.1907.

4.1.8.8. Methyl (20S, E)-10-(3-chlorophenyl)-8,17,22-trioxo-9-oxa-7,16,21-triazaspiro [5.16] docos-13-ene-20-carboxylate 8h: Off-white solid (yield 70%); m.p 105 – 106°C. ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 1.20 – 1.26 (m, 4 H), 1.35 – 1.76 (m, 4 H), 1.77 – 1.90 (m, 5 H), 2.26 – 2.49 (m, 6 H), 3.69 – 3.72 (s, 3 H), 3.381 – 3.88 (m, 2 H), 4.35 – 4.49 (m, 1 H), 5.46 – 5.70 (m, 2 H), 5.91 – 6.04 (m, 1 H), 6.08 – 6.15 (d, 1 H), 7.14 – 7.38 (m, 4 H), 7.87 – 7.96 (d, 1 H). HRMS (ESI) calcd for $C_{26}H_{35}ClN_3O_6$: $[M+H]^+$: 520.2214 Found: 520.2222.

4.1.8.9. Methyl (21S, E)-10-(3-chlorophenyl)-8,18,23-trioxo-9-oxa-7,17,22-triazaspiro [5.17] tricos-14-ene-21-carboxylate 8i: Off-white solid (yield 72%); m.p 87 – 88°C. ^{1}H

NMR (400 MHz, $CDCl_3$-*d*) δ ppm 1.20 – 1.26 (m, 4 H), 1.35 – 1.55 (m, 6 H), 1.55 – 1.74 (m, 5 H), 2.05 – 2.36 (m, 4 H), 2.37 – 2.55 (m, 2 H), 3.76 (d, *J*=2.34 Hz, 3 H), 3.80 – 3.87 (m, 2 H), 4.53 – 4.74 (m, 1 H), 5.59 – 5.75 (m, 2 H), 6.38 – 6.51 (d, 1 H), 6.51 – 6.61 (d, 1 H), 6.83 – 6.91 (d, 1 H), 7.13 – 7.39 (m, 4 H). HRMS (ESI) calcd for $C_{27}H_{37}ClN_3O_6$: [M +H] $^+$: 534.2371 Found: 534.2381.

4.1.8.10. Methyl (4S, 7S, Z)-4-isobutyl-2,5,10-trioxo-17-(3-phenylpropyl)-1-oxa-3,6,11-triazacycloheptadec-13-ene-7-carboxylate 8j: Off-white solid (yield 68%); m.p 72 – 73°C.^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 0.86 – 1.02 (d, 6 H), 1.46 – 1.86 (m, 7 H), 2.04 – 2.36 (m, 8 H), 2.52 – 2.60 (t, 2 H), 3.70 – 3.77 (s, 3 H), 3.82 – 3.93 (d, 2 H), 4.13 – 4.23 (m, 1 H), 4.63 – 4.80 (m, 1 H), 5.00 – 5.08 (t, 1 H), 5.33 – 5.63 (m, 2 H), 5.73 – 5.83 (d, 1H), 6.00 – 6.06 (d, 1 H), 6.93 – 6.70 (d, 1 H), 7.10 – 7.20 (d, 2 H), 7.22 – 7.32 (m, 3 H). HRMS (ESI) calcd for $C_{28}H_{42}N_3O_6$: [M+H] $^+$: 516.3074 Found: 516.3079.

4.1.8.11. Methyl (4S, 7S, Z)-4-isobutyl-2,5,10-trioxo-17-pentyl-1-oxa-3,6,11-triazacycloheptadec-13-ene-7-carboxylate 8k: Off-white solid **(**yield 54%); m.p 64 – 65°C. ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 0.85 – 0.92 (t, 2 H),0.93 – 1.04 (d, 6 H), 1.22 – 1.37 (m, 6 H), 1.43 – 1.92 (m, 6 H), 2.12 – 2.40 (m, 4 H), 2.52 – 2.64 (m, 2 H), 3.70 – 3.78 (s, 3 H), 3.80 – 3.90 (d, 2 H), 4.15 – 4.25 (m, 1 H), 4.50 – 4.60 (m, 1 H), 4.62 – 4.78 (m, 2 H), 4.91 – 5.24 (m, 1 H), 5.42 – 5.50 (m, 2 H), 5.84 – 5.89 (d, 1 H), 6.03 – 6.09 (d, 1 H), 6.31 – 6.37 (br. s, 1 H). HRMS (ESI) calcd for $C_{24}H_{42}N_3O_6$: [M+H] $^+$: 468.3074 Found: 468.3081.

4.1.8.12. Methyl (6S, 9S, E)-13-(3-chlorophenyl)-9-isobutyl-3,8,11-trioxo-1,12-dioxa-2,7,10-triazacyclooctadec-16-ene-6-carboxylate 33: Off-white solid (yield 52%); m.p 160 – 161°C. ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 0.84 – 0.99 (d, 6 H), 1.23 – 1.29 (t, 2 H), 1.49 – 2.57 (t, 8 H), 3.72 – 3.79 (s, 3 H), 3.82 – 3.90 (d, 1 H), 4.03 – 4. 17 (d, 2 H), 4.24 – 4.43 (m, 1 H), 4.59 – 4.74 (m, 1 H), 5.18 – 5.24 (d, 1 H), 5.52 – 5.67 (m, 2 H), 5.68 – 5.96 (m, 1 H), 6. 24 – 6. 32 (d, 1 H), 7.13 – 7.21 (s, 1 H), 7.25 – 7.36 (m, 3 H), 9.24 – 9.30 (d, 1 H). HRMS (ESI) calcd for $C_{25}H_{35}ClN_3O_7$: [M+H] $^+$: 524.2164 Found: 524.2171.

4.1.9. Synthesis of compounds 9a–k and 34. General procedure for the hydrogenation reaction—To a solution of the appropriate olefin ***8*** (1.0 mmol) in anhydrous ethanol (10 mL) was added palladium on carbon (10% Pd-C, 2.0 eq) and the mixture was stirred under a hydrogen atmosphere (a balloon was used) at room temperature and atmospheric pressure for 18 h. The mixture was filtered through Celite and the filtrate was concentrated *in vacuo* to yield a crude product which was purified by flash chromatography to yield compounds ***9a–k*** as white solids.

4.1.9.1. Methyl (4S, 7S)-4-isobutyl-2,5,10-trioxo-17-phenyl-1-oxa-3,6,11-triazacycloheptadecane-7-carboxylate 9a: White solid (yield 92%); m.p 85 – 86°C. ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 0.68 – 1.05 (d, 6 H), 1.40 – 1.73 (m, 5 H), 1.73 – 1.95 (m, 4 H), 1.95 – 2.21 (m, 6 H), 3.20 – 3.29 (t, 2 H), 3.64 – 3.76 (s, 3 H), 4.45 – 4.53 (m, 2 H), 5.76 – 5.90 (m, 1 H), 7.17 – 7.35 (m, 5 H), 7.40 – 7.50 (d, 1 H), 8.25 – 8.35 (d, 1 H), 8. 72 – 8.80 (d, 1 H). HRMS (ESI) calcd for $C_{25}H_{38}N_3O_6$: [M+H] $^+$: 476.2761 Found: 476.2768.

4.1.9.2. Methyl (4S, 7S)-17-benzyl-4-isobutyl-2,5,10-trioxo-1-oxa-3,6,11-triazacycloheptadecane-7-carboxylate 9b: White solid (yield 95%); m.p 190 – 191°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.81 – 0.97 (d, 6 H), 1.53 – 1.69 (m, 6 H), 1,91 – 2.02 (m, 5H), 2.20 – 2.31 (4 H), 2.70 – 2.93 (m, 2 H), 3.21 – 3.31 (t, 2 H), 3.73 – 3.80 (s, 3 H), 4.40 – 4.62 (m, 2 H), 4.88 – 5.04 (m, 1 H), 6.20 – 6.41 (br. d, 1 H), 6.91 – 7.10 (d, 1 H), 7.14 – 7.36 (m, 5 H), 7.85 – 7.92 (d, 1 H). HRMS (ESI) calcd for $C_{26}H_{40}N_3O_6$: $[M+H]^+$: 490.2917 Found: 490.2921.

4.1.9.3. Methyl (4S, 7S)-17-(3-chlorophenyl)-4-isobutyl-2,5,10-trioxo-1-oxa-3,6,11-triazacycloheptadecane-7-carboxylate 9c: White solid (yield 95%); m.p 149 – 150°C. ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 0.74 – 0.99 (d, 6 H), 1.42 – 1.83 (m, 3 H), 2.11 – 2.43 (m, 6 H), 2.47 – 2.64 (m, 2 H), 3.28 – 3.40 (m, 2 H), 3.69 – 3.81 (s, 3 H), 3.81 – 3.96 (t, 2 H), 4.50 – 5.60 (m, 1 H), 4.62 – 4.77 (m, 1 H), 5.91 – 6.03 (m, 1 H), 6.14 – 6.24 (d, 1 H), 6.96 – 7.34 (m, 4 H), 8.02 – 8.11 (d, 1 H), 8.60 – 8.65 (d, 1 H). HRMS (ESI) calcd for $C_{25}H_{37}ClN_3O_6$: $[M+H]^+$: 510.2371 Found: 510.2378.

4.1.9.4. Methyl (4S, 7S)-17-(3-chlorophenyl)-4-(cyclohexylmethyl)-2,5,10-trioxo-1-oxa-3,6,11-triazacycloheptadecane-7-carboxylate 9d: White solid (yield 95%); m.p (A: 71 – 72°C, B: 161 – 162°C).^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ 0.66 – 0.93 (m, 12 H), 0.95 – 2.32 (m, 10 H), 3.18 – 3.21 (t, 2 H), 3.63 – 3.81 (s, 3 H), 3.96 – 4.10 (t, 2 H), 4.00 – 4.20 (m, 1 H), 4.29 – 4.51 (m, 1 H), 4.84 – 5.01 (m, 1 H), 5.94 – 6.11 (m, 1 H), 6.22 – 6.31 (d, 1 H), 6.75 – 6.92 (d, 1 H), 7. 12 – 7.27 (m, 4 H), 8.25 – 8.40 (br. s, 1 H), 8.82 – 8.90 (d, 1 H). HRMS (ESI) calcd for $C_{28}H_{41}ClN_3O_6$: $[M+H]^+$: 550.2684 Found: 550.2689.

4.1.9.5. Methyl (4S, 7S)-18-(3-chlorophenyl)-4-isobutyl-2,5,10-trioxo-1-oxa-3,6,11-triazacyclooctadecane-7-carboxylate 9e: White solid (yield 94%); m.p 122 – 123°C. ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 0.74 – 0.99 (d, 6 H), 1.42 – 1.83 (m, 5 H), 2.11 – 2.43 (m, 6 H),2.47 – 2.64 (m, 2 H), 3.28 – 3.40 (m, 2 H), 3.69 – 3.81 (s, 3 H), 3.81 – 3.96 (t, 2 H), 4.50 – 5.60 (m, 1 H), 4.62 – 4.77 (m, 1 H), 5.91 – 6.03 (m, 1 H), 6.14 – 6.24 (d, 1 H), 6.96 – 7.34 (m, 4 H), 8.02 – 8.11 (d, 1 H), 8.60 – 8.65 (d, 1 H). HRMS (ESI) calcd for $C_{26}H_{39}ClN_3O_6$: $[M+H]^+$: 525.2527 Found: 524.2533.

4.1.9.6. Methyl (4S, 7S)-17-(3-chlorophenyl)-4-methyl-2,5,10-trioxo-1-oxa-3,6,11-triazacycloheptadecane-7-carboxylate 9f: White solid (yield 93%); m.p 131 – 132°C. ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 0.82 – 0.99 (m, 4 H), 1.40 (d, *J*=6.64 Hz, 3 H), 1.52 – 1.84 (m, 2 H), 1.82 – 2.06 (m, 2 H), 2.09 – 2.50 (m, 4 H), 3.12 – 3.31 (m, 2 H), 3.70 – 3.81 (s, 3 H), 4.65 – 4.77 (m, 1 H), 5.18 – 5.26 (m, 1 H), 5.84 – 5.93 (m, 1 H), 6.17 – 6.33 (br s, 1 H), 6.52 – 6.63 (d, 1 H), 7.10 – 7.30 (m, 4 H), 7.30 – 7.44 (m, 1 H). HRMS (ESI) calcd for $C_{22}H_{31}ClN_3O_6$: $[M+H]^+$: 468.1901 Found: 468.1911.

4.1.9.7. Methyl (4S, 7S)-18-(3-chlorophenyl)-4-methyl-2,5,10-trioxo-1-oxa-3,6,11-triazacyclooctadecane-7-carboxylate 9g: White solid (yield 93%); m.p 100 – 101°C. ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 0.75 – 0.96 (m, 4 H), 1.37 – 1.58 (d, m, 5 H), 1.65 – 1.81 (m, 2 H), 1.81 – 2.10 (m, 2 H), 2.10 – 2.54 (m, 4 H), 3.21 – 3.34 (t, 2 H), 3.75 (s, 3 H), 4.17 – 4.28 (m, 1 H), 4.56 – 4.70 (m, 1 H), 5.25 – 5.40 (br s, 1 H), 5.47 – 5.82 (m, 1 H), 6.14 –

6.29 (br s, 1 H), 7.01 – 7.51 (m, 4 H). 7.90 – 8.00 (br s, 1 H). HRMS (ESI) calcd for $C_{23}H_{33}ClN_3O_6$: $[M+H]^+$: 482.2058 Found: 482.2069.

4.1.9.8. Methyl (20S)-10-(3-chlorophenyl)-8,17,22-trioxo-9-oxa-7,16,21-triazaspiro [5.16] docosane-20-carboxylate 9h: White solid (yield 95%); m.p 105 – 106°C. ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 0.84 – 0.97 (m, 2 H), 1.09 – 1.40 (m, 7 H), 1.40 – 2.65 (m, 13 H), 3.10 – 3.28 (m, 2 H), 3.67 – 3.70 (s, 3 H), 4.31 – 4.44 (m, 1 H), 5.81 – 5.90 (m, 1 H), 6.47 – 6.62 (t, 1 H), 7.11 – 7.39 (m, 4 H), 8.26 – 8. 30 (br. S, 1 H), 8.64 – 8.79 (br. s, 1 H). HRMS (ESI) calcd for $C_{26}H_{37}ClN_3O_6$: $[M+H]^+$: 522.2371 Found: 522.2389.

4.1.9.9. Methyl (21S)-10-(3-chlorophenyl)-8,18,23-trioxo-9-oxa-7,17,22-triazaspiro [5.17] tricosane-21-carboxylate 9i: White solid (yield 95%); m.p 91 – 92°C. ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 0.83 – 0.91 (m, 2 H), 1.12 – 2.11 (m, 14 H), 2.18 – 2.40 (m, 8 H), 3.28 – 3.38 (m, 2 H), 3.76 (s, 3 H), 4.55 – 4.69 (m, 1 H), 5.52 – 5.61 (m, 1 H), 6.29 – 6.37 (br. s, 1 H), 6.44 – 6.59 (br. s, 1 H), 6.63 – 6.73 (br. s, 1 H), 7.13 – 7.39 (m, 4 H). HRMS (ESI) calcd for $C_{27}H_{39}ClN_3O_6$: $[M+H]^+$: 536.2527 Found: 536.2543.

4.1.9.10. Methyl (4S, 7S)-4-isobutyl-2,5,10-trioxo-17-(3-phenylpropyl)-1-oxa-3,6,11-triazacycloheptadecane-7-carboxylate 9j: White solid (yield 97%); m.p 200 – 201°C. ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 0.85 – 1.01 (d, 6 H), 1.15 – 1.81 (m, 10 H), 2.09 – 2.19 (m, 9 H), 2.54 – 2.67 (t, 2 H), 3.02 – 3.14 (t, 2 H), 3.68 – 3.77 (s, 3 H), 3.90 – 3.98 (d, 1 H), 4.66 – 4.75 (m, 1 H), 4.90 – 5.00 (m, 2 H), 6.07 – 6.16 (d, 1 H), 6.17 – 6.24 (d, 1 H), 7.10 – 7.20 (d, 2 H), 7.24 – 7.34 (m, 3 H). HRMS (ESI) calcd for $C_{28}H_{44}N_3O_6$: $[M+H]^+$: 518.3230 Found: 518.3253.

4.1.9.11. Methyl (4S, 7S)-4-isobutyl-2,5,10-trioxo-17-pentyl-1-oxa-3,6,11-triazacycloheptadecane-7-carboxylate 9k: White solid (yield 96%); m.p 203 – 204°C. ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 0.83 – 0.90 (t, 2 H),0.91 – 1.01 (d, 6 H), 1.20 – 1.34 (m, 8 H), 1.38 – 1.84 (m, 9 H), 2.24 – 2.51 (m, 4 H), 3.02 – 3.17 (br m, 1 H), 3.44 – 3.55 (br. s, 1 H), 3.69 – 3.78 (s, 3 H), 3.90 – 4.00 (d, 1 H), 4.65 – 4.75 (m, 2 H), 4.86 – 4.98 (d, 2 H), 5.84 – 5.89 (d, 1 H), 6.09 – 6.14 (d, 1 H), 6.16 – 6.24 (d, 1 H), 8.03 – 8.13 (br. s, 1 H). HRMS (ESI) calcd for $C_{24}H_{44}N_3O_6$: $[M+H]^+$: 470.3230 Found: 470.3271.

4.1.9.12. Methyl (6S, 9S)-13-(3-chlorophenyl)-9-isobutyl-3,8,11-trioxo-1,12-dioxa-2,7,10-triazacyclooctadecane-6-carboxylate 34: White solid (yield 92%); m.p 73 – 73°C. ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 0.87 – 1.00 (d, 6 H), 1.16 – 1.34 (m, 3 H), 1.36 – 1.78 (m, 9 H), 1.83 – 2.05 (t, 2 H), 2.14 – 2.30 (m, 2 H), 3.74 – 3.82 (s, 3 H), 3.83 – 4.16 (t, 2 H), 4.61 – 4.76 (m, 1 H), 5.10 – 5.19 (m, 1 H), 5.71 – 5.83 (m, 1 H), 7.15 – 7.23 (s, 1 H), 7.23 – 7.39 (m, 3 H), 9.12 – 9.19 (d, 1 H), 9.76 – 9.80 (d, 1 H). HRMS (ESI) calcd for $C_{25}H_{37}ClN_3O_7$: $[M+H]^+$: 526.2320 Found: 526.2332.

4.1.10. Synthesis of alcohols 10a–k and 35. General procedure—To a solution of an appropriate macrocyclic ester ***9*** (5 mmol) in anhydrous THF (30 mL) was added dropwise a solution of lithium borohydride in THF (2M in THF, 7.5 mL, 15 mmol) and the reaction mixture was stirred for 30 min at room temperature. Absolute ethyl alcohol (15 mL) was added and the reaction mixture was stirred at room temperature overnight. The reaction

mixture was then acidified by adding 1.0 M aqueous potassium bisulfate until the pH of the solution was ~3. Removal of the solvent left a residue which was taken up in ethyl acetate (150 mL). The organic layer was washed with brine (25 mL), dried over anhydrous sodium sulfate, filtered, and concentrated to yield compounds ***10a–k*** as white solids.

4.1.10.1. (4S, 7S)-7-(Hydroxymethyl)-4-isobutyl-17-phenyl-1-oxa-3,6,11-triazacycloheptadecane-2,5,10-trione 10a: White solid (yield 93%); m.p 144 – 145°C. ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 0.68 – 1.05 (d, 6 H), 1.40 – 1.73 (m, 5 H), 1.73 – 1.95 (m, 4 H), 1.95 – 2.21 (m, 6 H), 3.20 – 3.29 (t, 2 H), 3.35 – 3.46 (m, 2 H), 4.45 – 4.53 (m, 2 H), 5.76 – 5.90 (m, 1 H), 7.17 – 7.35 (m, 5 H), 7.40 – 7.50 (d, 1 H), 8.25 – 8.35 (d, 1 H), 8. 72 – 8.80 (d, 1 H). HRMS (ESI) calcd for $C_{24}H_{38}N_3O_5$: $[M+H]^+$: 448.2811 Found: 448.2834.

4.1.10.2. (4S, 7S)-17-Benzyl-7-(hydroxymethyl)-4-isobutyl-1-oxa-3,6,11-triazacycloheptadecane-2,5,10-trione 10b: White solid (yield 96%); m.p 214 – 215°C. ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 0.81 – 0.97 (d, 6 H), 1.53 – 1.69 (m, 6 H), 1,91 – 2.02 (m, 5H), 2.20 – 2.31 (4 H), 2.70 – 2.93 (m, 2 H), 3.21 – 3.31 (t, 2 H), 3.52 – 3.60 (m, 2 H), 4.40 – 4.62 (m, 3 H), 4.88 – 5.04 (m, 1 H), 6.20 – 6.41 (br. d, 1 H), 6.91 – 7.10 (d, 1 H), 7.14 – 7.36 (m, 5 H), 7.85 – 7.92 (d, 1 H). HRMS (ESI) calcd for $C_{25}H_{40}N_3O_5$: $[M+H]^+$: 462.2968 Found: 462.2984.

4.1.10.3. (4S, 7S)-17-(3-Chlorophenyl)-7-(hydroxymethyl)-4-isobutyl-1-oxa-3,6,11-triazacycloheptadecane-2,5,10-trione 10c: White solid (yield 95%); m.p 98 – 99°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.74 – 0.99 (d, 6 H), 1.42 – 1.83 (m, 3 H), 2.11 – 2.43 (m, 6 H), 2.47 – 2.64 (m, 2 H), 3.28 – 3.40 (m, 2 H), 3.4 – 3.5 (m, 2H), 3.81 – 3.96 (t, 2 H), 4.50 – 5.60 (m, 1 H), 4.62 – 4.77 (m, 1 H), 5.91 – 6.03 (m, 1 H), 6.14 – 6.24 (d, 1 H), 6.96 – 7.34 (m, 4 H), 8.02 – 8.11 (d, 1 H), 8.60 – 8.65 (d, 1 H). HRMS (ESI) calcd for $C_{24}H_{37}ClN_3O_5$: $[M+H]^+$: 482.2422 Found: 482.2444.

4.1.10.4. (4S, 7S)-17-(3-Chlorophenyl)-4-(cyclohexylmethyl)-7-(hydroxymethyl)-1-oxa-3,6,11-triazacycloheptadecane-2,5,10-trione 10d: White solid (yield 93%); m.p (A: 71 – 72°C, B: 161 – 162°C). ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ 0.66 – 0.93 (m, 12 H), 0.95 – 2.32 (m, 10 H), 3.18 – 3.21 (t, 2 H), 3.96 – 4.10 (t, 2 H), 4.00 – 4.20 (m, 1 H), 4.29 – 4.51 (m, 1 H), 4.84 – 5.01 (m, 1 H), 5.94 – 6.11 (m, 1 H), 6.22 – 6.31 (d, 1 H), 6.75 – 6.92 (d, 1 H),7. 12 – 7.27 (m, 4 H), 8.25 – 8.40 (br s, 1 H), 8.82 – 8.90 (d, 1 H). HRMS (ESI) calcd for $C_{27}H_{41}ClN_3O_5$: $[M+H]^+$: 522.2735 Found: 522.2744.

4.1.10.5. (4S, 7S)-18-(3-Chlorophenyl)-7-(hydroxymethyl)-4-isobutyl-1-oxa-3,6,11-triazacyclooctadecane-2,5,10-trione 10e: White solid (yield 95%); m.p 71 – 72°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.79 – 0.94 (d, 6 H), 1.15 – 1.30 (m, 4 H), 1.31 – 1.41 (m, 4 H), 1.61 – 1.81 (m, 5 H), 2.00 – 2.10 (m, 4 H), 3.53 – 3.77 (m, 5 H), 4.55 – 4.74 (m, 2 H), 5.75 – 5.82 (m, 1 H), 6.48 – 6.54 (t, 1 H), 7.21 – 7.46 (m, 4 H), 7.54 – 7.63 (d, 1 H), 7.73 – 7.83 (d, 1 H). HRMS (ESI) calcd for $C_{25}H_{39}ClN_3O_5$: $[M+H]^+$: 496.2578 Found: 496.2612.

4.1.10.6. (4S, 7S)-17-(3-Chlorophenyl)-7-(hydroxymethyl)-4-methyl-1-oxa-3,6,11-triazacycloheptadecane-2,5,10-trione 10f: White solid (yield 82%); m.p 154 – 155°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.75 – 0.91 (m, 2 H), 1.13 – 1.25 (m, 2 H), 1.39 – 1.41 (d, 3 H), 1.44 – 1.57 (m, 2 H), 1.57 – 1.71 (m, 2 H), 1.79 – 1.95 (m, 2 H), 2.11 – 2.32 (m, 2 H), 3.23 – 3.38 (m, 4 H), 3.72 – 3.90 (m, 1 H), 4.64 – 4.72 (m, 2 H), 5.82 – 5.89 (m, 1 H), 6.86 – 6.97 (br. s, 1 H), 7.22 – 7.45 (m, 4 H), 7.57 – 7.68 (d, 1 H), 7.72 – 7.83 (d, 1 H). HRMS (ESI) calcd for $C_{21}H_{31}ClN_3O_5$: $[M+H]^+$: 440.1952 Found: 440.1983.

4.1.10.7. (4S, 7S)-18-(3-Chlorophenyl)-7-(hydroxymethyl)-4-methyl-1-oxa-3,6,11-triazacyclooctadecane-2,5,10-trione 10g: White solid (yield 88%); m.p 192 – 193°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.75 – 0.96 (m, 4 H), 1.37 – 1.58 (d, m, 5 H), 1.65 – 1.81 (m, 2 H), 1.81 – 2.10 (m, 2 H), 2.10 – 2.54 (m, 4 H), 3.21 – 3.34 (t, 2 H), 3.45 – 3.50 (m, 3 H), 4.17 – 4.28 (m, 1 H), 4.56 – 4.70 (m, 1 H), 5.25 – 5.40 (br s, 1 H), 5.47 – 5.82 (m, 1 H), 6.14 – 6.29 (br s, 1 H), 7.01 – 7.51 (m, 4 H). 7.90 – 8.00 (br s, 1 H). HRMS (ESI) calcd for $C_{22}H_{33}ClN_3O_5$: $[M+H]^+$: 454.2109 Found: 454.2477.

4.1.10.8. (20S)-10-(3-Chlorophenyl)-20-(hydroxymethyl)-9-oxa-7,16,21-triazaspiro[5.16]docosane-8,17,22-trione 10h: White solid (yield 90%); m.p 107 – 108°C. ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 0.82 – 0.92 (m, 2 H), 1.12 – 1.54 (m, 9 H), 1.54 – 1.99 (m, 10 H), 2.16 – 2.43 (m, 6 H), 2.96 – 3.40 (m, 2 H), 3.44 – 3.70 (m, 2 H), 5.21 – 5.24 (s, 1 H), 5.54 – 5.65 (m, 1 H), 6.54 – 6.60 (br. s, 1 H), 6.71 – 6.84 (d, 1 H), 7.01 – 7.09 (d, 1 H), 7.11 – 7.38 (m, 4 H). HRMS (ESI) calcd for $C_{25}H_{37}ClN_3O_5$: $[M+H]^+$: 494.2422 Found: 494.2440.

4.1.10.9. (21S)-10-(3-Chlorophenyl)-21-(hydroxymethyl)-9-oxa-7,17,22-triazaspiro[5.17]tricosane-8,18,23-trione 10i: White solid (yield 94%); m.p 92 – 93°C. ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 0.84 – 0.92 (m, 2 H), 1.14 – 1.51 (m, 7 H), 1.51 – 1.99 (m, 10 H), 2.14 – 2.47 (m, 6 H), 2.94 – 3.41 (m, 2 H), 3.45 – 3.71 (m, 2 H), 5.19 – 5.21 (s, 1 H), 5.53 – 5.65 (m, 1 H), 6.49 – 6.60 (br. s, 1 H), 6.76 – 6.86 (d, 1 H), 6.94 – 7.04 (d, 1 H), 7.11 – 7.38 (m, 4 H). HRMS (ESI) calcd for $C_{26}H_{39}ClN_3O_5$: $[M+H]^+$: 508.2578 Found: 508.2601.

4.1.10.10. (4S, 7S)-7-(Hydroxymethyl)-4-isobutyl-17-(3-phenylpropyl)-1-oxa-3,6,11-triazacycloheptadecane-2,5,10-trione 10j: White solid (yield 98%); m.p 216 – 217°C. ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 0.85 – 1.01 (d, 6 H), 1.15 – 1.81 (m, 10 H), 2.09 – 2.19 (m, 9 H), 2.54 – 2.67 (t, 2 H), 3.02 – 3.14 (t, 2 H), 3.53 – 3.74 (m, 2 H), 3.90 – 3.98 (d, 1 H), 4.66 – 4.75 (m, 1 H), 4.90 – 5.00 (m, 2 H), 6.07 – 6.16 (d, 1 H), 6.17 – 6.24 (d, 1 H), 7.10 – 7.20 (d, 2 H), 7.24 – 7.34 (m, 3 H). HRMS (ESI) calcd for $C_{27}H_{44}N_3O_5$: $[M+H]^+$: 490.3281 Found: 490.3312.

4.1.10.11. (4S, 7S)-7-(Hydroxymethyl)-4-isobutyl-17-pentyl-1-oxa-3,6,11-triazacycloheptadecane-2,5,10-trione 10k: White solid (yield 98%); m.p 230°C (d). ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 0.85 – 0.93 (t, 2 H), 0.93 – 1.01 (d, 6 H), 1.20 – 1.34 (m, 8 H), 1.38 – 1.84 (m, 9 H), 2.24 – 2.51 (m, 4 H), 3.02 – 3.17 (br m, 1 H), 3.44 – 3.55 (br. s, 1 H), 3.54 – 3.73 (m, 2 H), 3.90 – 4.00 (d, 1 H), 4.65 – 4.75 (m, 2 H), 4.86 – 4.98 (d, 2 H), 5.00 – 5.06 (d, 1 H), 6.09 – 6.14 (d, 1 H), 6.29 – 6.39 (d, 1 H), 8.03 – 8.13 (br. s, 1 H). HRMS (ESI) calcd for $C_{23}H_{34}N_3O_5$: $[M+H]^+$: 442.3281 Found: 442.3319.

4.1.10.12. (6S, 9S)-13-(3-Chlorophenyl)-6-(hydroxymethyl)-9-isobutyl-1,12-dioxa-2,7,10-triazacyclooctadecane-3,8,11-trione 35: White solid (yield 97%); m.p 138 – 139°C. ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 0.83 – 0.97 (d, 6 H), 1.21 – 1.34 (m, 3 H), 1.41 – 1.99 (m, 9 H), 2.01 – 2.10 (t, 2 H), 2.17 – 2.30 (m, 2 H), 3.54 – 3.72 (t, 2 H), 3.80 – 4.16 (m, 2 H), 5.23 – 5.31 (m, 1 H), 5.47 – 5.57 (m, 1 H), 5.69 – 5.79 (m, 1 H), 6.57 – 6.63 (d, 1 H), 6.94 – 6.99 (d, 1 H), 7.15 – 7.20 (s, 1 H), 7.23 – 7.34 (m, 3 H), 9.33 – 9.39 (d, 1 H). HRMS (ESI) calcd for $C_{24}H_{37}ClN_3O_6$: $[M+H]^+$: 498.2371 Found: 498.2388.

4.1.11. Synthesis of macrocyclic aldehydes 11–21 and 36. General procedure

—An appropriate alcohol (0.6 mmol) was dissolved in anhydrous dichloromethane (20 mL) under a nitrogen atmosphere and cooled to 0° C. Dess-Martin periodinane (1.2 mmol, 2.0 eq.) was added to the reaction mixture with stirring. The ice bath was removed and the reaction mixture was stirred at room temperature for 3 h (monitoring by TLC indicated complete disappearance of the starting material). A solution of 40 mM sodium thiosulfate in saturated aqueous $NaHCO_3$ (50 mL) was added and the solution was stirred for another 15 min. The aqueous layer was removed and the organic layer was washed with saturated sodium bicarbonate (25 mL), water (2 × 25 mL) and brine (25 mL). The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated. The yellow residue was purified by flash chromatography (silica gel/methylene chloride/ethyl acetate/methanol) to yield the aldehyde.

4.1.11.1. (4S, 7S)-4-Isobutyl-2,5,10-trioxo-17-phenyl-1-oxa-3,6,11-triazacycloheptadecane-7-carbaldehyde 11: White solid (yield 60%); m.p 130 – 131°C. ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 0.68 – 1.05 (d, 6 H), 1.40 – 1.73 (m, 5 H), 1.73 – 1.95 (m, 4 H), 1.95 – 2.21 (m, 6 H), 3.20 – 3.29 (t, 2 H), 4.45 – 4.53 (m, 2 H), 5.76 – 5.90 (m, 1 H), 7.17 – 7.35 (m, 5 H), 7.40 – 7.50 (d, 1 H), 8.25 – 8.35 (d, 1 H), 8. 72 – 8.80 (d, 1 H), 9.55 – 9.64 (m, 1 H). HRMS (ESI) calcd for $C_{24}H_{36}N_3O_5$: $[M+H]^+$: 446.2655 Found: 446.2658.

4.1.11.2. (4S, 7S)-17-Benzyl-4-isobutyl-2,5,10-trioxo-1-oxa-3,6,11-triazacycloheptadecane-7-carbaldehyde 12: White solid (yield 55%); m.p 82 – 83°C. ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 0.81 – 0.97 (d, 6 H), 1.53 – 1.69 (m, 6 H), 1,91 – 2.02 (m, 5H), 2.20 – 2.31 (4 H), 2.70 – 2.93 (m, 2 H), 3.21 – 3.31 (t, 2 H), 4.40 – 4.62 (m, 2 H), 4.88 – 5.04 (m, 1 H), 6.20 – 6.41 (br. d, 1 H), 6.91 – 7.10 (d, 1 H), 7.14 – 7.36 (m, 5 H), 7.85 – 7.92 (d, 1 H), 9.53 – 9.62 (s, 1 H). HRMS (ESI) calcd for $C_{25}H_{38}N_3O_5$: $[M+H]^+$: 460.2811 Found: 460.2813.

4.1.11.3. (4S, 7S)-17-(3-Chlorophenyl)-4-isobutyl-2,5,10-trioxo-1-oxa-3,6,11-triazacycloheptadecane-7-carbaldehyde 13: White solid (yield 75%); m.p 90 – 91°C. ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 0.74 – 0.99 (d, 6 H), 1.42 – 1.83 (m, 5 H), 2.11 – 2.43 (m, 6 H),2.47 – 2.64 (m, 2 H), 3.28 – 3.40 (t, 2 H), 3.4 – 3.5 (m, 2H), 4.50 – 5.60 (m, 1 H), 4.62 – 4.77 (m, 1 H), 5.91 – 6.03 (m, 1 H), 6.14 – 6.24 (d, 1 H), 6.96 – 7.34 (m, 4 H), 8.02 – 8.11 (d, 1 H), 8.60 – 8.65 (d, 1 H), 9.54 – 9.61 (s, 1 H). HRMS (ESI) calcd for $C_{24}H_{34}ClN_3NaO_5$: $[M+Na]^+$: 502.2085 Found: 502.2071.

4.1.11.4. (4S, 7S)-17-(3-Chlorophenyl)-4-(cyclohexylmethyl)-2,5,10-trioxo-1-oxa-3,6,11-triazacycloheptadecane-7-carbaldehyde 14A: White solid (yield 70%); m.p 71 – 72°C, ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ 0.66 – 0.93 (m, 12 H), 0.95 – 2.32 (m, 10 H), 3.18 – 3.21 (t, 2 H), 3.96 – 4.10 (t, 2 H), 4.29 – 4.51 (m, 1 H), 4.84 – 5.01 (m, 1 H), 5.94 – 6.11 (m, 1 H), 6.75 – 6.92 (d, 1 H), 7. 12 – 7.27 (m, 4 H), 8.25 – 8.40 (br s, 1 H), 8.82 – 8.90 (d, 1 H), 9.48 – 9.58 (s, 1 H). HRMS (ESI) calcd for $C_{27}H_{39}ClN_3O_5$: $[M+H]^+$: 520.2578 Found: 520.2771.

4.1.11.5. (4S, 7S)-17-(3-Chlorophenyl)-4-(cyclohexylmethyl)-2,5,10-trioxo-1-oxa-3,6,11-triazacycloheptadecane-7-carbaldehyde 14B: White solid **(**yield 70%); m.p 75 – 76°C. ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ 0.66 – 0.93 (m, 12 H), 0.95 – 2.32 (m, 10 H), 3.18 – 3.21 (t, 2 H), 3.96 – 4.10 (t, 2 H), 4.29 – 4.51 (m, 1 H), 4.84 – 5.01 (m, 1 H), 5.94 – 6.11 (m, 1 H), 6.75 – 6.92 (d, 1 H), 7. 12 – 7.27 (m, 4 H), 8.25 – 8.40 (br s, 1 H), 8.82 – 8.90 (d, 1 H), 9.48 – 9.58 (s, 1 H). HRMS (ESI) calcd for $C_{27}H_{38}ClN_3NaO_5$: $[M+Na]^+$: 542.2398 Found: 542.2506.

4.1.11.6. (4S, 7S)-18-(3-Chlorophenyl)-4-isobutyl-2,5,10-trioxo-1-oxa-3,6,11-triazacyclooctadecane-7-carbaldehyde 15: White solid (yield 68%); m.p 69 – 70°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.79 – 0.94 (d, 6 H), 1.15 – 1.30 (m, 4 H), 1.31 – 1.41 (m, 4 H), 1.61 – 1.81 (m, 5 H), 2.00 – 2.10 (m, 4 H), 3.18 – 3.27 (t, 2 H), 4.55 – 4.74 (m, 2 H), 5.75 – 5.82 (m, 1 H), 6.48 – 6.54 (t, 1 H), 7.21 – 7.46 (m, 4 H), 7.54 – 7.63 (d, 1 H), 8.53 – 8.59 (d, 1 H), 9.57 – 9.63 (s, 1 H). HRMS (ESI) calcd for $C_{25}H_{37}ClN_3O_5$: $[M+H]^+$: 494.2422 Found: 494.2446.

4.1.11.7. (4S, 7S)-17-(3-Chlorophenyl)-4-methyl-2,5,10-trioxo-1-oxa-3,6,11-triazacycloheptadecane-7-carbaldehyde 16: White solid (yield 70%); m.p 84 – 85°C. ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 0.79 – 0.91 (m, 2 H), 1.13 – 1.36 (m, 2 H), 1.44 – 1.48 (d, 3 H), 1.76 – 2.01 (m, 4 H), 2.19 – 2.70 (m, 4 H), 3.27 – 3.43 (m, 2 H), 4.33 – 4.40 (m, 1 H), 4.63 – 4.71 (m, 1 H), 5.81 – 5.93 (m, 1 H), 6.06 – 6.16 (m, 1 H), 6.57 – 6.65 (d, 1 H), 6.92 – 7.01 (d, 1 H), 7.10 – 7.40 (m, 4 H), 9.56 – 9.64 (s, 1 H). HRMS (ESI) calcd for $C_{21}H_{29}ClN_3O_5$: $[M+H]^+$: 438.1796 Found: 438.2153.

4.1.11.8. (4S, 7S)-18-(3-Chlorophenyl)-4-methyl-2,5,10-trioxo-1-oxa-3,6,11-triazacyclooctadecane-7-carbaldehyde 17: White solid (yield 64%); m.p 123 – 124°C. ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 0.83 – 0.91 (m, 2 H), 1.17 – 1.38 (m, 4 H), 1.42 – 1.47 (d, 3 H), 1.80 – 2.00 (m, 4 H), 2.21 – 2.65 (m, 4 H), 3.30 – 3.41 (m, 2 H), 4.35 – 4.43 (m, 1 H), 4.61 – 4.71 (m, 1 H), 5.83 – 5.93 (m, 1 H), 6.06 – 6.14 (m, 1 H), 6.67 – 6.75 (d, 1 H), 6.81 – 6.91 (d, 1 H), 7.11 – 7.35 (m, 4 H), 9.61 – 9.71 (s, 1 H). HRMS (ESI) calcd for $C_{22}H_{31}ClN_3O5$: $[M+H]^+$: 452.1952 Found: 452.2315.

4.1.11.9. (20S)-10-(3-Chlorophenyl)-8,17,22-trioxo-9-oxa-7,16,21-triazaspiro[5.16]docosane-20-carbaldehyde 18: White solid (yield 63%); m.p 110 – 111°C. ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 0.91 – 1.41 (m, 9 H), 1.51 – 1.70 (m, 5 H), 2.12 – 2.16 (m, 2 H), 2.20 – 2.50 (m, 4 H), 2.87 – 3.03 (m, 2 H), 4.50 – 4.60 (m, 1 H), 5.02 – 5.10 (m, 1 H), 5.68 – 5.85 (m, 1 H), 6.30 – 6.41 (d, 1 H), 7.18 – 7.34 (m, 4 H), 7.69 – 7.74

(d, 1 H), 8.23 – 8.30 (d, 1 H), 9.58 – 9.65 (s, 1 H). HRMS (ESI) calcd for $C_{25}H_{35}ClN_3O_5$: [M+H] $^+$: 492.2265 Found: 492.2293.

4.1.11.10. (21S)-10-(3-Chlorophenyl)-8,18,23-trioxo-9-oxa-7,17,22-triazaspiro [5.17] tricosane-21-carbaldehyde 19: White solid (yield 65%); m.p 87 – 88°C. ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 0.90 – 1.36 (m, 9 H), 1.53 – 1.72 (m, 7 H), 2.10 – 2.14 (m, 2 H), 2.14 – 2.54 (m, 4 H), 2.86 – 3.02 (m, 2 H), 4.42 – 4.60 (m, 1 H), 5.00 – 5.11 (m, 1 H), 5.66 – 5.81 (m, 1 H), 6.15 – 6.23 (d, 1 H), 7.19 – 7.36 (m, 4 H), 7.89 – 7.94 (d, 1 H), 8.10 – 8.16 (d, 1 H), 9.55 – 9.62 (s, 1 H). HRMS (ESI) calcd for $C_{26}H_{37}ClN_3O_5$: [M+H] $^+$: 506.2422 Found: 506.2440.

4.1.11.11. (4S, 7S)-4-Isobutyl-2,5,10-trioxo-17-(3-phenylpropyl)-1-oxa-3,6,11-triazacycloheptadecane-7-carbaldehyde 20: White solid (yield 60%); m.p 163 – 164°C. ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 0.86 – 1.03 (d, 6 H), 1.15 – 1.81 (m, 10 H), 2.09 – 2.19 (m, 9 H), 2.54 – 2.67 (t, 2 H), 3.02 – 3.14 (t, 2 H), 3.95 – 4.05 (d, 1 H), 4.65 – 4.73 (m, 1 H), 4.90 – 5.00 (d, 2 H), 6.09 – 6.17 (d, 1 H), 6.36 – 6.44 (d, 1 H), 7.12 – 7.22 (d, 2 H), 7.24 – 7.32 (m, 3 H), 9.56 – 9.60 (s, 1 H). HRMS (ESI) calcd for $C_{27}H_{41}N_3O_5$: [M +H] $^+$: 487.3046 Found: 487.3051.

4.1.11.12. (4S, 7S)-4-Isobutyl-2,5,10-trioxo-17-pentyl-1-oxa-3,6,11-triazacycloheptadecane-7-carbaldehyde 21: White solid (yield 61%), m.p 172 – 173°C. ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 0.83 – 0.91 (t, 2 H),0.92 – 1.01 (d, 6 H), 1.20 – 1.34 (m, 8 H), 1.38 – 1.84 (m, 9 H), 2.24 – 2.51 (m, 4 H), 3.15 – 3.27 (m, 1 H), 3.39 – 3.49 (m, 1 H), 3.97 – 4.07 (m, 1 H), 4.63 – 4.73 (m, 2 H), 4.85 – 4.96 (m, 2 H), 4.96 – 5.03 (d, 1 H), 6.14 – 6.22 (d, 1 H), 6.49 – 6.56 (d, 1 H), 9.56 – 9.63 (s, 1 H). HRMS (ESI) calcd for $C_{23}H_{42}N_3O_5$: [M+H] $^+$: 440.3124 Found: 440.3163.

4.1.11.13. (6S, 9S)-13-(3-Chlorophenyl)-9-isobutyl-3,8,11-trioxo-1,12-dioxa-2,7,10-triazacyclooctadecane-6-carbaldehyde 36A: White solid (yield (30%); m.p 62 – 63°C. ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 0.83 – 1.00 (d, 6 H), 1.21 – 1.29 (m, 4 H), 1.44 – 1.96 (m, 7 H), 2.36 – 2.77 (m, 4 H), 4.00 – 4.22 (t, 2 H), 5.08 – 5.11 (m, 1 H), 5.45 – 5.60 (m, 1 H), 6.14 – 6.22 (d, 1 H), 6.49 – 6.56 (d, 1 H), 7.12 – 7.20 (s, 1 H),7.23 – 7.33 (m, 3 H),7.39 – 7.44 (d, 1 H), 9.59 – 9.63 (d, 1 H), 9.72 – 9.77 (s, 1 H). HRMS (ESI) calcd for $C_{24}H_{34}ClN_3O_6$: [M] $^+$: 495.2136 Found: 495.2279.

4.1.11.14. (6S, 9S)-13-(3-Chlorophenyl)-9-isobutyl-3,8,11-trioxo-1,12-dioxa-2,7,10-triazacyclooctadecane-6-carbaldehyde 36B: White solid (yield 50%); m.p 78 – 79°C. ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 0.81 – 0.99 (d, 6 H), 1.23 – 1.31 (m, 4 H), 1.44 – 1.94 (m, 7 H), 2.36 – 2.70 (m, 4 H), 3.58 – 3.64 (t, 2 H), 4.00 – 4.16 (m, 2 H), 4.25 – 4.34 (m, 1 H), 5.50 – 5.63 (m, 1 H), 6.60 – 6.70 (d, 1 H), 7.12 – 7.22 (s, 1 H),7.23 – 7.34 (m, 3 H),7.85 – 7.91 (d, 1 H), 8.22 – 8.27 (d, 1 H), 9.72 – 9.76 (s, 1 H). HRMS (ESI) calcd for $C_{24}H_{34}ClN_3O_6$: [M] $^+$: 495.2136 Found: 495.2283.

4.1.12. Synthesis of alcohols 22b and 22d. General procedure—To a solution of representative ester (5 mmol) in anhydrous THF (30 mL) was added lithium borohydride (2M in THF, 7.5 mL, 15 mmol) dropwise, followed by absolute ethyl alcohol (15 mL), and

the reaction mixture was stirred at room temperature overnight. The reaction mixture was then acidified by adding 5% HCl and the pH adjusted to ~2. Removal of the solvent left a residue which was taken up in ethyl acetate (100 mL). The organic layer was washed with brine (25 mL), dried over anhydrous sodium sulfate, filtered, and concentrated to yield compounds ***22b*** and ***22d*** as white solids.

4.1.12.1. (4S, 7S, E)-17-Benzyl-7-(hydroxymethyl)-4-isobutyl-1-oxa-3,6,11-triazacycloheptadec-13-ene-2,5,10-trione 22b: Sticky solid **(**yield 83%); ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 0.81 – 0.97 (d, 6 H), 1.53 – 1.69 (m, 4 H), 1,91 – 2.02 (m, 5 H), 2.70 – 2.93 (m, 2 H), 3.53 – 3.64 (m, 2 H), 3.80 – 3.94 (m, 2 H), 4.40 – 4.62 (m, 2 H), 4.88 – 5.04 (m, 1 H), 5.06 – 5.26 (m, 2 H), 5.68 – 5.87 (m, 2 H), 6.20 – 6.41 (br. d, 1 H), 6.91 – 7.10 (d, 1 H), 7.14 – 7.36 (m, 5 H), 7.85 – 7.92 (d, 1 H). HRMS (ESI) calcd for $C_{25}H_{38}N_3O_5$: [M +H] $^{+}$: 460.2811 Found: 460.2831.

4.1.12.2. (4S, 7S, E)-17-(3-Chlorophenyl)-4-(cyclohexylmethyl)-7-(hydroxymethyl)-1-oxa-3,6,11-triazacycloheptadec-13-ene-2,5,10-trione 22d: Sticky solid (yield 80%); ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 0.66 – 0.93 (m, 11 H), 0.95 – 2.32 (m, 10 H), 3.51 – 3.61 (m, 2 H), 3.74 – 3.86 (d, 2 H), 4.00 – 4.20 (m, 1 H), 4.29 – 4.51 (m, 1 H), 4.84 – 5.01 (m, 1 H), 5.42 – 5.83 (m, 2 H), 5.94 – 6.11 (m, 1 H), 6.22 – 6.31 (d, 1 H), 6.75 – 6.92 (d, 1 H), 7.04 – 7.27 (m, 4 H). HRMS (ESI) calcd for $C_{27}H_{38}ClN_3O_5$: [M+H] $^{+}$: 519.2500 Found: 519.2561.

4.1.13. Synthesis of aldehydes 23 and 24. General procedure—Representative alcohol (0.6 mmol) was dissolved in anhydrous dichloromethane (20 mL) under a nitrogen atmosphere and cooled to 0°C. Dess-Martin periodinane (0.75 g, 1.78 mmol, 3.0 eq.) was added to the reaction mixture with stirring. The ice bath was removed and the reaction mixture was stirred at room temperature for 3 h (monitoring by TLC indicated complete disappearance of the starting material). A solution of 40 mM sodium thiosulfate in saturated aqueous $NaHCO_3$ (50 mL) was added and the solution was stirred for another 15 min. The aqueous layer was removed and the organic layer was washed with sodium bicarbonate (25 mL), water (2 × 25 mL) and brine (25 mL). The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated. The yellow residue was purified by flash chromatography (silica gel/methylene chloride/ethyl acetate/methanol) to yield the desired aldehyde.

4.1.13.1 (4S, 7S, E)-17-Benzyl-4-isobutyl-2,5,10-trioxo-1-oxa-3,6,11-triazacycloheptadec-13-ene-7-carbaldehyde 23: White solid (yield 53%); m.p 88 – 89°C. ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 0.83 – 0.98 (d, 6 H), 1.55 – 1.70 (m, 4 H), 1,93 – 2.04 (m, 5 H), 2.71 – 2.93 (m, 2 H), 3.81 – 3.95 (m, 2 H), 4.41 – 4.61 (m, 2 H), 4.90 – 5.05 (m, 1 H), 5.08 – 5.27 (m, 2 H), 5.69 – 5.89 (m, 2 H), 6.20 – 6.37 (br. d, 1 H), 6.95 – 7.11 (d, 1 H), 7.14 – 7.36 (m, 5 H), 7.85 – 7.92 (d, 1 H), 9.54 – 9.67 (s, 1 H). HRMS (ESI) calcd for $C_{25}H_{36}N_3O_5$: [M+H] $^{+}$: 458.2655 Found: 458.2666.

4.1.13.2. (4S, 7S, E)-17-(3-Chlorophenyl)-4-(cyclohexylmethyl)-2,5,10-trioxo-1-oxa-3,6,11-triazacycloheptadec-13-ene-7-carbaldehyde 24: White solid (yield 59%); m.p 83 – 84°C.^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ ppm 0.68 – 0.95 (m, 11 H), 0.99 – 2.34 (m, 10

H), 3.74 – 3.86 (d, 2 H), 4.00 – 4.20 (m, 1 H), 4.29 – 4.51 (m, 1 H), 4.84 – 5.01 (m, 1 H), 5.42 – 5.83 (m, 2 H), 5.94 – 6.11 (m, 1 H), 6.22 – 6.31 (d, 1 H), 6.75 – 6.92 (d, 1 H), 7.04 – 7.27 (m, 4 H), 9.56 – 9.63 (s, 1 H). HRMS (ESI) calcd for $C_{27}H_{37}ClN_3O_5$: $[M+H]^+$: 518.2422 Found: 518.2430.

4.1.14. Synthesis of aldehyde bisulfite salts 25–29. General procedure—To a solution of aldehydes ***13, 18, 19***, *or* ***21*** (0.52 mmol) in dry ethyl acetate (3.5 mL) was added absolute ethanol (1.8 mL) with stirring, followed by a solution of sodium bisulfite (55 mg; 0.52 mmol) in water (0.5 mL). The reaction mixture was stirred for 3 h at 50 °C. The reaction mixture was allowed to cool to room temperature and then vacuum filtered. The solid was thoroughly washed with absolute ethanol and the filtrate was dried over anhydrous sodium sulfate, filtered, and concentrated to yield yellowish oil. The oily product was treated with ethyl ether (2 × 10 mL) to form white solid. The white solid was stirred with ethyl ether (5 mL) and ethyl acetate (2.5 mL) for 5 minutes. Careful removal of the solvent using a pipette left compound as a white solid.

4.1.14.1. Sodium ((4S, 7S)-17-(3-chlorophenyl)-4-isobutyl-2,5,10-trioxo-1-oxa-3,6,11-triazacycloheptadecan-7-yl) (hydroxy) methanesulfonate 25: White solid (yield 77%); m.p 145 – 146°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.81 – 0.97 (d, 6 H), 1.42 – 1.83 (m, 5 H), 2.11 – 2.43 (m, 6 H), 2.47 – 2.64 (m, 2 H), 3.28 – 3.40 (t, 2 H), 3.40 – 3.50 (m, 2H), 4.50 – 4.60 (m, 1 H), 4.62 – 4.77 (m, 1 H), 5.23 – 5.31 (br. s, 1 H), 5.62 – 5.71 (m, 1 H), 5.91 – 6.03 (m, 1 H), 6.14 – 6.24 (d, 1 H), 6.96 – 7.34 (m, 4 H), 8.02 – 8.11 (d, 1 H), 8.60 – 8.65 (d, 1 H). HRMS (ESI) calcd for $C_{24}H_{35}ClN_3O_8S^-$: $[M]^-$: 560.1839 Found: 560.1925.

4.1.14.2. Sodium ((20S)-10-(3-chlorophenyl)-8,17,22-trioxo-9-oxa-7,16,21-triazaspiro [5.16] docosan-20-yl) (hydroxy) methanesulfonate 26: White solid (yield 75%); m.p 148 – 149°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.07 – 1.23 (m, 7 H), 1.23 – 1.98 (m, 9 H), 2.09 – 2.27 (m, 6 H), 3.42 – 3.56 (m, 3 H), 5.12 – 5.18 (br. s, 1 H), 5.43 – 5.66 (m, 1 H), 5.78 – 5.82 (m, 1 H), 6.97 – 7.08 (d, 1 H), 7.08 – 7.19 (d, 1 H), 7.24 – 7.46 (m, 4 H), 8.11 – 8.17 (d, 1H). HRMS (ESI) calcd for $C_{25}H_{35}ClN_3O_8S^-$: $[M]^-$: 572.1839 Found: 572.1849.

4.1.14.3. Sodium ((21S)-10-(3-chlorophenyl)-8,18,23-trioxo-9-oxa-7,17,22-triazaspiro[5.17] tricosan-21-yl) (hydroxy) methanesulfonate 27: White solid (yield 65%); m.p 145 – 146°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.90 – 1.36 (m, 11 H), 1.53 – 1.72 (m, 7 H), 2.10 – 2.14 (m, 2 H), 2.14 – 2.54 (m, 4 H), 2.86 – 3.02 (m, 2 H), 4.42 – 4.60 (m, 1 H), 5.00 – 5.11 (m, 1 H), 5.13 – 5.20 (br. s, 1 H), 5.66 – 5.81 (m, 1 H), 6.15 – 6.23 (d, 1 H), 7.19 – 7.36 (m, 4 H), 7.89 – 7.94 (d, 1 H), 8.10 – 8.16 (d, 1 H). HRMS (ESI) calcd for $C_{26}H_{37}ClN_3O_8S^-$: $[M]^-$: 586.1995 Found: 586.2064.

4.1.14.4. Sodium hydroxyl ((4S, 7S)-4-isobutyl-2,5,10-trioxo-17-(3-phenylpropyl)-1-oxa-3,6,11-triazacycloheptadecan-7-yl) methanesulfonate 28: White solid (yield 70%); m.p 173 – 174°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.90 – 1.03 (d, 6 H), 1.15 – 1.81 (m, 10 H), 2.09 – 2.19 (m, 9 H), 2.53 – 2.64 (t, 2 H), 3.08 – 3.19 (t, 2 H), 3.50 – 3.65 m, 1 H), 4.65 – 4.73 (m, 1 H), 4.90 – 5.00 (d, 2 H), 5.21 – 5.30 (s, 1 H), 5.68 – 5.70 (d, 1 H), 6.36

– 6.44 (d, 1 H), 7.12 – 7.22 (d, 2 H), 7.24 – 7.32 (m, 3 H), 8.32 – 8.40 (d, 1 H). HRMS (ESI) calcd for $C_{26}H_{37}ClN_3O_8S^-$: [M] $^-$: 586.1995 Found: 586.2064.

4.1.14.5. Sodium hydroxyl ((4S, 7S)-4-isobutyl-2,5,10-trioxo-17-pentyl-1-oxa-3,6,11-triazacycloheptadecan-7-yl) methanesulfonate 29: White solid (yield 75%); m.p 143 – 144°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.85 – 0.92 (t, 2 H), 0.93 – 1.04 (d, 6 H), 1.21 – 1.35 (m, 8 H), 1.40 – 1.85 (m, 9 H), 2.22 – 2.50 (m, 4 H), 3.15 – 3.27 (m, 1 H), 3.39 – 3.49 (m, 1 H), 3.97 – 4.07 (m, 1 H), 4.63 – 4.73 (m, 2 H), 4.85 – 4.96 (m, 2 H), 4.96 – 5.03 (d, 1 H), 5.21 – 5.30 (br. s, 1 H), 5.67 – 5.71 (d, 1 H), 6.14 – 6.22 (d, 1 H), 6.49 – 6.56 (d, 1 H), 8.12 – 8.17 (d, 1 H). HRMS (ESI) calcd for $C_{23}H_{42}ClN_3O_8S^-$: [M] $^-$: 520.2698 Found: 520.2715.

4.1.15. Synthesis of compound 30.—A solution of aldehyde ***13*** (1.25 mmol) in ethyl acetate (10 mL) kept at 0° C was treated with acetic acid (90 mg; 1.44 mmol) followed by cyclopropyl isocyanide (92 mg; 1.375 mmol), and the reaction mixture was stirred at room temperature for 18 h. The solution was concentrated in vacuo and the residue was dissolved in methanol (10 mL) and treated with a solution of K_2CO_3 (0.4 g; 2.95 mmol) in water (7 mL). The reaction mixture was stirred at room temperature for 2 h. Methanol was evaporated off and the aqueous layer was extracted with ethyl acetate (3 × 20 mL). The combined organic layers were washed with 5% HCl (2 × 20 mL) and brine (25 mL). The organic layer was dried over anhydrous sodium sulfate and the solvent was removed to yield compound ***30*** as a off-white solid. This was used in the next step without further purification.

4.1.15.1. 2-((4S, 7S)-17-(3-Chlorophenyl)-4-isobutyl-2,5,10-trioxo-1-oxa-3,6,11-triazacycloheptadecan-7-yl)-N-cyclopropyl-2-hydroxyacetamide 30: Off-white solid (yield (57%); m.p 189 – 190°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.37 – 0.48 (m, 2 H), 0.51 – 0.63 (m, 2 H), 0.73 – 0.96 (d, 6 H), 1.06 – 1.86 (m, 11 H), 2.11 – 2.33 (m, 4 H), 2.60 – 2.75 (m, 1 H), 3.18 – 3.23 (t, 2 H), 3.97 – 4.16 (m, 1 H), 4.48 – 4.59 (m, 1 H), 4.87 – 4.94 (m, 1 H), 5.72 – 5.87 (m, 1 H), 6.26 – 6.44 (s, 1 H), 6.82 – 6.99 (d, 1 H), 6.99 – 7.08 (d, 1 H), 7.22 – 7.51 (m, 4 H), 7.60 – 7.65 (m, 1 H), 7.65 – 7.80 (d, 1 H). HRMS (ESI) calcd for $C_{28}H_{42}ClN_4O_6$: [M+H] $^+$: 565.2793 Found: 565.2811.

4.1.16. Synthesis of α-ketoamide 31—To a solution of **c**ompound ***30*** (0.62 mmol) in anhydrous dichloromethane (20 mL) cooled to 0° C and kept under a nitrogen atmosphere was added Dess-Martin periodinane reagent (0.53 g, 1.24 mmol, 2.0 eq) with stirring. The ice bath was removed and the reaction mixture was stirred at room temperature for 3 h. The reaction was monitored by TLC until the starting material disappeared. A solution of 10 % aqueous sodium thiosulfate (20 mL) was added and the solution was stirred for 15 min. The solution was poured into a separatory funnel and the aqueous layer was removed. The organic layer was washed with 10 % aqueous sodium thiosulfate (20 mL), followed by saturated aqueous sodium bicarbonate (2 × 20 mL), water (2 × 20 mL) and brine (20 mL). The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated leaving a yellow solid which was purified by flash chromatography (silica gel/methylene chloride/ethyl acetate/methanol) to yield ***31*** as a white solid.

4.1.16.1. 2-((4S, 7S)-17-(3-Chlorophenyl)-4-isobutyl-2,5,10-trioxo-1-oxa-3,6,11-triazacycloheptadecan-7-yl)-N-cyclopropyl-2-oxoacetamide 31: White solid (yield 50%); m.p 210°C (d). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.34 – 0.44 (m, 2 H), 0.49 – 0.60 (m, 2 H), 0.83 – 0.96 (d, 6 H), 1.12 – 1.84 (m, 11 H), 2.10 – 2.30 (m, 4 H), 2.63 – 2.75 (m, 1 H), 3.18 – 3.23 (t, 2 H), 4.00 – 4.16 (m, 1 H), 4.49 – 4.57 (m, 1 H), 4.86 – 4.92 (m, 1 H), 5.75 – 5.87 (m, 1 H), 6.80 – 6.90 (d, 1 H), 6.98 – 7.08 (d, 1 H), 7.21 – 7.50 (m, 4 H), 7.62 – 7.68 (m, 1 H), 7.70 – 7.80 (d, 1 H). HRMS (ESI) calcd for $C_{28}H_{40}ClN_4O_6$: $[M+H]^+$: 563.2636 Found: 563.2660.

4.2. Biochemical studies. FRET protease assays

The FRET protease assay 3CLpro was performed by preparing stock solutions of the substrate (Edans-DFHLQ/GP-Dabcyl) and inhibitor in DMSO and diluting into assay buffer which was comprised of 20mM HEPES buffer, pH 8, containing NaCl (200 mM), 0.4 mM EDTA, glycerol (60%), and 6 mM dithiothreitol (DTT). The protease was mixed with serial dilutions of each compound up to 100 μM or with DMSO in 25 μL of assay buffer and incubated at 37°C for 30 min, followed by the addition of 25 μL of assay buffer containing substrate. Fluorescence readings were obtained using an excitation wavelength of 360 nm and an emission wavelength of 460 nm on a fluorescence microplate reader (FLx800; Biotec, Winoosk, VT) 1 h following the addition of substrate. Relative fluorescence units (RFU) were determined by subtracting background values (substrate-containing well without protease) from the raw fluorescence values, as described previously [33,47–49]. The dose-dependent FRET inhibition curves were fitted with a variable slope by using GraphPad Prism software (GraphPad, La Jolla, CA) in order to determine the IC_{50} values of the inhibitors.

4.3. Cell-based inhibition assays

The effects of each inhibitor on virus replication were examined against NV or MNV in the NV replicon harboring cells (HG23 cells) or RAW264.7 cells, respectively [33]. Briefly, confluent and semi-confluent HG23 cells were incubated with medium containing DMSO (<0.1%) or each compound (up to 20 μM) for 48 h for NV. After the incubation, total RNA was extracted and viral genome was quantitated with real-time quantitative RT-PCR (qRT-PCR). For MNV, confluent RAW264.7 cells were inoculated with MNV at a multiplicity of infection (MOI) of 0.1 for 1 h, and the inoculum was replaced with medium containing DMSO (0.1%) or each compound (up to 20 μM). The virus-infected cells were further incubated for 24 h, and the replication of virus was measured by the 50% tissue culture infective dose ($TCID_{50}$) method. The EC_{50} values were determined by GraphPadPrism software [33].

4.4. Nonspecific cytotoxic effects

The cytotoxic dose for 50% cell death (CC_{50}) for each compound was determined for HG23 cells used in this study. Confluent cells grown in 96-well plates were treated with various concentrations (1 to 100 μM) of each compound for 48 h. Cell cytotoxicity was measured by a CytoTox 96 nonradioactive cytotoxicity assay kit (Promega, Madison, WI) and crystal

violet staining. The in vitro therapeutic index was calculated by dividing the CC_{50} by the EC_{50}.

4.5. X-ray crystallographic studies. Crystallization and Data Collection

Purified NV 3CL pro in 100 mM NaCl, 50 mM PBS pH 7.2, 1 mM DTT at a concentration of 10 mg/mL was used for preparation of the NV 3CLpro:inhibitor complexes. A 100 mM stock solution of the inhibitors was prepared in DMSO and the NV 3CLpro:inhibitor complex was prepared by mixing 9 μL of inhibitor (3 mM) with 291 μL (0.49 mM) of NV 3CLpro and incubating on ice for 1 h. The buffer was exchanged to 100 mM NaCl, 20 mM Tris pH 8.0 in a Vivaspin-20 (MWCO=5kDa, Vivaproducts, Inc.) concentrator and the sample was concentrated to 10.0 mg/mL for crystallization screening. All crystallization experiments were conducted Compact Jr. (Rigaku Reagents) sitting drop vapor diffusion plates at 20 °C using equal volumes of protein and crystallization solution equilibrated against 75 μL of the latter. Crystals of the inhibitor ***13*** complex (NV 3CLpro:***13***), that displayed a prismatic morphology, were obtained from the Index HT screen (Hampton Research) condition H12 (30% (w/v) PEG 2000 MME, 150 potassium bromide) in 1–2 days. Crystals of the complex with inhibitor ***21*** (NV 3CLpro:***21***) were also obtained from the Index HT G5 condition. Samples were transferred to a fresh drop composed of 80% crystallization solution and 20% PEG 400 and stored in liquid nitrogen. X-ray diffraction data were collected at the Advanced Photon Source IMCA-CAT beamline 17-ID using a Dectris Pilatus 6M pixel array detector.

4.6. Structure Solution and Refinement

Intensities were integrated using XDS [50–51] via Autoproc [52] and the Laue class analysis and data scaling were performed with Aimless [53] which suggested that the highest probability Laue class was $2/m$ and space group $C2$. The Matthew's coefficient [54] suggested that there was a single molecule in the asymmetric unit (Vm=1.8 $Å^3$/Da, % solvent=32%). Structure solution was conducted by molecular replacement with Phaser [55] using a previously determined structure of inhibitor bound NV 3CLpro (PDB: 3UR9) [33] as the search model. Structure refinement using and manual model building were conducted with Phenix [56] and Coot [57] respectively. Anisotropic atomic displacement parameters were refined for all atoms except solvent molecules for the NV 3CLpro:***13*** structure. Disordered side chains were truncated to the point for which electron density could be observed. Structure validation was conducted with Molprobity [58] and figures were prepared using the CCP4MG package [59].

4.7. Accession Codes

Coordinates and structure factors were deposited to the Worldwide Protein Databank (wwPDB) with the accession codes 5TG1 (NV 3CLpro:***13***) and 5TG2 (NV 3CLpro:***21***).

Acknowledgments

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Macromolecular Crystallography Association through a contract with Hauptman-Woodward Medical Research Institute. Use of the Advanced Photon Source was supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences under contract no. DE-AC02-06CH11357.

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- A novel series of macrocyclic inhibitors of norovirus 3CL protease inhibitors is reported.
- High resolution crystal structures of enzyme-inhibitor complexes have confirmed the mechanism of action.
- The structural determinants involved in binding have been delineated.
- The inhibitor design rationale was validated by x-ray crystallographic studies.

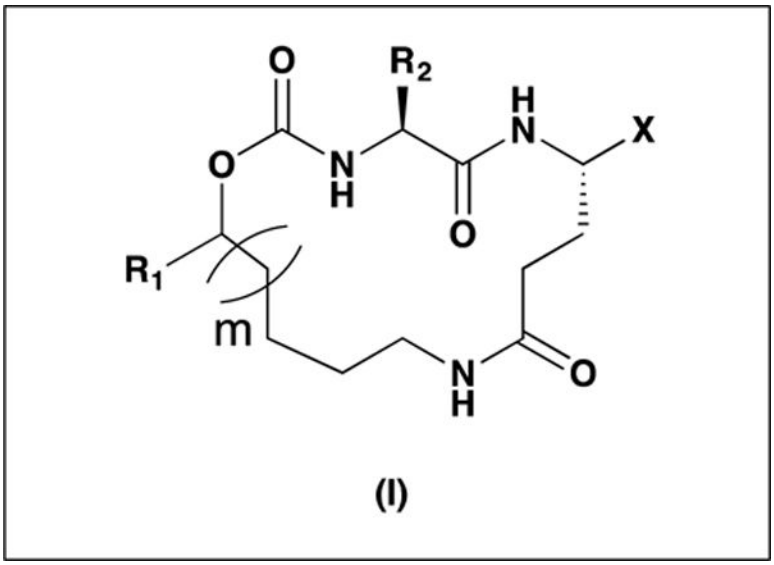


Fig. 1.
General structure of macrocyclic inhibitor ***(I)***

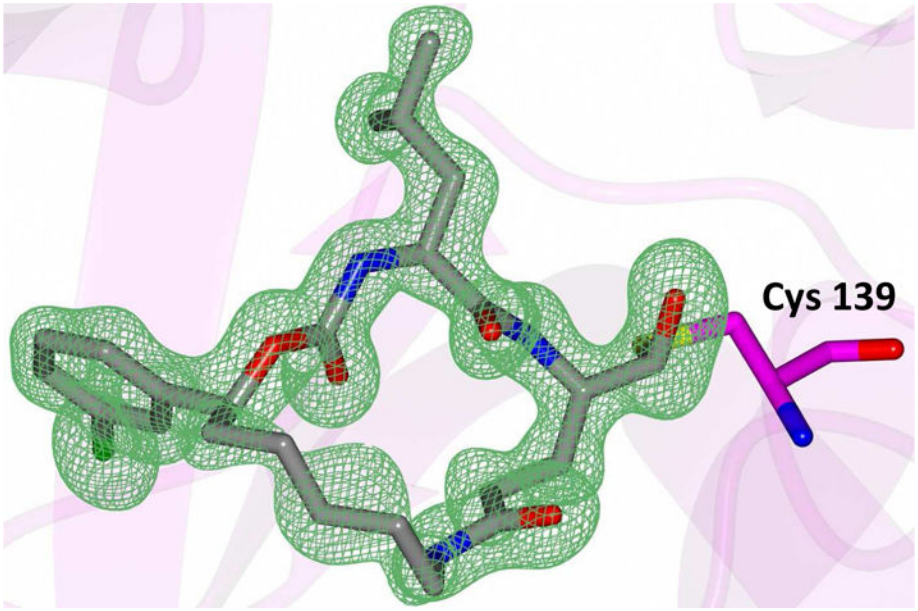


Fig. 2.

View of the F_o–F_c omit map for inhibitor ***13*** (green mesh) contoured at 3σ

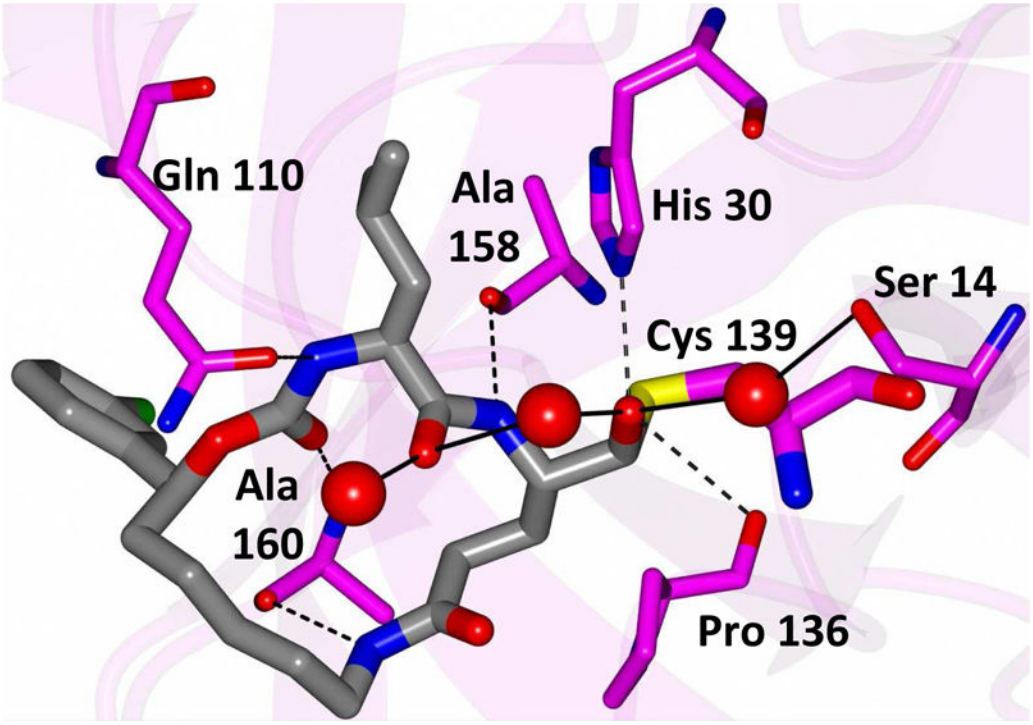


Fig. 3.

Hydrogen bond interactions (dashed lines) between NV 3CLpro and inhibitor ***13***. Contacts to water molecules are indicated by the solid lines

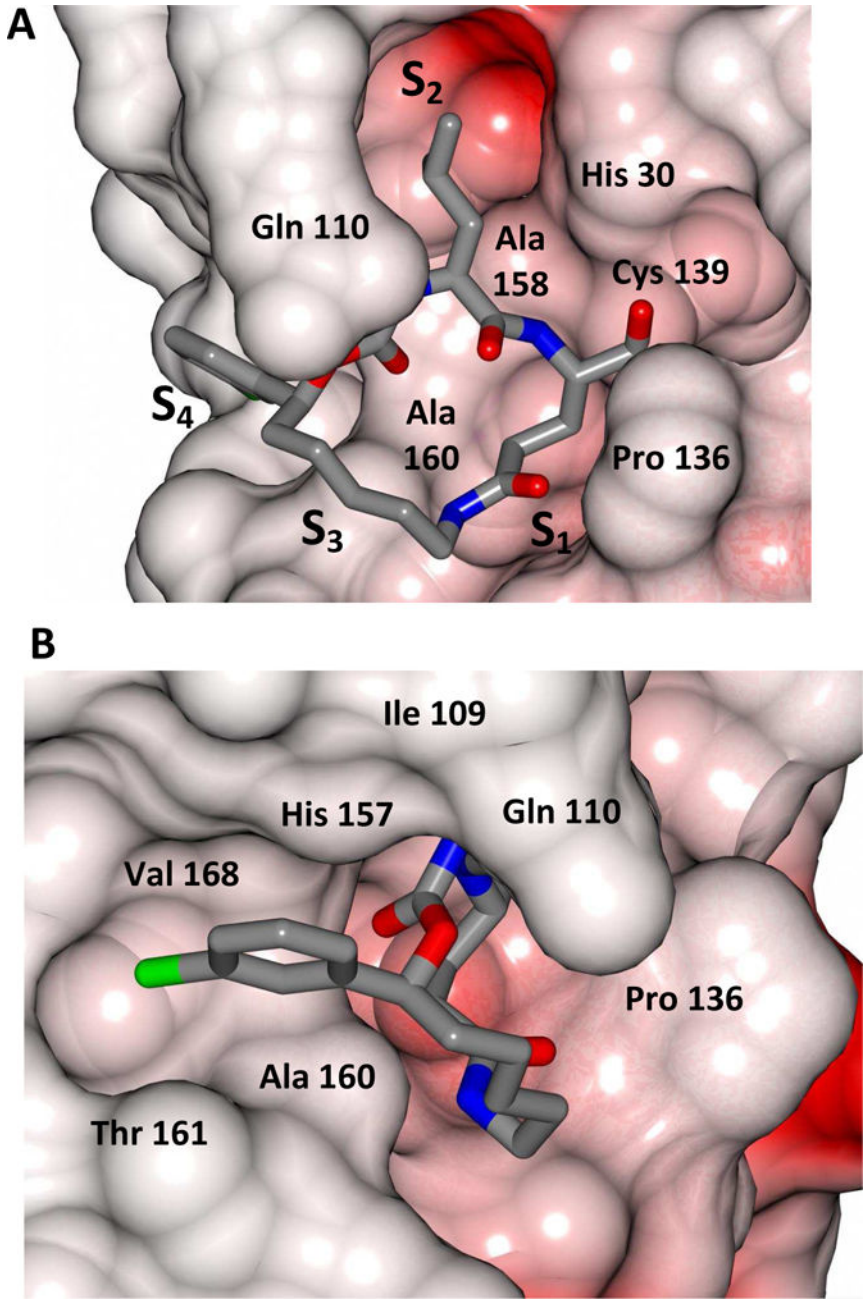


Fig. 4.

Two views showing the electrostatic surface representation NV 3CLpro binding site of inhibitor ***13.*** **A**) View of the active site and **B**) the *m*-chlorophenyl group in the hydrophobic S_4 pocket. View is rotated counterclockwise approximately 90° about the vertical axis relative to panel A.

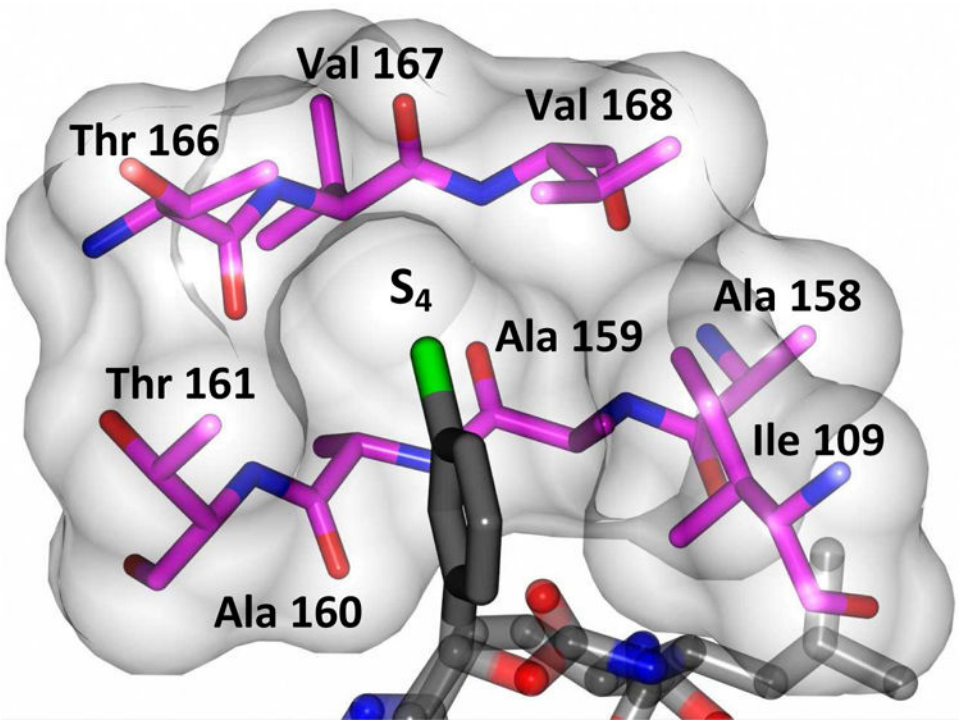


Fig. 5.

View of the m-chlorophenyl of inhibitor ***13*** which is positioned in a hydrophobic pocket of NV 3CLpro.

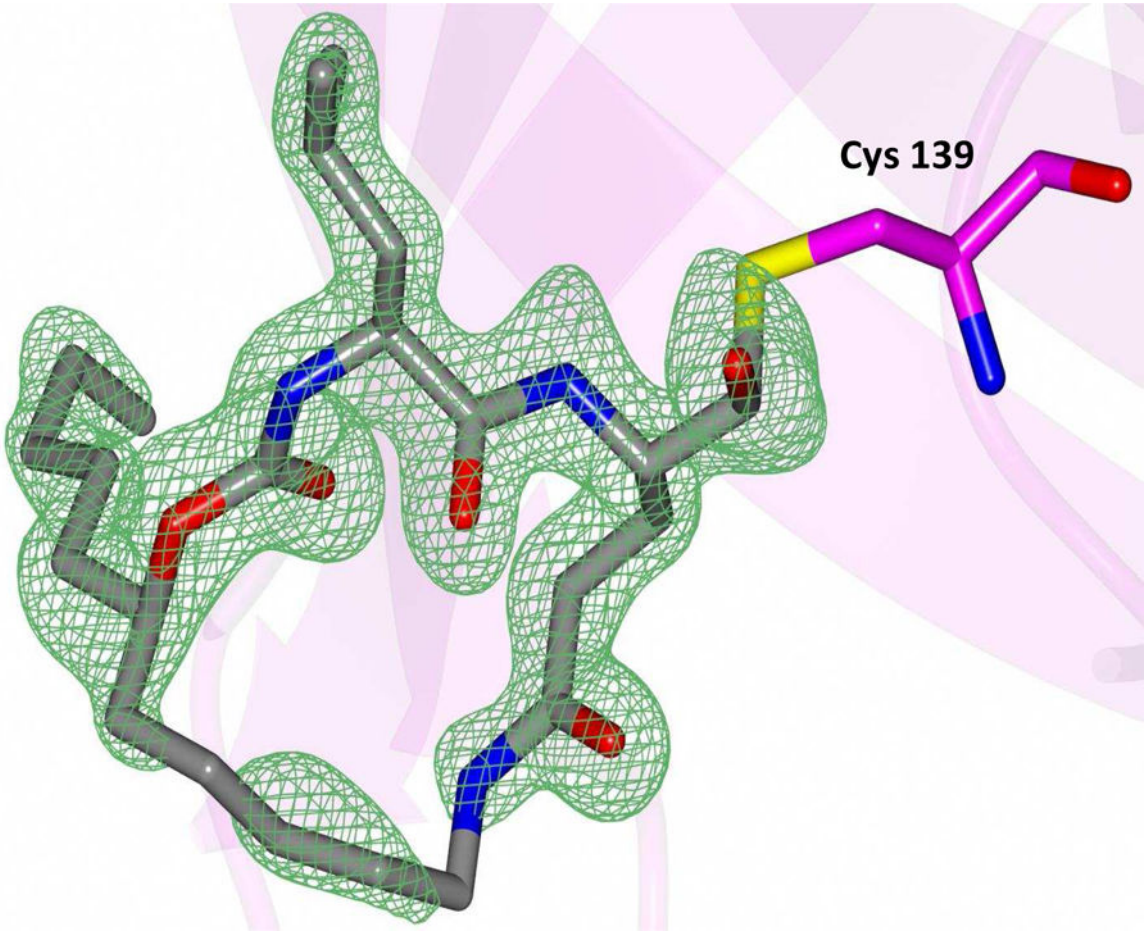


Fig. 6.

F_o–F_c omit map of inhibitor ***21*** (green mesh) contoured at 3σ.

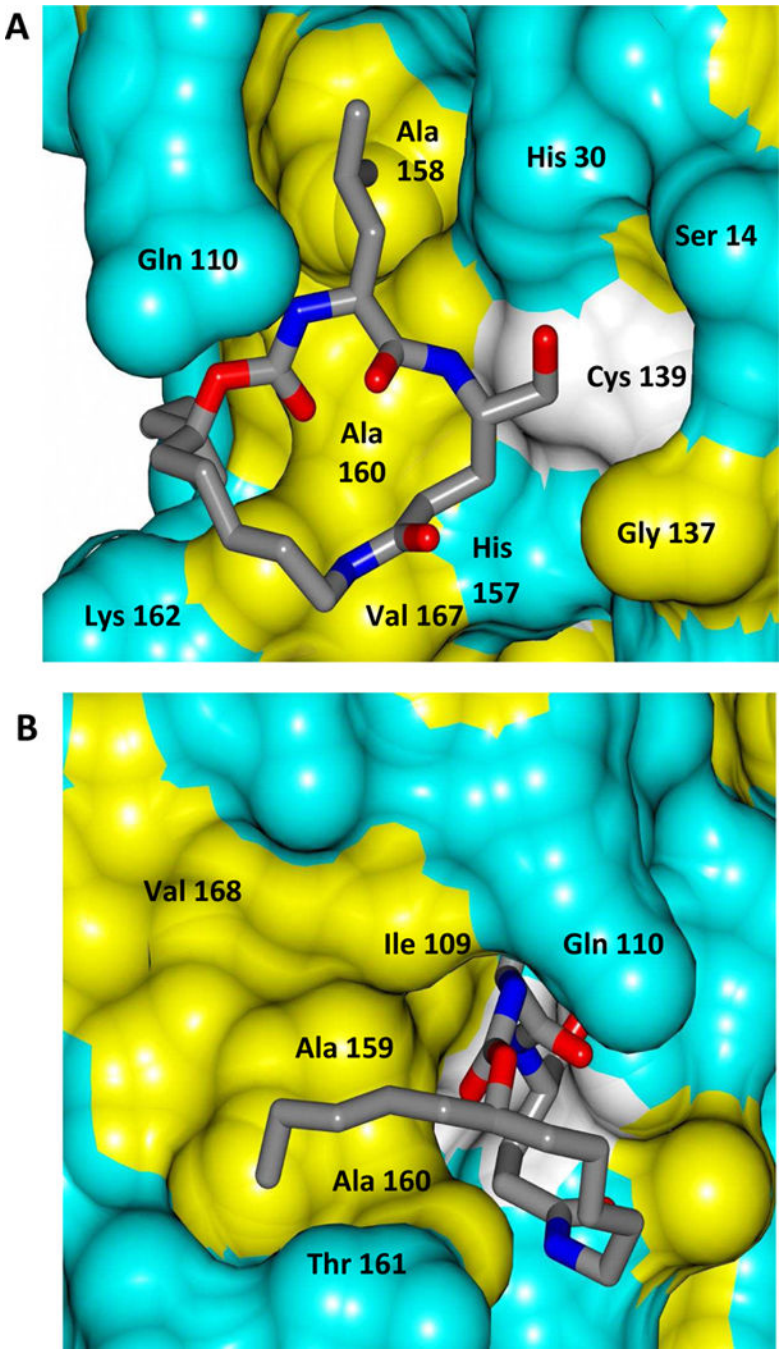


Fig. 7.

Surface representation of NV 3CL protease with bound inhibitor ***21*** site and with neighboring residues colored yellow (nonpolar), cyan (polar), and white (weakly polar). **A**) View of the inhibitor in the S_1/S_2 pocket and **B)** the S_4 pocket. View is rotated counterclockwise approximately 90° about the vertical axis relative to panel A.

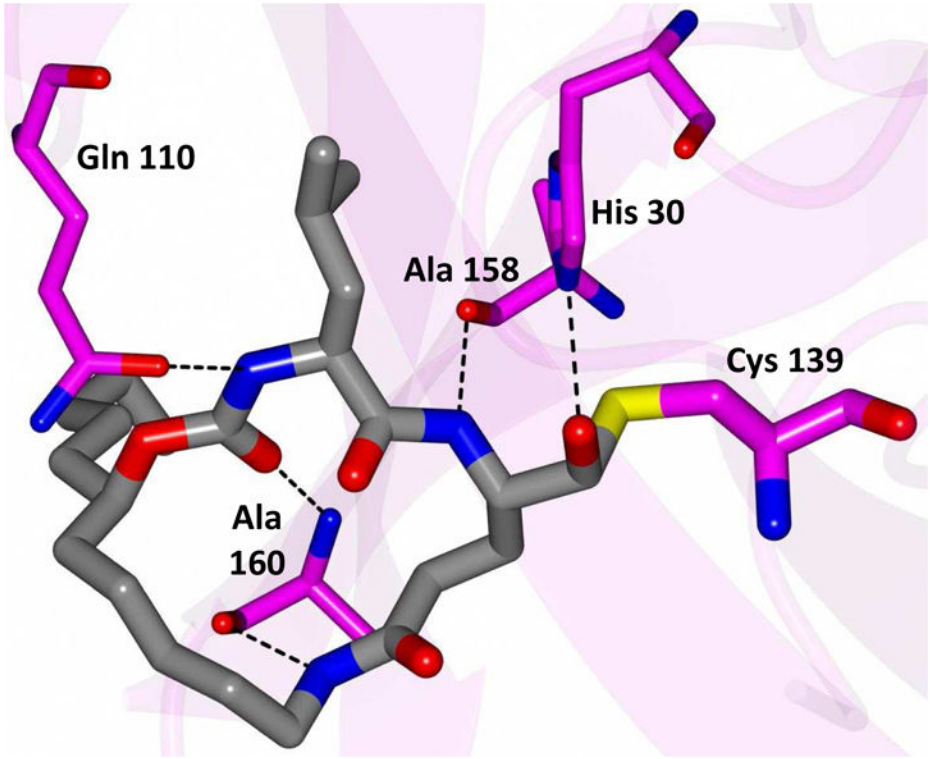


Fig. 8.
Hydrogen bond interactions (dashed lines) between inhibitor ***21*** and NV 3CL protease.

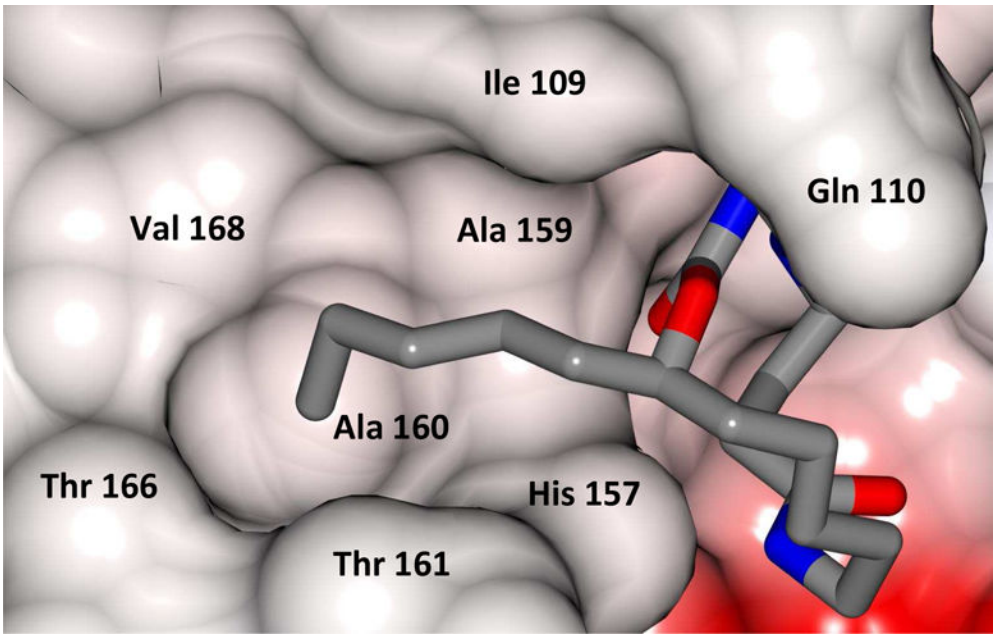
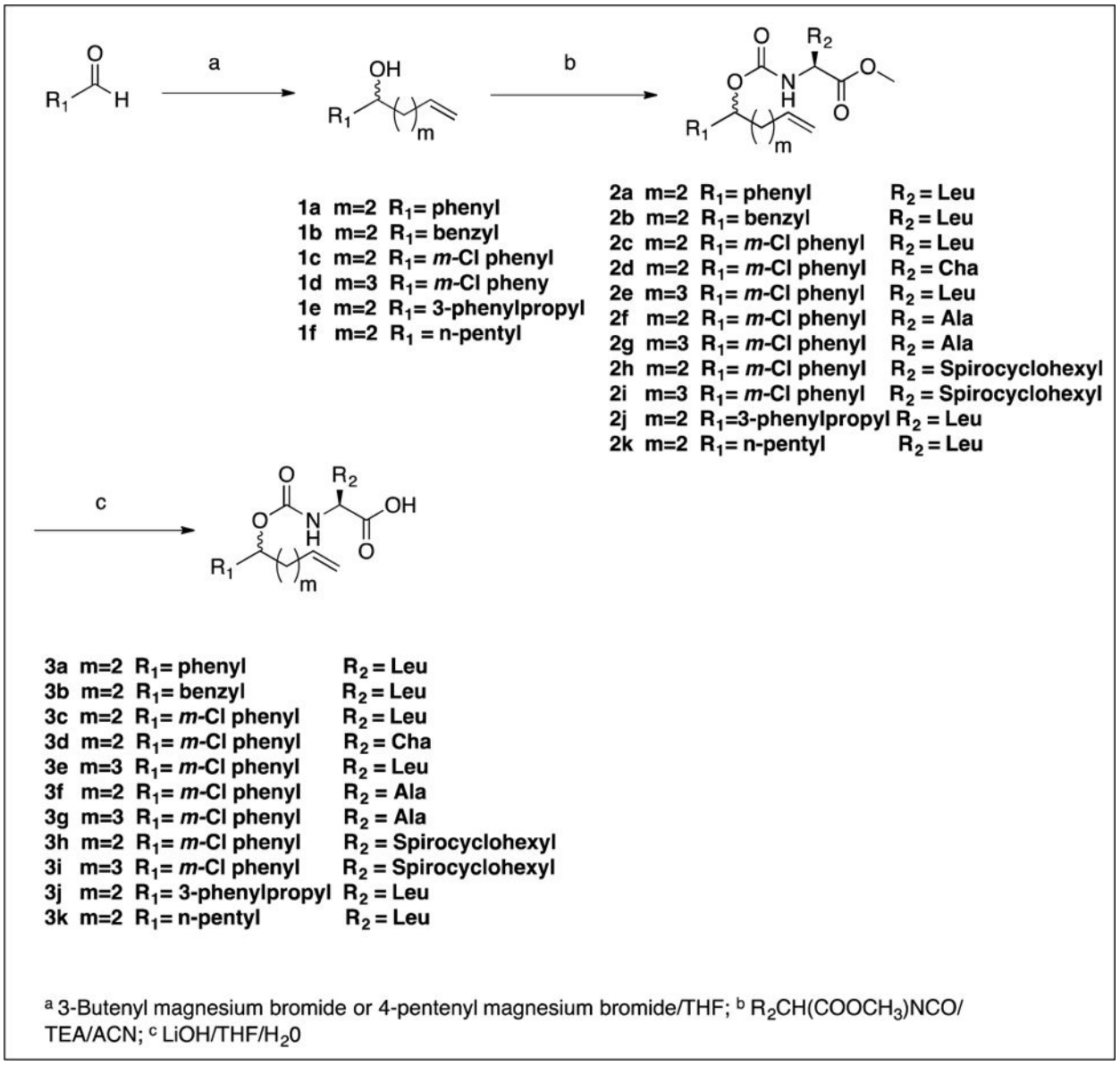
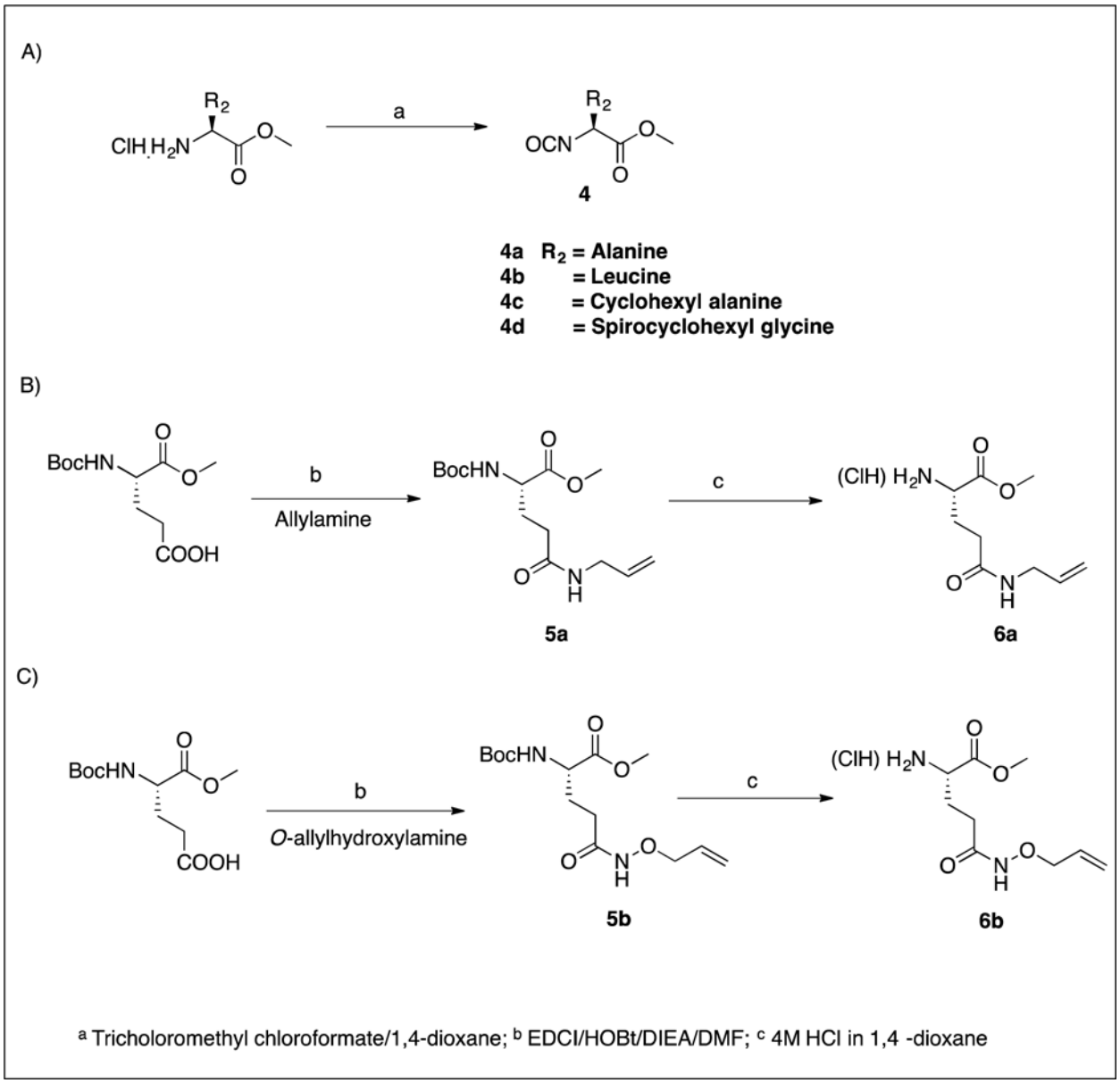


Fig. 9.

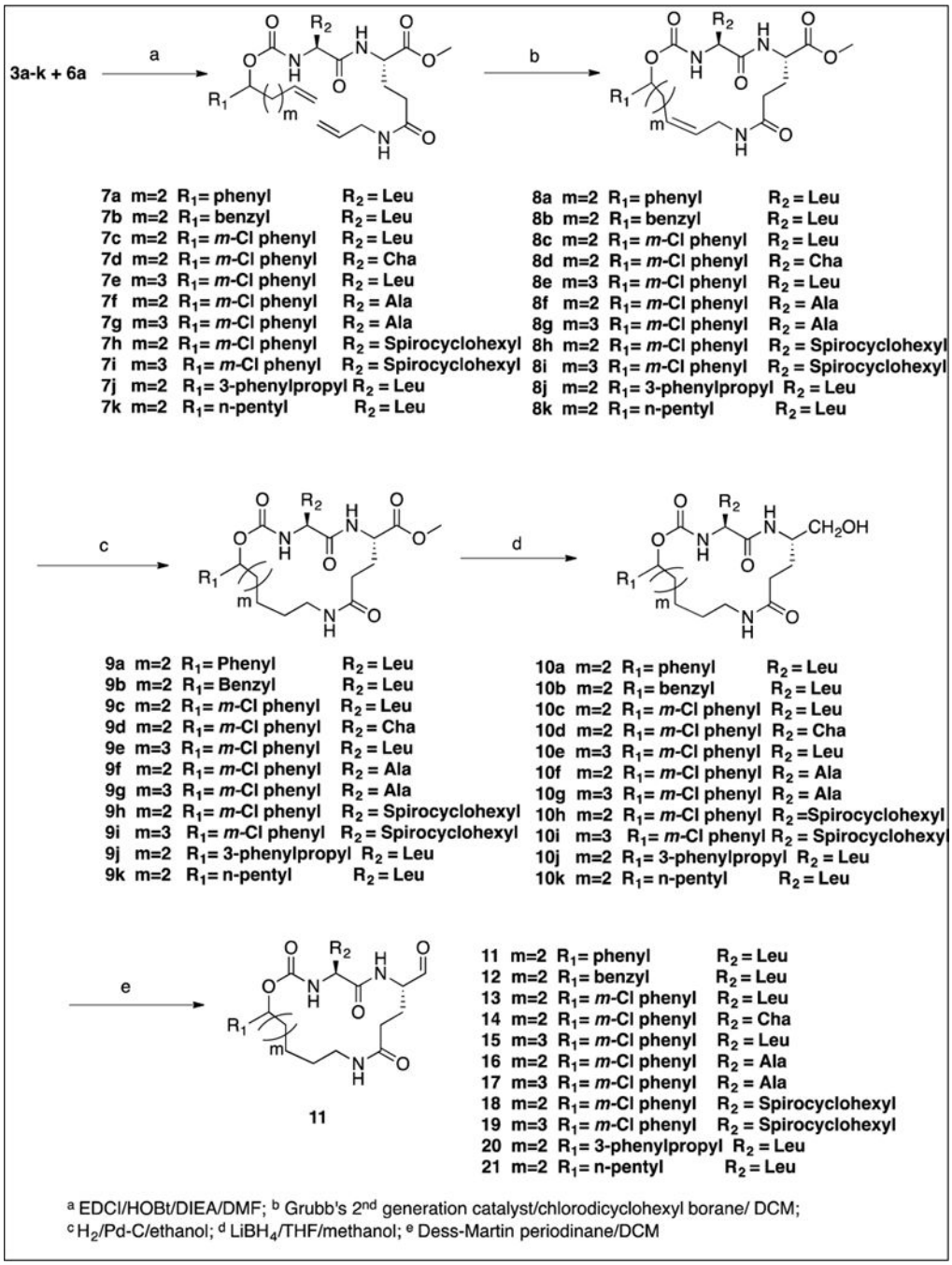
Electrostatic surface representation of NVPro showing the aliphatic chain of inhibitor ***21*** positioned in a hydrophobic S_4 pocket.



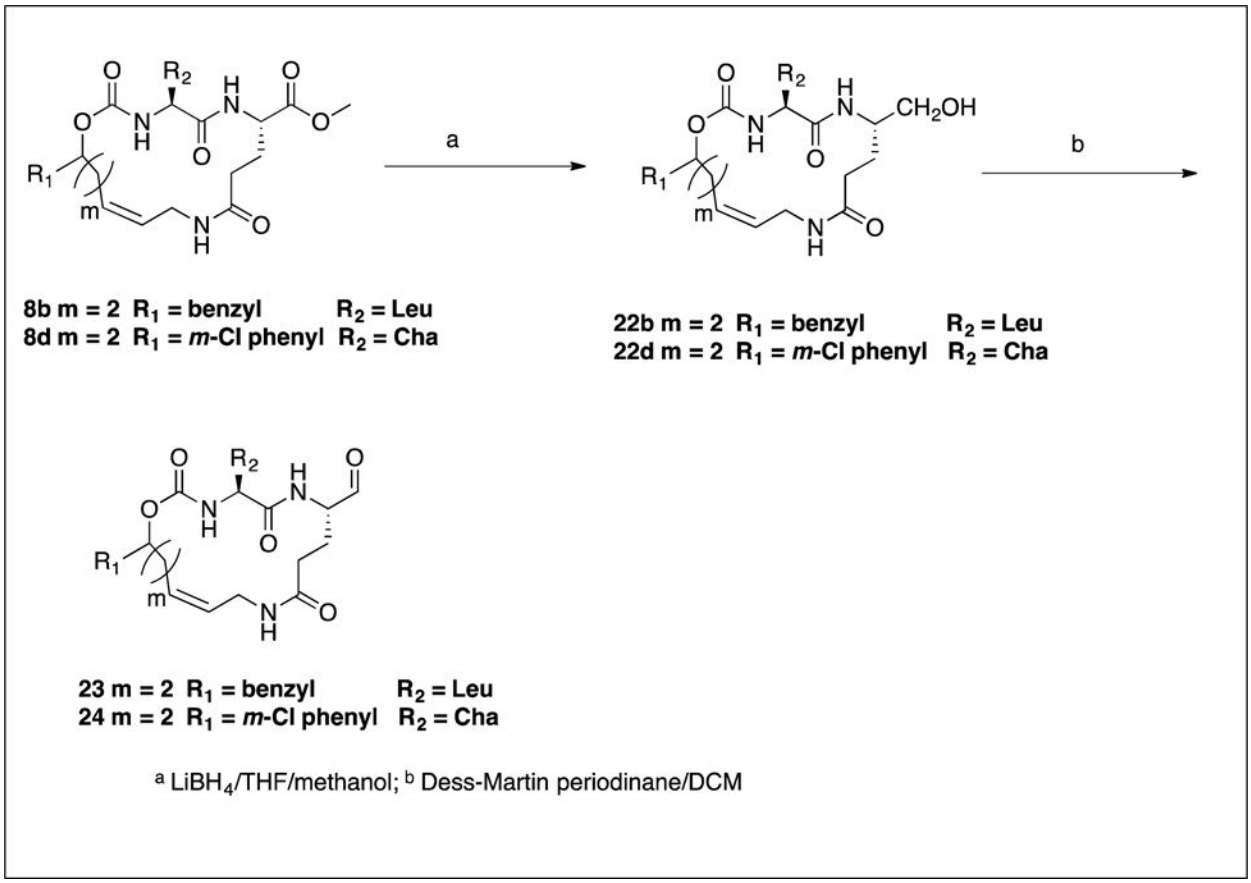
Scheme 1.



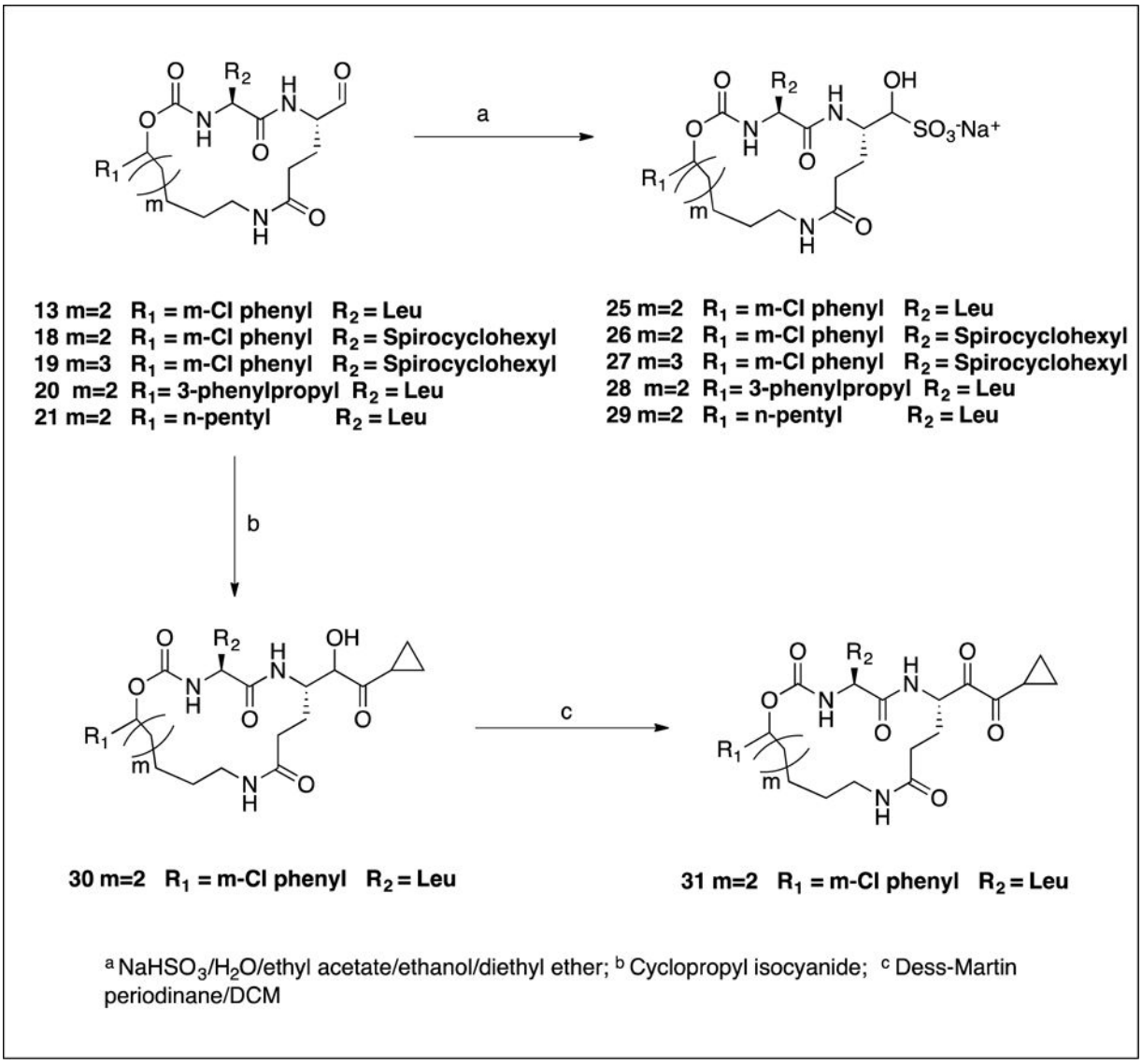
Scheme 2.



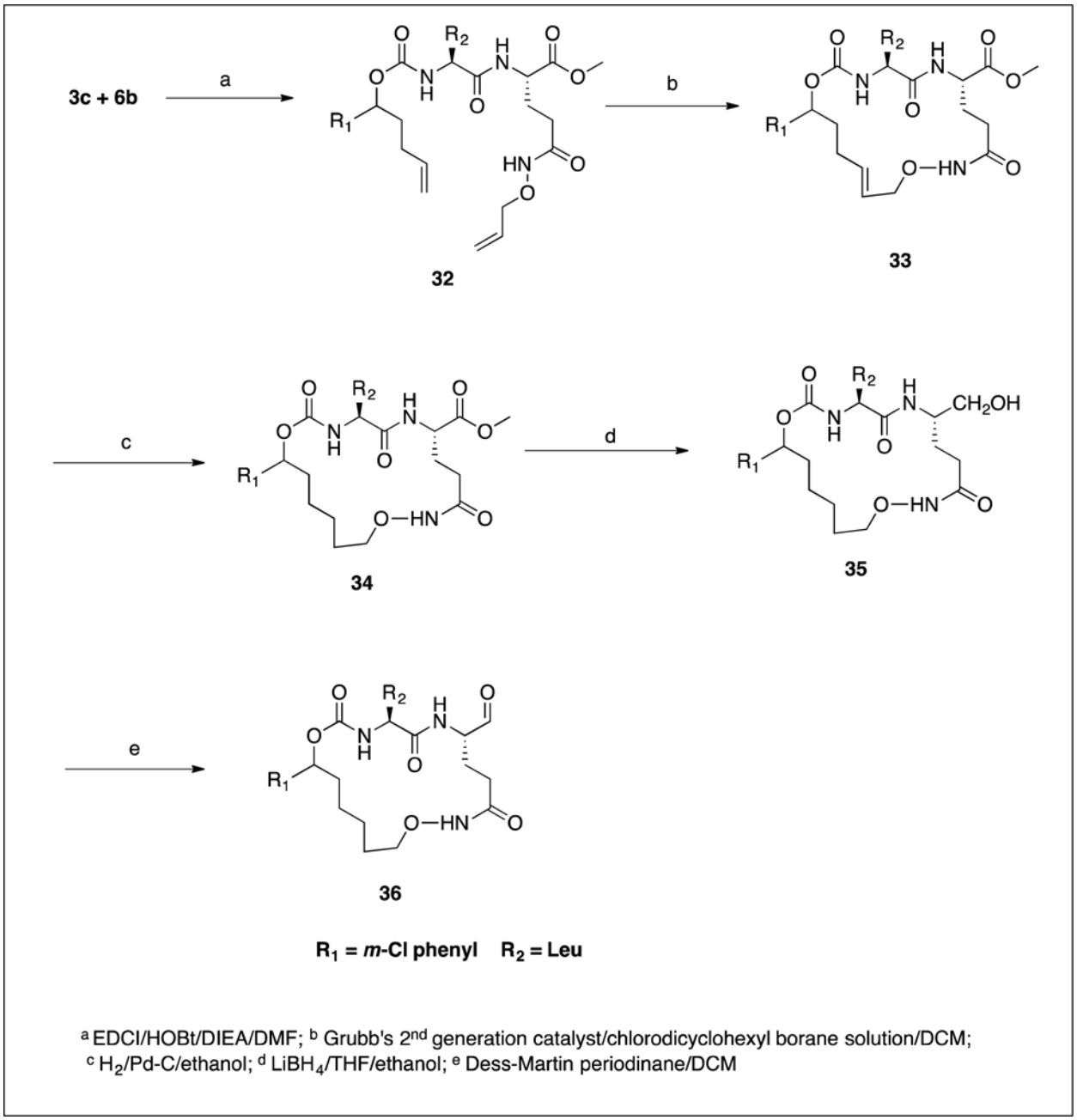
Scheme 3.



Scheme 4.



Scheme 5.



Scheme 6.

Eur J Med Chem. Author manuscript; available in PMC 2018 February 15.

Table 1

Activity and cell toxicity of compounds **11–12**, ***20, 28, 13, 25, 31, 14a–b, 15–18, 26, 19, 27, 21, 29***, and ***36A–B*** (all values are μM).

Compound	R_1	R_2	X	Ring Size	IC_{50} (μM) (NV)	EC_{50} (μM) (NV)	EC50 (μM) (MNV)	TC50 (EC50)
11	Phenyl	Leu	CHO	17	1.3	7.7	>20	>100
12	Benzyl	Leu	CHO	17	4.6	8.8	>20	>100
20	Propyl benzene	Leu	CHO	17	4.2	7.5	>20	71.5
28			$CH(OH)SO_3Na$		6.8	4.6	>20	69.2
13	*m*-Cl-phenyl	Leu	CHO	17	1.5	1.8	13.2	>100
25			$CH(OH)SO_3Na$		1.1	1.6	15.1	>100
31			(C=O)(C=O) Cyclopropyl		2.3	9.1	15.5	53.1
14(major)	*m*-Cl-phenyl	Cha	CHO	17	4.1	2.1	4.3	62.4
14(minor)					6.5	2.5	6.5	45.3
15	*m*-Cl-phenyl	Leu	CHO	18	6.3	5.4	>20	67.4
16	*m*-Cl-phenyl	Ala	CHO	17	>100	>20	>20	>100
17				18	62.3	9.5	>20	>100
18	*m*-Cl-phenyl	spirocyclohexyl	CHO	17	>100	>20	>20	62.4
26			$CH(OH)SO_3Na$		>100	>20	>20	48.5
19			CHO	18	>100	>20	>20	53.3
27			$CH(OH)SO_3Na$		>100	>20	>20	46.6
21	*n*-pentyl	Leu	CHO	17	3.2	3.1	12.1	72.7
29			$CH(OH)SO_3Na$		5.1	3.5	14.6	78.5
36A	*m*-Cl-phenyl	Leu	CHO	18	30.5	3.6	15.5	68.3
36B					46.7	5.1	13.4	73.8
23	Benzyl	Leu	CHO	17	2.5	3.1	>20	>100
24	*m*-Cl-phenyl	Cha	CHO	17	1.3	1.5	3.1	42.3

Table 2

Crystallographic data for NV 3CLpro:inhibitor ***13*** and ***21*** structures

	NV 3CLpro:13	NV 3CLpro:21
Data Collection		
Unit-cell parameters (Å, °)	a=64.40, b=36.49, c=61.47, β=112.5	a=67.02, b=36.70, c=61.72, β=110.2
Space group	$C2$	$C2$
Resolution (Å)[1]	31.83–1.40 (1.42–1.40)	32.79–1.75 (1.78–1.75)
Wavelength (Å)	1.0000	1.0000
Temperature (K)	100	100
Observed reflections	83,610	46,353
Unique reflections	25,218	14,350
$<I/\sigma(I)>$[1]	13.4 (1.9)	9.5 (1.9)
Completeness (%)[1]	96.8 (95.3)	99.6 (94.6)
Multiplicity[1]	3.3 (3.2)	3.2 (2.4)
R_{merge} (%)[1, 2]	4.8 (69.1)	8.2 (46.4)
R_{meas} (%)[1, 4]	5.8 (82.9)	9.8 (58.9)
R_{pim} (%)[1, 4]	3.1 (45.4)	5.4 (35.6)
$CC_{1/2}$[1, 5]	0.998 (0.704)	0.996 (0.780)
Refinement		
Resolution (Å) [1]	31.83–1.40	32.79–1.75
Reflections (working/test)[1]	24,030/1,183	13,624/710
R_{factor}/R_{free} (%)[1,3]	14.3/19.2	17.6/22.7
No. of atoms (Protein/Ligand/Water)	1,253/33/128	1,196/31/83
Model Quality		
R.m.s deviations		
Bond lengths (Å)	0.009	0.01
Bond angles (°)	1.035	0.998
Average B-factor (Å^2)		
All Atoms	19.2	20.4
Protein	17.9	19.7
Ligand	20.9	22
Water	31.4	29.9
Coordinate error(maximum likelihood) (Å)	0.15	0.19
Ramachandran Plot		
Most favored (%)	98.8	98.1
Additionally allowed (%)	1.2	1.9

[1] Values in parenthesis are for the highest resolution shell.

[2] $R_{merge} = \Sigma_{hkl}\Sigma_i |I_i(hkl) - <I(hkl)>| / \Sigma_{hkl}\Sigma_i I_i(hkl)$, where $I_i(hkl)$ is the intensity measured for the ith reflection and $<I(hkl)>$ is the average intensity of all reflections with indices hkl.

[3] $R_{factor} = \Sigma_{hkl} ||F_{obs}(hkl)| - |F_{calc}(hkl)|| / \Sigma_{hkl} |F_{obs}(hkl)|$; Rfree is calculated in an identical manner using 5% of randomly selected reflections that were not included in the refinement.

[4] R_{meas} = redundancy-independent (multiplicity-weighted) R_{merge}[53,60]. R_{pim} = precision-indicating (multiplicity-weighted) R_{merge}[61–62].

[5] $CC_{1/2}$ is the correlation coefficient of the mean intensities between two random half-sets of data [63–64].