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Novel potent macrocyclic inhibitors of the hepatitis C virus NS3 protease: Use of cyclopentane and cyclopentene P2-motifs

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Abstract—Several highly potent novel HCV NS3 protease inhibitors have been developed from two inhibitor series containing either a P2 trisubstituted macrocyclic cyclopentane- or a P2 cyclopentene dicarboxylic acid moiety as surrogates for the widely used *N*-acyl-(4*R*)-hydroxyproline in the P2 position. These inhibitors were optimized for anti HCV activities through examination of different ring sizes in the macrocyclic systems and further by exploring the effect of P4 substituent removal on potency. The target molecules were synthesized from readily available starting materials, furnishing the inhibitor compounds in good overall yields. It was found that the 14-membered ring system was the most potent in these two series and that the corresponding 13-, 15-, and 16-membered macrocyclic rings delivered less potent inhibitors. Moreover, the corresponding P1 acylsulfonamides had superior potencies over the corresponding P1 carboxylic acids. It is noteworthy that it has been possible to develop highly potent HCV protease inhibitors that altogether lack the P4 substituent. Thus the most potent inhibitor described in this work, inhibitor **20**, displays a K_i value of 0.41 nM and an EC_{50} value of 9 nM in the subgenomic HCV replicon cell model on genotype 1b. To the best of our knowledge this is the first example described in the literature of a HCV protease inhibitor displaying high potency in the replicon assay and lacking the P4 substituent, a finding which should facilitate the development of orally active small molecule inhibitors against the HCV protease.

1. Introduction

Hepatitis C virus (HCV) infection is predominantly a chronic viral disease afflicting over 170 million individuals worldwide,[1] representing a human epidemic nearly five times more prevalent than infections caused by the human immunodeficiency virus (HIV-1).[2]

The disease is associated with slowly progressive liver injury with the risk of patients developing cirrhosis and in some cases hepatocellular carcinoma.[3] HCV infection is now the leading cause for liver transplantation in Western countries,[4] and with infected individuals harboring the virus for a decade or more before symptoms emerge, the population requiring medical treatment will increase dramatically over the next 10–20 years.[2]

At present there is no vaccine against HCV infection and the current main therapy, PEGylated interferon-α in combination with ribavirin,[5] is associated with side effects and is effective in less than 50% of patients infected by the predominant HCV genotype 1.[6] Non-responders and relapsers are currently left with few treatment options.

The virally encoded NS3/4A serine protease has emerged as one of the most promising and intensively studied HCV drug targets. The NS3 protease is respon-

Keywords: HCV; NS3 protease; Macrocyclic inhibitors; Cyclopentane and Cyclopentene derived P2 templates.
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sible for cleavages of the downstream region of the polyprotein; thereby making it essential for viral replication.[7] The development of drug-like inhibitors has however proven to be a challenging task. Recently, highly promising inhibitors were reported by Llinàs-Brunet and co-workers[8–11] exemplified by the tetrapeptide inhibitor **A**[11] (Fig. 1), displaying promising low nanomolar activity against the NS3/4A protease.

This inhibitor incorporates a P2 *N*-acyl-(4*R*)-alkoxyproline moiety, a motif subsequently adopted in many reported and potent inhibitors.[12,13] Further refinement[14] of inhibitor **A**, and analogs thereof, mainly through optimization of the P2 quinoline substituent, and macrocyclization led to the discovery of **BILN 2061** (Ciluprevir; Boehringer Ingelheim)[9,10,14–17] (Fig. 1), a macrocyclic mimic of the peptide product inhibitor.

BILN 2061 was the first HCV protease inhibitor to enter clinical trials, and early results were highly promising showing a rapid and significant decline in plasma HCV RNA virus load in all treated patients infected with HCV genotype 1.[15] Subsequent clinical trials were halted apparently due to cardiac toxicity observed at high doses in rhesus monkeys.[18]

Currently there are two product-derived linear peptidomimetics, **VX-950**[19–22] and **SCH 503034**,[23–25] reported to be in phase II clinical trials. Previous work[26] from our laboratory on linear HCV NS3/4A

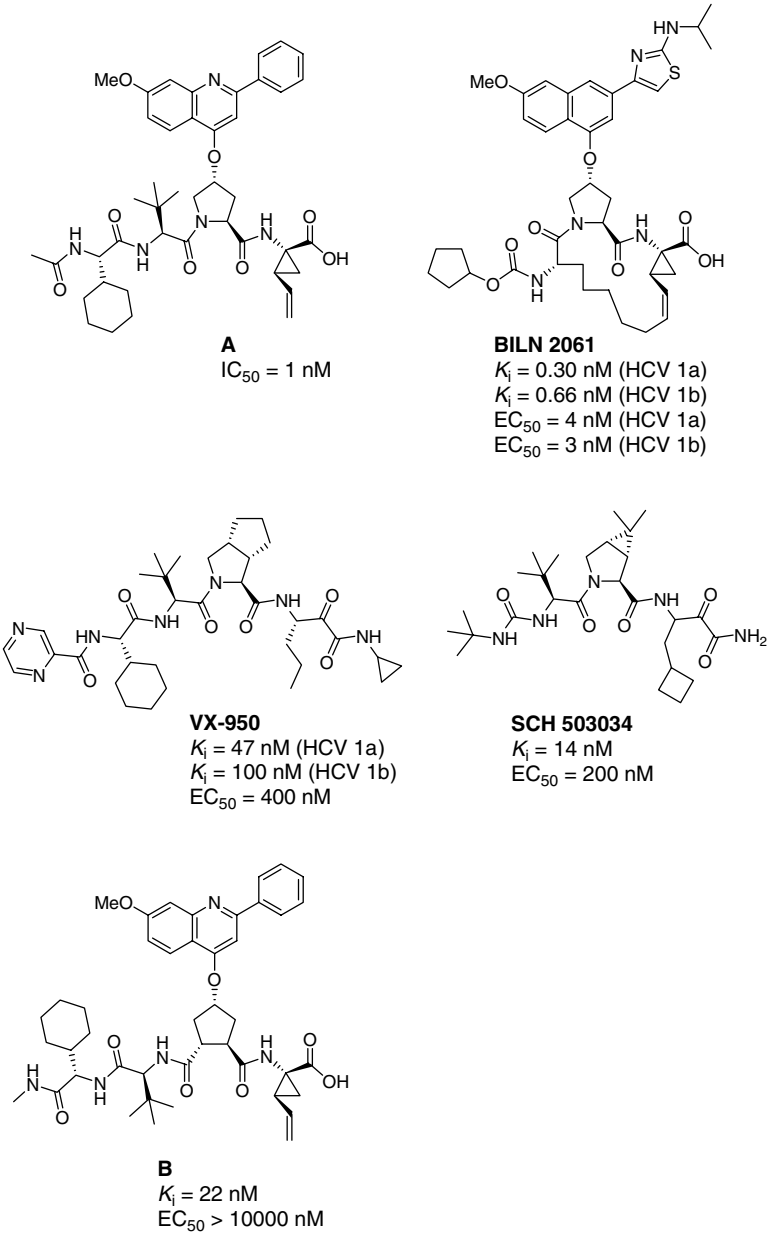


Figure 1. Two potent product-based HCV NS3 protease inhibitors incorporating a 4-hydroxyproline moiety and a C-terminal carboxylic acid (**A** and **BILN 2061**), and two promising linear peptidomimetics comprising the electrophilic α-ketoamide motif (**VX-950** and **SCH 503034**). **B** is a previously synthesized and promising compound in our series of cyclopentane-based inhibitors.

protease inhibitors has highlighted that a trisubstituted cyclopentane dicarboxylic acid could be a novel P2 mimic of the frequently used *N*-acyl-(4*R*)-hydroxyproline, exemplified by inhibitor **B** (Fig. 1). Encouraged by the observation that the sp^2-hybridized proline nitrogen could be replaced by an sp^3-hybridized carbon in the cyclopentane proline mimic series, we now report on the further optimization of this series with particular emphasis on macrocyclization studies and P4 truncations, aiming to improve both potency and cell based activity.

In another report[27] we have shown that a trisubstituted cyclopentene dicarboxylic acid, in which the 1-position of the cyclopentene adopts a planar configuration, may also be effective as a P2 *N*-acyl-(4*R*)-hydroxyproline mimic. Consequently we also report on the effect of macrocyclization and P4 truncation in this series.

The SAR from HCV NS3 protease inhibitors containing a P1 carboxylic acid with either a P3 hydrazine- or P4 NH-Boc-functionalized macrocyclic moiety clearly indicated a preference for 14-membered rings, exemplified by the P2 cyclopentane inhibitors **14b** (Scheme 2 and Table 1) and **16b** (Scheme 2 and Table 1), displaying K_i values of 31 nM and 6 nM, respectively, and the P2 cyclopentene inhibitor **24a** (Scheme 3 and Table 3) displaying a K_i value of 15 nM. Inhibitors **14b** and **16b** were subsequently converted into the corresponding acyl sulfonamides **17** (Scheme 2 and Table 2) and **18** (Fig. 2, Scheme 2, Table 2), exhibiting K_i values of 0.07 nM and 0.19 nM, and EC_{50} values, replicon assay genotype 1b, of 530 nM and 33 nM, respectively. After having determined the 14-membered ring size as the optimal we proceeded to investigate the inhibitory effects of compounds lacking a P4 substituent or just containing small P3 capping groups. Among these inhibitors **14e** (–H) and **14f** (–Me) (Table 1) delivered K_i values of 260 nM and 44 nM, respectively. Converting inhibitors **14e** and **14f** into their corresponding P1 acyl sulfonamides **19** (Scheme 2 and Table 2) and **20** (Fig. 2, Scheme 2 and Table 2) furnished K_i values of promising 2.2 and 0.41 nM. More impressive, inhibitor **20** delivered an EC_{50} replicon value of 9.1 nM. To the best of our knowledge this is the first reported example of a HCV protease inhibitor lacking a P4 substituent, and still maintaining a high potency in the replicon assay. Moreover inhibitor **20** exhibits good selectivity against the human serine proteases cathepsin B, chymotrypsin, and elastase.

2. Results and discussion

2.1. Chemistry

Scheme 1 depicts the synthesis of six P3 olefin building blocks employed in the coupling steps generating **12a–f** (Scheme 2) and **22a–b** (Scheme 3).

Two procedures were used in order to obtain the hydrazine building blocks. Heating commercially available 5-bromo-1-pentene (**1a**) and 6-bromo-1-hexene (**1b**) with *tert*-butyl carbazate at 100 °C in DMF afforded the alkylated products **2a** and **2b** in 72% and 75% yields, respectively.[28] In the second approach a two-step reductive amination protocol was employed. Commercial 6-heptenol (**3a**) and 7-octenol (**3b**) were oxidized, using a catalytic amount of tetrapropylammonium perruthenate (TPAP) and 4 Å molecular sieves in dichloromethane (DCM) with *N*-methylmorpholine *N*-oxide as reoxidizing agent.[29] The resulting volatile aldehydes **4a** and **4b**, obtained in 74% and 98% yields, respectively, were treated with *tert*-butyl carbazate in MeOH containing 3 Å molecular sieves furnishing the hydrazones, which were subsequently reduced to their corresponding hydrazine cyanoborane adducts using sodium cyanoborohydride in acetic acid/THF 1:1. Final hydrolysis of the borane adducts with sodium hydroxide in MeOH provided hydrazines **5a** and **5b** in 45% and 38% total yields, respectively, over the three steps.[30]

The direct alkylation protocol using a large excess of *tert*-butyl carbazate was found to work surprisingly well in our hands delivering the monoalkylated hydrazine building blocks **2a** and **2b** in good yields.

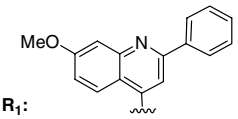
Treating commercial 5-hexenol (**6**) with pyridine and methanesulfonyl chloride in DCM gave mesylate **7** (91%), which was subjected to two different reaction conditions. Stirring **7** in a solution of aqueous ammonia and MeOH gave amine **8** in 96% yield, whereas adding the mesylate to a solution of *N*-Boc-methylamine (**9**) and sodium hydride in DMF gave compound **10** in 69% yield (Scheme 1).

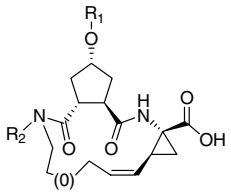
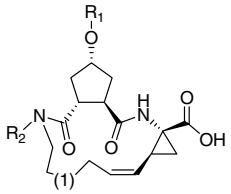
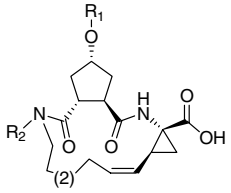
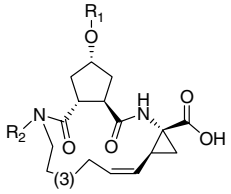
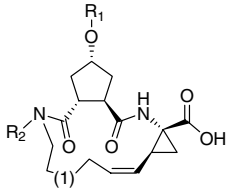
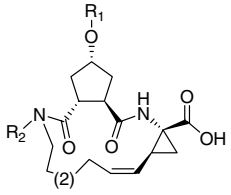
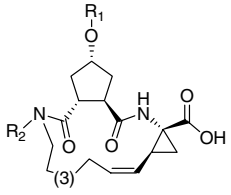
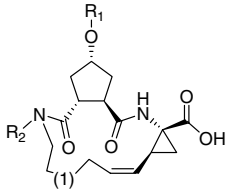
The synthesis of the macrocyclic inhibitors containing the P2 cyclopentane moiety is depicted in Scheme 2, where the synthesis of building block **11** has previously been reported.[26,31]

Hydrolysis of the *tert*-butyl ester in **11** using TFA and triethylsilane in DCM followed by subsequent coupling to amines **2a**, **2b**, **5a**, **5b**, **8** or deprotected **10** (subjected to HCl in dioxane prior to coupling) employing *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU) and *N*,*N*-diisopropylethylamine (DIPEA) in DMF furnished dienes **12a–f** in yields ranging from 64 to 85%. Dienes **12a–f** were then subjected to ring-closing olefin metathesis, using 2nd generation Hoveyda-Grubbs ruthenium catalyst in refluxing DCM,[9] providing compounds **13a–f** in 10–79% yield. The yields of compounds **13a–f** depended on the ring size of the products, with the 13-membered ring (**13a**) being the most difficult to form.

Ethyl esters **13a–f** were subsequently hydrolyzed with lithium hydroxide in refluxing THF/MeOH/H_2O 2:1:1 providing target compounds **14a–f** (Table 1) in 32–100% yields. Treating compounds **13b–d** with TFA and triethylsilane in DCM afforded hydrazines **15b–d** in yields ranging from 63 to 74%. Hydrolysis of the ethyl esters, *vide supra*, produced inhibitors **16b–d** (Table 1) in 46–71% yields (Scheme 2).

Table 1. Effect of ring size and P3 capping groups on inhibition of NS3/4A and EC_{50} in the replicon assay for macrocyclic P2 cyclopentane P1 carboxylic acid inhibitors

R1: 

Compound	Structure	R_2 P3 capping group	Ring size	K_i (nM) HCV NS3 1a	EC_{50} (μM) HCV NS3 1b
14a		Boc-NH	13	130	>10
14b		Boc-NH	14	31	>10
14c		Boc-NH	15	710	>10
14d		Boc-NH	16	>10,000	>10
16b		H_2N	14	6	7.6
16c		H_2N	15	120	>10
16d		H_2N	16	>10,000	>10
14e		H	14	260	>10

(*continued on next page*)

Table 1 (*continued*)

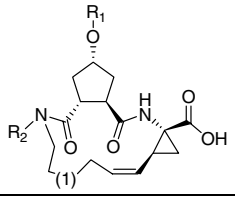
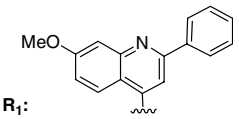
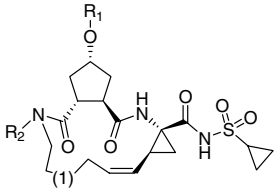
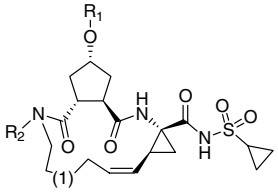
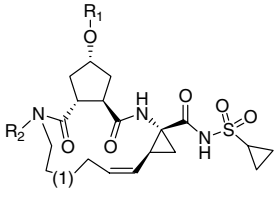
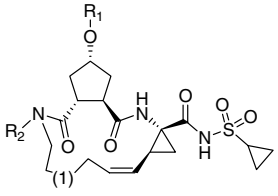
Compound	Structure	R_2 P3 capping group	Ring size	K_i (nM) HCV NS3 1a	EC_{50} (μM) HCV NS3 1b
14f		Me	14	44	2.2

Table 2. Effect of ring size and P3 capping groups on inhibition of NS3/4A and EC_{50} in the replicon assay for macrocyclic P2 cyclopentane P1 cyclopropyl acyl sulfonamide inhibitors

R_1: 

Compound	Structure	R_2 P3 capping group	Ring size	K_i (nM) HCV NS3 1a	EC_{50} (μM) HCV NS3 1b
17		Boc-NH	14	0.07	0.53
18		H_2N	14	0.19	0.033
19		H	14	2.2	4.4
20[a]		Me	14	0.41	0.0091

[a] Selectivity data: K_i = 2200, >5000, and >5000 nM for the human serine proteases cathepsin B, chymotrypsin, and elastase, respectively.

For the introduction of the cyclopropylsulfonamide carboxylic acid moiety, compounds **14b**, **14e**, and **14f** were preactivated with *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC) in DCM. Subsequent reaction of the preactivated compounds with cyclopropanesulfonic acid amide and DBU in DCM gave target compounds **17**, **19**, and **20** in 23–80% yields. Boc-removal of **17**, vide supra, furnished target compound **18** in 95% yield (Scheme 2 and Table 2).

For the synthesis of the inhibitors incorporating the P2 cyclopentene moiety the diastereomeric building block **21** (Scheme 3), synthesized as previously reported,[27,31–33] was employed. Coupling of carboxylic acid **21** to amines **2b** and **5a**, vide supra, furnished dienes **22a** and **22b** in 81% and 79% yields, respectively. Ring-closing metathesis using 2nd generation Hoveyda-Grubbs catalyst produced macrocycles **23a** and **23b** in 81% and 53% yields, respectively. Final hydrolysis of both the *tert*-butyl ester and the Boc group in **23a** and **23b** with TFA and triethylsilane in DCM generated inhibitors **24a** and **24b** (Scheme 3 and Table 3) (diastereomeric mixtures) in 38% and 47% yields, respectively.

2.1.1. Biological data and structure–activity relationships. All target compounds, summarized in Tables 1–3, were screened against the HCV NS3 1a protease to determine K_i values and in the cellular subgenomic genotype 1b replicon assay to determine EC_{50} values. All the inhibitors synthesized have *Z* configured double bonds, as deter-

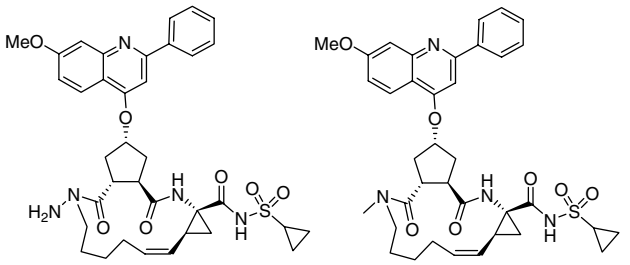
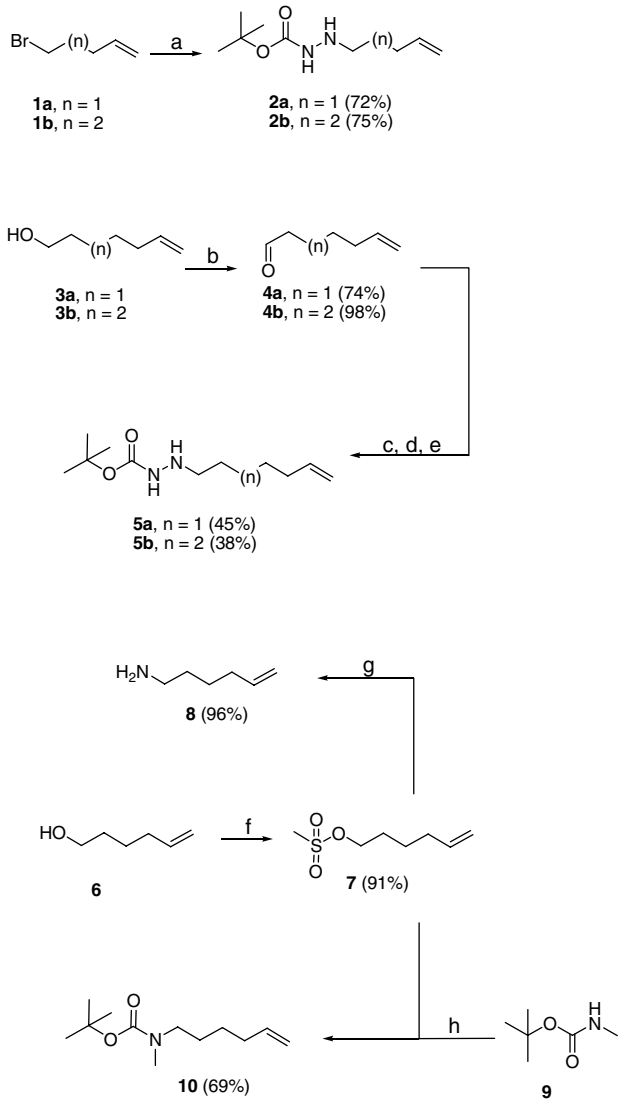


Figure 2. Two 14-membered ring acyl sulfonamide inhibitors from the new series of macrocyclic cyclopentane-based inhibitors; P3 hydrazine compound **18** and P3 *N*-methyl-capped compound **20** displaying K_i values of 0.19 nM and 0.41 nM and EC_{50} replicon 1b values of 33 nM and 9.1 nM, respectively.



Scheme 1. Reagents and conditions: (a) *tert*-butyl carbazate, DMF, 100 °C; (b) *N*-methylmorpholine *N*-oxide, TPAP, molecular sieves (4 Å), CH_2Cl_2; (c) *tert*-butyl carbazate, molecular sieves (3 Å), MeOH; (d) $NaBH_3CN$, AcOH/THF, 1:1; (e) NaOH (2M), MeOH; (f) MsCl, pyridine, DCM; (g) NH_3 (aq), MeOH; (h) NaH, DMF.

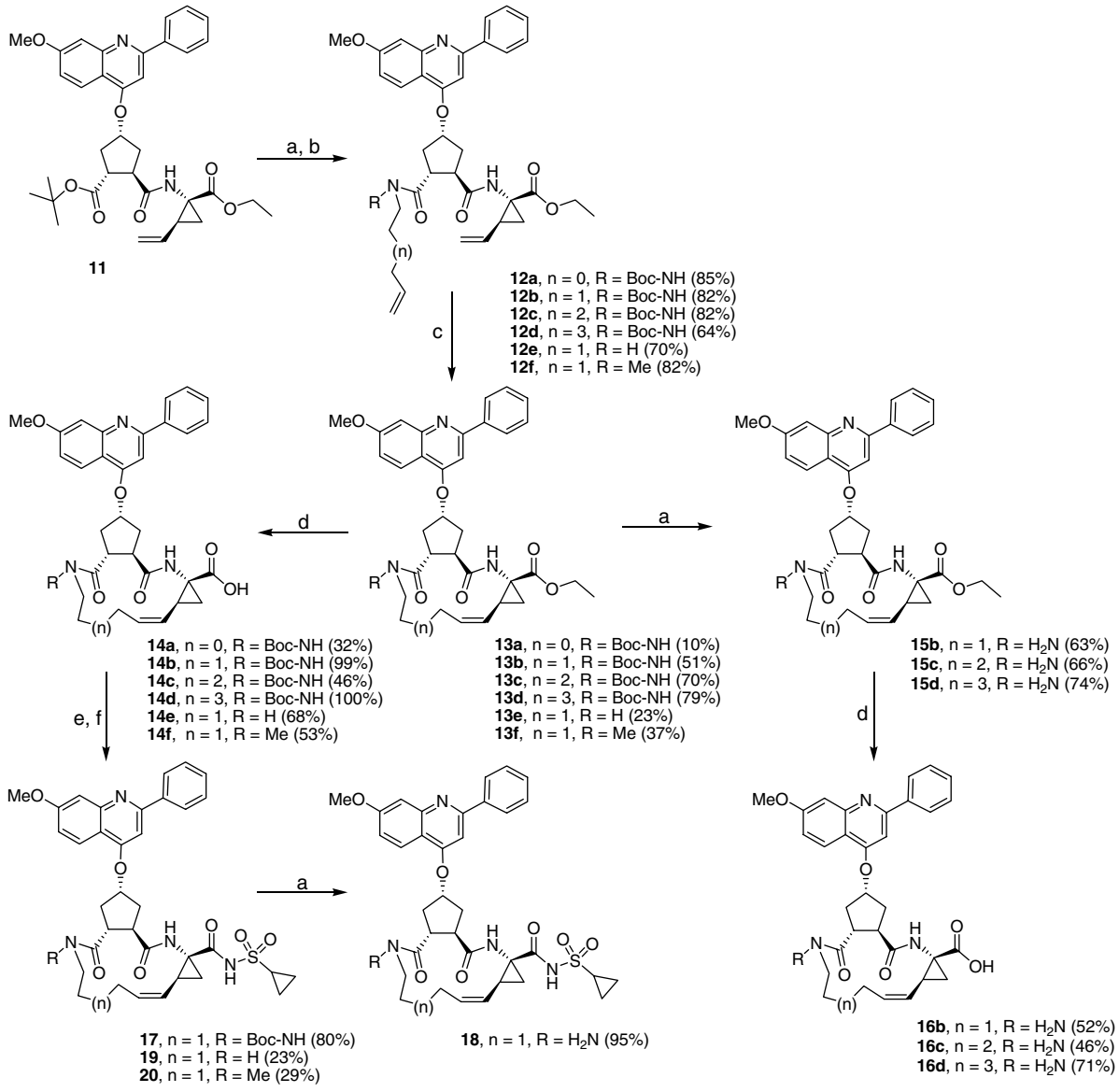
mined by NMR-analysis, and they contain the P2 2-phenyl-7-methoxy-4-quinoline substituent, a motif frequently employed[11,14,16,26,27,34] as P2 extension in potent HCV NS3 protease inhibitors, for example, compounds **A**[11] and **B**[26] (Fig. 1). Besides being crucial in shielding the catalytic machinery from exposure to solvent,[35,36] the P2 aryl substituent likely interacts favorably with the helicase domain of the NS3 protease.[37–39] The beneficial effects from macrocyclization are likely resulting from favorable conformational restrictions of these inhibitors providing a good overall fit into the active site.

From examination of the P2 cyclopentane-derived inhibitors and **BILN 2061** it is evident that the former inhibitors differ from **BILN 2061** by, for example, having an sp^3-hybridized carbon at the 1-position in the P2 cyclopentane, whereas the corresponding position in the proline of **BILN 2061** is an sp^2-hybridized nitrogen. Moreover, the cyclopentane-derived inhibitors have a reversed direction of the amino acid chain extending from the P2 group. In view of these substantial differences it was not evident whether potent macrocyclic inhibitors could be obtained at all from this investigatory series and furthermore which macrocyclic ring size would be favored. To investigate this we decided to prepare and evaluate each of the 13–16 membered rings.

The 13-membered macrocycle **14a** (Table 1) exhibits a moderate K_i value of 130 nM whereas compound **14b**, containing a 14-membered ring, is over four times more potent than the corresponding 13-membered ring exhibiting a promising K_i value of 31 nM. The 15- and 16-membered macrocyclic compounds **14c** and **14d** exhibit K_i values of 710 nM and >10 μM, respectively, indicating that the 14-membered rings possess the overall best fit into the S1–S3 pocket for these P2 cyclopentane-derived inhibitors.

The same trend held true for the P4 truncated series where the P4 Boc groups of inhibitors **14b–d** were replaced with the small but polar P3 amino extension in inhibitors **16b–d**. The 14-, 15-, and 16-membered macrocyclic inhibitors **16b–d** deliver K_i values of 6 nM, 120 nM, and >10 μM, respectively. Furthermore, the P3 amino 14-membered macrocyclic inhibitor **16b** is five times more potent than the corresponding P4 Boc-amino 14-membered macrocyclic inhibitor **14b**, displaying K_i values of 6 nM and 31 nM, respectively. Whilst neither of inhibitors **14b** and **16b** is highly potent it points to the possibility that potent inhibitors can be delivered from compounds that do not interact with the S4 sub pocket of the NS3 protease. Thus to further explore small P3 substituents in the 14-membered macrocyclic series, inhibitors **14e**, lacking a P4 group, and **14f**, having a P3 methyl cap, were prepared delivering K_i values of 260 nM and 44 nM, respectively (Table 1).

In spite of having promising potencies in the enzyme assay these P1 carboxylic acid inhibitors give rise to poor activities in the relevant cell-based HCV replicon assay. It has previously been reported that extensions of the P1 carboxylic acid, furnishing the corresponding acyl sulfonamides, sometimes result in improved activities in



Scheme 2. Reagents and conditions: (a) TFA, Et_3SiH, CH_2Cl_2; (b) **2a** or **2b** or **5a** or **5b** or **8** or **10** (subjected to HCl in dioxane prior to coupling), HATU, DIPEA, DMF; (c) Hoveyda-Grubbs Catalyst 2nd Gen. CH_2Cl_2, reflux; (d) LiOH (1M), THF/MeOH/H_2O 2:1:1, reflux; (e) EDC, DCM; (f) cyclopropanesulfonic acid amide, DBU, DCM.

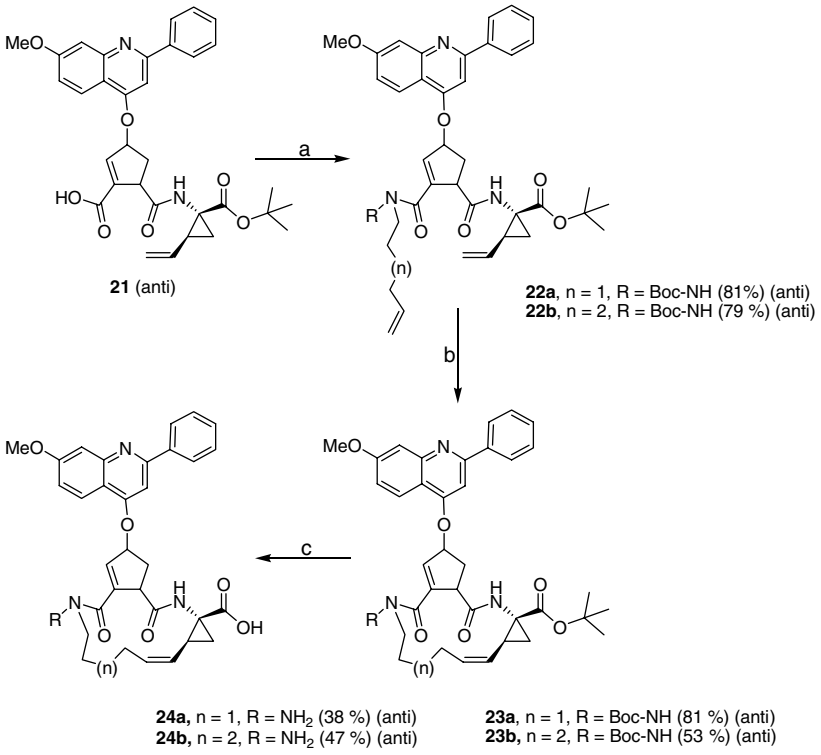
both enzyme and cell-based assays of HCV NS3 inhibitors.[12,34,40,41]

By introducing a cyclopropyl acyl sulfonamide into the 14-membered macrocyclic inhibitors **14b** and **16b** (Table 2) a substantial improvement in K_i values to 0.07 nM and 0.19 nM, respectively, was obtained for inhibitors **17** and **18**. In addition, moderate to highly potent activities in the subgenomic replicon assay can be seen, with EC_{50} values of 530 nM for **17** and promising 33 nM for **18**. For inhibitor **19**, lacking a P4 group, and the P3-methyl-capped inhibitor **20** the corresponding K_i values were 2.2 nM and 0.41 nM, respectively. In the subgenomic cell-based replicon assay an EC_{50} value of 4.4 μM was obtained for inhibitor **19** and an impressive EC_{50} replicon value of 9.1 nM for inhibitor **20** (Table 2).

The diastereomeric P2 cylopentene derivatives **24a** and **24b** share with the P2 proline the same planar configuration at the 1-position. Nonetheless, the 14-membered macrocyclic inhibitor **24a** is more potent than the 15-membered macrocycle **24b** following the same trend as for the P2 cyclopentane inhibitor series. However, inhibitor **24a** is somewhat less potent than **16b**. The inconvenient synthesis route for the P2 cyclopentene inhibitors which furnishes diastereomeric mixtures makes them less attractive than the corresponding P2 cylopentane derivatives. Moreover, the potential of irreversible Michael acceptor properties with increased risk for adverse events in vivo renders this inhibitor class less attractive for further development.[27]

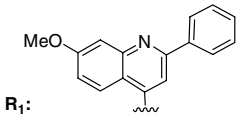
In contrast, the P2 cyclopentane inhibitor class shows great promise for further refinements.

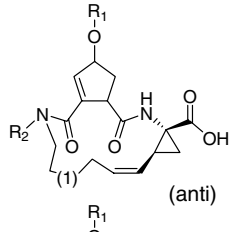
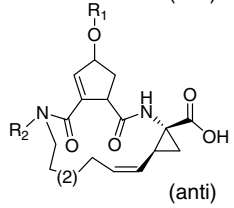
Modeling was used to rationalize the SAR observed for the various P4 substituents and P3 extensions. Conformational analysis indicates that the moderate potency



Scheme 3. Reagents and conditions: (a) **2b** or **5a**, DIPEA, HATU, DMF; (b) Hoveyda-Grubbs Catalyst 2nd Gen., CH_2Cl_2, reflux; (c) TFA, Et_3SiH, CH_2Cl_2.

Table 3. Effect of ring size on inhibition of NS3/4A and EC_{50} in the replicon assay for macrocyclic P2 cyclopentene P1 carboxylic acid inhibitors



Compound	Structure	R_2 P3 capping group	Ring size	K_i (nM) HCV NS3 1a	EC_{50} (μM) HCV NS3 1b
24a	 (anti)	H_2N	14	15	5.4
24b	 (anti)	H_2N	15	110	4.5

seen with the P3-H in both series (**14e** and **19**) may result from an internal hydrogen bond, between the P3-H and the P2 carbonyl, forcing the macrocycle to a conformational change upon binding to the active site. Conformational studies were also used to explain how inhibitor **16b** having the small P3-NH_2 extension is five times more potent than inhibitor **14b** having the larger P4-NHBoc. Analysis of these inhibitors indicates that the conformations of the reversed amides may differ. Thus inhibitor **16b** could be docked into the active site in any of the two rotations around the reversed amide bond while inhibitor **14b** fits only in the higher energy rotamer state.

Using a previously published crystal structure of the full length NS3[39] compound **20** was docked and minimized in the active site of NS3 (Fig. 3). The model of compound **20** shows interactions with both the protease and the helicase parts of NS3. The protease interactions are the most important and the inhibitor forms hydrogen bonds to five residues in the active site of the protease.

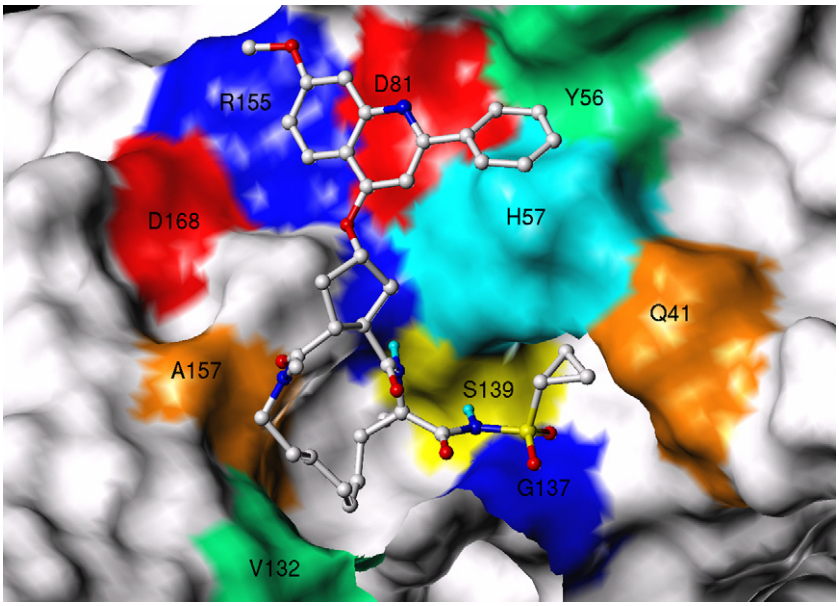


Figure 3. Model of compound **20** in the active site of the NS3 protease. The NS3 crystal structure 1cu1 from PDB was used for modeling and Arg 155 was rotated to accommodate the P2 quinoline substituent.

The cyclopropyl acyl sulfonamide has been shown to add potency to the inhibitors and this can be explained from the interactions of this group to the NS3 protease. In this model the sulfone oxygens of the acyl sulfonamide bind to the oxyanion hole, that is, the NH of both Gly 137 and Ser 139. The flexible side chain of Lys 136 has possibilities to interact both with one of the sulfone oxygens and with the amide carbonyl of the acyl sulfonamide. The cyclopropyl group has close contact interactions with Gln 41 and the catalytic His 57 in the S1′ sub pocket.

The amides flanking the P2 cyclopentyl have main chain hydrogen bonds to Arg 155 and Ala 157. The aliphatic P1–P3 linker of the macrocycle has close contact interactions with the side chain of Val 132.

With the introduction of an extending P2 substituent there seems to be an inhibitor induced conformational change of the Arg 155 and Asp 168 side chains leading to the formation of a salt bridge.[16] This conformational change makes it possible for the methoxy quinoline to make a favorable interaction with the guanidine of Arg 155. The quinoline is also shielding the catalytic interaction between His 57 and Asp 81, while the extending phenyl seems to add interactions and stabilize the position of the P2 substituent by aromatic stacking with Tyr 56. The P2 substituent also has interactions with some residues from the NS3 helicase, mainly the side chains of Pro 482, Met 485, Val 524, and Gln 526.

3. Conclusion

Trisubstituted cyclopentane- and cyclopentene dicarboxylic acid moieties have successfully been used to replace the commonly employed *N*-acyl-(4*R*)-hydroxyproline, and have been incorporated in the P2 position of macrocyclic functionalized HCV NS3 inhibitors. Utilizing ring-closing metathesis, inhibitors of different ring sizes were synthesized and evaluated. The ring size with the best fit, 14-membered macrocycles, delivered inhibitors with sub nanomolar activity, for example, inhibitors **17**, **18**, and **20**, with K_i values of 0.07, 0.19, and 0.41 nM, respectively and with EC_{50} values of 530, 33, and 9.1 nM, respectively, in the subgenomic replicon assay.

The excellent potencies found, together with the observation that this class of inhibitors can display potent activities in the replicon assay in spite of P4 truncation, make further refinements of the compounds presented herein highly warranted.

4. Experimental

4.1. HCV NS3 protease enzyme assay[42]

The enzyme-based inhibition assay with HCV protease was performed using recombinant full length NS3 enzyme (Poliakov et al.)[43] and NS4A (KKGSVVIVGRIVLSGK, Gunnar Lindeberg, Department of Medicinal Chemistry, Uppsala University, Sweden) in final concentrations of 3.5 nM and 14 μM, respectively. The test compounds were dissolved and diluted in DMSO and were added to the assay buffer containing 50 mM Hepes, pH 7.5, 40% glycerol, 0.1% CHAPS, and 10 mM DTT. The maximum final DMSO concentration in the assay was 1%. After a pre-incubation for 30 min at room temperature, the enzyme reaction was started by adding the FRET substrate Ac-Asp-Glu-Asp(EDANS)-Glu-Glu-Abu-ψ-[COO)Ala-Ser-Lys(DABCYL)-NH2 (RET S1, AnaSpec, San Jose, CA. USA) to a final concentration of 2 μM. The enzyme activity was continously measured over time (20 min) in a fluorescence reader (Fluorocan Ascent, ThermoLab systems,

Stockholm, Sweden) with 355 nm as excitation and 500 nm as emission wavelengths, respectively.

IC_{50} values were calculated by non-linear fitting into the equation $(1 - v_i/v_o) = (I)/((I) + IC_{50})$ and the K_i value was calculated from the IC_{50} value using the equation $K_i = IC_{50}/(1 + S/K_m)$ assuming a competitive enzyme inhibition.

4.2. HCV replicon assay

The Huh-7 cell line containing HCV subgenomic genotype 1b replicon with firefly luciferase (Lohmann et al. 1999) was cultured in DMEM (Dulbecco's minimal essential medium, Life Technologies) supplemented with 10% fetal calf serum (Life Technologies), 100 U/ml penicillin, 100 μg/ml streptomycin, and 0.5 mg/ml G418 (Geneticin, Life Technologies). Replicon cells were trypsinized and seeded into 96-well microplates (1500 cells/well). After 24 h, test compounds, fivefold serially diluted in cell culture medium, were added to the subconfluent cells. The compound stock solutions were made in DMSO and the final concentration of DMSO in the assay never exceeded 1%.

The cells were incubated with compounds for 48 h and were then lysed and the luciferase activity in each well was determined using Luciferase Assay HTS Kit (Bio Thema) and a Berthold luminometer.

The EC_{50} value represents the concentration of the compound that decreases the luciferase signal with 50% compared to the untreated control.

4.3. Modeling

All modeling experiments were performed using SYBYL 7.3 (Tripos Inc. 1699 South Hanley Road, St. Louis, Missouri, 63144, USA). Inhibitor **20** was modeled in the full length NS3 crystal structure 1cu1[39] from PDB, where Arg 155 had been rotated to mimic the inhibitor induced conformational change taking place upon inhibitor binding.[16]

4.4. General methods

NMR-spectra were recorded on a Varian 300 MHz instrument using $CDCl_3$, CD_3OD or $(CD_3)_2SO$ as solvents. TMS was used as reference. In recorded NMR-spectra with diasteromeric mixtures the presumed diasteromeric peaks were put into brackets with the general formula [n.nn and n.nn, (x, yH)] for ^1H NMR and [nn.n and nn.n] for ^{13}C NMR, respectively. Optical rotations were measured using a Perkin-Elmer 141 polarimeter. TLC was carried out on Merck precoated $60F_{254}$ plates using UV-light and charring with ethanol/sulfuric acid/*p*-anisaldehyde/acetic acid 90:3:2:1, and a solution of 0.5% ninhydrin in ethanol for visualization. Flash column chromatography was performed using silica gel 60 (0.040–0.063 mm, Merck). Organic phases were dried over anhydrous magnesium sulfate. Concentrations were performed under diminished pressure (1–2 kPa) at a bath temperature of 40 °C. Isocratic HPLC was performed on a preparative C-18 column. Gradient HPLC-MS was performed on a Gilson system (Column: Phenomenex C-18 250×15 mm and Phenomenex C-18 150×4.6 mm for preparative and analytical runs, respectively; Pump: Gilson gradient pump 322; UV/vis-detector: Gilson 155; MS detector: Thermo Finnigan Surveyor MSQ; Gilson Fraction Collector FC204) using methanol with 0.1% formic acid and deionized water with 0.1% formic acid as mobile phase. MALDI-TOF-spectra were recorded on a Voyager-DE STR Biospectrometry Workstation using α-cyano-4-hydroxycinnamic acid as a matrix and reference.

4.5. LC-MS purity measurements

4.5.1. Chromatography system A. Column: Phenomenex C18 150×4.6 mm; Pump: Gilson gradient pump 322; UV/VIS-detector: Gilson 155; MS detector: Thermo Finnigan Surveyor MSQ; Software: Gilson UniPoint 4.0 and Xcalibur 1.3. Gradient: methanol 40–100% over 10 min at 1 mL/min followed by 100% for 5 min at 1 mL/min. To all solvents formic acid (0.1% v/v) was added. Peaks were detected at 254 nm.

4.5.2. Chromatography system B. As system A except: Gradient: acetonitrile 0–100% over 10 min at 1 mL/min followed by 100% for 5 min at 1 mL/min.

4.5.3. Chromatography system C. As system A except: Gradient: acetonitrile 5–99% over 3 min followed by 100% for 3 min. Ammonium acetate buffer was used instead of formic acid.

4.5.4. Chromatography system D. As system A except: Gradient: acetonitrile 30–80% over 3 min followed by 100% for 3 min. Ammonium acetate buffer was used instead of formic acid.

4.6. General synthetic procedures

4.6.1. General procedure A: *tert*-butyl ester deprotection and amide bond formation. Scaffold molecule **11** (135 mg, 0.225 mmol) and triethylsilane (71 μL, 0.45 mmol) were dissolved in DCM (2 mL) and trifluoroacetic acid (TFA) (2 mL) was added. The mixture was stirred for 2 h and thereafter concentrated and co-concentrated with toluene. The residue was dissolved in DMF (3 mL) and the amine (60 mg, 0.26 mmol) and DIPEA (118 μL, 0.677 mmol) were added. The temperature was lowered to 0 °C and the coupling reagent *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU) (94 mg, 0.25 mmol) was added. The solution was allowed to stir for 30 min at 0 °C and then for ~16 h (overnight) at room temperature. The solvent was removed by heating the reaction flask in a water bath under diminished pressure. The residue was thereafter dissolved in ethyl acetate and the organic phase was washed with brine, dried, filtered, and concentrated. The crude product was purified by isocratic HPLC.

4.6.2. General procedure B: olefin ring-closing metathesis (RCM). A solution of the diene (158 mg, 0.209 mmol) in dry DCM (25 mL) was bubbled with argon for 5 min.

To the stirred solution under argon atmosphere was then added a solution of 2nd Generation Hoveyda-Grubbs Catalyst (14 mg, 0.022 mmol) in dry DCM (5 mL). The mixture was stirred at reflux under argon atmosphere for ~16 h and concentrated. The crude product was purified by isocratic HPLC.

4.6.3. General procedure C: deprotection of Boc group. The Boc protected hydrazine (33 mg, 0.046 mmol) and triethylsilane (15 μL, 0.094 mmol) were dissolved in DCM (2 mL) after which was added trifluoroacetic acid (TFA) (2 mL). The mixture was stirred for 3 h and was subsequently concentrated and co-concentrated with toluene. The crude product was purified by isocratic HPLC.

4.6.4. General procedure D: ester hydrolysis. To a solution of the ethyl ester (27 mg, 0.037 mmol) in THF/MeOH/H_2O 2:1:1 (5 mL) was added 1 M LiOH (296 μL, 0.296 mmol). The solution was stirred at reflux for ~5 h. After acidification to approximately pH 3–4 with 1 M HCl and concentration the residue was purified by isocratic HPLC.

4.6.5. General procedure E: sulfonamide coupling. To a solution of the acid (36 mg, 0.053 mmol) in dry DCM (1.5 mL) was added EDC (21 mg, 0.11 mmol). The mixture was stirred at room temperature overnight and diluted with DCM, washed with water, dried, filtered and concentrated. The residual was dissolved in dry DCM (1.5 mL) and cyclopropanesulfonic acid amide (14 mg, 0.12 mmol) and DBU (20 μL, 0.13 mmol) were added. The mixture was stirred at room temperature overnight and diluted with DCM, washed with 10% aqueous citric acid and brine, dried, filtered, and concentrated. The crude product was purified by isocratic HPLC.

4.6.6. General procedure F: oxidation of olefin alcohol. To a solution of the olefin alcohol (1.00 mL, 7.44 mmol) and *N*-methylmorpholine *N*-oxide (1.31 g, 11.2 mmol) in DCM (17 mL) were added ground molecular sieves (3.5 g, 4 Å). The mixture was stirred for 10 min at room temperature under nitrogen atmosphere before tetrapropylammonium perruthenate (TPAP) (131 mg, 0.370 mmol) was added. After stirring for an additional 2.5 h the solution was filtered through Celite. The solvent was carefully evaporated and the remaining liquid was purified by flash column chromatography.

4.6.7. General procedure G1: synthesis of olefin hydrazine. To a mixture of the volatile aldehyde (68 mg, 0.61 mmol) and *tert*-butyl carbazate (81 mg, 0.61 mmol) in MeOH (5 mL) was added ground molecular sieves (115 mg, 3 Å). The mixture was stirred for 3 h, filtered through Celite and concentrated.

The residue was dissolved in dry THF (3 mL) and AcOH (3 mL). $NaBH_3CN$ (95 mg, 1.5 mmol) was added and the solution was stirred overnight. The reaction mixture was diluted with saturated aqueous $NaHCO_3$ (6 mL) and ethyl acetate (6 mL). The organic phase was washed with brine and saturated aqueous $NaHCO_3$, dried over $MgSO_4$, filtered, and concentrated. The cyanoborane adduct was hydrolyzed by treatment with 2 M NaOH (1.9 mL) in MeOH (3 mL). The mixture was stirred for 2 h and the MeOH was evaporated. H_2O (5 mL) and DCM (5 mL) were added and the organic phase was separated. The water phase was extracted three additional times with DCM. The combined organic phases were dried, filtered, concentrated, and the crude residue was purified by flash column chromatography.

4.6.8. General procedure G2: synthesis of olefin hydrazine. The bromo olefin (200 μL, 1.604 mmol) and *tert*-butyl carbazate (1.50 g, 11.4 mmol) were stirred at 100 °C in DMF for 5 h. The solvent was removed by heating the reaction flask in a water bath under diminished pressure. The residue was thereafter dissolved in ethyl acetate and the organic phase was washed with brine, dried, filtered, concentrated and the crude residue was purified by flash column chromatography.

4.7. Synthetic experimentals

4.7.1. *N*′-Pent-4-enyl-hydrazinecarboxylic acid *tert*-butyl ester (2a). Hydrazine **2a** was synthesized according to General procedure G2 using 5-bromo-1-pentene (**1a**). Purification by flash column chromatography (toluene/ethyl acetate 6:1 + 1% triethylamine) gave the product (72%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.46 (s, 9H), 1.49–1.62 (m, 2H), 2.05–2.16 (m, 2H), 2.80–2.89 (m, 2H), 3.93 (bs, 1H), 4.91–5.08 (m, 2H), 5.72–5.88 (m, 1H), 6.81 (bs, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 27.1, 28.3, 31.2, 51.4, 80.2, 114.8, 138.3, 156.9.

4.7.2. *N*′-Hex-5-enyl-hydrazinecarboxylic acid *tert*-butyl ester (2b). Hydrazine **2b** was synthesized according to General procedure G2 using 6-bromo-1-hexene (**1b**). Purification by flash column chromatography (toluene/ethyl acetate 6:1 + 1% triethylamine) gave the product (75%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.36–1.56 (m, 4H), 1.46 (s, overlapped, 9H), 2.01–2.12 (m, 2H), 2.79–2.88 (m, 2H), 3.91 (bs, 1H), 4.90–5.06 (m, 2H), 5.71–5.88 (m, 1H), 6.62 (bs, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 26.2, 27.1, 28.2, 33.4, 51.7, 80.1, 114.4, 138.4, 156.7.

4.7.3. Hept-6-enal (4a). Alcohol **3a** was oxidized according to General procedure F. Flash column chromatography (CH_2Cl_2) gave the crude and volatile aldehyde **4a** (74%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.35–1.50 (m, 2H), 1.59–1.72 (m, 2H), 2.01–2.14 (m, 2H), 2.39–2.49 (m, 2H), 4.91–5.08 (m, 2H), 5.71–5.88 (m, 1H), 9.76 (t, J = 1.8 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 21.5, 28.3, 33.4, 43.7, 114.8, 138.2, 202.5.

4.7.4. Oct-7-enal (4b). Alcohol **3b** was oxidized according to General procedure F. Flash column chromatography ($CHCl_3$) gave the volatile aldehyde **4b** (98%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.26–1.48 (m, 4H), 1.56–1.71 (m, 2H), 1.97–2.11 (m, 2H), 2.36–2.47 (m, 2H), 4.87–5.05 (m, 2H), 5.70–5.89 (m, 1H), 9.77 (t, J = 1.9 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 21.9, 28.6, 33.5, 43.9, 114.5, 138.7, 202.8.

4.7.5. *N′*-Hept-6-enyl-hydrazinecarboxylic acid *tert*-butyl ester (5a). Hydrazine **5a** was synthesized according to General procedure G1 using aldehyde **4a**. Purification by flash column chromatography (toluene/ethyl acetate 9:1 + 1% triethylamine) gave the product (45%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.25–1.51 (m, 6H), 1.43 (s, overlapped, 9H), 1.96–2.07 (m, 2H), 2.75–2.85 (m, 2H), 3.90 (bs, 1H), 4.86–5.04 (m, 2H), 5.69–5.85 (m, 1H), 6.31 (bs, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 26.5, 27.6, 28.3, 28.7, 33.6, 51.9, 80.2, 114.3, 138.8, 156.7.

4.7.6. *N′*-Oct-7-enyl-hydrazinecarboxylic acid *tert*-butyl ester (5b). Hydrazine **5b** was synthesized according to General procedure G1 using aldehyde **4b**. Purification by flash column chromatography (toluene/ethyl acetate 9:1 + 1% triethylamine) gave the product (38%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.24–1.57 (m, 8H), 1.46 (s, overlapped, 9H), 1.98–2.10 (m, 2H), 2.78–2.88 (m, 2H), 3.91 (bs, 1H), 4.88–5.06 (m, 2H), 5.71–5.89 (m, 1H), 6.49 (bs, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 26.8, 27.7, 28.3, 28.7, 28.9, 33.6, 51.9, 80.1, 114.1, 138.9, 156.7.

4.7.7. Methanesulfonic acid hex-5-enyl ester (7). To a stirred solution of 5-hexenol (**6**) (500 μL, 4.24 mmol) in dry pyridine (1.25 mL, 15.5 mmol) and dry CH_2Cl_2 (25 mL) at 0 °C was added methanesulfonyl chloride. After stirring for 24 h at room temperature the mixture was concentrated and co-concentrated with toluene. Flash column chromatography (toluene/ethyl acetate 9:1) gave **7** (91%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.46–1.58 (m, 2H), 1.71–1.83 (m, 2H), 2.05–2.17 (m, 2H), 3.00 (s, 3H), 4.23 (t, J = 6.5 Hz, 2H), 4.95–5.08 (m, 2H), 5.71–5.86 (m, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 24.7, 28.5, 33.0, 37.4, 69.9, 115.2, 137.9.

4.7.8. Hex-5-enylamine (8). A solution of **7** (250 mg, 1.40 mmol), ~28% NH_3 aqueous solution (10 mL), and MeOH (10 mL) was stirred for 48 h. Thereafter H_2O (10 mL) was added and the solution was extracted with CH_2Cl_2 (3 × 15 mL). The combined organic extracts were dried, filtered, and carefully concentrated. Purification by flash column chromatography (ethyl acetate/methanol 4:1) gave the volatile amine **8** (134 mg, 96%) as a yellowish oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.29 (bs, 2H), 1.35–1.54 (m, 4H), 2.02–2.12 (m, 2H), 2.66–2.73 (m, 2H), 4.91–5.06 (m, 2H), 5.74–5.89 (m, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 26.2, 33.3, 33.6, 42.1, 114.5, 138.8.

4.7.9. Hex-5-enyl-methyl-carbamic acid *tert*-butyl ester (10). A cooled (0 °C) solution of *N*-boc-methylamine (**9**) (7.55 g, 57.6 mmol) and sodium hydride (60.5 mmol) in DMF (80 mL) was stirred for 1 h. To the cooled solution was slowly added compound **7** (11.28 g, 63.30 mmol) and the mixture was allowed to stir overnight. EtOAc was added and the organic phase was washed with citric acid and brine, dried, filtered, and concentrated. Purification by flash column chromatography (toluene/ethyl acetate 9:1) gave **10** (69%) as a colorless oil ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.32–1.40 (m, 2H), 1.44 (s, 9H), 1.46–1.54 (m, 2H), 2.02–2.10 (m, 2H), 2.82 (s, 3H), 3.19 (t, J = 7.2 Hz, 2H), 4.91–5.03 (m, 2H), 5.71–5.85 (m, 1H).

4.7.10. (1*R*,2*R*,4*R*)-2-((1*R*,2*S*)-1-Ethoxycarbonyl-2-vinyl-cyclopropylcarbamoyl)-4-(7-methoxy-2-phenyl-quinolin-4-yloxy)-cyclopentanecarboxylic acid *tert*-butyl ester (11). Compound **11** was synthesized according to Refs. 26 and 31. NMR in accordance with Ref. 26. $[\alpha]_D^{22}$ − 22 (*c* 1.0, $CHCl_3$); HPLC-MS: $(M+H)^+$ calcd: 601.3, found: 601.4.

4.7.11. (1*R*,2*S*)-1-{[(1*R*,2*R*,4*R*)-2-(*N′*-*tert*-Butoxycarbonyl-*N*-pent-4-enyl-hydrazinocarbonyl)-4-(7-methoxy-2-phenyl-quinolin-4-yloxy)-cyclopentanecarbonyl]-amino}-2-vinyl-cyclopropanecarboxylic acid ethyl ester (12a). Scaffold molecule **11** was coupled to hydrazine **2a** according to General procedure A. Purification by HPLC (MeOH/H_2O 85:15 + 0.2% triethylamine) gave tripeptide diene **12a** (85%) as a colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.23 (t, J = 7.1 Hz, 3H), 1.30–1.52 (m, 1H), 1.46 (s, overlapped, 9H), 1.57–1.73 (m, 2H), 1.82–1.92 (m, 2H), 1.96–2.15 (m, 4H), 2.17–2.36 (m, 2H), 2.40–2.56 (m, 1H), 2.57–2.74 (m, 1H), 3.30–3.54 (m, 2H), 3.68–3.86 (b, 1H), 3.92 (s, 3H), 4.15 (q, J = 7.1 Hz, 2H), 4.92–5.05 (m, 2H), 5.06–5.19 (m, 2H), 5.20–5.32 (m, 1H), 5.62–5.84 (m, 2H), 6.58 (bs, 1H), 6.94 (s, 1H), 7.06–7.15 (m, 1H), 7.36–7.55 (m, 4H), 7.99–8.14 (m, 3H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 14.2, 23.0, 25.7, 28.1, 30.7, 32.8, 35.2, 37.3, 40.3, 44.5, 47.0, 47.8, 55.4, 61.5, 77.9, 81.7, 98.2, 107.3, 115.0, 115.2, 118.0, 123.0, 127.4, 128.6, 129.0, 133.1, 137.5, 140.3, 151.1, 154.2, 159.0, 160.6, 161.1, 170.5, 174.0, 176.2. MALDI-TOF: $(M+H)^+$ calcd: 727.4, found: 727.5.

4.7.12. (1*R*,2*S*)-1-{[(1*R*,2*R*,4*R*)-2-(*N′*-*tert*-Butoxycarbonyl-*N*-hex-5-enyl-hydrazinocarbonyl)-4-(7-methoxy-2-phenyl-quinolin-4-yloxy)-cyclopentanecarbonyl]-amino}-2-vinyl-cyclopropanecarboxylic acid ethyl ester (12b). Scaffold molecule **11** was coupled to hydrazine **2b** according to General procedure A. Purification by HPLC (MeOH/H_2O 85:15 + 0.2% triethylamine) gave tripeptide diene **12b** (82%) as a colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.23 (t, J = 7.1 Hz, 3H), 1.32–1.50 (m, 3H), 1.46 (s, overlapped, 9H), 1.51–1.64 (m, 2H), 1.83–1.92 (m, 2H), 1.99–2.14 (m, 4H), 2.18–2.36 (m, 2H), 2.43–2.58 (m, 1H), 2.59–2.73 (m, 1H), 3.35–3.52 (m, 2H), 3.70–3.86 (b, 1H), 3.93 (s, 3H), 4.15 (q, J = 7.1 Hz, 2H), 4.89–5.03 (m, 2H), 5.08–5.20 (m, 2H), 5.21–5.32 (m, 1H), 5.64–5.83 (m, 2H), 6.51 (bs, 1H), 6.95 (s, 1H), 7.09 (dd, J = 2.5, 9.1 Hz, 1H), 7.37–7.52 (m, 4H), 8.00–8.10 (m, 3H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 14.1, 23.0, 25.9, 25.9, 28.1, 32.5, 32.9, 33.2, 35.2, 37.3, 40.3, 44.5, 47.1, 48.0, 55.3, 61.4, 77.9, 81.7, 98.2, 107.2, 114.7, 115.2, 117.9, 123.0, 127.4, 128.6, 129.0, 133.2, 138.2, 140.2, 151.1, 154.2, 159.0, 160.6, 161.1, 170.5, 174.0, 176.2. MALDI-TOF: $(M+H)^+$ calcd: 741.4, found: 741.4.

4.7.13. (1*R*,2*S*)-1-{[(1*R*,2*R*,4*R*)-2-(*N′*-*tert*-Butoxycarbonyl-*N*-hept-6-enyl-hydrazinocarbonyl)-4-(7-methoxy-2-phenyl-quinolin-4-yloxy)-cyclopentanecarbonyl]-amino}-2-vinyl-cyclopropanecarboxylic acid ethyl ester (12c). Scaffold molecule **11** was coupled to hydrazine **5a**

according to General procedure A. Purification by HPLC (MeOH/H_2O 90:10 + 0.2% triethylamine) gave tripeptide diene **12c** (82%) as a colorless solid. ^1H NMR (300 MHz, $CDCl_3$, 40 °C) δ 1.22 (t, J = 7.1 Hz, 3H), 1.28–1.42 (m, 6H), 1.46 (s, 9H), 1.52–1.62 (m, 2H), 1.82–1.91 (m, 1H), 1.96–2.16 (m, 3H), 2.18–2.34 (m, 2H), 2.42–2.56 (m, 1H), 2.58–2.72 (m, 1H), 3.42 (m, 3H), 3.66–3.84 (m, 1H), 3.92 (s, 3H), 4.15 (q, J = 7.1 Hz, 2H), 4.88–5.02 (m, 2H), 5.07–5.18 (m, 2H), 5.20–5.32 (m, 1H), 5.63–5.84 (m, 2H), 6.62 (bs, 1H), 6.94 (s, 1H), 7.09 (dd, J = 2.6, 9.2 Hz, 1H), 7.36–7.51 (m, 4H), 7.99–8.10 (m, 3H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 14.3, 23.0, 26.4, 26.6, 28.3, 28.6, 33.2, 33.5, 35.6, 37.6, 40.6, 44.7, 47.1, 48.6, 55.5, 61.5, 81.9, 98.4, 107.9, 114.5, 115.6, 118.1, 123.2, 127.6, 128.3, 128.7, 129.1, 133.5, 138.7, 140.7, 151.5, 154.5, 159.2, 160.9, 161.5, 170.5, 174.2, 176.3. MALDI-TOF: $(M+H)^+$ calcd: 755.4, found: 755.6.

4.7.14. (1*R*,2*S*)-1-{[(1*R*,2*R*,4*R*)-2-(*N'*-*tert*-Butoxycarbonyl-*N*-oct-7-enyl-hydrazinocarbonyl)-4-(7-methoxy-2-phenyl-quinolin-4-yloxy)-cyclopentanecarbonyl]-amino}-2-vinyl-cyclopropanecarboxylic acid ethyl ester (12d). Scaffold molecule **11** was coupled to hydrazine **5b** according to General procedure A. Purification by HPLC (MeOH/H_2O 90:10 + 0.2% triethylamine) gave tripeptide diene **12d** (64%) as a colorless solid. ^1H NMR (300 MHz, $CDCl_3$) δ 1.14–1.55 (m, 13H), 1.43 (s, overlapped, 9H), 1.80–1.89 (m, 1H), 1.90–2.02 (m, 3H), 2.04–2.16 (m, 1H), 2.18–2.33 (m, 3H), 2.38–2.52 (m, 1H), 2.53–2.69 (m, 1H), 3.25–3.54 (m, 2H), 3.88 (s, 3H), 4.11 (q, J = 7.1 Hz, 2H), 4.84–4.98 (m, 2H), 5.03–5.16 (m, 2H), 5.18–5.29 (m, 1H), 5.58–5.81 (m, 2H), 6.82–6.96 (m, 2H), 7.04 (dd, J = 2.6, 9.2 Hz, 1H), 7.34–7.48 (m, 4H), 7.94–8.06 (m, 3H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 14.1, 22.9, 26.5, 28.0, 28.5, 28.6, 32.5, 33.5, 35.3, 37.2, 40.2, 44.3, 45.7, 47.0, 48.3, 55.3, 61.3, 77.9, 98.2, 107.3, 114.1, 115.2, 117.8, 123.0, 127.4, 128.5, 128.8, 129.0, 133.2, 138.7, 140.2, 151.1, 154.2, 159.0, 160.6, 161.1, 170.4, 174.0, 176.1. HPLC-MS: $(M+H)^+$ calcd: 769.4, found: 769.5.

4.7.15. (1*R*,2*S*)-1-{[(1*R*,2*R*,4*S*)-2-Hex-5-enylcarbamoyl-4-(7-methoxy-2-phenyl-quinolin-4-yloxy)-cyclopentanecarbonyl]-amino}-2-vinyl-cyclopropanecarboxylic acid ethyl ester (12e). Scaffold molecule **11** was coupled to amine **8** according to General procedure A. Purification by HPLC (MeOH/H_2O 80:20 + 0.2% triethylamine) gave diene **12e** (70%) as a colorless solid. ^1H NMR (300 MHz, $CDCl_3$) δ 1.21 (t, J = 7.1 Hz, 3H), 1.29–1.50 (m, 6H), 1.83–1.92 (m, 1H), 1.93–2.06 (m, 2H), 2.09–2.20 (m, 1H), 2.34–2.47 (m, 1H), 2.48–2.62 (m, 1H), 2.92–3.07 (m, 1H), 3.08–3.19 (m, 1H), 3.20–3.33 (m, 2H), 3.89 (s, 3H), 4.06–4.20 (m, 2H), 4.89–5.03 (m, 2H), 5.07–5.19 (m, 2H), 5.23–5.33 (m, 1H), 5.65–5.82 (m, 2H), 6.28 (b, 1H), 6.93 (s, 1H), 7.02–7.12 (m, 1H), 7.37 (b, 1H), 7.40–7.54 (m, 4H), 7.94–8.06 (m, 3H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 14.8, 23.6, 26.7, 29.4, 33.8, 36.4, 36.7, 40.3, 40.9, 47.9, 48.1, 56.0, 61.9, 78.7, 98.8, 108.0, 115.4, 115.8, 118.6, 118.6, 126.6, 128.1, 129.3, 129.8, 134.1, 138.8, 140.9, 151.8, 159.7, 161.2, 161.8, 170.8, 173.7, 175.2. HPLC-MS: $(M+H)^+$ calcd: 626.3, found: 626.2.

4.7.16. (1*R*,2*S*)-1-{[(1*R*,2*R*,4*R*)-2-(Hex-5-enyl-methyl-carbamoyl)-4-(7-methoxy-2-phenyl-quinolin-4-yloxy)-cyclopentanecarbonyl]-amino}-2-vinyl-cyclopropanecarboxylic acid ethyl ester (12f). Scaffold molecule **11** was coupled to amine **10** (subjected to HCl in dioxane prior to coupling) according to General procedure A. Purification by HPLC (MeOH/H_2O 80:20 + 0.2% triethylamine) gave diene **12f** (82%) as a colorless solid. ^1H NMR (300 MHz, $CDCl_3$) δ 1.17 (t, J = 7.1 Hz, 3H), 1.26–1.46 (m, 4H), 1.47–1.67 (m, 2H), 1.78–1.88 (m, 1H), 1.95–2.17 (m, 3H), 2.31–2.35 (m, 1H), 2.43–2.59 (m, 1H), 2.71–2.91 (m, 1H), 2.98 (s, 3H), 3.17–3.49 (m, 3H), 3.54–3.73 (m, 1H), 3.92 (s, 3H), 4.01–4.18 (m, 2H), 4.85–5.12 (m, 3H), 5.20–5.36 (m, 2H), 5.63–5.83 (m, 2H), 6.92–7.02 (m, 1H), 7.04 (s, 1H), 7.13 (d, J = 9.1 Hz, 1H), 7.23-7.43 (m, 3H), 7.50 (s, 1H), 7.92 (d, J = 8.0 Hz, 2H), 8.00–8.08 (m, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 14.3, 23.2, 26.0, 26.5, 28.1, 33.4, 35.4, 37.2, 40.2, 45.6, 45.8, 48.3, 49.9, 55.9, 61.2, 79.2, 99.1, 104.5, 115.1, 115.2, 117.8, 119.4, 123.7, 128.0, 128.9, 130.3, 133.7, 138.1, 138.4, 147.8, 157.8, 162.7, 162.9, 170.0, 173.1, 174.2. HPLC-MS: $(M+H)^+$ calcd: 640.3, found: 640.3.

4.7.17. (*Z*)-(1*R*,4*R*,6*S*,14*R*,16*R*)-12-*tert*-Butoxycarbonylamino-16-(7-methoxy-2-phenyl-quinolin-4-yloxy)-2,13-dioxo-3,12-diaza-tricyclo[12.3.0.0^{4,6}]heptadec-7-ene-4-carboxylic acid ethyl ester (13a). Diene **12a** was cyclized according to General procedure B. Purification by HPLC (MeOH/H_2O 90:10 + 0.2% triethylamine) provided macrocyclic compound **13a** (10%) as a colorless solid. ^1H NMR (300 MHz, $CDCl_3$) δ 1.14–1.30 (m, 4H), 1.32–1.58 (m, 2H), 1.51 (s, overlapped, 9H), 1.64–1.80 (m, 2H), 1.83–2.12 (m, 2H), 2.13–2.28 (m, 1H), 2.28–2.41 (m, 1H), 2.66–2.80 (m, 2H), 3.00–3.17 (m, 1H), 3.40–3.64 (m, 1H), 3.65–3.80 (m, 1H), 3.96 (s, 3H), 4.04–4.28 (m, 2H), 4.34–4.49 (m, 1H), 5.10–5.24 (m, 1H), 5.20–5.36 (m, 1H), 5.53–5.70 (m, 1H), 6.27–6.39 (m, 1H), 6.90–7.02 (m, 1H), 7.08–7.18 (m, 1H), 7.36–7.58 (m, 4H), 7.82–7.92 (m, 1H), 7.93–8.15 (m, 3H). MALDI-TOF: $(M+H)^+$ calcd: 699.3, found: 699.3.

4.7.18. (*Z*)-(1*R*,4*R*,6*S*,15*R*,17*R*)-13-*tert*-Butoxycarbonylamino-17-(7-methoxy-2-phenyl-quinolin-4-yloxy)-2,14-dioxo-3,13-diaza-tricyclo[13.3.0.0^{4,6}]octadec-7-ene-4-carboxylic acid ethyl ester (13b). Diene **12b** was cyclized according to General procedure B. Purification by HPLC (MeOH/H_2O 90:10 + 0.2% triethylamine) provided macrocyclic compound **13b** (51%) as a colorless solid. ^1H NMR (300 MHz, $CDCl_3$) δ 1.23 (t, J = 7.1 Hz, 3H), 1.30–1.56 (m, 3H), 1.49 (s, overlapped, 9H), 1.60–1.80 (m, 2H), 1.81–1.96 (m, 1H), 1.96–2.08 (m, 2H), 2.10–2.35 (m, 3H), 2.53–2.77 (m, 2H), 2.96–3.14 (m, 1H), 3.50–3.68 (m, 2H), 3.95 (s, 3H), 4.07–4.24 (m, 2H), 4.40–4.56 (m, 1H), 5.08–5.20 (m, 1H), 5.20–5.29 (m, 1H), 5.68–5.80 (m, 1H), 6.06–6.24 (bs, 1H), 6.95 (s, 1H), 7.11 (d, J = 9.1 Hz, 1H), 7.40–7.54 (m, 4H), 7.95 (bs, 1H), 8.03 (d, J = 6.6 Hz, 2H), 8.08 (d, J = 9.1 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 14.3, 22.6, 25.6, 26.1, 26.3, 27.9, 28.2, 32.8, 37.4, 42.0, 45.3, 47.3, 47.5, 55.5, 61.5, 77.8, 81.8, 98.4, 107.4, 115.3, 118.1, 123.2, 125.7, 127.6, 128.7, 129.1, 135.6, 140.5, 151.5, 154.1, 159.2, 160.9, 161.3, 170.4, 173.5,

177.8. MALDI-TOF: $(M+H)^+$ calcd: 713.4, found: 713.4.

4.7.19. (*Z*)-(1*R*,4*R*,6*S*,16*R*,18*R*)-14-*tert*-Butoxycarbonylamino-18-(7-methoxy-2-phenyl-quinolin-4-yloxy)-2,15-dioxo-3,14-diaza-tricyclo[14.3.0.0^{4,6}]nonadec-7-ene-4-carboxylic acid ethyl ester (13c). Diene **12c** was cyclized according to General procedure B. Purification by HPLC ($MeOH/H_2O$ 90:10 + 0.2% triethylamine) provided macrocyclic compound **13c** (70%) as a colorless solid. ^1H NMR (300 MHz, CD_3OD) δ 1.03–1.22 (m, 1H), 1.28 (t, $J = 7.1$ Hz, 3H), 1.32–1.44 (m, 4H), 1.49 (s, 9H), 1.55–1.73 (m, 2H), 1.81–1.91 (m, 1H), 2.04–2.28 (m, 3H), 2.30–2.52 (m, 3H), 2.53–2.70 (m, 1H), 2.86–3.00 (m, 1H), 3.34–3.44 (m, 1H), 3.46–3.62 (m, 1H), 3.95 (s, 3H), 4.19 (q, $J = 7.1$ Hz, 2H), 4.32–4.48 (m, 1H), 5.20–5.33 (m, 1H), 5.34 (bs, 1H), 5.58–5.70 (m, 1H), 7.10 (s, 1H), 7.14 (dd, $J = 2.5$, 9.1 Hz, 1H), 7.39 (d, $J = 2.5$ Hz, 1H), 7.45–7.55 (m, 3H), 8.00 (d, $J = 8.0$ Hz, 2H), 8.17 (d, $J = 9.3$ Hz, 1H); ^{13}C NMR (75.5 MHz, CD_3OD) δ 14.6, 23.4, 27.5, 27.7, 28.0, 28.5, 30.7, 36.1, 38.1, 42.5, 45.6, 56.0, 62.7, 79.9, 82.8, 100.2, 107.4, 116.6, 119.1, 124.5, 126.5, 128.9, 129.8, 130.5, 135.8, 141.5, 152.2, 156.4, 161.3, 162.5, 163.1, 171.9, 175.8, 179.0. MALDI-TOF: $(M+H)^+$ calcd: 727.4, found: 727.5.

4.7.20. (*Z*)-(1*R*,4*R*,6*S*,17*R*,19*R*)-15-*tert*-Butoxycarbonylamino-19-(7-methoxy-2-phenyl-quinolin-4-yloxy)-2,16-dioxo-3,15-diaza-tricyclo[15.3.0.0^{4,6}]icos-7-ene-4-carboxylic acid ethyl ester (13d). Diene **12d** was cyclized according to General procedure B. Purification by HPLC ($MeOH/H_2O$ 90:10 + 0.2% triethylamine) provided macrocyclic compound **13d** (79%) as a colorless solid. ^1H NMR (300 MHz, $CDCl_3$) δ 1.06–1.30 (m, 6H), 1.31–1.68 (m, 6H), 1.48 (s, overlapped, 9H), 1.89–2.06 (m, 2H), 2.09–2.38 (m, 4H), 2.54–2.69 (m, 2H), 3.00–3.14 (m, 1H), 3.40–3.55 (m, 2H), 3.93 (s, 3H), 4.04–4.24 (m, 2H), 4.28–4.44 (m, 1H), 5.07–5.25 (m, 2H), 5.37–5.50 (m, 1H), 6.39 (b, 1H), 6.94 (s, 1H), 7.09 (dd, $J = 2.6$, 9.1 Hz, 1H), 7.38–7.51 (m, 4H), 7.94–8.06 (m, 4H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 14.3, 23.8, 25.9, 26.7, 26.9, 27.6, 28.1, 28.6, 34.0, 37.7, 41.0, 44.9, 46.8, 47.3, 55.4, 61.4, 77.9, 81.6, 98.3, 107.4, 115.3, 118.0, 123.1, 125.5, 127.5, 128.6, 129.0, 135.3, 140.5, 151.3, 153.8, 159.2, 160.7, 161.2, 170.9, 173.8, 177.0. HPLC-MS: $(M+H)^+$ calcd: 741.4, found: 741.5.

4.7.21. (*Z*)-(1*R*,4*R*,6*S*,15*R*,17*S*)-17-(7-Methoxy-2-phenyl-quinolin-4-yloxy)-2,14-dioxo-3,13-diaza-tricyclo[13.3.0. 0^{4,6}]-octadec-7-ene-4-carboxylic acid ethyl ester (13e). Diene **12e** was cyclized according to General procedure B. Purification by HPLC ($MeOH/H_2O$ 85:15 + 0.2% triethylamine) provided macrocyclic compound **13e** (23%) as a green-white solid. ^1H NMR (300 MHz, $(CD_3)_2SO$) δ 1.13 (t, $J = 7.1$ Hz, 3H), 1.18–1.30 (m, 1H), 1.36–1.46 (m, 3H), 1.47–1.60 (m, 2H), 1.68–1.86 (m, 1H), 2.00–2.10 (m, 2H), 2.11–2.28 (m, 4H), 2.52–2.64 (m, 1H), 2.66–2.79 (m, 1H), 2.81–2.95 (m, 1H), 2.95–3.10 (m, 1H), 3.44–3.58 (m, 1H), 3.92 (s, 3H), 4.03 (q, $J = 7.1$ Hz, 2H), 5.34–5.45 (m, 2H), 5.62–5.75 (m, 1H), 7.18 (dd, $J = 2.2$, 9.1 Hz, 1H), 7.33 (s, 1H), 7.35–7.45 (m, 1H), 7.38 (d, $J = 2.5$ Hz, overlapped, 1H), 7.46–7.58 (m, 3H), 8.05 (d, $J = 9.1$ Hz, 1H), 8.19–8.29 (m, 2H); ^{13}C NMR (75.5 MHz, $(CD_3)_2SO$) δ 14.1, 22.1, 25.7, 26.5, 27.8, 28.3, 34.9, 35.8, 37.7, 47.3, 47.9, 55.4, 60.3, 78.2, 98.1, 107.3, 114.8, 117.6, 123.0, 126.7, 127.2, 128.5, 129.4, 132.5, 139.1, 150.4, 157.7, 160.4, 160.8, 169.8, 172.0, 172.9. MALDI-TOF: $(M+H)^+$ calcd: 598.3, found: 598.5.

4.7.22. (*Z*)-(1*R*,4*R*,6*S*,15*R*,17*R*)-17-(7-Methoxy-2-phenyl-quinolin-4-yloxy)-13-methyl-2,14-dioxo-3,13-diaza-tricyclo[13.3.0.0^{4,6}]octadec-7-ene-4-carboxylic acid ethyl ester (13f). Diene **12f** was cyclized according to General procedure B. Purification by HPLC ($MeOH/H_2O$ 85:15 + 0.2% triethylamine) provided macrocyclic compound **13f** (37%) as a green-white solid. ^1H NMR (300 MHz, $CDCl_3$) δ 1.19 (t, $J = 7.1$ Hz, 3H), 1.23–1.33 (m, 4H), 1.34–1.41 (m, 2H), 1.54–1.69 (m, 1H), 1.70–1.89 (m, 1H), 1.91–2.03 (m, 1H), 2.04–2.16 (m, 1H), 2.18–2.30 (m, 1H), 2.31–2.50 (m, 1H), 2.58–2.76 (m, 2H), 2.80–2.96 (m, 1H), 3.08 (s, 3H), 3.31–3.49 (m, 1H), 3.75–3.89 (m, 1H), 3.99 (s, 3H), 4.02–4.19 (m, 1H), 4.58–4.73 (m, 1H), 5.15–5.25 (m, 1H), 5.27–5.41 (m, 1H), 5.58–5.71 (m, 1H), 6.98 (s, 1H), 7.09 (s, 1H), 7.21 (d, $J = 6.1$ Hz, 1H), 7.33–7.54 (m, 3H), 7.89 (b, 1H), 7.96–8.11 (m, overlapped, 1H), 8.07 (d, overlapped, $J = 9.3$ Hz, 2H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 14.6, 21.2, 22.5, 24.8, 25.1, 26.5, 28.5, 29.8, 32.4, 34.1, 36.6, 42.1, 45.2, 45.9, 47.2, 56.2, 61.1, 79.2, 99.3, 104.2, 115.0, 119.9, 123.7, 125.2, 128.4, 129.2, 129.5, 130.9, 135.7, 157.7, 163.2, 169.4, 173.7, 174.6. HPLC-MS: $(M+H)^+$ calcd: 612.3, found: 612.3.

4.7.23. (*Z*)-(1*R*,4*R*,6*S*,14*R*,16*R*)-12-*tert*-Butoxycarbonylamino-16-(7-methoxy-2-phenyl-quinolin-4-yloxy)-2,13-dioxo-3,12-diaza-tricyclo[12.3.0. 0^{4,6}]heptadec-7-ene-4-carboxylic acid (14a). Compound **13a** was hydrolyzed according to General procedure D. Purification by HPLC ($MeOH/H_2O$ 70:30 + 0.2% trifluoroacetic acid) provided **14a** (32%) as a colorless solid. $[\alpha]_D^{22} + 15.0$ (*c* 0.1, MeOH); ^1H NMR (300 MHz, CD_3OD) δ 1.18–1.38 (m, 2H), 1.49 (s, 9H), 1.66–1.86 (m, 1H), 1.91–2.01 (m, 1H), 2.04–2.22 (m, 3H), 2.24–2.38 (m, 2H), 2.48–2.65 (m, 1H), 2.66–2.81 (m, 1H), 2.90–3.02 (m, 1H), 3.46–3.58 (m, 1H), 3.59–3.70 (m, 1H), 4.07 (s, 3H), 4.28–4.43 (m, 1H), 5.34–5.41 (m, 1H), 5.60–5.72 (m, 2H), 7.43–7.58 (m, 3H), 7.68–7.82 (m, 3H), 7.98–8.09 (m, 2H), 8.42 (d, $J = 9.1$ Hz, 1H); HRMS calcd $(M+H)^+$: 671.3081; found 671.3079. LC-MS Purity System A: $t_R = 7.78$ min, 97%; System B: $t_R = 7.85$ min, 99%.

4.7.24. (*Z*)-(1*R*,4*R*,6*S*,15*R*,17*R*)-13-*tert*-Butoxycarbonylamino-17-(7-methoxy-2-phenyl-quinolin-4-yloxy)-2,14-dioxo-3,13-diaza-tricyclo[13.3.0.0^{4,6}]octadec-7-ene-4-carboxylic acid (14b). Compound **13b** was hydrolyzed according to General procedure D. Purification by HPLC ($MeOH/H_2O$ 70:30 + 0.2% trifluoroacetic acid) provided **14b** (99%) as a colorless solid. $[\alpha]_D^{22} + 15.3$ (*c* 0.2, MeOH); ^1H NMR (300 MHz, CD_3OD) δ 1.22–1.42 (m, 4H), 1.47 (s, 9H), 1.61–1.75 (m, 2H), 1.75–2.02 (m, 2H), 2.08–2.20 (m, 1H), 2.20–2.29 (m, 1H), 2.30–2.54 (m, 2H), 2.62–2.78 (m, 1H), 2.80–2.93 (m, 1H), 3.37–3.51 (m, 1H), 3.52–3.70 (m, 1H), 4.06 (s, 3H),

4.35–4.48 (m, 1H), 5.34–5.45 (m, 1H), 5.60–5.70 (m, 1H), 5.71–5.83 (m, 1H), 7.41–7.51 (m, 2H), 7.53 (s, 1H), 7.66–7.81 (m, 3H), 7.92–8.07 (m, 2H), 8.42 (d, J = 9.3 Hz, 1H); ^{13}C NMR (75.5 MHz, CD_3OD) δ 23.4, 26.6, 27.6, 28.5, 28.7, 28.8, 35.8, 37.0, 42.7, 46.7, 56.9, 82.7, 83.4, 100.5, 102.3, 116.1, 121.7, 126.4, 127.3, 129.8, 130.8, 133.3, 133.8, 134.9, 143.5, 156.7, 158.0, 166.5, 168.3, 173.3, 175.9, 178.2. HRMS calcd $(M+H)^+$: 684.3237; found 684.3251. LC-MS Purity System A: t_R = 8.27 min, 97%; System B: t_R = 7.99 min, 98%.

4.7.25. (*Z*)-(1*R*,4*R*,6*S*,16*R*,18*R*)-14-*tert*-Butoxycarbonylamino-18-(7-methoxy-2-phenyl-quinolin-4-yloxy)-2,15-dioxo-3,14-diaza-tricyclo[14.3.0.0^{4,6}]nonadec-7-ene-4-carboxylic acid (14c). Compound **13c** was hydrolyzed according to General procedure D. Purification by HPLC ($MeOH/H_2O$ 80:20 and $MeOH/H_2O$ 90:10) provided **14c** (46%) as a colorless solid. $[\alpha]_D^{22}$ + 7.9 (c 0.2, MeOH); ^{1}H NMR (300 MHz, CD_3OD) δ 1.06–1.24 (m, 1H), 1.26–1.42 (m, 3H), 1.48 (s, 9H), 1.52–1.73 (m, 3H), 1.80–1.90 (m, 1H), 2.02–2.15 (m, 1H), 2.15–2.40 (m, 4H), 2.43–2.54 (m, 1H), 2.54–2.68 (m, 1H), 2.88–3.00 (m, 1H), 3.35–3.48 (m, 1H), 3.49–3.66 (m, 1H), 3.96 (s, 3H), 4.32–4.48 (m, 1H), 5.25–5.42 (m, 2H), 5.56–5.68 (m, 1H), 7.14 (s, 1H), 7.17 (dd, J = 2.5, 9.1 Hz, 1H), 7.40 (d, J = 2.2 Hz, 1H), 7.46–7.58 (m, 3H), 8.00 (d, J = 8.0 Hz, 2H), 8.19 (d, J = 9.1 Hz, 1H); ^{13}C NMR (75.5 MHz, CD_3OD) δ 23.6, 26.8, 27.8, 28.3, 28.5, 30.5, 35.8, 38.1, 43.0, 45.5, 56.0, 80.2, 82.7, 100.4, 106.9, 116.6, 119.2, 124.7, 127.4, 129.0, 129.8, 130.7, 134.8, 140.9, 151.6, 156.5, 161.1, 163.0, 163.4, 173.8, 175.7, 179.3. HRMS calcd $(M+H)^+$: 699.3394; found 699.3409. LC-MS Purity System A: t_R = 8.50 min, 100%; System B: t_R = 8.23 min, 100%.

4.7.26. (*Z*)-(1*R*,4*R*,6*S*,17*R*,19*R*)-15-*tert*-Butoxycarbonylamino-19-(7-methoxy-2-phenyl-quinolin-4-yloxy)-2,16-dioxo-3,15-diaza-tricyclo[15.3.0.0^{4,6}]icos-7-ene-4-carboxylic acid (14d). Compound **13d** was hydrolyzed according to General procedure D. Purification by HPLC ($MeOH/H_2O$ 70:30 + 0.2% trifluoroacetic acid) provided **14d** (100%) as a colorless solid. $[\alpha]_D^{22}$ + 25.2 (c 0.3, MeOH); ^{1}H NMR (300 MHz, CD_3OD) δ 1.04–1.51 (m, 6H), 1.46 (s, overlapped, 9H), 1.55–1.69 (m, 2H), 1.70–1.79 (m, 2H), 2.10–2.34 (m, 4H), 2.35–2.53 (m, 2H), 2.63–2.80 (m, 1H), 2.86–2.98 (m, 1H), 3.38–3.54 (m, 1H), 3.55–3.71 (m, 1H), 4.06 (s, 3H), 4.19–4.38 (m, 1H), 5.26–5.36 (m, 1H), 5.52–5.70 (m, 2H), 7.40–7.50 (m, 2H), 7.54 (s, 1H), 7.66–7.80 (m, 3H), 8.02 (d, J = 6.6 Hz, 2H), 8.41 (d, J = 9.3 Hz, 1H); ^{13}C NMR (75.5 MHz, CD_3OD) δ 24.2, 27.3, 27.5, 28.1, 28.5, 29.0, 30.6, 37.0, 41.6, 45.4, 47.6, 56.9, 82.7, 83.6, 100.5, 102.3, 116.2, 121.7, 126.4, 127.0, 129.8, 130.8, 133.5, 133.8, 135.6, 143.6, 156.6, 158.0, 166.6, 168.3, 169.7, 173.7, 175.5, 178.2. HRMS calcd $(M+H)^+$: 713.3550; found 713.3541. LC-MS Purity System A: t_R = 8.43 min, 100%; System B: t_R = 7.68 min, 100%.

4.7.27. (*Z*)-(1*R*,4*R*,6*S*,15*R*,17*S*)-17-(7-Methoxy-2-phenyl-quinolin-4-yloxy)-2,14-dioxo-3,13-diaza-tricyclo-[13.3.0.0^{4,6}]octadec-7-ene-4-carboxylic acid (14e). Compound **13e** was hydrolyzed according to General procedure D. Purification by HPLC ($MeOH/H_2O$ 70:30 + 0.2% trifluoroacetic acid) provided **14e** (68%) as a colorless solid. $[\alpha]_D^{22}$ + 25.3 (c 0.2, MeOH); ^{1}H NMR (300 MHz, CD_3OD) δ 1.21–1.41 (m, 1H), 1.42–1.54 (m, 2H), 1.55–1.71 (m, 2H), 1.75–1.85 (m, 1H), 1.86–2.01 (m, 1H), 2.12–2.24 (m, 1H), 2.25–2.35 (m, 1H), 2.37–2.56 (m, 3H), 2.60–2.74 (m, 1H), 2.80–2.95 (m, 1H), 3.01–3.15 (m, 1H), 3.16–3.29 (m, 1H), 3.62–3.78 (m, 1H), 4.07 (s, 3H), 5.41–5.53 (m, 1H), 5.60–5.70 (m, 1H), 5.76–5.89 (m, 1H), 7.46 (d, J = 9.3 Hz, 1H), 7.50–7.58 (m, 2H), 7.66–7.82 (m, 3H), 8.00–8.10 (m, 2H), 8.44 (d, J = 9.3 Hz, 1H); ^{13}C NMR (75.5 MHz, CD_3OD) δ 23.5, 27.7, 28.2, 29.0, 29.3, 36.1, 36.8, 39.7, 42.6, 56.9, 83.8, 100.4, 102.3, 116.2, 121.7, 126.6, 127.8, 129.8, 130.8, 133.4, 133.8, 134.9, 143.5, 158.1, 166.6, 168.6, 173.5, 175.1, 175.7. HRMS calcd $(M+H)^+$: 570.2604; found 570.2603. LC-MS Purity System A: t_R = 4.76 min, 98%; System B: t_R = 7.08 min, 98%.

4.7.28. (*Z*)-(1*R*,4*R*,6*S*,15*R*,17*R*)-17-(7-Methoxy-2-phenyl-quinolin-4-yloxy)-13-methyl-2,14-dioxo-3,13-diaza-tricyclo[13.3.0.0^{4,6}]octadec-7-ene-4-carboxylic acid (14f). Compound **13f** was hydrolyzed according to General procedure D. Purification by HPLC ($MeOH/H_2O$ 70:30 + 0.2% trifluoroacetic acid) provided **14f** (53%) as a colorless solid. $[\alpha]_D^{22}$ + 47.1 (c 0.2, MeOH); ^{1}H NMR (300 MHz, CD_3OD) δ 1.25–1.37 (m, 2H), 1.45 (dd, J = 5.1, 9.8 Hz, 1H), 1.55–1.64 (m, 1H), 1.66–1.72 (m, 1H), 1.73–1.83 (m, 2H), 1.84–1.94 (m, 1H), 2.10 (app. q, J = 8.9 Hz, 1H), 2.24–2.31 (m, 1H), 2.32–2.44 (m, 2H), 2.64–2.75 (m, 1H), 2.84–2.98 (m, 1H), 3.08 (s, 3H), 3.37–3.45 (m, 1H), 3.49–3.60 (m, 1H), 3.94 (s, 3H), 4.40–4.49 (m, 1H), 5.29–5.40 (m, 2H), 5.60–5.70 (m, 1H), 7.13 (s, 1H), 7.15 (d, J = 9.4 Hz, 1H), 7.28 (s, 1H), 7.49–7.58 (m, 3H), 8.00 (d, J = 7.8 Hz, 2H), 8.10 (d, J = 9.0 Hz, 1H); ^{13}C NMR (75.5 MHz, CD_3OD) δ 21.9, 24.6, 26.2, 27.6, 27.9, 33.7, 34.0, 35.4, 45.4, 46.4, 54.8, 78.6, 99.1, 104.9, 115.0, 118.1, 123.3, 125.7, 127.7, 128.6, 129.7, 133.2, 138.6, 149.2, 159.3, 162.0, 162.2, 174.4, 175.4. HRMS calcd $(M+H)^+$: 584.2761; found 584.2763. LC-MS Purity System C: t_R = 2.74 min, 97%; System D: t_R = 2.37 min, 95%.

4.7.29. (*Z*)-(1*R*,4*R*,6*S*,15*R*,17*R*)-13-Amino-17-(7-methoxy-2-phenyl-quinolin-4-yloxy)-2,14-dioxo-3,13-diaza-tricyclo[13.3.0.0^{4,6}]octadec-7-ene-4-carboxylic acid ethyl ester (15b). The Boc group of **13b** was removed according to General procedure C. Purification by HPLC ($MeOH/H_2O$ 90:10 + 0.2% triethylamine) gave **15b** (63%) as a colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.08–1.25 (m, 2H), 1.21 (t, J = 7.1 Hz, overlapped, 3H), 1.28–1.45 (m, 2H), 1.55–1.70 (m, 1H), 1.71–1.85 (m, 1H), 1.86–2.02 (m, 3H), 2.03–2.12 (m, 1H), 2.20–2.32 (m, 1H), 2.33–2.45 (m, 1H), 2.62–2.72 (m, 1H), 2.73–2.85 (m, 1H), 2.85–2.97 (m, 1H), 3.60–3.73 (m, 1H), 3.85 (bs, 2H), 3.96 (s, 3H), 4.02–4.16 (m, 2H), 4.67–4.82 (m, 1H), 5.12–5.25 (m, 2H), 5.58–5.70 (m, 1H), 6.95–7.01 (m, 2H), 7.11 (d, J = 9.2 Hz, 1H), 7.41–7.60 (m, 4H), 8.02–8.10 (m, 3H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 14.6, 22.5, 25.0, 25.1, 25.4, 28.2, 32.7, 37.3, 42.2, 45.7, 46.7, 47.3, 55.8, 61.2, 78.1, 98.8, 107.2, 115.5, 118.6, 123.4, 125.6, 127.9, 128.4, 129.0, 129.6, 135.6, 140.0, 150.9, 159.1, 161.5, 161.8, 169.6,

174.2, 177.5. MALDI-TOF: $(M+H)^+$ calcd: 613.3, found: 613.6.

4.7.30. (*Z*)-(1*R*,4*R*,6*S*,16*R*,18*R*)-14-Amino-18-(7-methoxy-2-phenyl-quinolin-4-yloxy)-2,15-dioxo-3,14-diaza-tricyclo[14.3.0.0^{4,6}]nonadec-7-ene-4-carboxylic acid ethyl ester (15c). The Boc group of **13c** was removed according to General procedure C. Purification by HPLC ($MeOH/H_2O$ 90:10 + 0.2% triethylamine) gave **15c** (66%) as a colorless solid. ^1H NMR (300 MHz, $CDCl_3$) δ 1.01–1.20 (m, 3H), 1.24 (t, $J = 7.1$ Hz, 3H), 1.28–1.49 (m, 3H), 1.52–1.67 (m, 1H), 1.68–1.90 (m, 1H), 1.93–2.04 (m, 1H), 2.05–2.19 (m, 4H), 2.20–2.31 (m, 1H), 2.62–2.85 (m, 3H), 3.56–3.75 (m, 1H), 3.95 (s, 3H), 4.01 (bs, 2H), 4.09–4.22 (m, 2H), 4.58–4.72 (m, 1H), 5.09–5.22 (m, 2H), 5.50–5.65 (m, 1H), 6.55 (bs, 1H), 6.99 (s, 1H), 7.10 (dd, $J = 2.5$, 9.1 Hz, 1H), 7.40–7.55 (m, 4H), 8.00–8.11 (m, 3H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 14.4, 23.0, 25.6, 26.2, 27.5, 28.8, 33.0, 37.4, 41.7, 45.3, 45.6, 50.4, 55.1, 61.1, 78.0, 98.5, 107.3, 115.4, 118.1, 123.3, 125.8, 127.6, 128.7, 129.2, 134.0, 140.3, 151.1, 159.2, 161.0, 161.3, 170.1, 174.4, 176.9. MALDI-TOF: $(M+H)^+$ calcd: 627.3, found: 627.6.

4.7.31. (*Z*)-(1*R*,4*R*,6*S*,17*R*,19*R*)-15-Amino-19-(7-methoxy-2-phenyl-quinolin-4-yloxy)-2,16-dioxo-3,15-diaza-tricyclo[15.3.0.0^{4,6}]icos-7-ene-4-carboxylic acid ethyl ester (15d). The Boc group of **13d** was removed according to General procedure C. Purification by HPLC ($MeOH/H_2O$ 90:10 + 0.2% triethylamine) gave **15d** (74%) as a colorless solid. ^1H NMR (300 MHz, $CDCl_3$) δ 1.05–1.29 (m, 3H), 1.20 (t, $J = 7.1$ Hz, 3H), 1.30–1.42 (m, 3H), 1.43–1.56 (m, 2H), 1.54–1.80 (m, 1H), 1.85–1.98 (m, 2H), 1.99–2.18 (m, 3H), 2.19–2.34 (m, 2H), 2.53–2.68 (m, 1H), 2.70–2.83 (m, 1H), 2.85–3.00 (m, 1H), 3.57–3.71 (m, 1H), 3.79 (bs, 2H), 3.94 (s, 3H), 4.00–4.19 (m, 2H), 4.52–4.66 (m, 1H), 5.10–5.26 (m, 2H), 5.50–5.62 (m, 1H), 6.93–7.01 (m, 2H), 7.09 (dd, $J = 2.5$, 9.7 Hz, 1H), 7.38–7.54 (m, 4H), 7.97–8.11 (m, 3H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 14.3, 23.5, 26.0, 26.1, 26.2, 27.0, 27.5, 29.5, 33.7, 37.8, 41.0, 44.7, 45.9, 49.4, 55.5, 60.9, 77.6, 98.4, 107.4, 115.4, 118.0, 123.2, 125.6, 127.5, 128.7, 129.1, 135.4, 140.5, 151.2, 159.1, 160.8, 161.3, 170.1, 174.2, 176.7. HPLC-MS: $(M+H)^+$ calcd: 641.3, found: 641.4.

4.7.32. (*Z*)-(1*R*,4*R*,6*S*,15*R*,17*R*)-13-Amino-17-(7-methoxy-2-phenyl-quinolin-4-yloxy)-2,14-dioxo-3,13-diaza-tricyclo[13.3.0.0^{4,6}]octadec-7-ene-4-carboxylic acid (16b). Compound **15b** was hydrolyzed according to General procedure D. Purification by HPLC ($MeOH/H_2O$ 70:30 + 0.2% trifluoroacetic acid) provided **16b** (52%) as a colorless solid. $[\alpha]_D^{22} + 11.3$ (*c* 0.1, MeOH); ^1H NMR (300 MHz, CD_3OD) δ 1.24–1.39 (m, 2H), 1.40–1.50 (m, 2H), 1.52–1.72 (m, 1H), 1.73–1.84 (m, 1H), 1.86–1.98 (m, 1H), 1.99–2.11 (m, 1H), 2.12–2.24 (m, 1H), 2.30–2.44 (m, 2H), 2.44–2.57 (m, 1H), 2.81–2.92 (m, 1H), 2.95–3.10 (m, 1H), 3.40–3.53 (m, 1H), 4.06 (s, 3H), 4.10–4.25 (m, 1H), 4.48–4.61 (m, 1H), 5.28–5.40 (m, 1H), 5.62–5.84 (m, 2H), 7.41–7.56 (m, 3H), 7.67–7.82 (m, 3H), 8.00–8.09 (m, 2H), 8.40 (d, $J = 9.3$ Hz, 1H); ^{13}C NMR (75.5 MHz, CD_3OD) δ 23.2, 26.4, 26.7, 28.6, 28.9, 35.2, 37.2, 42.8, 47.0, 48.0, 56.9, 83.2, 100.5, 102.3, 116.1, 121.7, 126.4, 127.1, 129.8, 130.8, 133.4, 133.8, 135.0, 143.5, 158.1, 166.6, 168.6, 172.8, 176.5, 177.7. HRMS calcd $(M+H)^+$: 585.2713; found 585.2703. LC-MS Purity System A: $t_R = 5.14$ min, 100%; System B: $t_R = 6.62$ min, 100%.

4.7.33. (*Z*)-(1*R*,4*R*,6*S*,16*R*,18*R*)-14-Amino-18-(7-methoxy-2-phenyl-quinolin-4-yloxy)-2,15-dioxo-3,14-diaza-tricyclo[14.3.0.0^{4,6}]nonadec-7-ene-4-carboxylic acid (16c). Compound **15c** was hydrolyzed according to General procedure D. Purification by HPLC ($MeOH/H_2O$ 70:30 + 0.2% trifluoroacetic acid) provided **16c** (46%) as a colorless solid. $[\alpha]_D^{22} - 2.4$ (*c* 0.2, MeOH); ^1H NMR (300 MHz, CD_3OD) δ 1.05–1.28 (m, 1H), 1.30–1.48 (m, 2H), 1.44–1.56 (m, 2H), 1.57–1.72 (m, 1H), 1.76–1.91 (m, 2H), 2.05–2.26 (m, 2H), 2.27–2.43 (m, 2H), 2.52–2.66 (m, 1H), 2.81–2.95 (m, 2H), 3.20–3.38 (m, overlapped, 1H), 3.44–3.60 (m, 1H), 4.01 (s, 3H), 4.21–4.35 (m, 1H), 4.45–4.60 (m, 1H), 5.20–5.31 (m, 1H), 5.43–5.75 (m, 2H), 7.46 (d, $J = 9.2$ Hz, 1H), 7.50 (s, 1H), 7.53 (s, 1H), 7.68–7.82 (m, 3H), 8.00–8.09 (m, 2H), 8.42 (d, $J = 9.2$ Hz, 1H); ^{13}C NMR (75.5 MHz, CD_3OD) δ 23.5, 26.8, 27.3, 27.7, 28.5, 30.3, 35.3, 37.8, 42.6, 45.5, 48.0, 50.5, 56.9, 83.5, 100.4, 102.3, 116.2, 121.7, 126.6, 127.0, 129.8, 130.8, 133.4, 133.8, 135.2, 143.5, 158.1, 160.9, 161.4, 166.6, 168.6, 172.7, 176.0, 178.1. HRMS calcd $(M+H)^+$: 599.2869; found 599.2889. LC-MS Purity System A: $t_R = 6.09$ min, 100%; System B: $t_R = 7.61$ min, 100%.

4.7.34. (*Z*)-(1*R*,4*R*,6*S*,17*R*,19*R*)-15-Amino-19-(7-methoxy-2-phenyl-quinolin-4-yloxy)-2,16-dioxo-3,15-diaza-tricyclo[15.3.0.0^{4,6}]icos-7-ene-4-carboxylic acid (16d). Compound **15d** was hydrolyzed according to General procedure D. Purification by HPLC ($MeOH/H_2O$ 70:30 + 0.2% trifluoroacetic acid) provided **16d** (71%) as a colorless solid. $[\alpha]_D^{22} + 31.9$ (*c* 0.4, MeOH); ^1H NMR (300 MHz, CD_3OD) δ 1.08–1.57 (m, 9H), 1.67–1.75 (m, 1H), 2.02–2.18 (m, 2H), 2.19–2.33 (m, 2H), 2.34–2.50 (m, 2H), 2.81–2.92 (m, 1H), 2.94–3.08 (m, 1H), 3.35–3.52 (m, 1H), 4.06 (s, 3H), 4.11–4.24 (m, 1H), 4.38–4.50 (m, 1H), 5.22–5.32 (m, 1H), 5.53–5.70 (m, 2H), 7.42–7.56 (m, 3H), 7.67–7.80 (m, 3H), 8.00–8.07 (m, 2H), 8.39 (d, $J = 9.3$ Hz, 1H); ^{13}C NMR (75.5 MHz, CD_3OD) δ 24.1, 27.2, 27.5, 27.7, 28.8, 29.1, 30.7, 36.6, 37.8, 41.2, 46.0, 47.5, 49.9, 56.9, 83.5, 100.5, 102.3, 116.2, 121.7, 126.5, 127.1, 129.8, 130.8, 133.5, 133.8, 135.6, 143.6, 158.1, 166.6, 168.5, 173.5, 176.0, 177.6. HRMS calcd $(M+H)^+$: 613.3026; found 613.3038. LC-MS Purity System A: $t_R = 6.87$ min, 100%; System B: $t_R = 6.98$ min, 100%.

4.7.35. [(*Z*)-(1*R*,4*R*,6*S*,15*R*,17*R*)-4-Cyclopropanesulfonylaminocarbonyl-17-(7-methoxy-2-phenyl-quinolin-4-yloxy)-2,14-dioxo-3,13-diaza-tricyclo[13.3.0.0^{4,6}]octadec-7-en-13-yl]-carbamic acid *tert*-butyl ester (17). Compound **14b** was coupled to cyclopropanesulfonic acid amide according to General procedure E. Purification by HPLC ($MeOH/H_2O$ 70:30 + 0.2% trifluoroacetic acid) provided **17** (80%) as a colorless solid. $[\alpha]_D^{22} + 35.0$ (*c* 0.1, MeOH); ^1H NMR (300 MHz, CD_3OD) δ 0.84–0.99 (m, 1H), 1.00–1.20 (m, 3H), 1.22–1.40 (m, 4H), 1.46 (s, 9H), 1.54–1.64 (m, 1H), 1.64–1.77 (m, 2H), 1.78–2.00

(m, 2H), 2.21–2.37 (m, 2H), 2.43–2.60 (m, 2H), 2.67–2.84 (m, 1H), 2.86–3.00 (m, 1H), 3.38–3.53 (m, 1H), 3.53–3.71 (m, 1H), 4.06 (s, 3H), 4.40–4.55 (m, 1H), 5.12–5.25 (m, 1H), 5.58–5.78 (m, 2H), 7.38 (s, 1H), 7.47 (dd, J = 2.5, 9.3 Hz, 1H), 7.55 (s, 1H), 7.65–7.81 (m, 3H), 8.02 (d, J = 6.6 Hz, 2H), 8.37 (d, J = 9.3 Hz, 1H); ^{13}C NMR (75.5 MHz, CD_3OD) δ 6.5, 6.7, 22.0, 25.7, 26.5, 28.1, 28.5, 31.9, 33.0, 36.0, 36.6, 44.8, 45.1, 56.9, 82.7, 83.3, 100.7, 102.3, 116.0, 121.8, 125.9, 126.2, 129.8, 130.8, 133.5, 133.8, 143.7, 156.9, 158.1, 166.5, 168.1, 171.0, 177.3, 180.4. HRMS calcd $(M+H)^+$: 788.3329; found 788.3296. LC-MS Purity System A: t_R = 6.59 min, 98%; System B: t_R = 6.56 min, 97%.

4.7.36. Cyclopropanesulfonic acid [(*Z*)-(1*R*,4*R*,6*S*,15*R*,17*R*)-13-amino-17-(7-methoxy-2-phenyl-quinolin-4-yloxy)-2,14-dioxo-3,13-diaza-tricyclo[13.3.0.0^{4,6}]octadec-7-ene-4-carbonyl]-amide (18). The Boc group of **17** was removed according to General procedure C. Purification by gradient HPLC-MS gave **18** (95%) as a colorless solid. $[\alpha]_D^{22}$ + 20.0 (*c* 0.1, MeOH); ^{1}H NMR (300 MHz, CD_3OD) δ 0.83–0.98 (m, 1H), 1.00–1.17 (m, 3H), 1.22–1.48 (m, 4H), 1.52–1.61 (m, 1H), 1.64–1.78 (m, 2H), 1.84–1.98 (m, 1H), 1.99–2.14 (m, 1H), 2.23–2.39 (m, 2H), 2.41–2.59 (m, 2H), 2.76–2.85 (m, 1H), 2.87–2.99 (m, 1H), 3.06–3.19 (m, 1H), 3.53–3.69 (m, 1H), 4.05 (s, 3H), 4.51–4.64 (m, 1H), 5.10–5.22 (m, 1H), 5.58–5.74 (m, 2H), 7.41–7.50 (m, 2H), 7.54 (s, 1H), 7.65–7.80 (m, 3H), 8.03 (d, J = 6.0 Hz, 2H), 8.37 (d, J = 9.6 Hz, 1H); ^{13}C NMR (75.5 MHz, CD_3OD) δ 6.5, 6.8, 21.9, 25.6, 25.8, 28.5, 31.8, 33.2, 36.4, 36.7, 45.2, 47.0, 56.9, 83.3, 100.6, 102.3, 116.0, 121.7, 125.9, 126.2, 129.8, 130.8, 133.5, 137.8, 143.7, 158.2, 166.5, 168.3, 170.9, 176.6, 181.1. HRMS calcd $(M+H)^+$: 688.2805; found 688.2809. LC-MS Purity System A: t_R = 4.59 min, 98%; System B: t_R = 5.85 min, 98%.

4.7.37. Cyclopropanesulfonic acid [(*Z*)-(1*R*,4*R*,6*S*,15*R*,17*S*)-17-(7-methoxy-2-phenyl-quinolin-4-yloxy)-2,14-dioxo-3,13-diaza-tricyclo[13.3.0. 0^{4,6}]octadec-7-ene-4-carbonyl]-amide (19). Compound **14e** was coupled to cyclopropanesulfonic acid amide according to General procedure E. Purification by HPLC ($MeOH/H_2O$ 70:30 + 0.2% trifluoroacetic acid) provided **19** (23%) as a colorless solid. $[\alpha]_D^{22}$ + 37.3 (*c* 0.1, MeOH); ^{1}H NMR (300 MHz, $(CD_3)_2SO$) δ 0.95–1.15 (m, 3H), 1.23–1.35 (m, 1H), 1.40–1.60 (m, 4H), 1.67–1.81 (m, 1H), 1.86–1.97 (m, 1H), 1.99–2.10 (m, 1H), 2.11–2.25 (m, 1H), 2.33–2.43 (m, 1H), 2.60–2.69 (m, 2H), 2.70–2.80 (m, 2H), 2.88–2.95 (m, 1H), 2.96–3.12 (m, 2H), 3.38–3.46 (m, overlapped, 1H), 3.47–3.59 (m, 1H), 3.94 (s, 3H), 5.16–5.24 (m, 1H), 5.47–5.56 (m, 1H), 5.61–5.71 (m, 1H), 7.30 (b, 1H), 7.43 (s, 1H), 7.51–7.65 (m, 4H), 7.70 (b, 1H), 8.11 (d, J = 9.4 Hz, 1H), 8.18–8.25 (m, 2H), 8.87 (s, 1H), 11.47 (b, 1H); ^{13}C NMR (75.5 MHz, $(CD_3)_2SO$) δ 6.1, 6.3, 21.4, 25.6, 26.7, 28.0, 31.1, 32.4, 35.5, 37.3, 43.1, 43.3, 48.2, 48.5, 56.2, 72.9, 79.6, 98.7, 111.1, 115.0, 119.1, 124.0, 126.0, 128.5, 129.3, 132.3, 141.1, 152.5, 157.6, 169.7, 171.7, 172.0, 175.0, 177.9. HRMS calcd $(M+H)^+$: 673.2696; found 673.2690. LC-MS Purity System C: t_R = 2.55 min, 99%; System D: t_R = 2.09 min, 99%.

4.7.38. Cyclopropanesulfonic acid [(*Z*)-(1*R*,4*R*,6*S*,15*R*,17*R*)-17-(7-methoxy-2-phenyl-quinolin-4-yloxy)-13-methyl-2,14-dioxo-3,13-diaza-tricyclo[13.3.0.0^{4,6}]octadec-7-ene-4-carbonyl]-amide (20). Compound **14f** was coupled to cyclopropanesulfonic acid amide according to General procedure E. Purification by HPLC ($MeOH/H_2O$ 70:30 + 0.2% trifluoroacetic acid) provided **20** (29%) as a colorless solid. $[\alpha]_D^{22}$ + 59.3 (*c* 0.1, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90–1.01 (m, 1H), 1.01–1.12 (m, 1H), 1.14–1.22 (m, 1H), 1.24–1.35 (m, 2H), 1.36–1.52 (m, 2H), 1.53–1.61 (m, 1H), 1.62–1.73 (m, 1H), 1.76–1.86 (m, 1H), 1.87–1.97 (m, 2H), 2.03–2.14 (m, 2H), 2.32–2.46 (m, 1H), 2.51–2.63 (m, 2H), 2.64–2.75 (m, 1H), 2.85–2.95 (m, 1H), 2.98 (s, 3H), 3.27–3.48 (m, 2H), 4.00 (s, 3H), 4.53–4.64 (m, 1H), 4.99–5.09 (m, 2H), 5.58–5.68 (m, 1H), 6.76 (s, 1H), 7.06 (dd, J = 2.5, 9.2 Hz, 1H), 7.41 (b, 1H), 7.43–7.55 (m, 3H), 7.71 (s, 1H), 7.82 (d, J = 9.4 Hz, 1H), 7.92 (d, J = 7.0 Hz, 2H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 6.0, 6.6, 21.0, 24.0, 26.0, 27.6, 31.0, 33.8, 34.9, 44.0, 44.9, 46.8, 47.2, 48.1, 55.6, 77.8, 98.4, 106.8, 114.9, 118.3, 123.2, 124.3, 127.5, 128.8, 128.9, 129.6, 133.1, 140.0, 150.9, 159.5, 160.6, 161.5, 172.4, 172.9. HRMS calcd $(M+H)^+$: 687.2853; found 687.2848. LC-MS Purity System C: t_R = 2.81 min, 100%; System D: t_R = 2.36 min, 99%.

4.7.39. ((3*R*,5*R*) and (3*S*,5S))-5-((1*R*,2*S*)-1-*tert*-Butoxycarbonyl-2-vinyl-cyclopropylcarbamoyl)-3-(7-methoxy-2-phenyl-quinolin-4-yloxy)-cyclopent-1-enecarboxylic acid (21). Compound **21** was synthesized according to Refs. 27 and 31–33.

4.7.40. (1*R*,2*S*)-1-{[((1*R*,4*R*) and (1*S*,4*S*))-2-(*N*′-*tert*-Butoxycarbonyl-*N*-hex-5-enyl-hydrazinocarbonyl)-4-(7-methoxy-2-phenyl-quinolin-4-yloxy)-cyclopent-2-enecarbonyl]-amino}-2-vinyl-cyclopropanecarboxylic acid *tert*-butyl ester (22a). To a cooled solution (0 °C) of a diastereomeric mixture of compound **21** (28 mg, 0.049 mmol), hydrazine **2b** (16 mg, 0.074 mmol), and DIPEA (13 μL, 0.074 mmol) in DMF (1 mL) was added HATU (28 mg, 0.074 mmol). The mixture was stirred for 3 h and concentrated. The residue was dissolved in DCM and the organic phase was washed with water, dried with $MgSO_4$, filtered, and concentrated. Purification by gradient HPLC-MS gave a diasteromeric mixture of **22a** (31 mg, 81%) as a pale brown syrup. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.17–1.61 (m, 24H), 1.68–1.89 (m, 2H), 1.98–2.16 (m, 2H), 2.24–2.41 (m, 1H), 2.99–3.16 (m, 1H), 3.35–3.52 (m, 1H), 3.65–3.83 (m, 1H), 3.96 (s, 3H), 4.07–4.16 (m, 1H), 4.88–5.04 (m, 2H), 5.11–5.19 (m, 1H), 5.24–5.38 (m, 1H), 5.66–5.85 (m, 2H), 5.93–6.03 (m, 1H), 6.34–6.46 (m, 1H), 6.71–6.86 (m, 1H), 7.03 (s, 1H), 7.09–7.19 (m, 1H), 7.45–7.64 (m, 4H), 7.93–8.07 (m, 3H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 22.3, 26.0, 26.5, 27.0, 28.2, 32.6, 33.3, 34.2, 38.8, 39.1, 40.6, 43.9, 45.5, 45.9, 48.5, 51.6, 52.3, 55.8, 83.7, 98.9, 105.1, 114.9, 119.0, 123.4, 128.0, 128.9, 130.1, 133.3, 133.7, 138.3, 149.3, 154.5, 158.9, 162.3, 164.5, 169.5, 173.8. HPLC-MS: $(M+H)^+$ calcd: 767.4, found: 767.4.

4.7.41. (1*R*,2*S*)-1-{[((1*R*,4*R*) and (1*S*,4*S*))-2-(*N*′-*tert*-Butoxycarbonyl-*N*-hept-6-enyl-hydrazinocarbonyl)-4-(7-methoxy-2-phenyl-quinolin-4-yloxy)-cyclopent-2-enecarbonyl]-amino}-2-vinyl-cyclopropanecarboxylic acid *tert*-butyl ester (22b). Compound **22b** (diastereomeric mixture) (79%) was prepared from **21** according to the method of the preparation of **22a** using hydrazine **5a** instead of hydrazine **2b**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.16–1.63 (m, 24H), 1.69–1.92 (m, 2H), 1.96–2.16 (m, 4H), 2.23–2.38 (m, 1H), 2.99–3.15 (m, 1H), 3.34–3.51 (m, 1H), 3.62–3.83 (m, 1H), 3.95 (s, 3H), 4.06–4.16 (m, 1H), 4.88–5.03 (m, 2H), 5.10–5.18 (m, 1H), 5.25–5.37 (m, 1H), 5.66–5.85 (m, 2H), 5.90–6.00 (m, 1H), 6.37–6.45 (m, 1H), 6.62–6.73 (m, 1H), 7.02–7.14 (m, 2H), 7.38–7.54 (m, 4H), 7.95–8.08 (m, 3H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 22.9, 23.0, 26.3, 26.5, 27.7, 28.2, 28.3, 28.4, 28.6, 32.7, 33.5, 33.6, 34.0, 34.2, 40.7, 41.1, 48.7, 52.2, [55.5 and 55.6], 83.0, 98.2, [107.5 and 107.6], [114.3 and 114.4], [118.1 and 118.2], 123.0, [127.6 and 127.7], 128.7, 129.2, [133.4 and 133.6], [138.7 and 138.9], 140.4, 151.3, 154.7, [159.3 and 159.4], 161.0, 161.3, 169.5, 172.5. HPLC-MS: $(M+H)^+$ calcd: 781.4, found: 781.5.

4.7.42. (*Z*)-((1*R*,4*R*,6*S*,17*R*) and (1*S*,4*R*,6*S*,17*S*))-13-*tert*-Butoxycarbonylamino-17-(7-methoxy-2-phenyl-quinolin-4-yloxy)-2,14-dioxo-3,13-diaza-tricyclo[13.3.0.0^{4,6}]octa-deca-7,15-diene-4-carboxylic acid *tert*-butyl ester (23a). To a solution of compound **22a** (23 mg, 0.030 mmol) (diastereomeric mixture) in DCM (4 mL) was added 2nd Generation Hoveyda-Grubbs Catalyst (1.1 mg, 0.018 mmol) and the reaction mixture was refluxed for 24 h with two extra additions of the catalyst. The mixture was concentrated and purified by gradient HPLC-MS to give a diastereomeric mixture of **23a** (18 mg, 81%) as a syrup. ^{1}H NMR (300 MHz, CD_3OD) δ 0.73–0.97 (m, 5H), 1.15–1.74 (m, 20H), 1.95–2.59 (m, 4H), 2.91–3.04 (m, 1H), 3.84–4.05 (m, 4H), 4.18–4.38 (m, 1H), 5.21–5.47 (m, 1H), 5.61–5.79 (m, 1H), 6.01–6.11 (m, 1H), 6.58–6.70 (m, 1H), 7.10–7.28 (m, 2H), 7.42–7.66 (m, 4H), 7.94–8.11 (m, 3H). HPLC-MS: $(M+H)^+$ calcd: 739.4, found: 739.4.

4.7.43. (*Z*)-((1*R*,4*R*,6*S*,18*R*) and (1*S*,4*R*,6*S*,18*S*))-14-*tert*-Butoxycarbonylamino-18-(7-methoxy-2-phenyl-quinolin-4-yloxy)-2,15-dioxo-3,14-diaza-tricyclo[14.3.0.0^{4,6}]nonadeca-7,16-diene-4-carboxylic acid *tert*-butyl ester (23b). Compound **23b** (53%) was prepared from **22b** according to the method of the preparation of **23a**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.16–1.51 (m, 18H), 1.58–1.89 (m, 8H), 1.99–2.34 (m, 5H), 3.13–3.39, (m, 2H), 3.95 (s, 3H), 4.18–4.28 (m, 1H), 4.36–4.49 (m, 1H), 5.16–5.34 (m, 1H), 5.59–5.70 (m, 1H), 5.90–5.99 (m, 1H), 6.20–6.32 (m, 1H), 7.02–7.13 (m, 2H), 7.39–7.55 (m, 4H), 7.97–8.08 (m, 3H), 8.80 (bs, 1H). HPLC–MS: $(M+H)^+$ calcd: 753.4, found: 753.5.

4.7.44. (*Z*)-(1*R*,4*R*,6*S*,17*R*) and (1*S*,4*R*,6*S*,17*S*)-13-Amino-17-(7-methoxy-2-phenyl-quinolin-4-yloxy)-2,14-dioxo-3,13-diaza-tricyclo[13.3.0.0^{4,6}]octadeca-7,15-diene-4-carboxylic acid (24a). To a cooled (0 °C) solution of compound **23a** (18 mg, 0.024 mmol) (diastereomeric mixture) in DCM (2 mL) were added triethylsilane (11.4 μL, 0.072 mmol) and TFA (1 mL). The mixture was stirred for 4 h and concentrated. Purification by gradient HPLC-MS gave compound **24a** (5.4 mg, 38%) (diastereomeric mixture) as a white powder after lyophilization. ^{1}H NMR (300 MHz, $CDCl_3$/MeOD (1:1, v/v)) δ 1.24-1.86 (m, 6H), 1.99–2.17 (m, 2H), 2.49–2.68 (m, 2H), 2.84–3.01 (m, 2H), 4.05 (s, 3H), 4.18–4.32 (m, 1H), 5.45–5.73 (m, 2H), 6.15–6.27 (m, 1H), 6.37–6.48 (m, 1H), 6.91–6.99 (m, 1H), 7.07–7.13 (m, 1H), 7.38–7.84 (m, 5H), 7.98–8.12 (m, 3H). HRMS calcd $(M+H)^+$: 583.2567; found 583.2557. LC-MS Purity System A: t_R = 7.41 min, 100%; System B: t_R = 6.58 min, 99%.

4.7.45. (*Z*)-(1*R*,4*R*,6*S*,18*R*) and (1*S*,4*R*,6*S*,18*S*)-14-Amino-18-(7-methoxy-2-phenyl-quinolin-4-yloxy)-2,15-dioxo-3,14-diaza-tricyclo[14.3.0.0^{4,6}]nonadeca-7,16-diene-4-carboxylic acid (24b). Compound **24b** (47%) was prepared from **23b** according to the method of the preparation of **24a**. ^{1}H NMR (300 MHz, $CDCl_3$/CD_3OD (1:1 v/v)) δ 1.08–1.71 (m, 8H), 1.96–2.26 (m, 4H), 2.41–2.52 (m, 2H), 2.80–2.95 (m, 2H), 3.94 (s, 3H), 4.18–4.34 (m, 1H), 5.34–5.62 (m, 2H), 5.96–6.05 (m, 1H), 6.51–6.60 (m, 1H), 7.03–7.20 (m, 2H), 7.35–7.56 (m, 3H), 7.71–7.78 (m, 1H), 7.92–8.07 (m, 2H). HRMS calcd $(M+H)^+$: 597.2712; found 597.2713. LC-MS Purity System A: t_R = 7.22 min, 100%; System B: t_R = 6.43 min, 100%.

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References and notes

1. White, P. W.; Llinas-Brunet, M.; Bos, M. *Prog. Med. Chem.* **2006**, *44*, 65–107.
2. Ni, Z. J.; Wagman, A. S. *Curr. Opin. Drug Discov. Devel.* **2004**, *7*, 446–459.
3. Rehermann, B.; Nascimbeni, M. *Nat. Rev. Immunol.* **2005**, *5*, 215–229.
4. Tan, S. L.; Pause, A.; Shi, Y. U.; Sonenberg, N. *Nat. Rev. Drug Discov.* **2002**, *1*, 867–881.
5. Gordon, C. P.; Keller, P. A. *J. Med. Chem.* **2005**, *48*, 1–20.
6. Strader, D. B.; Wright, T.; Thomas, D. L.; Seeff, L. B. *Hepatology* **2004**, *39*, 1147–1171.
7. Dymock, B. W.; Jones, P. S.; Wilson, F. X. *Antiviral Chem. Chemother.* **2000**, *11*, 79–96.
8. Poupart, M. A.; Cameron, D. R.; Chabot, C.; Ghiro, E.; Goudreau, N.; Goulet, S.; Poirier, M.; Tsantrizos, Y. S. *J. Org. Chem.* **2001**, *66*, 4743–4751.
9. Goudreau, N.; Brochu, C.; Cameron, D. R.; Duceppe, J. S.; Faucher, A. M.; Ferland, J. M.; Grand-Maitre, C.; Poirier, M.; Simoneau, B.; Tsantrizos, Y. S. *J. Org. Chem.* **2004**, *69*, 6185–6201.
10. Rancourt, J.; Cameron, D. R.; Gorys, V.; Lamarre, D.; Poirier, M.; Thibeault, D.; Llinàs-Brunet, M. *J. Med. Chem.* **2004**, *47*, 2511–2522.

11. Llinàs-Brunet, M.; Bailey, M. D.; Ghiro, E.; Gorys, V.; Halmos, T.; Poirier, M.; Rancourt, J.; Goudreau, N. *J. Med. Chem.* **2004**, *47*, 6584–6594.
12. Goudreau, N.; Llinàs-Brunet, M. *Expert Opin. Investig. Drugs* **2005**, *14*, 1129–1144.
13. Narjes, F.; Koch, U.; Steinkuhler, C. *Expert Opin. Investig. Drugs* **2003**, *12*, 153–163.
14. Llinàs-Brunet, M.; Bailey, M. D.; Bolger, G.; Brochu, C.; Faucher, A. M.; Ferland, J. M.; Garneau, M.; Ghiro, E.; Gorys, V.; Grand-Maitre, C.; Halmos, T.; Lapeyre-Paquette, N.; Liard, F.; Poirier, M.; Rheaume, M.; Tsantrizos, Y. S.; Lamarre, D. *J. Med. Chem.* **2004**, *47*, 1605–1608.
15. Lamarre, D.; Anderson, P. C.; Bailey, M.; Beaulieu, P.; Bolger, G.; Bonneau, P.; Bos, M.; Cameron, D. R.; Cartier, M.; Cordingley, M. G.; Faucher, A. M.; Goudreau, N.; Kawai, S. H.; Kukolj, G.; Lagace, L.; LaPlante, S. R.; Narjes, H.; Poupart, M. A.; Rancourt, J.; Sentjens, R. E.; St George, R.; Simoneau, B.; Steinmann, G.; Thibeault, D.; Tsantrizos, Y. S.; Weldon, S. M.; Yong, C. L.; Llinàs-Brunet, M. *Nature* **2003**, *426*, 186–189.
16. Tsantrizos, Y. S.; Bolger, G.; Bonneau, P.; Cameron, D. R.; Goudreau, N.; Kukolj, G.; LaPlante, S. R.; Llinàs-Brunet, M.; Nar, H.; Lamarre, D. *Angew. Chem., Int. Ed.* **2003**, *42*, 1355–1360.
17. Faucher, A. M.; Bailey, M. D.; Beaulieu, P. L.; Brochu, C.; Duceppe, J. S.; Ferland, J. M.; Ghiro, E.; Gorys, V.; Halmos, T.; Kawai, S. H.; Poirier, M.; Simoneau, B.; Tsantrizos, Y. S.; Llinàs-Brunet, M. *Org. Lett.* **2004**, *6*, 2901–2904.
18. Reiser, M.; Hinrichsen, H.; Benhamou, Y.; Reesink, H. W.; Wedemeyer, H.; Avendano, C.; Riba, N.; Yong, C. L.; Nehmiz, G.; Steinmann, G. G. *Hepatology* **2005**, *41*, 832–835.
19. Lin, C.; Gates, C. A.; Rao, B. G.; Brennan, D. L.; Fulghum, J. R.; Luong, Y. P.; Frantz, J. D.; Lin, K.; Ma, S.; Wei, Y. Y.; Perni, R. B.; Kwong, A. D. *J. Biol. Chem.* **2005**, *280*, 36784–36791.
20. Lin, K.; Perni, R. B.; Kwong, A. D.; Lin, C. *Antimicrob. Agents Chemother.* **2006**, *50*, 1813–1822.
21. Reesink, H. W.; Zeuzem, S.; Weegink, C. J.; Forestier, N.; van Vliet, A.; de Rooij, J. V.; McNair, L. A.; Purdy, S.; Chu, H. M.; Jansen, P. L. *Hepatology* **2005**, *42*, 234A–235A.
22. Malcolm, B. A.; Liu, R.; Lahser, F.; Agrawal, S.; Belanger, B.; Butkiewicz, N.; Chase, R.; Gheyas, F.; Hart, A.; Hesk, D.; Ingravallo, P.; Jiang, C.; Kong, R.; Lu, J.; Pichardo, J.; Prongay, A.; Skelton, A.; Tong, X.; Venkatraman, S.; Xia, E.; Girijavallabhan, V.; Njoroge, F. G. *Antimicrob. Agents Chemother.* **2006**, *50*, 1013–1020.
23. Lin, C.; Lin, K.; Luong, Y. P.; Rao, B. G.; Wei, Y. Y.; Brennan, D. L.; Fulghum, J. R.; Hsiao, H. M.; Ma, S.; Maxwell, J. P.; Cottrell, K. M.; Perni, R. B.; Gates, C. A.; Kwong, A. D. *J. Biol. Chem.* **2004**, *279*, 17508–17514.
24. Tong, X.; Chase, R.; Skelton, A.; Chen, T.; Wright-Minogue, J.; Malcolm, B. A. *Antiviral Res.* **2006**, *70*, 28–38.
25. Venkatraman, S.; Bogen, S. L.; Arasappan, A.; Bennett, F.; Chen, K.; Jao, E.; Liu, Y. T.; Lovey, R.; Hendrata, S.; Huang, Y.; Pan, W.; Parekh, T.; Pinto, P.; Popov, V.; Pike, R.; Ruan, S.; Santhanam, B.; Vibulbhan, B.; Wu, W.; Yang, W.; Kong, J.; Liang, X.; Wong, J.; Liu, R.; Butkiewicz, N.; Chase, R.; Hart, A.; Agrawal, S.; Ingravallo, P.; Pichardo, J.; Kong, R.; Baroudy, B.; Malcolm, B.; Guo, Z.; Prongay, A.; Madison, V.; Broske, L.; Cui, X.; Cheng, K. C.; Hsieh, Y.; Brisson, J. M.; Prelusky, D.; Korfmacher, W.; White, R.; Bogdanowich-Knipp, S.; Pavlovsky, A.; Bradley, P.; Saksena, A. K.; Ganguly, A.; Piwinski, J.; Girijavallabhan, V.; Njoroge, F. G. *J. Med. Chem.* **2006**, *49*, 6074–6086.
26. Johansson, P.-O.; Bäck, M.; Kvarnström, I.; Jansson, K.; Vrang, L.; Hamelink, E.; Hallberg, A.; Rosenquist, Å.; Samuelsson, B. *Bioorg. Med. Chem.* **2006**, *14*, 5136–5151.
27. Thorstensson, F.; Wångsell, F.; Kvarnström, I.; Vrang, L.; Hamelink, E.; Jansson, K.; Hallberg, A.; Rosenquist, Å.; Samuelsson, B. *Bioorg. Med. Chem.* **2007**, *15*, 827–838.
28. Hansen, T. K. *Tetrahedron Lett.* **1999**, *40*, 9119–9120.
29. Farquhar, D.; Cherif, A.; Bakina, E.; Nelson, J. A. *J. Med. Chem.* **1998**, *41*, 965–972.
30. Callabretta, R.; Giordano, C.; Gallina, C.; Morea, V.; Scandurra, R. *Eur. J. Med. Chem.* **1995**, *30*, 931–941.
31. Rosenquist, Å.; Kvarnsröm, I.; Svensson, S. C. T.; Classon, B.; Samuelsson, B. *Acta. Chem. Scand.* **1992**, *46*, 1127–1129.
32. Dolby, L. J.; Esfandiari, S.; Ellinger, C. A.; Marshall, K. S. *J. Org. Chem.* **1971**, *36*, 1277–1285.
33. Molander, G. A.; Quirmbach, M. S.; Silva, L. F.; Spencer, K. C.; Balsells, J. *Org. Lett.* **2001**, *3*, 2257–2260.
34. Rönn, R.; Sabnis, Y. A.; Gossas, T.; Åkerblom, E.; Danielson, U. H.; Hallberg, A.; Johansson, A. *Bioorg. Med. Chem.* **2006**, *14*, 544–559.
35. Barbato, G.; Cicero, D. O.; Cordier, F.; Narjes, F.; Gerlach, B.; Sambucini, S.; Grzesiek, S.; Matassa, V. G.; De Francesco, R.; Bazzo, R. *EMBO J.* **2000**, *19*, 1195–1206.
36. Goudreau, N.; Cameron, D. R.; Bonneau, P.; Gorys, V.; Plouffe, C.; Poirier, M.; Lamarre, D.; Llinàs-Brunet, M. *J. Med. Chem.* **2004**, *47*, 123–132.
37. Johansson, A.; Hubatsch, I.; Åkerblom, E.; Lindeberg, G.; Winiwarter, S.; Danielson, U. H.; Hallberg, A. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 203–206.
38. Johansson, A.; Poliakov, A.; Åkerblom, E.; Lindeberg, G.; Winwarter, S.; Samuelsson, B.; Danielson, U. H.; Hallberg, A. *Bioorg. Med. Chem.* **2002**, *10*, 3915–3922.
39. Yao, N.; Reichert, P.; Taremi, S. S.; Prosise, W. W.; Weber, P. C. *Structure* **1999**, *7*, 1353–1363.
40. Johansson, A.; Poliakov, A.; Åkerblom, E.; Wiklund, K.; Lindeberg, G.; Winiwarter, S.; Danielson, U. H.; Samuelsson, B.; Hallberg, A. *Bioorg. Med. Chem.* **2003**, *11*, 2551–2568.
41. Örtqvist, P.; Peterson, S. D.; Åkerblom, E.; Gossas, T.; Sabnis, Y. A.; Fransson, R.; Lindeberg, G.; Helena Danielson, U.; Karlen, A.; Sandström, A. *Bioorg. Med. Chem.* **2007**, *15*, 1448–1474.
42. The enzyme inhibition assays was performed by Professor Pei Zhen Tao at The Department of Virology, Institute of Medicinal. Technology, Beijing, China.
43. Poliakov, A.; Hubatsch, I.; Shuman, C. F.; Stenberg, G.; Danielson, U. H. *Protein Expr. Purif.* **2002**, *25*, 363–371.