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Improved P2 phenylglycine-based hepatitis C virus NS3 protease inhibitors with alkenylic prime-side substituents

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ABSTRACT

Phenylglycine has proved to be a useful P2 residue in HCV NS3 protease inhibitors. A novel π–π-interaction between the phenylglycine and the catalytic H57 residue of the protease is postulated. We hypothesized that the introduction of a vinyl on the phenylglycine might strengthen this π–π-interaction. Thus, herein is presented the synthesis and inhibitory potency of a series of acyclic vinylated phenylglycine-based HCV NS3 protease inhibitors. Surprisingly, inhibitors based on both D- and L-phenylglycine were found to be effective inhibitors, with a slight preference for the D-epimers. Furthermore, prime-side alkenylic extension of the C-terminal acylsulfonamide group gave significantly improved inhibitors with potencies in the nanomolar range (~35 nM), potencies which were retained on mutant variants of the protease.

1. Introduction

Hepatitis C virus (HCV) is considered as a major and growing global health problem. HCV causes inflammation of the liver and is carried by an estimated 170 million people worldwide.[1] An HCV infection can lead to serious conditions such as acute liver disease, cirrhosis, or liver cancer.[2] As a consequence, HCV is the leading cause of liver transplantation. Reinfection of the new liver is often a problem, and liver transplantation does therefore not lead to termination of an HCV infection.[3] The current treatment regimen consists of a combination of ribavirin and pegylated interferon-α. Unfortunately, the therapy is ineffective for about 40–50% of patients with chronic HCV infection, and it has several adverse effects.[4] Hence, development of new therapies is of great importance.

HCV contains single stranded (+)-RNA that is released inside the infected hepatocyte and translated into a polyprotein. The polyprotein contains about 3000 amino acids and is proteolytically cleaved into 10 viral proteins: C, E1, E2, p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B. NS3 is an enzyme with dual functionalities. It has serine protease as well as helicase/NTPase activities. This viral protease is responsible for the cleavage between NS3/4A, NS4A/4B, NS4B/5A, and NS5A/5B[5] and has proven to be essential for survival of HCV in vivo.[6] Supplementary to the proteolytical properties of the NS3 protease, it has also been shown to be involved in processes to attenuate and evade the host cell's natural immune defense.[7]

Of several potential anti-HCV drug targets, the NS5B polymerase and the NS3 protease have so far been the most extensively examined points of attack. An advantage with NS3 protease inhibitors is the potential two mechanisms of action, as mentioned, leading to a restored host immune response as well as inhibition of HCV replication. Thus, a number of NS3 protease inhibitors have been developed, for example, BILN-2061,[8] ciluprevir (Fig. 1). It was the first HCV NS3 protease inhibitor to enter clinical trials, but it was later withdrawn due to heart toxicity in animals. At present, several inhibitors are being evaluated in clinical trials[9] (Fig. 1).

HCV NS3 protease inhibitors can be divided into two major classes, according to their mechanism of action. The product-based HCV NS3 protease inhibitors, exemplified by BILN-2061, TMC435, RG7227, and MK-7009 (Fig. 1), are based on the findings that NS3 protease is inhibited by its own cleavage product.[15,16] These inhibitors are characterized by an acidic functionality such as a carboxylic acid or an acyl sulfonamide at the C-terminal. The other class, the electrophilic HCV NS3 protease inhibitors, exemplified by VX-950 and SCH 503034 (Fig. 1), are reversibly, but covalently, modifying the catalytic serine residue of the protease.[17] Although the two classes of inhibitors are very different, both from a structural and mechanistical point of view, a common feature in the inhibitors is a P2 proline or a proline mimic.

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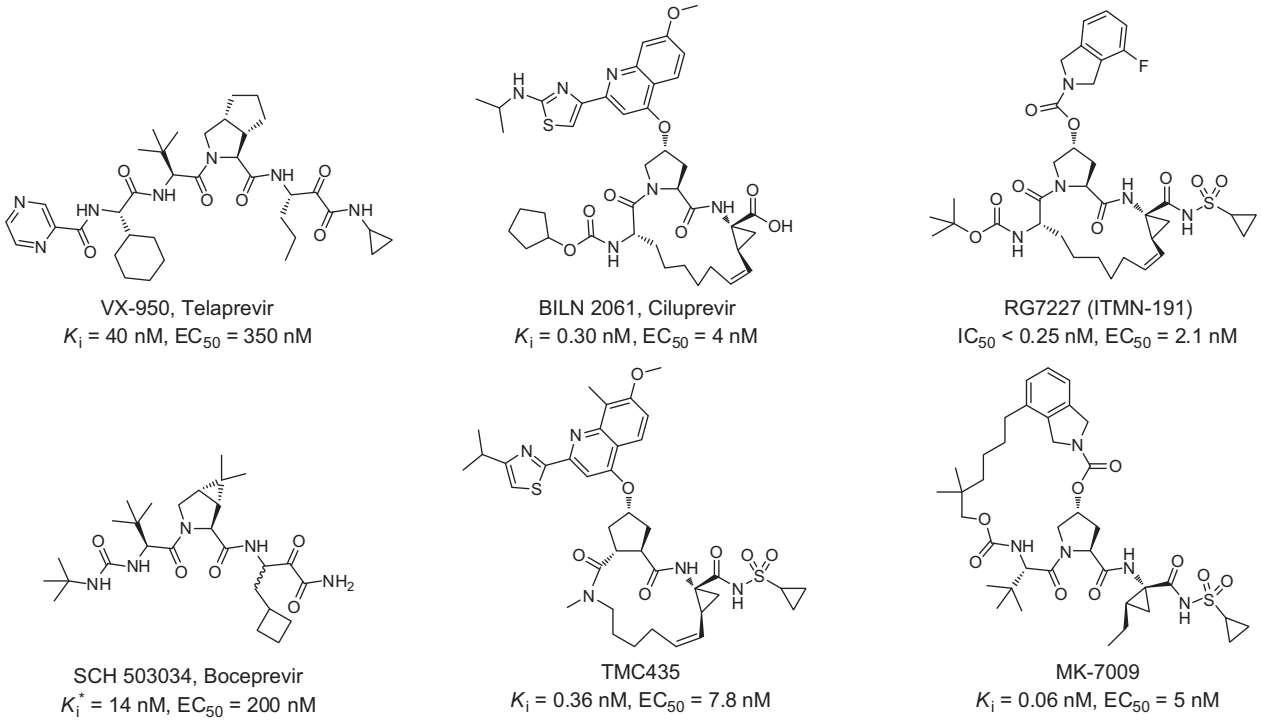


Figure 1. Selection of HCV NS3 protease inhibitors that have entered clinical trials. VX-950,[10] SCH 503034,[11] BILN-2061,[8] TMC435,[12] RG7227, formerly referred to as ITMN-191,[13] and MK-7009.[14]

The large population size and high mutability of HCV, have led to the emergence of several genotypes and quasispecies of the virus.[18] Moreover, HCV has been shown to evolve into resistant forms to one or more types of protease inhibitors both in vitro and in vivo. The proline residue in the P2 position has been shown to be the basis for the cross-resistance shown by the two classes of inhibitors for A156 mutated NS3 variants.[19,20] Consequently, there is a big need for new HCV NS3 protease inhibitors based on non-proline P2 moieties which can possess a different binding mode.

In the search for novel HCV NS3 protease inhibitors, we previously found phenylglycine (Phg) as a useful P2 residue, with a possible π-stacking to the catalytic H57 residue, exemplified by compound **A** in Figure 2.[21] Moreover, a phenylglycine-based inhibitor has shown to retain its potency to the R155Q, A156T, and D168V mutants of the NS3 protease.[22] Phenylglycine-based inhibitors were previously found to differ in structure–activity relationship to the well investigated proline-based NS3 protease inhibitors. Fragments that are optimized for the proline-based inhibitors, for example, the vinyl-ACCA in the P1 position did not improve potency for phenylglycine-based inhibitors.[21] The overall results indicated that phenylglycine-based inhibitors bind to the protease in a different fashion than the proline-based ones — a fact which we believe can be utilized in the development of inhibitors with a unique resistance profile. However, this necessitates the search for new optimized phenylglycine-based inhibitors.

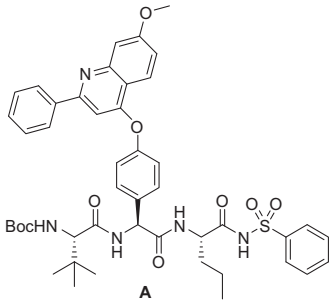


Figure 2. Compound **A**,[21] K_i = 230 nM.

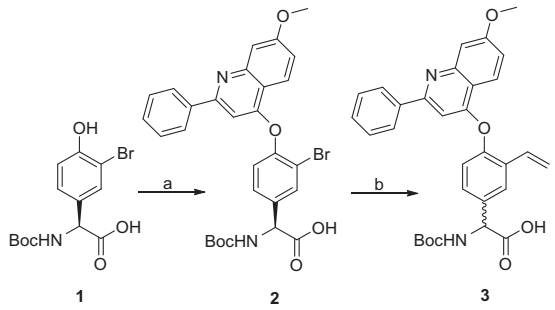
The aromatic nature of phenylglycine not only convey possible π-interactions, but also allows for chemical decorations. We hypothesized that introduction of a vinyl substituent on the phenylglycine could increase the π-stacking that has been proposed as an important interaction between the phenylglycine and the H57 residue.[21] A vinyl can also serve as a handle in forthcoming metathesis reactions.

Thus, we herein present the synthesis, structure–activity relationships and biochemical evaluation on both wild-type and mutated enzyme of a series of vinyl substituted phenylglycine-based HCV NS3 protease inhibitors with different acylsulfonamides at the C-terminal. The inhibitor modifications explored herein led to optimized phenylglycine-based inhibitors. The same modifications were less effective in a proline-based inhibitor. These results further strengthen the hypothesis of a different binding mode of phenylglycine-based inhibitors which can be advantageous in the development of unique HCV NS3 protease inhibitors.

2. Results

2.1. Chemistry

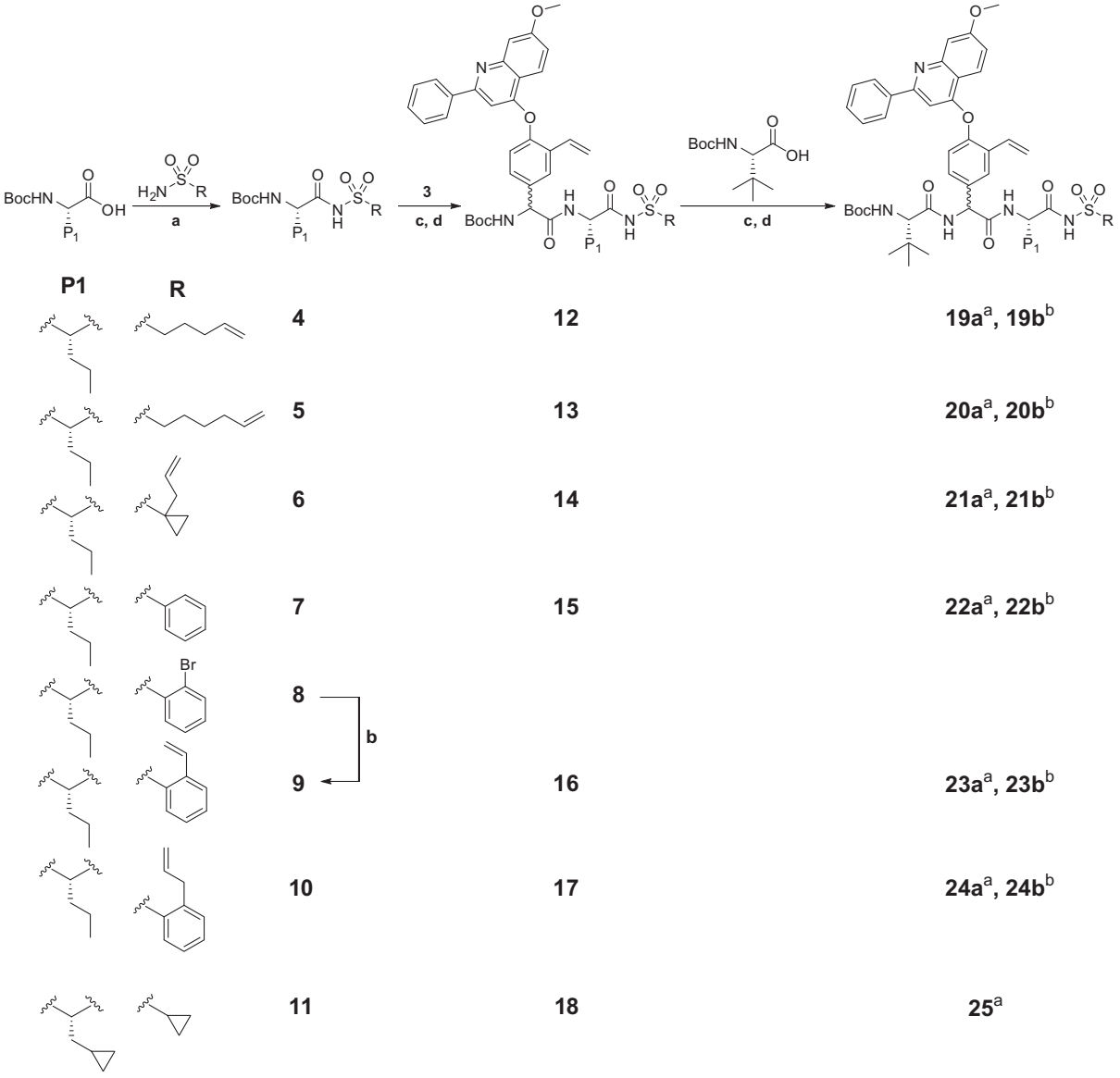
Compound **1**[23] (Scheme 1) was synthesized according to described procedures,[23–25] starting by brominating 4-hydroxy-L-phenylglycine with bromine and hydrobromic acid in acetic acid. Only monobrominated 3-bromo-4-hydroxy-L-phenylglycine was obtained, in our case as the hydrobromide salt. The amine was Boc-protected,[24] and then separated from non-brominated compound on silica.[25] A nucleophilic aromatic substitution reaction using **1**, 4-chloro-7-methoxy-2-phenylquinoline[26] and potassium-*tert*-butoxide in DMSO resulted in the heteroaryl ether **2** (Scheme 1) after 6½ weeks of stirring at 64 °C. The extremely long



Scheme 1. Reagents and conditions: (a) 4-chloro-7-methoxy-2-phenylquinoline, KO*t*Bu, DMSO, 64 °C, 43%; (b) 2,4,6-trivinylcycloboroxane pyridine complex, $Pd(OAc)_2$, $[(tBu)_3PH]BF_4$, K_2CO_3, DME, H_2O, microwave heating at 100 °C for 15 min, 34%.

reaction time was preferred over a shorter reaction with higher temperature due to unwanted removal of the Boc-protecting group and decarboxylation. The vinyl was introduced by a microwave assisted Suzuki reaction[27] using 2,4,6-trivinylcycloboroxane pyridine complex resulting in a partly racemized product (Scheme 1, compound **3**). An enantiomeric mixture of L and D in about a 2:1 ratio was obtained, as proved by chiral HPLC analysis of **3** and also diastereomeric analysis by HPLC after a forthcoming coupling (vide infra). The tendency of phenylglycine racemization during Suzuki reactions has been described previously.[28]

The P1–P1′ building blocks (**4**–**11**) were all synthesized (Scheme 2) from commercially available Boc protected norvaline (Boc-L-Nva) (compounds **4**–**10**), or Boc protected L-cyclopropylalanine (Cpa) (compound **11**). The amino acids were coupled with sulfonamides that were either obtained commercially or synthesized following literature procedures[26,29–31] using carbonyldiimidazole (CDI) and 1,8-diazabicycloundecene (DBU) as base in dry THF resulting in compounds **4**–**8** and **10**, **11**. The amino acids were activated by CDI at 60 °C or room temperature for 1 h before addition of sulfonamide and DBU at room temperature. The reactions where the amino acids were activated under heat were completed within 2 h. This also resulted in a small amount of racemization at the P1 α-carbon as proved by the formation of epimers detected in compounds **19**–**25**. The *ortho* vinylated P1–P1′ building block (compound **9**)

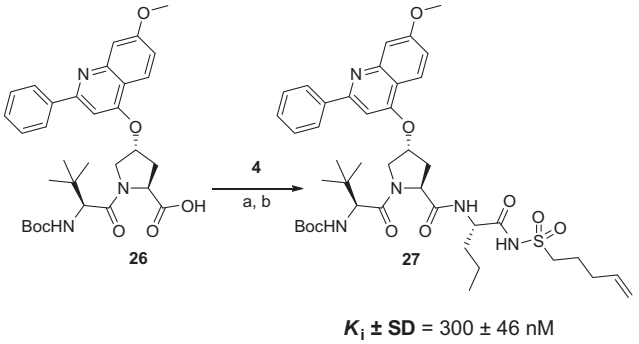


Scheme 2. Reagents and conditions: (a) CDI, THF, 60 °C/rt, DBU, rt, 15–84% ; (b) 2,4,6-trivinylcycloboroxane pyridine complex, $Pd(OAc)_2$, $[(tBu)_3PH]BF_4$, K_2CO_3, DME, H_2O, microwave heating at 100 °C for 15 min, 66%; (c) 4.0 M HCl in dioxane; (d) HATU, DIEA, DMF, rt [a]L-Phg, [b]D-Phg, 20–56%.

was made from the corresponding *ortho* brominated compound (compound **8**) through a microwave assisted Suzuki reaction.[27]

Deprotection of **4**–**7** and **9**–**11** with 4.0 M HCl in dioxane and coupling with the P2-building block (compound **3**) using *N*-[(dimethylamino)-1*H*-1,2,3-triazolo-[4,5-*b*]pyridin-1-yl-methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (HATU) and *N*,*N*-diisopropylethylamine (DIEA) in DMF resulted in diastereomeric mixtures of compounds **12**–**18** (Scheme 2), due to the previously mentioned racemization on the phenylglycine residue during the Suzuki reaction.

After N-deprotection, compounds **12**–**18** were coupled with Boc-*t*-Leu, using HATU and DIEA in DMF. This resulted in compounds **19**–**25** (Scheme 2) in a diastereomeric mixture of two isomers with the phenylglycine in L-form (compounds **19a–24a**, **25**), and D-form (compounds **19b**–**24b**), respectively. The two isomers could be separated by preparative HPLC on a reversed phase column. The major isomer eluted earlier than the minor isomer. The ratio between the major and minor isomers was approximately 2:1, in analogy with the results shown by chiral HPLC analysis of compound **3**. This indicates that the major isomer corresponds to the L-Phg isomer since the synthesis started with L-Phg (compound **1**). Throughout the series we have also observed in the ^{1}H NMR spectra that the phenylglycine α-proton is shifted downfield in the L-Phg isomers (compounds **19a**–**24a**, **25**) to the corresponding shift for the D-Phg isomers (compounds **19b**–**24b**). The difference in shift of the Phg α-proton in epimeric pairs has been described before by Salituro and Townsend,[24] and is in analogy with our findings. The degree of RP-HPLC separation of the L- and D-isomers of the final compounds varied a lot. The yields of the final compounds depended on how well the isomers separated and are therefore indefinitely calculated, and sometimes deceptively low. Some inhibitors were better separated in TFA buffer than formic acid buffer. With TFA present in the eluent the inhibitors were obtained as TFA-salt.



Scheme 3. Reagents and condition: (a) 4.0 M HCl/dioxane; (b) HATU, DIEA, DMF, rt, 21%.

Compound **26** (Scheme 3), synthesized according to a previously published procedure[26] was coupled with the N-deprotected HCl salt of **4**. This yielded compound **27** (Scheme 3) which was used as a reference compound comprising proline in the P2 position.

2.2. Biochemical evaluation

Compounds **19**–**25** were biochemically evaluated and K_i-values determined with an activity-based inhibition assay containing the

Table 1
Enzyme inhibition constants (K_i-values) and inhibition of replication (EC_{50}) measured on wild-type enzyme and in a subgenomic HCV replicon assay, respectively

R	Compound	K_i ± SD (nM)	EC_{50} (μM)	R	Compound	K_i ± SD (nM)	EC_{50} (μM)
	19a	63 ± 9	>10		**19b**	48 ± 2	8.9
	20a	120 ± 26	>10		**20b**	100 ± 14	4.7
	21a	330 ± 60	nd		**21b**	190 ± 40	nd
	22a	290 ± 40	nd		**22b**	130 ± 20	nd
	23a	120 ± 20	>10		**23b**	47 ± 6	5.4
	24a	140 ± 40	>10		**24b**	35 ± 2	3
	25[a]	500 ± 130	nd				

SD, standard deviation.
nd, not determined.
[a] P1 Cpa.

full-length NS3 protein and a peptide corresponding to the activating region of NS4A[32] (Table 1). Additionally, compounds **19a**, **19b**, **20a**, **20b**, **23a**, and **23b** were evaluated on A156T and D168V mutant forms of the protease[22] (Table 2). Inhibition constants were measured and vitality values calculated. Vitality values describe the influence the mutant substitution has on the enzyme when the inhibitor is present. A vitality value >1 means that the mutation is favorable for the enzyme in the presence of inhibitor, while a vitality value <1 means that the mutation is disadvantageous. Compounds **19a**, **19b**, **20a**, **20b**, **23a**, **23b**, **24a**, **24b**, and **26** were also tested for EC_{50}-values in a replicon system using the Huh-7 cell line containing subgenomic HCV RNA genotype 1b replicon with firefly luciferase (Table 1).[33]

3. Discussion

Aiming at developing novel HCV NS3 protease inhibitors, distinct from the already well explored P2 proline-based inhibitors, we decided to further optimize P2 phenylglycine-based inhibitors that had shown a potential from previous studies.[21] Phenylglycine-based inhibitors seem to bind with a slightly different binding mode as compared with proline-based inhibitors and is thus not suitable with building blocks that are optimized for the proline inhibitors. Hence, new inhibitors based on vinyl decorated D- and L-phenylglycine with C-terminal acylsulfonamide substituents of varying sizes and lengths were synthesized. The P1′ sulfonamide substituents ranged from straight alkenylic chains of five (compounds **19a** and **19b**) or six carbons (compounds **20a** and **20b**) to cyclopropyl substituents, either allyl substituted (compounds **21a** and **21b**) or non-substituted (compound **25**). A variety of phenyl substituents were also made, ranging from the unsubstituted phenyl (compounds **22a** and **22b**) to phenyl that were *ortho* substituted with a vinyl (compounds **23a** and **23b**) or an allyl (compounds **24a** and **24b**).

The biochemical evaluation revealed that the new series of inhibitors were equipotent or more potent to the previous series with the best inhibitor possessing a K_i value of 35 nM (compared to compound **A**, with a K_i of 230 nM). Within both epimeric series (**a** and **b**), all inhibitors encompassing substituents with a vinylic- or allylic elongation were slightly more potent than their non-elongated counterparts. The inhibitors with linear alkenylic substituents (compounds **19a**, **19b**, **20a**, and **20b**) in P1′ and elongated phenyl substituents (compounds **23a**, **23b**, **24a**, and **24b**) proved to be the most successful inhibitors in the series. Among the linear alkenylic substituents, the substituents with five carbons (compounds **19a** K_i = 63 nM and **19b** K_i = 48 nM) were preferred over the substituents with six carbons (compounds **20a** K_i = 120 nM and **20b** K_i = 100 nM). For the elongated phenyl substituents, the differences in activities were not large going from *ortho*-vinylic (compounds **23a** K_i = 120 nM and **23b** K_i = 47 nM) to *ortho*-allylic (compounds **24a** K_i = 140 nM and **24b** K_i = 35 nM). Although, the elongated phenyl substituents were 2–4 times more potent than the non-elongated phenyl substituents (compounds **22a** K_i = 290 nM and **22b** K_i = 130 nM). The same increase in potency was seen when the cyclopropyl was extended with an allyl (compound **21a** K_i = 330 nM), which gave a 1.5-fold more potent compound than the non-elongated cyclopropyl (compound **25** K_i = 500 nM), even though these compounds have different P1 substituents and cannot be fairly compared. Cyclopropyl is commonly used as a P1′ substituent in proline-based inhibitors (see Fig. 1). However, in this series the compounds containing an elongated cyclopropyl (compounds **21a** and **21b**) or a cyclopropyl (compound **25**) in P1′ were among the least potent.

Generally, there was not very much difference in inhibitory potency between the epimeric pairs (**a** and **b**). Surprisingly, the D-Phg isomers (**b**-compounds) were slightly more potent than the L-Phg isomers (**a**-compounds) throughout the whole series. According to modeling, both the D-form and the L-form can fit into the binding pocket with a slight adjustment in the backbone position (Fig. 3). Modeling of compounds **19a** and **19b** also shows that both the L- and D-form might have a good π-overlap with H57. Although, the phenylglycine seems to contribute more to the π-overlap for the L-form, while the vinyl is the major π-contributor in the D-form.

Comparison of the equipotent compounds **22a** and **A** (Fig. 2) shows that the vinyl substitution on the phenylglycine does not have a large impact on the inhibitory potency, at least not for L-Phg compounds. Since the vinyl is more involved in the π-overlap with H57 according to modeling, the impact may be larger for the D-form as indicated by the twofold potency improvement from 230 nM (compound **A**) and 290 nM (compound **22a**) to 130 nM for **22b**. In the model (Fig. 3), the sulfonamide pentenylic substituent is positioned in between the residues K136 and Q41 and thereby maximizing hydrophobic contacts. Modeling also suggests that the elongated inhibitors can adopt a c-clamp shape in analogy with an X-ray crystal structure of a P2–P4 macrocyclic HCV NS3 protease inhibitor presented by Venkatraman et al.[34] This shape immobilizes K136 and enhances binding efficiency.

The D-Phg inhibitors **19b**, **20b**, **23b**, and **24b** were the only inhibitors that showed measurable inhibitory effects in the cell-based assay. Considering the fact that the inhibitors in this series are acyclic and flexible, it must be noted that these inhibitors still shows micromolar cell activity. By comparing the activities of compounds **19a** (K_i = 63 nM), **19b** (K_i = 48 nM), and **27** (300 nM) it is

Table 2
Inhibition constants and vitality values evaluated with A156T and D168V substituted NS3

Compound	A156T		D168V	
	K_i ± SD (nM)	*V*	K_i ± SD (nM)	*V*
19a	180 ± 20	0.9	140 ± 10	1.0
19b	86 ± 20	0.9	380 ± 30	6.2
20a	380 ± 80	1.6	390 ± 70	2.5
20b	270 ± 60	1.4	530 ± 40	3.0
23a	250 ± 70	1.1	450 ± 60	2.8
23b	180 ± 10	0.9	230 ± 30	2.0

SD, standard deviation.
V, vitality values calculated as described in Ref. 21.

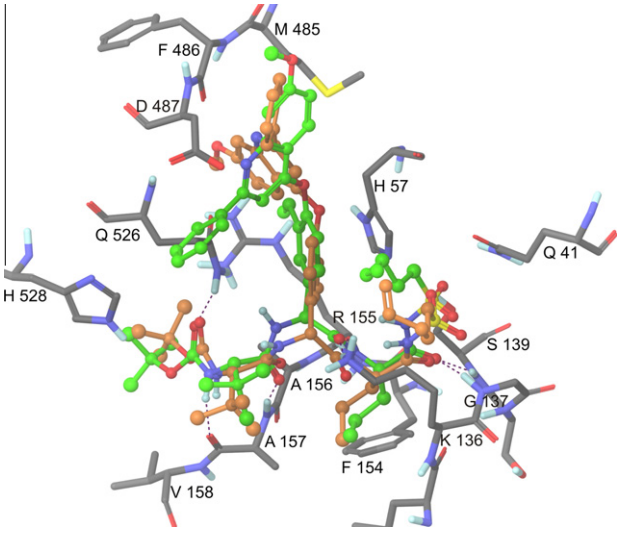


Figure 3. Overlay of compound **19a** (green) and **19b** (orange) docked in the full-length NS3 protease binding site (PDB code 1CU1). The protease residues are displayed as sticks.

shown that the phenylglycine-based inhibitors are fivefold as potent as the corresponding proline-based analog. Thus, elongated P1′ alkenylic- and phenyl acylsulfonamides seems to potentiate the inhibition potency of phenylglycine-based inhibitors, as did vinyl substitution for the D-Phg epimer.

Contrary to some P2 proline-based inhibitors,[22] biochemical evaluation on mutated forms of NS3 show that the inhibitory effects of the tested inhibitors herein, **19a**, **19b 20a**, **20b**, **23a**, and **23b** are more or less unaffected by the A156T mutation as shown by vitality values around 1, and slightly less efficient on the D168V mutations compared to wild-type (V = 1–6). As a comparison it could be mentioned that the vitality values for BILN-2061 (Fig. 1) is 1600 for A156T and 3200 for D168V. The pentenylic inhibitor **19b** retains potency against A156T (K_i = 180 nM, V = 0.90) while it loses potency against D168V (K_i = 380 nM, V = 6.2). Interestingly, compound **19a** retains potency to both A156T and D168V. The larger drop in potency on D168V for **19b** compared to **19a** must be attributed to loss of important interactions or increased steric conflict on V168 from the D-Phg **19b**. Altogether, these results further strengthen the hypothesis that phenylglycine-based inhibitors have a resistance profile differing from proline-based inhibitors.

4. Conclusion

In summary, an attempt to optimize P2 phenylglycine-based HCV NS3 protease inhibitors by incorporating a vinyl fragment resulted in a series of acyclic tripeptidic inhibitors. Due to the phenylglycine's tendency to racemize, the inhibitors were obtained both as the L- and D-Phg epimer. The epimers proved to be of equal potency, with a slight preference for the D-Phg epimer. Notably, some of the inhibitors possessed cell-activities below 10 μM. Some inhibitors were evaluated on A156T and D168V substituted enzyme and showed the important ability to retain their potency against these mutant variants. C-terminal acylsulfonamides with alkenylic substituents proved to be beneficial in combination with the vinylated P2 phenylglycine, yielding inhibitors with inhibition constants of 35 nM. The same elongated alkenylic acylsulfonamide was not advantageous on a P2 proline-based inhibitor which was five times less potent. This indicates that the P2 phenylglycine-based inhibitors bind in a different fashion compared to the P2 proline-based inhibitors, and has different optimization points. This will be explored in further optimization of inhibitors with an anticipated unique resistance profile.

5. Experimental

5.1. Chemistry

Reagents and solvents were obtained commercially and used without further purification. Thin layer chromatography (TLC) was performed on aluminum sheets precoated either with silica gel 60 F_{254} (0.2 mm, Merck) or aluminum oxide 60 F_{254} neutral (0.2 mm, Merck). Chromatographic spots were visualized using UV detection and/or 2% ninhydrin in ethanol solution followed by heating. Column chromatography was performed using silica gel 60 (40–63 μm, Merck) or aluminum oxide 90 standardized normal (63–200 μm, Merck) or silica gel 60 RP-18 (40–63 μm, Merck). Analytical HPLC-MS was performed on a Gilson-Finnigan ThermoQuest AQA system equipped with a C18 (Onyx Monolithic C18 (50 × 4.6 mm) or a C4 (Hichrom ACE C4 (5 μm, 50 × 4.6 mm)) column using MeCN/H_2O (0.05% HCOOH) as the mobile phase with UV (254 nm) MS (ESI) detection or on a Gilson Thermo-Finnigan Surveyor MSQ system using MeCN/H_2O (0.05% HCOOH) as the mobile phase with ELSD detection or on a system equipped with a C8 (Zorbax SB-C8 (4.8 × 50 mm)) column using UV (220 or 230 nm) detection. Preparative HPLC-MS was performed on a Gilson-Finnigan ThermoQuest AQA system equipped with a C8 (Zorbax SB-C8 (5 μm, 150 × 21.2 mm)) column using MeCN/H_2O (0.05% HCOOH) as the mobile phase with UV (254 nm) and MS (ESI) detection or a system equipped with a C8 (Zorbax SB-C8 (21.2 × 150 mm)) column using UV (220 or 230 nm) detection and MeCN/H_2O (0.05% HCOOH) or MeCN/H_2O (0.09% TFA) as the mobile phase. Chiral analysis was performed on a HPLC system equipped with a Reprosil CHIRAL-NR (250 × 4.6 mm, 8 μm) column. The purities of the inhibitors were determined by RP-HPLC on the following systems (UV detection at 220 nm): System 1 (Zorbax SB-C8, 4.8 × 5 mm, MeCN/H_2O with 0.05% HCOOH) and system 2 (Zorbax SB-C8, 4.8 × 5 mm, MeCN/H_2O with 25 mM NH_4OAc, pH 6.3). Microwave reactions were performed in a SmithSyntheSizer™ or in an Initiator™ single-mode microwave cavity producing controlled irradiation at 2450 MHz. NMR spectra were recorded on a Varian Mercury plus spectrometer (^1H at 399.8 MHz, ^{13}C at 100.5 MHz) at ambient temperature. Chemical shifts (δ) are reported in ppm, indirectly referenced to tetrametylsilane (TMS) via the solvent signal (^1H: $CHCl_3$ δ 7.26, CD_2HOD δ 3.31; ^{13}C: $CDCl_3$ δ 77.16, CD_3OD δ 49.00). Exact molecular masses were determined on Micromass Q-Tof2 mass spectrometer equipped with an electrospray ion source.

5.2. Compound 2

Compound **1** (2.08 g, 6.00 mmol), 4-chloro-7-methoxy-2-phenylquinoline (0.810 g, 3.00 mmol) and KO*t*Bu (1.34 g, 12.0 mmol) were dried over night in vacuo over P_2O_5. Dry DMSO (H_2O < 0.005%) (10.5 mL) was added to a vial containing **1** and KO*t*Bu. The vial was sealed under nitrogen and stored for 40 min before 4-chloro-7-methoxy-2-phenylquinoline dissolved in DMSO (7.5 mL) was added. The reaction was heated at 64 °C and stirred for 6½ weeks until no more product was formed. The reaction mixture was filtered, diluted with water (230 mL), and extracted with diethyl ether to remove unreacted 4-chloro-7-methoxy-2-phenylquinoline. The aqueous phase was acidified with 1 M HCl (6.0 mL) to pH 5.3. Centrifugal filtration of the solid, yielded **2** (753 mg, 43%) as a beige powder. To obtain a concentration of **2**, high enough to record a ^{13}C NMR spectrum, TFA had to be added to the NMR sample. Therefore, the ^{13}C NMR shifts of **2** is reported as a TFA-salt. ^1H NMR (CD_3OD): δ 8.30 (d, J = 9.2 Hz, 1H), 7.86 (d, J = 2.1 Hz, 1H), 7.83–7.76 (m, 2H), 7.57 (dd, J = 2.1, 8.4 Hz, 1H), 7.50 (d, J = 2.5 Hz, 1H), 7.48–7.43 (m, 3H), 7.38 (d, J = 8.4 Hz, 1H), 7.30 (dd, J = 9.2, 2.5 Hz, 1H), 6.72 (s, 1H), 5.24 (s, 1H), 4.00 (s, 3H), 1.46 (s, 9H). ^{13}C NMR (CD_3OD, as TFA-salt): δ 173.0, 167.7, 167.0, 158.6, 157.5, 150.5, 144.9, 140.8, 134.8, 133.8, 133.5, 130.8, 130.4, 129.7, 126.0, 124.5, 122.7, 116.6, 115.5, 103.0, 101.2, 81.2, 58.2, 57.1, 28.7. MS calcd for $C_{29}H_{27}BrN_2O_6$ $[M+H]^+$ 580.1, found: 579.2, 581.2.

5.3. Compound 3

In a microwave process vial, **2** (381 mg, 0.659 mmol), 2,4,6-trivinylcycloboroxane pyridine complex (294 mg, 1.32 mmol), $Pd(OAc)_2$ (15.8 mg, 0.0712 mmol), $[(tBu)_3PH]BF_4$ (38.2 mg, 0.132 mmol), K_2CO_3 (547 mg, 3.96 mmol), DME (13.2 mL), and water (3.96 mL) were mixed. The vial was sealed under N_2 and exposed to microwave heating at 100 °C for 15 min. After centrifugal filtration, the DME was evaporated. Water (16 mL) was added and the mixture was acidified with 1 M HCl to pH 1. The mixture was extracted with ethyl acetate and the organic phase was collected and evaporated. Purification by preparative HPLC-MS gave **3** (118 mg, 34%) as a yellow solid. ^1H NMR (CD_3OD): δ 8.29 (d, J = 9.1 Hz, 1H), 7.84 (d, J = 2.4 Hz, 1H), 7.76–7.73 (m, 2H), 7.49–7.46 (m, 2H), 7.43–7.40 (m, 3H), 7.28 (dd, J = 9.1, 2.5 Hz, 1H), 7.20 (d, J = 8.4 Hz, 1H), 6.77 (dd, J = 17.5,

11.3 Hz, 1H), 6.67 (s, 1H), 5.89 (dd, J = 17.5, 1.2 Hz, 1H), 5.26–5.22 (m, 2H), 3.99 (s, 3H), 1.46 (s, 9H). ^{13}C NMR (CD_3OD) δ 174.3, 164.5, 164.0, 160.6, 157.4, 151.6, 151.4, 139.6, 138.2, 131.9, 131.1, 130.9, 129.9, 129.9, 128.7, 127.5, 124.1, 123.2, 120.4, 117.9, 115.8, 106.7, 102.0, 80.9, 59.2, 56.2, 28.7. MS calcd for $C_{31}H_{30}N_2O_6$ $[M+H]^+$ 527.2, found: 527.3.

5.4. General procedure for synthesis of compounds 4–8, 10–11

Boc-L-Nva, CDI and sulfonamides were dried in vacuo over P_2O_5 over night. Boc-L-Nva or Boc-L-cyclopropylalanine and CDI were dissolved in dry THF under N_2 atmosphere.

Method A: The solution was stirred at room temperature for 1 h before the sulfonamide, dissolved in dry THF was added together with DBU. The reaction was left over night.

Method B: The solution was stirred at 60 °C for 1 h before the sulfonamide, dissolved in dry THF was added together with DBU. The reaction was stirred in room temperature for 2 h.

Method A and B: The solvent was evaporated and CH_2Cl_2 was added. The solution was washed with 5% citric acid solution before the organic phase was evaporated.

Purification: Most of the compounds were firstly purified on silica gel to remove rests of non-reacted amino acid. During a second purification on aluminum oxide the sulfonamide was washed out with the eluent (CH_2Cl_2/MeOH) while the product was adsorbed to the aluminum oxide. By adding 1% formic acid to the eluent, the pure product could be eluted from the aluminum oxide.

5.5. Compound 4

Prepared according to the general procedure described above, using method B. Boc-L-Nva (435 mg, 2.00 mmol), pent-4-ene-1-sulfonamide, prepared according to a previously described method[29] (895 mg, 6.00 mmol), CDI (649 mg, 4.00 mmol), DBU (900 μL, 6.01 mmol), and dry THF (48 mL). The product was purified on silica gel (CH_2Cl_2/MeOH 95:5), then on an aluminum oxide column (CH_2Cl_2/MeOH 97:3, then CH_2Cl_2/MeOH/HCOOH 95:5:1) and was yielded as a white solid (545 mg, 78%). ^{1}H NMR ($CDCl_3$): δ 9.50 (s, 1H), 5.73 (m, 1H), 5.13 (s, 1H), 5.08–5.01 (m, 2H), 4.20–4.09 (m, 1H), 3.45–3.38 (m, 2H), 2.22–2.15 (m, 2H), 1.96–1.86 (m, 2H), 1.85–1.73 (m, 1H), 1.66–1.54 (m, 1H), 1.44 (s, 9H), 1.43–1.32 (m, 2H), 0.93 (t, J = 7.2 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ 172.1, 156.3, 136.3, 116.7, 81.3, 55.0, 52.7, 33.5, 31.9, 28.4, 22.4, 18.9, 13.7. MS calcd for $C_{15}H_{28}N_2O_5S$ $[M+H]^+$ 349.2, found: 349.1.

5.6. Compound 5

Prepared according to the general procedure previously described, using method B. Boc-L-Nva (387 mg, 1.78 mmol), hex-5-ene-1-sulfonamide, prepared as described previously[29] (871 mg, 5.34 mmol), CDI (577 mg, 3.56 mmol), DBU (798 μL, 5.33 mmol), and dry THF (54 mL). Purification on silica gel (CH_2Cl_2/MeOH 95:5) followed by aluminum oxide (CH_2Cl_2/MeOH 95:5, then CH_2Cl_2/MeOH/HCOOH 95:5:1) gave **5** as a white sticky solid (515 mg 80%). ^{1}H NMR ($CDCl_3$): δ 5.75 (m, 1H), 5.03 (m, 1H), 4.99 (m, 1H), 4.96 (m, 1H), 4.11 (br s, 1H), 3.45–3.40 (m, 2H), 2.11–2.05 (m, 2H), 1.87–1.79 (m, 2H), 1.66–1.49 (m, 4H), 1.46 (s, 9H), 1.44–1.34 (m, 2H), 0.94 (t, J = 7.1 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ 171.8, 156.3, 137.6, 115.6, 81.5, 55.1, 53.3, 33.2, 33.1, 28.4, 27.4, 22.6, 18.9, 13.8. MS calcd for $C_{16}H_{30}N_2O_5S$ $[M+H]^+$ 363.2, found: 363.8.

5.7. Compound 6

Prepared following method A, described in the general procedure. Boc-L-Nva (480 mg, 2.21 mmol), 1-allylcycloproane-1-sulfonamide, prepared the same way as described previously,[30] (237 mg, 1.47 mmol), CDI (715 mg, 4.41 mmol), DBU (662 μL, 4.41 mmol), and dry THF (21 mL). Purification on silica gel (CH_2Cl_2/MeOH 97:3) and then aluminum oxide (CH_2Cl_2/MeOH 95:5, then CH_2Cl_2/MeOH/HCOOH 95:5:1) gave **6** (59 mg, 15%). ^{1}H NMR ($CDCl_3$): δ 9.29 (s, 1H), 5.69 (m, 1H), 5.14–5.10 (m, 2H), 5.09 (s, 1H), 4.15 (m, 1H), 2.64 (dd, J = 3.2, 7.4 Hz, 2H), 1.81 (m, 1H), 1.70–1.54 (m, 3H), 1.45 (s, 9H), 1.44–1.37 (m, 2H), 0.97–0.93 (m, 2H), 0.93 (t, J = 7.3 Hz, 3H). ^{13}C NMR ($CDCl_3$): δ 170.8, 156.3, 132.4, 119.4, 81.1, 55.1, 40.1, 34.7, 33.6, 28.4, 18.9, 13.8, 11.2, 11.1. MS calcd for $C_{16}H_{28}N_2O_5S$ $[M+H]^+$ 361.2, found: 361.0.

5.8. Compound 7

Prepared according to method B described in the general procedure. Boc-L-Nva (65.2 mg, 0.300 mmol), benzenesulfonamide (142 mg, 0.900 mmol), CDI (97.3 mg, 0.600 mmol), DBU (135 μL, 0.903 mmol), and dry THF (9 mL). After purification on silica gel (CH_2Cl_2/MeOH 95:5) followed by aluminum oxide (CH_2Cl_2/MeOH 95:5, then CH_2Cl_2/MeOH/HCOOH 95:5:1) and then silica gel RP-C18 (MeCN/H_2O 30:70, then MeCN/H_2O 50:50), **7** was obtained as a white solid (47 mg, 44%). ^{1}H NMR ($CDCl_3$): δ 7.99 (d, J = 7.6 Hz, 2H), 7.57 (m, 1H), 7.50–7.45 (m, 2H), 3.96 (br s, 1H), 1.62 (m, 1H), 1.35 (s, 9H), 1.24–1.17 (m, 2H), 0.82 (t, J = 7.4 Hz, 3H). ^{13}C NMR ($CDCl_3$): δ 171.6, 156.1, 139.1, 133.7, 128.9, 128.2, 80.7, 54.4, 34.3, 28.2, 18.4, 13.6. MS calcd for $C_{16}H_{25}N_2O_5S$ $[M+H]^+$ 357.1, found: 357.1.

5.9. Compound 8

Prepared following method B in the general procedure described previously. Boc-L-Nva (615 mg, 2.83 mmol), 1-bromobenzenesulfonamide (2.0 g, 8.48 mmol), CDI (918 mg, 5.66 mmol), DBU (1260 μL, 8.48 mmol), and dry THF (70 mL). Purification on silica gel (CH_2Cl_2/MeOH 95:5) then aluminum oxide (CH_2Cl_2/MeOH 95:5, then CH_2Cl_2/MeOH/HCOOH 95:5:1) yielded the product as a white solid (1.0 g, 84%). ^{1}H NMR (CD_3OD): δ 8.23 (dd, J = 2.3, 7.4 Hz, 1H), 7.80 (dd, J = 1.7, 7.4 Hz, 1H), 7.59–7.50 (m, 2H), 4.07 (dd, J = 5.0, 9.1 Hz, 1H), 1.69 (m, 1H), 1.51 (m, 1H), 1.41 (s, 9H), 1.34–1.24 (m, 2H), 0.90 (t, J = 7.4 Hz, 3H). ^{13}C NMR (CD_3OD): δ 173.5, 157.8, 139.7, 136.4, 135.9, 134.2, 128.8, 121.0, 80.7, 56.1, 34.7, 28.7, 19.9, 13.9. MS calcd for $C_{16}H_{23}BrN_2O_5S$ $[M+H]^+$ 436.1, found: 435.1, 437.1.

5.10. Compound 9

In a microwave process vial, **8** (330 mg, 0.758 mmol), 2,4,6-trivinylcycloboroxane pyridine complex (338 mg, 1.52 mmol), $Pd(OAc)_2$ (18.0 mg, 0.0748 mmol), $[(t\text{Bu})_3\text{PH}]BF_4$ (44 mg, 0.152 mmol), K_2CO_3 (944 mg, 6.83 mmol), DME (12 mL), and water (3.6 mL) were mixed. The vial was sealed under N_2 and the vessel was heated by microwaves to 100 °C for 15 min. DME was evaporated, water (16 mL) was added and the mixture was acidified with 1 M HCl to pH 1. After extraction with ethyl acetate and evaporation of the organic phase, the product was purified on preparative RP-HPLC-MS giving **9** (192 mg, 66%) as a white solid. ^{1}H NMR (CD_3OD): δ 8.09 (m, 1H), 7.72 (m, 1H), 7.63 (m, 1H), 7.54 (dd, J = 10.9, 17.3 Hz, 1H), 7.45 (m, 1H), 5.79 (d, J = 17.3 Hz, 1H), 5.48 (dd, J = 1.3, 10.9 Hz, 1H), 3.98 (dd, J = 5.2, 8.8 Hz, 1H), 1.57 (m, 1H), 1.43 (m, 1H), 1.39 (s, 9H), 1.25–1.19 (m, 2H), 0.86 (t, J = 7.4 Hz, 3H). ^{13}C NMR (CD_3OD) δ 173.4, 157.7, 138.6, 137.3, 135.0, 134.5, 131.9, 128.8, 128.7, 119.5, 80.7, 56.0, 34.8, 28.6, 19.8, 13.9. MS calcd for $C_{18}H_{26}N_2O_5S$ $[M+H]^+$ 383.1, found: 383.1.

5.11. Compound 10

Prepared according to method A described in the general procedure earlier. Boc-L-Nva (163 mg, 0.750 mmol), 2-allylbenzenesulf-

onamide (99 mg, 0.502 mmol), prepared according to a previously described procedure,[31] CDI (243 mg, 1.50 mmol), DBU (225 μL, 1.50 mmol), and dry THF (10.5 mL). Purification on silica gel (EtOAc/*i*-hexane 40:60) yielded **10** (113 mg, 38%). ^{1}H NMR (CD_3OD): δ 8.09 (dd, J = 1.5, 8.0 Hz, 1H), 7.57 (dt, J = 1.4, 7.5 Hz, 1H), 7.41–7.36 (m, 2H), 6.04 (m, 1H), 5.11 (m, 1H), 5.09 (m, 1H), 3.98 (dd, J = 5.4, 8.7 Hz, 1H), 3.89 (dd, J = 6.8, 6.1 Hz, 1H), 3.81 (dd, J = 6.5, 16.2 Hz, 1H), 1.65–1.44 (m, 2H), 1.38 (s, 9H), 1.34–1.26 (m, 2H), 0.90 (t, J = 7.4 Hz, 3H). ^{13}C NMR (CD_3OD) δ 172.8, 156.6, 139.8, 137.5, 136.4, 133.5, 131.3, 130.9, 126.2, 116.1, 79.4, 55.0, 36.4, 33.7, 27.5, 18.7, 12.8. MS calcd for $C_{19}H_{28}N_2O_5S$ $[M+H]^+$ 397.2, found: 397.1.

5.12. Compound 11

Prepared according to method A described in the general procedure. L-cyclopropylalanine was Boc protected in the same way as earlier described.[24] Boc-L-cyclopropylalanine (830 mg, 3.62 mmol), cyclopropylsulfonamide (878 mg, 7.25 mmol) prepared as described previously,[26] CDI (1.18 mg, 7.27 mmol), DBU (1100 μL, 7.35 mmol), and dry THF (30 mL). Purification on RP-C18 silica (MeCN/H_2O 1:2 to MeCN/H_2O 1:1) followed by silica gel (CH_2Cl_2/MeOH 95:5) yielded **11** as a white solid (549 mg, 46%). ^{1}H NMR (CD_3OD): δ 4.12 (m, 1H), 2.96 (m, 1H), 1.66 (m, 1H), 1.52 (m, 1H), 1.45 (s, 9H), 1.30–1.18 (m, 2H), 1.14–1.06 (m, 2H), 0.80 (m, 1H), 0.56–0.44 (m, 2H), 0.21–0.10 (m, 2H). ^{13}C NMR (CD_3OD): δ 174.4, 157.8, 80.7, 56.9, 37.9, 31.8, 28.7, 8.4, 6.4, 6.2, 5.2, 4.8. MS calcd for $C_{14}H_{24}N_2O_5S$ $[M+H]^+$ 333.1, found: 333.0. Anal. Calcd for $C_{14}H_{24}N_2O_5S$: C, 50.58; H, 7.28; N, 8.43. Found: C, 50.77; H, 7.24; N, 8.45.

5.13. General procedure for synthesis of compounds 12–18

Removal of the Boc-protecting group of compounds **4**–**7** and **9**–**11** were performed by dissolving them in 4.0 M HCl in 1,4-dioxane (10 mL/mmol) and stirring until no starting material could be detected by LC–MS. The solvent was evaporated and the HCl salt of the product was used without further purification in the forthcoming couplings. Compound **3**, HATU, and the HCl salt of the N-deprotected **4**–**7** and **9**–**11** were dissolved in DMF. DIEA was added until the pH exceeded 10. The reactions were stirred until no starting material could be detected by LC–MS. After addition of EtOAc and washing with 0.1 M $NaHSO_4$ followed by 0.035 M $NaHSO_4$, the organic phase was evaporated.

5.14. Compound 12

Prepared according to the general procedure using **3** (100 mg, 0.190 mmol) the N-deprotected HCl salt of **4** (65.5 mg, 0.230 mmol), HATU (87.4 mg, 0.230 mmol), DIEA (190 μL, 1.11 mmol), and DMF (2 mL). Purification on silica gel (CH_2Cl_2/MeOH 95:5) yielded **12** as a 2:1 diastereomeric mixture (124 mg, 86%). ^{1}H NMR (CD_3OD, major isomer reported): δ 8.28 (d, J = 9.2 Hz, 1H), 7.87 (d, J = 2.3 Hz, 1H), 7.79–7.75 (m, 2H), 7.51 (m, 1H), 7.48 (d, J = 2.5 Hz, 1H), 7.46–7.41 (m, 3H), 7.27 (dd, J = 2.5, 9.2 Hz, 1H), 7.21 (d, J = 8.4 Hz, 1H), 6.79 (dd, J = 11.2, 17.7 Hz, 1H), 6.66 (s, 1H), 5.96 (dd, J = 1.0, 17.6 Hz, 1H), 5.67 (m, 1H), 5.35 (s, 1H), 5.28 (dd, J = 1.1, 11.2 Hz, 1H), 4.99–4.89 (m, 2H), 4.35 (dd, J = 5.5, 8.5 Hz, 1H), 3.99 (s, 3H), 3.43–3.25 (m, 2H), 2.10–2.03 (m, 2H), 1.91–1.62 (m, 4H), 1.48–1.38 (m, 2H), 1.46 (s, 9H), 0.95 (t, J = 7.3 Hz, 3H). ^{13}C NMR (CD_3OD, major isomer reported) δ 174.0, 172.9, 164.0, 163.7, 160.9, 157.6, 152.3, 151.9, 140.4, 138.0, 132.0, 130.9, 130.9, 130.8, 130.0, 129.9, 128.7, 127.6, 123.9, 123.3, 120.2, 118.0, 116.6, 115.8, 107.5, 101.8, 81.1, 59.0, 56.1, 55.3, 53.0, 34.6, 32.9, 28.7, 23.6, 20.1, 14.0. MS calcd for $C_{41}H_{48}N_4O_8S$ $[M+H]^+$ 757.3, found: 757.3.

5.15. Compound 13

Prepared according to the general procedure using **3** (30.0 mg, 0.0570 mmol), the N-deprotected HCl salt of **5** (26.9 mg, 0.09 mmol), HATU (25.8 mg, 0.0680 mmol), DIEA (64.4 μL, 0.370 mmol), and DMF (1 mL). Purification on silica gel (CH_2Cl_2/MeOH 93:7) gave a 4:3 diastereomeric mixture of **13** as a white solid (34 mg, 49%). ^{1}H NMR (CD_3OD, [both isomers reported]): δ [8.25 (d, J = 9.2 Hz), 8.24 (d, J = 9.2 Hz), 1H], [7.88 (d, J = 2.4 Hz), 7.86 (d, J = 2.4 Hz), 1H], [7.79–7.69 (m, 2H)], [7.52–7.46 (m, 2H)], [7.43–7.39 (m, 3H)], [7.26–7.16 (m,2H)], [6.77 (dd, J = 11.3, 17.5 Hz, 1H)], [6.65 (s), 6.61 (s), 1H], [5.94 (dd, J = 1.2, 17.5 Hz), 5.93 (dd, J = 1.2, 17.5 Hz), 1H], [5.68 (m, 1H)], 5.35 (s, 1H), [5.27 (dd, J = 1.4, 11.2 Hz), 5.26 (dd, J = 1.4, 11.2 Hz), 1H], [4.92–4.82 (m, 2H)], [4.33 (m, 1H)], 3.97 (s, 3H), [3.36–3.16 (m, 2H)], [2.11–1.92 (m, 2H)], [1.84–1.56 (m, 6H)], 1.45 (s, 9H), [1.41–1.34 (m, 2H)], [0.94 (t, J = 7.4 Hz), 0.78 (t, J = 7.4 Hz), 3H]. ^{13}C NMR (CD_3OD, major isomer reported): δ 174.6, 172.8, 169.9, 163.9, 157.4, 152.4, 151.9, 140.6, 139.0, 137.6, 132.1, 131.0, 130.7, 130.0, 129.9, 128.7, 128.6, 127.6, 123.9, 123.3, 120.1, 117.9, 115.8, 115.5, 107.6, 101.8, 81.1, 59.0, 56.1, 55.5, 53.5, 34.8, 34.1, 28.7, 28.4, 23.8, 20.0, 14.0. MS calcd for $C_{42}H_{50}N_4O_8S$ $[M+H]^+$ 771.3, found: 771.4.

5.16. Compound 14

Prepared according to the general procedure using **3** (80.0 mg, 0.151 mmol), the N-deprotected HCl salt of **6** (38.5 mg, 0.130 mmol), HATU (69.1 mg, 0.182 mmol), DIEA (140 μL, 0.820 mmol), and DMF (1.5 mL). Purification on silica gel (CH_2Cl_2/MeOH 95:5) yielded **14** in a 5:3 diastereomeric mixture (84 mg, 84%). ^{1}H NMR (CD_3OD, major isomer reported): δ 8.28 (d, J = 9.3 Hz, 1H), 7.89 (d, J = 2.3 Hz, 1H), 7.79–7.73 (m, 2H), 7.51 (m, 1H), 7.48 (d, J = 2.5 Hz, 1H), 7.45–7.41 (m, 3H), 7.27 (dd, J = 2.5, 9.2 Hz, 1H), 7.21 (d, J = 8.4 Hz, 1H), 6.79 (dd, J = 11.3, 17.7 Hz, 1H), 6.67 (s, 1H), 6.29 (dd, J = 1.1, 11.2 Hz, 1H), 5.97 (dd, J = 1.1, 17.7 Hz, 1H), 5.67 (m, 1H), 5.36 (s, 1H), 5.11–4.94 (m, 2H), 4.37 (dd, J = 5.4, 8.8 Hz, 1H), 4.00 (s, 3H), 2.63–2.49 (m, 2H), 1.85–1.61 (m, 2H), 1.60–1.39 (m, 2H), 1.47 (s, 9H), 1.39–1.22 (m, 2H), 0.96 (t, J = 7.3 Hz, 3H), 0.84–0.77 (m, 2H). ^{13}C NMR (CD_3OD, major isomer reported) δ 173.2, 172.8, 163.9, 163.6, 161.1, 157.6, 152.5, 152.0, 140.7, 137.6, 134.3, 132.0, 130.9, 130.7, 129.9, 129.9, 128.7, 127.6, 123.9, 123.3, 120.2, 119.2, 118.0, 115.8, 107.6, 101.9, 81.1, 59.0, 56.1, 55.3, 41.0, 35.8, 34.8, 28.7, 20.0, 14.0, 12.3, 11.0. MS calcd for $C_{42}H_{48}N_4O_8S$ $[M+H]^+$ 769.3, found: 769.1.

5.17. Compound 15

Prepared according to the general procedure using **3** (29.2 mg, 0.0555 mmol), the N-deprotected HCl salt of **7** (24.2 mg, 0.0886 mmol), HATU (25.3 mg, 0.0665 mmol), DIEA (63.9 μL, 0.366 mmol), and DMF (0.8 mL). Purification on preparative HPLC-MS (MeCN/H_2O (0.05% HCOOH)) yielded **15** as a diastereomeric mixture (26 mg, 61%). ^{1}H NMR (CD_3OD, major isomer reported): δ 8.31 (d, J = 9.15 Hz, 1H), 7.92 (m, 1H), 7.80–7.75 (m, 3H), 7.53–7.47 (m, 3H), 7.46–7.39 (m, 5H), 7.35 (dd, J = 2.4, 8.6 Hz, 1H), 7.30 (dd, J = 2.6, 9.4 Hz, 1H), 7.13 (d, J = 8.4 Hz, 1H), 6.76 (dd, J = 11.1, 17.4 Hz, 1H), 6.64 (s, 1H), 5.89 (d, J = 17.4 Hz, 1H), 5.27 (dd, J = 1.3, 11.4 Hz, 2H), 4.31 (dd, J = 5.5, 8.6 Hz, 1H), 4.01 (s, 3H), 1.78–1.55 (m, 2H), 1.45 (s, 9H), 1.37–1.26 (m, 2H), 0.88 (t, J = 7.5 Hz, 3H). ^{13}C NMR (CD_3OD, major isomer reported) 173.7, 172.6, 164.0, 163.7, 161.0, 156.3, 152.4, 151.9, 141.6, 140.6, 137.2, 134.2, 132.0, 130.9, 130.8, 129.9, 129.8, 129.7, 128.9, 128.7, 127.7, 123.9, 123.3, 120.2, 118.0, 115.8, 107.5, 101.9, 82.1, 59.0, 56.2, 55.4, 34.8, 28.7, 19.8, 14.0. MS calcd for $C_{42}H_{44}N_4O_8S$ $[M+H]^+$ 765.3, found: 765.5.

5.18. Compound 16

Prepared according to the general procedure using **3** (100 mg, 0.190 mmol), the N-deprotected HCl salt of **9** (95.5 mg, 0.300 mmol), HATU (87.4 mg, 0.230 mmol), DIEA (218 μL, 1.25 mmol), and DMF (3.5 mL). Purification on preparative RP-HPLC-MS ($MeCN/H_2O$ (0.05% HCOOH)) yielded **16** as a white solid in a 3:2 diastereomeric mixture (108 mg, 72%). ^1H NMR (CD_3OD, [both diastereomers reported]) δ [8.27 (d, J = 9.1 Hz), 8.26 (d, J = 9.1 Hz), 1H], [8.07 (dd, J = 1.4, 8.0 Hz), 7.97 (dd, J = 1.4, 8.0 Hz), 1H], [7.84–7.71 (m), 3H], [7.65–7.45 (m), 5H], [7.43–7.33 (m), 4H], [7.29–7.23 (m), 1H], [7.16 (d, J = 8.5 Hz), 7.09 (d, J = 8.5 Hz), 1H], [6.75 (t, J = 10.4 Hz), 6.71 (t, J = 10.4 Hz), 1H], [6.62 (s), 6.60 (s), 1H], [5.87 (dd, J = 6.7, 17.6 Hz), 1H], [5.71 (m), 1H], [5.44–5.38 (m), 1H], [5.31–5.20 (m), 2H], [4.38 (m), 1H], [3.99 (s), 3.98 (s), 3H], [1.75–1.66 (m), 1H], [1.63–1.53 (m), 1H], [1.44 (s), 9H], [1.36–1.25 (m), 2H], [0.87, (t, J = 7.3 Hz), 0.70 (t, J = 7.3 Hz), 3H]. ^{13}C NMR (CD_3OD) δ [173.7], [173.1], [172.5], [164.3, 164.2], [163.8], [160.7, 160.6], [157.3], [151.8], [151.6,], [139.7], [138.3], [138.1], [137.4], [134.8, 134.7], [134.4, 134.3], [132.0, 131.9], [131.5, 131.4], [131.0, 130.9], [130.7], [129.9], [128.8, 128.7], [128.5], [127.6, 127.5], [124.0], [123.3, 123.2], [120.3], [119.0, 118.8], [118.2, 118.1], [115.7], [107.0, 106.9], [101.9, 101.8], [81.1], [59.6, 58.9], [56.2], [55.1, 54.9], [34.9], [28.7], [19.8, 19.6], [14.0, 13.8]. MS calcd for $C_{44}H_{46}N_4O_8S$ $[M+H]^+$ 791.3, found: 791.7.

5.19. Compound 17

Prepared according to the general procedure using **3** (68.0 mg, 0.129 mmol), the N-deprotected HCl salt of **10** (43.2 mg, 0.130 mmol), HATU (59.0 mg, 0.155 mmol), DIEA (120 μL, 0.702 mmol), and DMF (1.3 mL). Purification on silica gel (CH_2Cl_2/MeOH 95:5) yielded **17** as a white solid in a 3:1 diastereomeric mixture (86 mg, 82%). ^1H NMR (CD_3OD, major isomer reported) δ 8.29 (d, J = 9.1 Hz, 1H), 7.98 (dd, J = 1.4, 7.9 Hz, 1H), 7.79–7.74 (m, 3H), 7.49 (d, J = 2.5 Hz, 1H), 7.45–7.41 (m, 4H), 7.36 (ddd, J = 1.4, 7.5, 7.5 Hz, 1H), 7.32 (m, 1H), 7.28 (dd, J = 2.5, 9.1 Hz, 1H), 7.20 (m, 1H), 7.05 (d, J = 8.4 Hz, 1H), 6.71 (dd, J = 11.2, 17.7 Hz, 1H), 6.61 (s, 1H), 6.00 (m, 1H), 5.83 (dd, J = 1.2, 17.6 Hz, 1H), 5.29 (s, 1H), 5.21 (dd, J = 1.1, 11.2 Hz, 1H), 4.98–4.91 (m, 2H), 4.34 (dd, J = 5.1, 8.6, 1H), 4.00 (s, 3H), 3.84 (dd, J = 6.5, 16.1 Hz, 1H), 3.71 (dd, J = 6.3, 16.1 Hz, 1H), 1.80–1.53 (m, 2H), 1.44 (s, 9H), 1.44–1.27 (m, 2H), 0.91 (t, J = 7.4 Hz, 3H). ^{13}C NMR (CD_3OD, major isomer reported) δ 172.5, 171.3, 162.9, 162.5, 159.8, 156.3, 151.1, 150.6, 139.5, 139.2, 138.2, 136.6, 136.1, 133.0, 131.3, 130.7, 130.4, 129.6, 128.7, 128.5, 127.6, 127.5, 126.4, 126.0, 122.8, 122.0, 119.1, 116.9, 115.9, 114.6, 106.2, 100.7, 79.9, 57.7, 55.0, 54.1, 36.4, 33.9, 27.5, 18.7, 12.9. MS calcd for $C_{45}H_{49}N_4O_8S$ $[M+H]^+$ 805.3, found: 805.5.

5.20. Compound 18

Prepared according to the general procedure described using **3** (15.3 mg, 0.0290 mmol), the N-deprotected HCl salt of **11** (7.79 mg, 0.0290 mmol), HATU (13.2 mg, 0.0348 mmol), DIEA (27.4 μL, 0.157 mmol), and DMF (0.7 mL). Purification on silica gel (CH_2Cl_2/MeOH 97:3) yielded the pure L-Phg diastereomer of **17** (6 mg, 28%) as well as a diastereomeric mixture of the L-Phg form and the D-Phg form (8.7 mg, 41%). ^1H NMR (CD_3OD, major isomer reported): δ 8.30 (d, J = 9.2 Hz, 1H), 7.88 (d, J = 2.3 Hz, 1H), 7.78 (m, 1H), 7.52–7.48 (m, 2H), 7.46–7.42 (m, 2H), 7.29 (dd, J = 2.4, 9.1 Hz, 1H), 7.23 (d, J = 8.4 Hz, 1H), 6.79 (dd, J = 11.2, 17.7 Hz, 1H), 6.66 (s, 1H), 5.97 (dd, J = 1.1, 17.7 Hz, 1H), 5.36 (s, 1H), 5.29 (dd, J = 1.1, 11.1 Hz, 1H), 4.45 (dd, J = 6.3, 7.6 Hz, 1H), 4.00 (s, 3H), 2.99 (s, 1H), 2.86 (d, J = 0.8 Hz, 1H), 1.79 (m, 1H), 1.61 (m, 1H), 1.47 (s, 9H), 1.30 (m, 1H), 1.22 (m, 1H), 1.11 (m, 1H), 1.01–0.93 (m, 2H), 0.85 (m, 1H), 0.51–0.43 (m, 2H), 0.20–0.09 (m, 2H). ^{13}C NMR (CD_3OD, major isomer reported): δ 173.3, 172.7, 164.9, 164.0, 163.6, 161.1, 152.5, 152.0, 140.7, 137.3, 132.1, 131.0, 130.7, 130.0, 129.9, 128.7, 127.6, 124.0, 123.4, 120.2, 117.9, 115.8, 107.6, 101.8, 81.1, 59.1, 56.1, 55.9, 38.8, 31.8, 28.7, 8.4, 6.4, 6.1, 5.3, 5.0. MS calcd for $C_{40}H_{44}N_4O_8S$ $[M+H]^+$ 741.3, found: 741.1.

5.21. General procedure for preparation of compounds 19–25

For removal of the Boc-protecting group, compounds **12–18** were dissolved in a 4.0 M HCl in dioxane solution (10 mL/mmol). The solutions were stirred until starting material no longer could be visualized by LC–MS. The solvent was evaporated and the N-deprotected HCl salts of 12–17 were used without further purification. They were mixed with Boc-*t*-Leu, HATU, DIEA and DMF and stirred until no starting material could be visualized by LC–MS. After addition of ethyl acetate, washing with 0.1 M sodium acetate buffer, pH 4, the organic phase was evaporated. Purification and partial separation of the diastereomers (compounds **19**–**24**) was made by preparative HPLC.

5.22. Compounds 19a and 19b

Prepared according to the general procedure using the N-deprotected HCl salt of **12** (16.8 mg, 0.0243 mmol), Boc-*t*-Leu (11.1 mg, 0.0479 mmol), HATU (22.0 mg, 0.0578 mmol), DIEA (32.0 μL, 0.187 mmol), and DMF (0.7 mL). Purification on preparative HPLC ($MeCN/H_2O$ (0.05% HCOOH)) yielded **19a** (8.2 mg, 39%) and **19b** (3.5 mg, 17%) as white solids. **19a**: ^1H NMR (CD_3OD): δ 8.29 (d, J = 9.2 Hz, 1H), 7.92 (d, J = 2.2 Hz, 1H), 7.78–7.75 (m, 2H), 7.53 (dd, J = 2.4, 8.5 Hz, 1H), 7.49 (d, J = 2.5 Hz, 1H), 7.47–7.42 (m, 3H), 7.28 (dd, J = 2.5, 9.2 Hz, 1H), 7.20 (d, J = 8.5 Hz, 1H), 6.79 (dd, J = 11.2, 17.6 Hz, 1H), 6.65 (s, 1H), 6.00 (d, J = 18.0 Hz, 1H), 5.71–5.60 (m, 1H), 5.69 (s, 1H), 5.28 (dd, J = 1.2, 11.2 Hz, 1H), 4.97–4.88 (m, 2H), 4.35 (dd, J = 5.4, 8.8 Hz, 1H), 4.03 (s, 1H), 4.00 (s, 3H), 3.38–3.16 (m, 2H), 2.10–2.02 (m, 2H), 1.89–1.65 (m, 4H), 1.55–1.41 (m, 2H), 1.42 (s, 9H), 1.02 (s, 9H), 0.96 (t, J = 7.3 Hz, 3H). ^{13}C NMR (CD_3OD): 173.9, 173.2, 171.9, 164.0, 163.7, 161.0, 157.9, 152.4, 151.9, 140.6, 138.0, 136.9, 132.0, 130.9, 130.7, 130.2, 139.9, 128.7, 127.8, 123.9, 123.3, 120.2, 118.1, 116.6, 115.8, 107.5, 101.9, 80.7, 63.8, 57.3, 56.1, 55.3, 53.0, 35.3, 34.6, 32.9, 28.7, 27.2, 23.6, 20.1, 14.0. HRMS calcd for $C_{47}H_{59}N_5O_9S$ $[M+H]^+$ 870.4112, found: 870.4097. HPLC purity (system 1: 93%, system 2: 98%). **19b**: ^1H NMR (CD_3OD): δ 8.31 (d, J = 9.1 Hz, 1H), 7.90 (d, J = 2.3 Hz, 1H), 7.76–7.72 (m, 2H), 7.51 (dd, J = 2.3, 8.3 Hz, 1H), 7.49 (d, J = 2.5 Hz, 1H), 7.46–7.42 (m, 3H), 7.30 (dd, J = 2.5, 9.2 Hz, 1H), 7.24 (d, J = 8.3 Hz, 1H), 6.80 (dd, J = 11.2, 17.7 Hz, 1H), 6.60 (s, 1H), 5.96 (d, 17.7 Hz, 1H), 5.73 (m, 1H), 5.61 (s, 1H), 5.31 (dd, J = 1.2, 11.1 Hz, 1H), 5.05–4.90 (m, 2H), 4.32 (dd, J = 5.4, 8.9 Hz, 1H), 4.01 (s, 3H), 3.97 (s, 1H), 3.44–3.33 (m, 2H), 2.18–2.11 (m, 2H), 1.95–1.65 (m, 4H), 1.44 (s, 9H), 1.38–1.24 (m, 2H), 0.96 (s, 9H), 0.84 (t, J = 7.3 Hz, 3H). ^{13}C NMR (CD_3OD): 173.9, 173.2, 172.4, 164.0, 163.7, 161.1, 157.9, 152.5, 152.1, 140.8, 138.0, 137.3, 132.2, 130.8, 130.7, 130.7, 129.8, 128.6, 128.0, 123.9, 123.5, 120.3, 118.0, 116.6, 115.8, 107.5, 101.8, 80.7, 63.9, 58.1, 56.1, 55.4, 53.1, 35.4, 34.4, 33.0, 28.8, 27.2, 23.6, 19.9, 13.8. HRMS calcd for $C_{47}H_{59}N_5O_9S$ $[M+H]^+$ 870.4112, found: 870.4095. HPLC purity (system 1: 95%, system 2: 99%).

5.23. Compounds 20a and 20b

Prepared according to the general procedure using the N-deprotected HCl salt of **13** (29.2 mg, 0.0422 mmol), Boc-*t*-Leu (27.7 mg, 0.119 mmol), HATU (53.2 mg, 0.140 mmol), DIEA (71.1 μL, 0.408 mmol), and DMF (0.9 mL). Purification on preparative HPLC ($MeCN/H_2O$ (0.05% HCOOH)) yielded **20a** (6 mg, 17%) and **20b** (3.5 mg, 10%) as white solids. **20a**: ^1H NMR (CD_3OD): δ 8.29 (d,

J = 9.2 Hz, 1H), 7.92 (d, 2.4 Hz, 1H), 7.79–7.75 (m, 2H), 7.54 (dd, *J* = 2.4, 8.5 Hz, 1H), 7.49 (d, *J* = 2.4 Hz, 1H), 7.47–7.42 (m, 3H), 7.28 (dd, *J* = 2.4, 9.1 Hz, 1H), 7.20 (d, *J* = 8.3 Hz, 1H), 6.79 (dd, *J* = 11.2, 17.7 Hz, 1H), 6.65 (s, 1H), 5.99 (d, *J* = 17.7 Hz, 1H), 5.73–5.62 (m, 2H), 5.28 (dd, *J* = 1.0, 11.2 Hz, 1H), 4.93–4.87 (m, 2H), 4.35 (dd, *J* = 5.0, 8.6 Hz, 1H), 4.06–3.98 (m, 4H), 3.29–3.12 (m, 2H), 2.00–1.92 (m, 2H), 1.85–1.64 (m, 4H), 1.50–1.43 (m, 2H), 1.42 (s, 9H), 1.41–1.28 (m, 2H), 1.02 (s, 9H), 0.96 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (CD_3OD): 175.2, 173.2, 171.7, 163.9, 163.6, 161.1, 157.9, 152.6, 152.0, 140.8, 139.1, 136.9, 132.0, 130.9, 130.7, 130.1, 129.9, 128.7, 127.7, 123.9, 123.3, 120.1, 118.0, 115.8, 115.5, 107.7, 101.8, 80.7, 63.8, 57.4, 56.1, 55.7, 53.4, 35.3, 35.0, 34.2, 28.7, 28.5, 27.2, 23.9, 20.1, 14.0. HRMS calcd for $C_{48}H_{61}N_5O_9S$ [M+]H$^+$ 884.4268, found: 884.4260. HPLC purity (system 1: 96%, system 2: 94%) **20b**: ^{1}H NMR (CD_3OD): δ 8.31 (d, *J* = 9.2 Hz, 1H), 7.90 (d, *J* = 2.4 Hz, 1H), 7.76–7.73 (m, 2H), 7.53–7.48 (m, 2H), 7.46–7.43 (m, 3H), 7.29 (dd, *J* = 2.4, 9.1 Hz, 1H), 7.24 (d, 8.3 Hz, 1H), 6.80 (dd, *J* = 11.2, 17.7 Hz, 1H), 6.60 (s, 1H), 5.96 (d, *J* = 17.7 Hz, 1H), 5.74 (m, 1H), 5.62 (s, 1H), 5.31 (dd, *J* = 1.0, 11.2 Hz, 1H), 5.01–4.48 (m, 2H), 4.33 (dd, *J* = 5.1, 8.7 Hz, 1H), 4.01 (s, 3H), 3.96 (s, 1H), 3.44–3.33 (m, 2H), 2.10–1.97 (m, 2H), 1.85–1.65 (m, 4H), 1.51–1.39 (m, 11H), 1.35–1.24 (m, 2H), 0.95 (s, 9H), 0.83 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (CD_3OD) δ 173.8, 173.2, 172.3, 163.9, 163.6, 161.1, 157.9, 152.6, 152.1, 140.9, 139.1, 137.4, 132.2, 130.9, 130.7, 130.7, 129.8, 128.6, 128.0, 123.9, 123.5, 120.2, 118.0, 115.8, 115.6, 107.7, 101.8, 80.7, 64.0, 58.1, 56.1, 55.5, 53.5, 35.4, 34.7, 34.2, 28.8, 28.5, 27.2, 23.9, 19.9, 13.9. HRMS calcd for $C_{48}H_{61}N_5O_9S$ [M+H]$^+$ 884.4268, found: 884.4262. HPLC purity (system 1: 97%, system 2: 99%).

5.24. Compounds 21a and 21b

Prepared according to the general procedure using the N-deprotected HCl salt of **14** (14.0 mg, 0.0200 mmol), Boc-*t*-Leu (9.25 mg, 0.0400 mmol), HATU (18.2 mg, 0.048 mmol), DIEA (26.6 μL, 0.156 mmol), and DMF (0.7 mL). Three purification on preparative HPLC (MeCN/H_2O (0.05% HCOOH)) had to be performed to yield **21a** (2.7 mg, 15.3%) and **21b** (0.8 mg, 4.5%) as white solids. **21a**: ^{1}H NMR (CD_3OD): δ 8.30 (d, *J* = 9.1 Hz, 1H), 7.94 (d, *J* = 2.3 Hz, 1H), 7.78–7.75 (m, 2H), 7.54 (dd, *J* = 2.3, 8.5 Hz, 1H), 7.49 (d, *J* = 2.5 Hz, 1H), 7.46–7.41 (m, 3H), 7.29 (dd, *J* = 2.5, 9.1 Hz, 1H), 7.21 (d, *J* = 8.4 Hz, 1H), 6.79 (dd, *J* = 11.3, 17.7 Hz, 1H), 6.65 (s, 1H), 6.01 (d, *J* = 17.7 Hz, 1H), 5.72–5.61 (m, 1H), 5.69 (s, 1H), 5.29 (dd, *J* = 1.2, 11.2, 1H), 5.05–4.93 (m, 2H), 4.37 (dd, *J* = 5.2, 9.0 Hz, 1H), 4.03 (s, 1H), 4.01 (s, 3H), 2.64–2.50 (m, 2H), 1.84–1.63 (m, 2H), 1.54–1.28 (m, 4H), 1.49 (s, 9H), 1.02 (s, 9H), 0.97 (t, *J* = 7.3 Hz, 3H), 0.84–0.79 (m, 2H). ^{13}C NMR (CD_3OD): δ 173.2, 172.7, 171.8, 163.9, 163.6, 161.2, 156.2, 152.6, 152.0, 140.8, 136.9, 134.3, 132.1, 130.9, 130.7, 129.9, 128.7, 127.5, 123.9, 123.2, 120.2, 119.3, 118.1, 115.8, 107.6, 101.9, 80.7, 63.8, 57.3, 56.1, 55.2, 41.0, 35.8, 35.3, 35.3, 34.7, 28.7, 27.2, 20.1, 14.0, 12.3, 10.9. HRMS calcd for $C_{48}H_{59}N_5O_9S$ [M+H]$^+$ 882.4112, found: 882.4131. HPLC purity (system 1: 96%, system 2: 96%). **21b**: ^{1}H NMR (CD_3OD): 8.31 (d, *J* = 9.1 Hz, 1H), 7.91 (d, *J* = 2.4 Hz, 1H), 7.77–7.72 (m, 2H), 7.52 (dd, *J* = 2.1, 8.4 Hz, 1H), 7.49 (d, *J* = 2.5 Hz, 1H), 7.45–7.42 (m, 3H), 7.29 (dd, *J* = 2.5, 9.2 Hz, 1H), 7.24 (d, *J* = 8.4 Hz, 1H), 6.80 (dd, *J* = 11.1, 17.7 Hz, 1H), 6.59 (s, 1H), 5.96 (d, *J* = 17.7 Hz, 1H), 5.75 (m, 1H), 5.62 (s, 1H), 5.31 (dd, *J* = 1.2, 11.1 Hz, 1H), 5.09–5.00 (m, 2H), 4.36 (dd, *J* = 5.2, 9.0 Hz, 1H), 4.01 (s, 3H), 3.97 (s, 1H), 2.65 (dt, *J* = 1.3, 7.3 Hz, 2H), 1.85–1.62 (m, 2H), 1.56 (m, 1H), 1.47–1.31 (m, 2H), 1.43 (s, 9H), 1.33–1.23 (m, 2H), 0.96 (s, 9H), 0.88 (m, 1H), 0.83 (t, *J* = 7.4 Hz, 3H). HRMS calcd for $C_{48}H_{59}N_5O_9S$ [M+H]$^+$ 882.4112, found: 882.4094. HPLC purity (system 1: 91%, system 2: 96%).

5.25. Compounds 22a and 22b

Prepared according to the general procedure using the N-deprotected HCl salt of **15** (11.9 mg, 0.0179 mmol), Boc-*t*-Leu (6.60 mg, 0.0285 mmol), HATU (8.30 mg, 0.0218 mmol), DIEA (20.8 μL, 0.119 mmol), and DMF (0.5 mL). Purification on preparative HPLC (MeCN/H_2O (0.05% HCOOH)) yielded **22a** (1.6 mg, 19%) and **22b** (2.2 mg, 29%) as white solids. **22a**: ^{1}H NMR (CD_3OD): δ 8.32 (d, *J* = 9.0 Hz, 1H), 7.92–7.87 (m, 2H), 7.83 (d, *J* = 2.4 Hz, 1H), 7.79–7.75 (m, 2H), 7.50 (d, *J* = 2.4 Hz, 1H), 7.48–7.37 (m, 5H), 7.30 (dd, *J* = 2.3, 9.0 Hz, 1H), 7.13 (d, *J* = 8.6 Hz, 1H), 6.77 (dd, *J* = 11.7, 18.2 Hz, 1H), 6.63 (s, 1H), 6.51–6.46 (m, 1H), 5.97–5.89 (m, 1H), 5.64 (s, 1H), 5.27 (dd, *J* = 1.2, 11.3 Hz, 1H), 4.31 (dd, *J* = 5.8, 8.8 Hz, 1H), 4.02 (s, 4H), 1.77–1.54 (m, 2H), 1.42 (s, 9H), 1.39–1.28, (m, 2H), 1.00 (s, 9H), 0.89 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR ($CDCl_3$): δ 173.1, 171.1, 163.9, 163.6, 161.2, 157.4, 152.6, 151.8, 140.9, 137.1, 132.9, 131.9, 130.9, 130.6, 130.0, 129.8, 129.3, 128.7, 128.4, 127.5, 123.9, 123.2, 120.1, 118.2, 118.0, 115.9, 111.2, 107.7, 101.9, 80.6, 63.9, 57.1, 56.5, 56.1, 35.8, 35.5, 28.7, 27.3, 19.8, 14.1. HRMS calcd for $C_{48}H_{55}N_5O_9S$ [M+H]$^+$ 878.3799, found: 878.3807. HPLC purity (system 1: 94%, system 2: 92%) **22b**: ^{1}H NMR (CD_3OD): δ 8.31 (d *J* = 9.3 Hz, 1H), 7.95–7.91 (m, 2H), 7.84 (m, 1H), 7.76–7.72 (m, 2H), 7.50–7.41 (m, 7H), 7.29 (dd, *J* = 2.6, 9.2 Hz, 1H), 7.19 (d, *J* = 9.0 Hz), 6.77 (dd, *J* = 11.3, 17.7 Hz, 1H), 6.59 (s, 1H), 5.89 (d, *J* = 17.3 Hz, 1H), 5.61 (s, 1H), 5.27 (d, *J* = 11.2 Hz, 1H), 4.30 (dd, *J* = 5.1, 8.6 Hz, 1H), 4.01 (s, 3H), 3.98–3.94 (m, 1H), 1.80–1.51 (m, 2H), 1.42 (s, 9H), 1.32–1.26 (m, 2H), 0.95 (s, 9H), 0.73 (t, *J* = 7.7 Hz, 3H). HRMS calcd for $C_{48}H_{55}N_5O_9S$ [M+H]$^+$ 878.3799, found: 878.3804. HPLC purity (system 1: 95%, system 2: 98%)

5.26. Compounds 23a and 23b

Prepared according to the general procedure using the N-deprotected HCl salt of **16** (11.6 mg, 0.0160 mmol), Boc-*t*-Leu (11.1 mg, 0.0479 mmol), HATU (21.8 mg, 0.0573 mmol), DIEA (28.4 μL, 0.163 mmol), and DMF (0.9 mL). Purification on preparative HPLC (MeCN/H_2O (0.05% HCOOH)) yielded **23a** (2.1 mg, 14%) and **23b** (1.4 mg, 9.6%) as white solids. **23a**: ^{1}H NMR (CD_3OD): δ 8.32 (dd, *J* = 1.4, 9.2 Hz, 1H), 7.98 (dd, *J* = 1.4, 8.0 Hz, 1H), 7.81 (d, *J* = 2.4 Hz, 1H), 7.77–7.72 (m, 2H), 7.59 (m, 1H), 7.56–7.50 (m, 2H), 7.49–7.41 (m, 4H), 7.36 (dd, *J* = 2.3, 8.5 Hz, 1H), 7.32 (ddd, *J* = 1.2, 2.5, 9.0 Hz, 1H), 7.27 (t, *J* = 7.8 Hz, 1H), 7.09 (d, *J* = 8.3 Hz, 1H), 6.72 (dd, *J* = 11.2, 17.5 Hz, 1H), 6.61 (s, 1H), 5.89 (d, *J* = 17.6 Hz, 1H), 5.69 (dd, *J* = 1.2, 17.4 Hz, 1H), 5.65 (s, 1H), 5.41 (dd, *J* = 1.2, 10.9 Hz, 1H), 5.24 (d, *J* = 11.2 Hz, 1H), 4.36 (dd, *J* = 4.8, 8.8 Hz, 1H), 4.02 (s, 4H), 1.69 (m, 1H), 1.55 (m, 1H), 1.41 (s, 9H), 1.37–1.28 (m, 2H), 0.99 (s, 9H), 0.89 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (CD_3OD): δ 173.1, 172.6, 171.6, 164.5, 164.0, 160.7, 157.9, 151.7, 151.6, 139.7, 138.5, 137.7, 136.9, 134.7, 134.6, 131.9, 131.7, 131.0, 130.6, 130.0, 128.8, 128.7, 128.6, 127.8, 124.1, 123.2, 120.5, 119.3, 118.3, 115.8, 106.8, 106.2, 102.0, 80.6, 63.8, 57.2, 56.3, 54.9, 35.3, 34.8, 28.7, 27.2, 19.9, 13.9. HRMS calcd for $C_{50}H_{57}N_5O_9S$ [M+H]$^+$ 904.3955, found: 904.3973. HPLC purity (system 1: 96%, system 2: 97%). **23b**: ^{1}H NMR (CD_3OD): δ 8.32 (d, *J* = 9.1 Hz, 1H), 8.08 (dd, *J* = 1.5, 8.1 Hz, 1H), 7.84 (d, *J* = 2.5 Hz, 1H), 7.77–7.72 (m, 2H), 7.66 (m, 1H), 7.56 (dd, *J* = 11.1, 17.4 Hz, 2H), 7.50 (d, *J* = 2.4 Hz, 1H), 7.48–7.39 (m, 5H), 7.31 (dd, *J* = 2.6, 9.2 Hz, 1H), 7.20 (d, *J* = 8.4 Hz, 1H), 6.76 (dd, *J* = 11.3, 17.6 Hz, 1H), 6.59 (s, 1H), 5.88 (dd, *J* = 1.1, 17.6 Hz, 1H), 5.76 (dd, *J* = 1.2, 17.2 Hz, 1H), 5.56 (s, 1H), 5.46 (dd, *J* = 1.2, 11.0 Hz, 1H), 5.28 (dd, *J* = 1.1, 11.1 Hz, 1H), 4.34 (dd, *J* = 4.8, 9.0 Hz, 1H), 4.01 (s, 3H), 3.95 (s, 1H), 1.67 (m, 1H), 1.54 (m, 1H), 1.41 (s, 9H), 1.18–1.06 (m, 2H), 0.94 (s, 9H), 0.73 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (CD_3OD): δ 173.2, 172.8, 172.0, 164.4, 164.0, 160.8, 157.8, 151.9, 151.7, 140.0, 138.6, 137.8, 137.5, 134.8, 134.6, 132.2, 131.8, 131.0,

130.7, 130.6, 130.0, 128.7, 128.7, 128.6, 127.9, 124.1, 123.4, 120.5, 119.4, 118.2, 115.7, 106.9, 101.9, 80.7, 63.9, 58.0, 56.2, 54.8, 35.4, 34.5, 28.8, 27.1, 19.7, 13.8. HRMS calcd for $C_{50}H_{57}N_5O_9S$ $[M+H]^+$ 904.3955, found: 904.3940. HPLC purity (system 1: 96%, system 2: 97%).

5.27. Compounds 24a and 24b

Prepared according to the general procedure using the N-deprotected HCl salt of **17** (18.5 mg, 0.0250 mmol), Boc-*t*-Leu (11.6 mg, 0.0501 mmol), HATU (22.8 mg, 0.0600 mmol), DIEA (33.3 μL, 0.195 mmol), and DMF (0.7 mL). Purification by preparative HPLC (MeCN/H_2O (0.1% TFA)) yielded **24a** (8.6 mg, 37%) as TFA-salt. Repurification of the remaining diastereomeric mixture by preparative HPLC (MeCN/H_2O (0.05% HCOOH)) yielded **24b** (1.84 mg, 8%) as a white solid. **24a**: ^{1}H NMR (CD_3OD (TFA-salt)): δ 8.57 (d, J = 9.3 Hz, 1H), 7.96 (dd, J = 1.4, 8.2 Hz, 1H), 7.81 (dd, J = 2.4, 9.5 Hz, 1H), 7.78 (d, J = 1.6 Hz, 1H), 7.68 (m, 1H), 7.62–7.60 (m, 4H), 7.58 (dd, J = 2.3, 9.3 Hz, 1H), 7.45 (ddd, J = 1.4, 6.9, 8.3 Hz, 1H), 7.37 (dd, J = 2.2, 8.4 Hz, 1H), 7.28 (dd, J = 1.0, 6.9 Hz, 1H), 7.22 (dt, J = 2.0, 8.3 Hz, 1H), 7.18 (d, J = 8.4 Hz, 1H), 6.72 (s, 1H), 6.68 (dd, J = 11.2, 17.7 Hz, 1H), 5.99 (m, 1H), 5.89 (dd, 0.9, 17.7 Hz, 1H), 5.60 (s, 1H), 5.28 (dd, J = 0.9, 11.2 Hz, 1H), 5.02–4.97 (m, 2H), 4.33 (dd, J = 5.2, 8.9 Hz, 1H), 4.11 (s, 3H), 3.98 (s, 1H), 3.86 (dd, J = 6.6, 16.3 Hz, 1H), 3.74 (dd, J = 6.6, 16.2 Hz, 1H), 1.74–1.55 (m, 2H), 1.42 (s, 9H), 1.41–1.40 (m, 2H), 1.00 (s, 9H), 0.93 (t, J = 7.2 Hz, 3H). ^{13}C NMR (CD_3OD): δ 173.1, 172.4, 171.5, 168.5, 166.8, 158.5, 157.9, 150.5, 145.1, 141.0, 138.6, 138.2, 137.6, 134.6, 133.7, 133.6, 132.6, 132.0, 131.7, 130.8, 130.4, 130.0, 129.6, 128.4, 127.3, 125.8, 123.0, 122.5, 119.5, 117.3, 115.5, 102.9, 101.5, 80.6, 63.8, 57.2, 57.0, 54.9, 37.5, 35.3, 34.5, 28.7, 27.2, 20.0, 13.9. HRMS calcd for $C_{51}H_{59}N_5O_9S$ $[M+H]^+$ 918.4112, found: 918.4131. HPLC purity (system 1: 97%, system 2: 98%). **24b**: ^{1}H NMR (CD_3OD): δ 8.31 (d, J = 9.2 Hz, 1H), 8.08 (dd, J = 1.2, 8.0 Hz, 1H), 7.82 (s, 1H), 7.75 (m, 2H), 7.50 (d, J = 2.5 Hz, 1H), 7.45–7.40 (m, 5H), 7.36–7.28 (m, 3H), 7.17 (d, J = 8.2 Hz, 1H), 6.75 (dd, J = 11.3, 17.8 Hz, 1H), 6.58 (s, 1H), 6.01 (m, 1H), 5.87 (dd, J = 1.0, 17.7 Hz, 1H), 5.56 (s, 1H), 5.26 (dd, J = 0.9, 11.3 Hz, 1H), 5.10–5.00 (m, 2H), 4.34 (dd, J = 4.9, 8.9, 1H), 4.01 (s, 3H), 3.94 (s, 1H), 3.88 (dd, J = 6.5, 15.7 Hz, 1H), 3.81 (dd, J = 6.5, 15.9 Hz, 1H), 1.72 (m, 1H), 1.61 (m, 1H), 1.42 (s, 9H), 1.25–1.11 (m, 2H), 0.94 (s, 9H), 0.77 (t, J = 7.0 Hz, 3H). ^{13}C NMR (CD_3OD): δ 173.1, 171.9, 164.0, 163.7, 161.1, 157.8, 152.5, 152.0, 140.8, 140.7, 140.0, 137.9, 137.4, 134.1, 132.5, 132.2, 131.7, 130.8, 130.7, 130.6, 129.9, 128.7, 128.0, 127.2, 123.9, 123.5, 120.3, 118.1, 117.1, 115.8, 107.5, 101.8, 80.7, 64.0, 58.1, 56.1, 54.6, 37.7, 35.4, 34.9, 28.8, 27.2, 19.8, 13.9. The Nva carbonyl carbon could not be found due to low concentration of NMR sample. HRMS calcd for $C_{49}H_{57}N_5O_9S$ $[M+H]^+$ 918.4112, found: 918.4124. HPLC purity (system 1: 98%, system 2: 98%).

5.28. Compound 25

Prepared according to the general procedure using the N-deprotected HCl salt of **18** (5.14 mg, 0.00760 mmol), Boc-*t*-Leu (8.00 mg, 0.0152 mmol), HATU (6.92 mg, 0.0182 mmol), DIEA (11.7 μL, 0.0669 mmol), and DMF (0.5 mL). After purification on preparative HPLC (MeCN/H_2O (0.05% HCOOH)), **25** was yielded as a white solid (2.48 mg, 38%). ^{1}H NMR (CD_3OD) δ 8.31 (d, J = 9.2 Hz, 1H), 7.93 (d, J = 2.3 Hz, 1H), 7.79–7.75 (m, 2H), 7.55 (dd, J = 2.2, 8.4 Hz, 1H), 7.49 (d, J = 2.5 Hz, 1H), 7.48–7.42 (m, 3H), 7.29 (dd, J = 2.5, 9.2 Hz, 1H), 7.23 (d, J = 8.3 Hz, 1H), 6.79 (dd, J = 11.2, 17.6 Hz, 1H), 6.64 (s, 1H), 6.00 (d, J = 17.6 Hz, 1H), 5.70 (s, 1H), 5.29 (d, J = 11.4 Hz, 1H), 4.86 (s, 1H), 4.45 (dd, J = 7.8, 6.3 Hz, 1H), 4.01 (s, 3H), 2.85 (m, 1H), 1.79 (m, 1H), 1.60 (m, 1H), 1.42 (s, 9H), 1.23–1.16 (m, 1H), 1.14–1.07 (m, 1H), 1.01 (s, 9H), 0.97–0.80 (m, 3H), 0.54–0.42 (m, 2H), 0.22–0.10 (m, 2H). ^{13}C NMR (CD_3OD (TFA-salt)): δ 173.3, 173.0, 171.6, 169.0, 167.1, 158.4, 155.5, 150.5, 144.4, 138.6, 133.9, 133.1, 132.2, 131.8, 130.9, 130.2, 129.6, 126.0, 123.1, 122.7, 199.5, 115.5, 103.1, 100.8, 80.7, 63.8, 57.2, 57.1, 55.8, 37.5, 35.3, 31.9, 28.7, 27.2, 8.4, 6.4, 6.2, 5.3, 5.0. HRMS calcd for $C_{46}H_{55}N_5O_9S$ $[M+H]^+$ 854.3799, found: 854.3787. HPLC purity (system 1: 95%, system 2: 99%)

5.29. Compound 27

Compound **4** (14 mg, 0.0401 mmol) was dissolved in 4.0 M HCl/dioxane and stirred for 4 h. The solvent was then evaporated. The HCl salt of **4** was stirred with **26**[26] (15.4 mg, 0.0267 mmol), HATU (12.3 mg, 0.0324 mmol), DIEA (22.9 μL, 0.132 mmol), and DMF (0.7 mL) for 2 h. EtOAc was added and the solution was washed with sodium acetate buffer, pH 4. Purification on silica gel (EtOAc/*i*-hexane 2:1), then preparative HPLC (MeCN/H_2O (0.05% HCOOH)) gave **27** (4.6 mg, 21%) as a white solid. ^{1}H NMR (CD_3OD): δ 8.15 (d, J = 9.0 Hz, 1H), 8.08–8.05 (m, 2H), 7.63–7.56 (m, 3H), 7.43 (d, J = 2.5 Hz, 1H), 7.36 (s, 1H), 7.15 (dd, J = 2.5, 9.0 Hz, 1H), 5.78 (m, 1H), 5.60 (s, 1H), 5.09–4.98 (m, 2H), 4.75 (m, 1H), 4.60 (m, 1H), 4.30 (dd, J = 5.1, 8.9 Hz, 1H), 4.23 (s, 1H), 4.06 (m, 1H), 3.98 (s, 3H), 3.36 (m, 2H), 2.77 (m, 1H), 2.42 (m, 1H), 2.21–2.15 (m, 2H), 1.98–1.61 (m, 3H), 1.59–1.42 (m, 2H), 1.28 (s, 9H), 1.04 (s, 9H), 0.99 (s, 1H), 0.95 (t, J = 7.3 Hz, 3H). ^{13}C NMR (CD_3OD): δ 174.2, 173.6, 173.4, 164.0, 160.7, 158.0, 151.7, 139.4, 138.1, 138.1, 131.4, 130.1, 129.3, 124.9, 119.9, 116.5, 116.5, 105.6, 100.7, 80.5, 78.9, 60.7, 60.0, 56.2, 55.3, 55.2, 53.1, 36.0, 35.9, 34.8, 33.0, 28.6, 26.9, 23.7, 20.0, 14.0. HRMS calcd for $C_{42}H_{57}N_5O_9S$ $[M+H]^+$ 808.3955, found: 808.3969. HPLC purity (system 1: 95%, system 2: 98%).

5.30. Enzyme inhibition

The protease activity of the full-length HCV NS3 protein (protease-helicase/NTPase) was measured using a FRET-assay as previously described.[32,35] In short, 1 nM enzyme was incubated for 10 min at 30 °C in 50 mM HEPES, pH 7.5, 10 mM DTT, 40% glycerol, 0.1% *n*-octyl-β-D-glucoside, 3.3% DMSO with 25 μM of the peptide cofactor 2K-NS4A (KKGVVIVGRIVLSGK), and inhibitor. The reaction was started by the addition of 0.5 μM substrate (Ac-DED(Edans)EEAbuψ[COO]ASK(Dabcyl)-NH_2) obtained from AnaSpec Inc. (San Jose, USA). Non-linear regression analysis of the data was made using Grafit 5.0.13 (Erithacus software limited). Evaluation of anti replicative effect in cells was performed as described by Lohmann et al.[33]

5.31. Computational methodology

All the ligands were built in maestro and geometry optimization is carried out using OPLS-2005 force field. The crystal structure of HCV bifunctional protease helicase (1CU1) is used in the study. This NS3 protein complex consists of the C-terminal helicase domain and the N-terminal protease domain with a covalently linked NS4A cofactor. The protease active site is occupied by the substrate (NS3 C-terminus which is part of the helicase domain). The active site was made available for the docking studies by deleting these terminal residues (624–631) occupying the active site.

All the crystallographic waters were removed and the protein was prepared for docking using the protein preparation tool implemented in the Schrodinger Suite 2008.[36] The generated structures were subjected to restrained minimization using the OPLS-2005 force field with 'normal' BatchMin cutoffs (7.0 Å VDW; 12.0 Å ELE). To account for the conformational changes in the protein, Induced fit docking is carried out using FLO (also called QXP).[37] A truncated protein structure that included only amino acids within 9 Å from the residues 624–631 (C-terminus) was used in the docking studies using FLO. Protein flexibility is accounted by allowing

crucial amino acid residues in the binding pocket to move freely up to 0.2 Å. Movement larger than 0.2 Å was penalized by 20.0 kJ/mol/Å^2. Flexible residues occupying the active site viz., R155, Q526 and K136 were given full conformational freedom. Constraints were applied to the inhibitor to restrict its translation away from the active site. For each inhibitor, 10 unique binding poses were generated using 2000 Monte Carlo perturbation cycles. These poses were further subjected to 20 steps of simulated annealing followed by energy minimization. Each cycle involves 400 rapid Monte Carlo steps generating unique conformations within an energy window of 50 kJ/mol/Å and an RMSD > 0.5 Å. Each of the complexes was submitted to a 3 fs dynamics at 600 K after 3000 fs of equilibration steps. The maximum movement of an atom in any single step was limited to 0.1 Å. Hydrogen vibrations were damped by assigning an atomic weight of 10. Among the 10 best poses generated, the most plausible binding mode conformation for each compound was selected based on visual inspection.

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