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Exploration of acyl sulfonamides as carboxylic acid replacements in protease inhibitors of the hepatitis C virus full-length NS3

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Abstract—The hepatitis C virus (HCV) NS3 protease has emerged as a promising anti-HCV drug target. Herein, we present an investigation of NS3 inhibitors comprising the acyl sulfonamide functionality. A series of tetra- and tripeptide based acyl sulfonamide inhibitors and their structure–activity relationships from both enzymatic and cell-based in vitro assays are presented. In summary, the acidity of the acyl sulfonamide functionality, the character of the P1 side chain, and the acyl sulfonamide substituent were found to be important for the inhibitory potencies.

1. Introduction

The hepatitis C virus (HCV) is a major health problem with an estimated 170 million people infected worldwide. Approximately 80% of the infected individuals develop a chronic infection that might progress into cirrhosis and eventually liver cancer.[1] Unfortunately, low efficacy and severe side effects have hampered the success of the present therapy regime and the need of improved antiviral agents is critical.[2]

The virally encoded non-structural protein 3 (NS3) is a bifunctional protein with both serine protease and helicase/NTPase activities that is crucial for the delivery of essential viral components.[3] Studies have validated the role of the protease in the replication of the virus and an NS3 protease inhibitor has recently been proven to be effective in reducing the viral load in humans.[4,5]

Steinkühler et al. and Llinàs-Brunet et al. observed that the HCV NS3 protease is subjected to N-terminal product inhibition and potent hexapeptides derived from the P6-P1 residues of the natural substrates were identified.[6,7] These hexapeptides subsequently served as lead structures and guided the design of less peptide-like inhibitors ultimately leading to the discovery of BILN 2061, a cyclic inhibitor with a C-terminal carboxylate group and with a powerful anti-HCV activity in human.[5] The C-terminal carboxylate seems crucial for both potency and selectivity of the product-based HCV NS3 protease inhibitors.[8,9]

We recently found that the C-terminal carboxylate could successfully be replaced by carboxylic acid bioisosteres such as the tetrazole and the phenyl acyl sulfonamide in hexa- and pentapeptide inhibitors (Fig. 1).[10,11] The acyl sulfonamide inhibitors displayed an impressive increase in the potency as compared to the corresponding carboxylic acids. Furthermore, the acyl sulfonamide inhibitors exhibited an exceptional specificity for the NS3 protease.[12] Campbell and co-workers at Bristol-Myers Squibb have recently devoted substantial efforts in developing acyl sulfonamide based NS3 protease inhibitors.[13–18]

We felt encouraged to further explore the potential of the acyl sulfonamide in HCV NS3 protease inhibitors. Herein, we report a more detailed investigation of the promising acyl sulfonamide functionality in tetra- and tripeptide based inhibitors with both enzymatic and cell-based inhibition data. Systematic alterations of the acyl sulfonamide group were conducted and the influence of the P1 side chain was assessed. Notably, we

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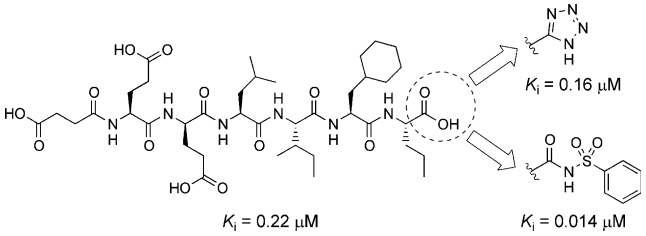


Figure 1. Impact of replacing the C-terminal carboxylic acid for a tetrazole or a phenyl acyl sulfonamide.[11]

demonstrate that not only the acidity of the acyl sulfonamide is important for inhibitory potency but also suggest that the two sulfonyl oxygens per se are involved in interactions with the enzyme.

2. Chemistry

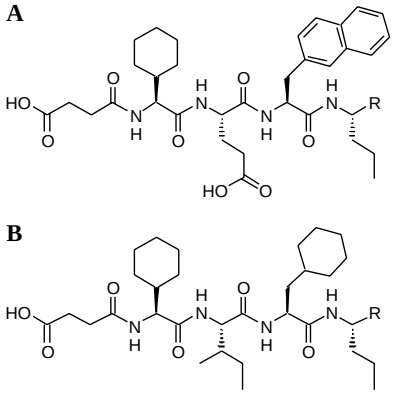
The inhibitors included in the SAR study (Tables 1–3) required preparation via different routes. The tetrapeptide inhibitors **1** and **4** with a C-terminal carboxylic acid were prepared using the standard Fmoc/*t*-Bu SPPS methodology.[19] The acyl sulfonamide containing tetrapeptides **2**, **3**, **5**, and **6** were prepared by an inverse SPPS method where tri-*tert*-butoxysilyl/fluorenylmethyl ester protected amino acids were used (Table 1).[20]

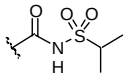
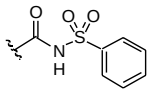
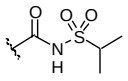
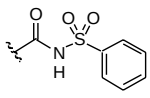
The tripeptide acyl sulfonamide inhibitors encompassing a P1 norvaline (Nva) side chain were synthesized by a convergent route and provided compounds with C-terminal carboxylic acid, alkyl/aryl acyl sulfonamide, benzylamide, phenyl β-ketosulfone, and *N*-methylated phenyl acyl sulfonamide. Thus, the P1-P1′ building block (Scheme 1) and the key intermediate **15**[13] comprising the P3-P2 residues were prepared for ultimate attachment via amide bond formation to suppress epimerization at the Nva α-carbon (Scheme 2). Synthesis of **15** has been previously described by others.[13]

The P1-P1′ building blocks were prepared as described in Scheme 1. The amide **7** was synthesized in solution using *O*-(benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) as coupling reagent and benzylamine; a reaction that delivered 90% yield. After Boc deprotection, the desired hydrochloride **8** was obtained quantitatively. The β-ketosulfone **10** was successfully prepared in 85% yield from Boc-L-Nva-OMe using two equiv of methyl phenyl sulfone and four equiv of *n*-butyl lithium.[21,22] The subsequent Boc deprotection gave **11** in quantitative yield. Partial racemization occurred during synthesis of **11** (approximately 20% according to ^{1}H NMR of the final inhibitor **24**). The acyl sulfonamide building blocks **12a**–**e** were prepared in 36–82% yields from Boc-L-Nva and the corresponding sulfonamides using 1,1-carbonyldiimidazole (CDI) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU).[11,23,24] Analysis of the final inhibitors revealed that partial racemization had occurred under these conditions. Initially, the reaction was heated, which resulted in a significant degree of racemization (Method B in Section 6, ~10% racemization according to HPLC). However, at room temperature, this racemization was considerably suppressed (Method A in Section 6, <5% racemization according to HPLC). The *N*-methylated acyl sulfonamide **12f** was prepared from **12c** in 81% yield using methyl iodide and Cs_2CO_3 at 65 °C. Boc deprotection of the acyl sulfonamide P1-P1′ building blocks gave the desired hydrochlorides **13a**–**f** in 93–100% yields. The hydrochlorides **13b** and **d** were converted to **14b** and **d** in quantitative yields using propylene oxide.[25]

The Nva containing tripeptide inhibitors were finally prepared as described in Scheme 2. The methyl ester **16** was synthesized from **15**[13] and commercially available HCl·L-Nva-OMe, which after ester hydrolysis using LiOH gave the carboxylic acid inhibitor **17** in 87% yield (2 steps). The inhibitors **18**–**25** were prepared by coupling of **15**[13] with the corresponding P1-P1′ building blocks

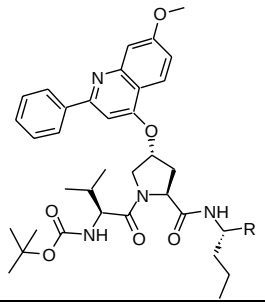
Table 1. Inhibition constants of tetrapeptide inhibitors of HCV NS3

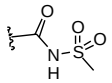
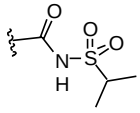
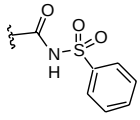
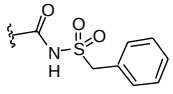
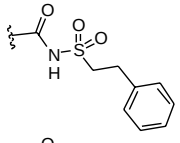
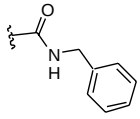
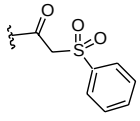
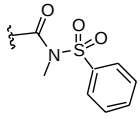


Peptide structure	Compound	R	$K_i \pm SD^a$ (μM)
A	**1**		46 ± 3
	2		12 ± 1
	3[b]		8.4 ± 0.8
B	**4**		28 ± 3
	5		9.7 ± 0.8
	6		5.3 ± 1.0

SD, standard deviation.
[a] Evaluated in an enzymatic assay comprising the full-length NS3 protein.
[b] 60:40 mixture of diastereomers at Nva.

Table 2. Inhibition constants and EC_{50} values of tripeptide inhibitors of HCV NS3: exploring the acyl sulfonamide functionality



Compound	R	$K_i \pm SD$[a] (μM)	EC_{50}[b] (μM)
17		0.76 ± 0.10	>10
18		0.77 ± 0.05	>10
19		0.58 ± 0.07	>10
20		0.30 ± 0.04	>10
21		0.18 ± 0.01	4.6
22		0.42 ± 0.10	>10
23		5.2 ± 1.0	>10
24[c]		2.7 ± 0.6	>10
25[d]		3.7 ± 0.4	n.d.

SD, standard deviation.; n.d., not determined.
[a] Evaluated in an enzymatic assay comprising the full-length NS3 protein.
[b] Evaluated in a HCV Replicon assay. Mean value of two determinations.
[c] 80:20 mixture of diastereomers at Nva.
[d] Did not bind irreversible to the enzyme.

using HBTU or *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU). In case of diastereomers (see above), they were successfully separated on silica gel and the major diastereomer in each case was subjected to biochemical evaluation (except for **24** that was tested as an 80:20 mixture of diastereomers). The *N*-methylated acyl sulfonamide **25** is susceptible to nucleophilic attack at the acyl sulfonamide carbonyl carbon and is thus moderately stable in solvents such as water or methanol.[26] Fortunately, we found it to be completely stable for 2 h in the buffer system used in the enzymatic assay, which facilitated biochemical evaluation.

The inhibitors containing the 1-amino-cyclopropanecarboxylic acid (ACCA) or the (1*R*, 2*S*)-1-amino-2-vinyl-cyclopropanecarboxylic acid (vinyl-ACCA), with no risk of epimerization, were prepared in a linear fashion (Scheme 2). The ester protected P1 amino acid was coupled to **15**[13] using HBTU to give **26**[13] and **27** in 84% and 76% yields, respectively. Ester deprotection with LiOH gave carboxylic acid inhibitors **28**[13] and **29**[27] in good yields. The acyl sulfonamide inhibitors **30**–**32** were prepared in moderate yields (75%, 60%, and 63%, respectively) from the corresponding acids utilizing preactivation with HATU and *N*,*N*-diisopropylethylamine (DIEA) followed by addition of the sulfonamide, 4-dimethylaminopyridine (DMAP), and DBU.[28]

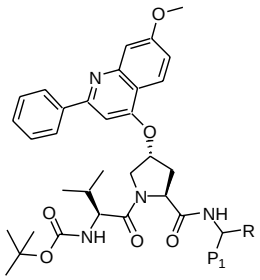
3. Biochemical evaluation

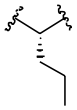
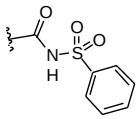
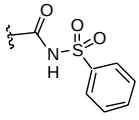
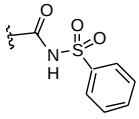
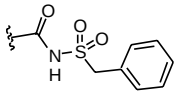
The K_i-values for inhibitors **1**–**6**, **17**–**25**, and **28**–**32** were determined in an in vitro assay comprising the full-length NS3 protein and the central part of NS4A as cofactor (Tables 1–3).[29,30] EC_{50}-values for compounds **17**–**24** and **28**–**32** were determined in an in vitro HCV replicon assay comprising human hepatic Huh-7 cells transfected with subgenomic HCV RNA (Tables 2 and 3).[31]

4. Results and discussion

After having introduced the acyl sulfonamide functionality in hexa- and pentapeptide inhibitors[10,11], we felt prompted to address the issue whether this group could serve as a proper carboxylic acid replacement also on shorter scaffolds. Two different tetrapeptide sequences originating from our tetrapeptide library and an optimized tripeptide scaffold developed by the researchers at Boehringer Ingelheim were chosen.[30,32] The isopropyl and the phenyl acyl sulfonamides were evaluated on the two tetrapeptide sequences (Table 1), whereas a more detailed investigation of acyl sulfonamide substituents (Me, *i*Pr, Ph, Bn, and phenethyl) was performed on the tripeptide scaffold (Table 2). Also in these two series the acyl sulfonamide group rendered inhibitors with improved potencies compared to the corresponding carboxylic acids, although the methyl acyl sulfonamide **18** was found to be equipotent to the carboxylic acid **17**. The response to the bioisosteric replacement is most pronounced when the acyl sulfonamides have substituents containing an aromatic ring system (**20**–**22**). Molecular modeling suggested three putative binding modes of the acyl sulfonamide substituent. The preference for aromatic groups could be

Table 3. Inhibition constants and EC_{50} values of tripeptide inhibitors of HCV NS3: influence from the P1 side chain



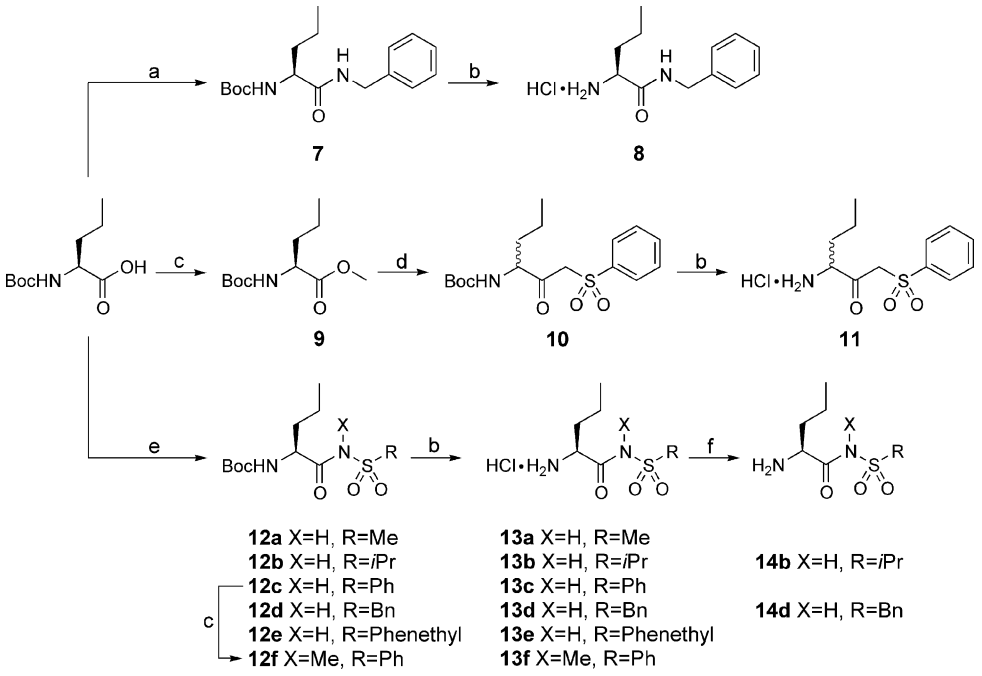
Compound	P1	R	$K_i \pm SD$[a] (μM)	EC_{50}[b] (μM)
17			0.76 ± 0.10	>10
20			0.30 ± 0.04	>10
28			0.32 ± 0.03	>10
30			0.055 ± 0.007	3.2
29			0.030 ± 0.006	1.1
31			0.00076 ± 0.00026	0.040
32			0.018 ± 0.003	0.28

SD, standard deviation.
[a] Evaluated in an enzymatic assay comprising the full-length NS3 protein.
[b] Evaluated in a HCV Replicon assay. Mean value of two determinations.

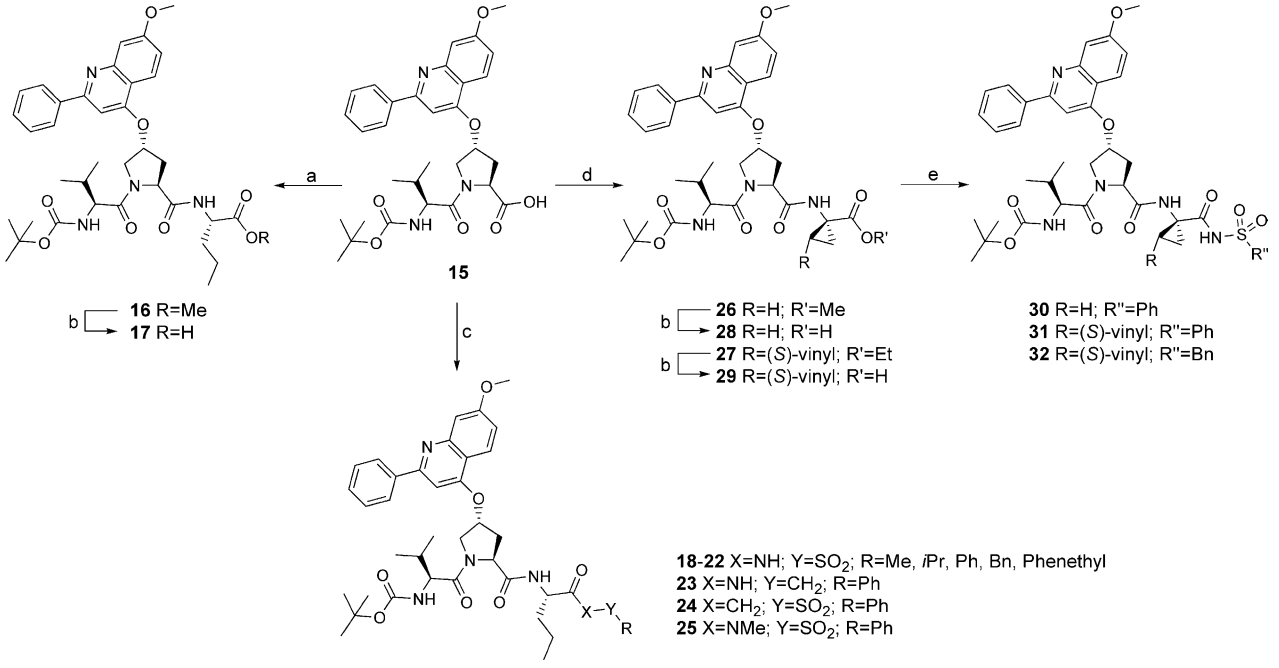
explained by favorable pi-stacking interactions between the phenyl ring and the side chain of Q41, as suggested by molecular modeling of **20** (Fig. 2B), and which is in line with the X-ray crystal structure of an α-ketoamide inhibitor encompassing a phenyl ring in a position similar to that reported by Liu et al.[33] However, the C-terminal phenyl group could also be seen docked in two other orientations. One in which it stacks over the aliphatic side chain of K136 as previously proposed for amide inhibitors by Colarusso et al.,[34] and the other where the C-terminal phenyl lies in the S1′ site between the backbones of K136, G137 on one side and Q41, S42 on the other.

The benzyl substituent (**21**, $K_i = 0.18$ μM) was found to be optimal in the tripeptide series. Elongation with one additional carbon spacer did not improve the potency (**22**, $K_i = 0.42$ μM).

To find out whether the acidity or the presence of the sulfonyl oxygens was important for efficient inhibitory potency of the acyl sulfonamides, three non-acidic analogs of **20** were synthesized, the amide **23**, the β-ketosulfone **24**, and the *N*-methylated acyl sulfonamide **25** (Table 2). The amide **23** is 17-fold less potent than the corresponding acyl sulfonamide **20**, which emphasizes the importance of the sulfone group in the acyl sulfonamides for efficient inhibition. The β-ketosulfone **24** and the *N*-methylated acyl sulfonamide **25** both displayed approximately 10 times lower potency as compared to **20**, suggesting that the acidity of the acyl sulfonamides is important for efficient enzyme inhibi-



Scheme 1. Reagents: (a) benzylamine, HBTU, DIEA, DMF; (b) HCl in EtOAc or 1,4-dioxane; (c) MeI, Cs_2CO_3, DMF; (d) $MeSO_2Ph$, *n*-BuLi, THF; (e) H_2NSO_2R, CDI, DBU, THF; (f) propylene oxide, EtOH or EtOH/MeOH.



Scheme 2. Reagents: (a) HCl·L-Nva-OMe, HBTU, DIEA, DMF; (b) LiOH, THF, MeOH, H_2O; (c) **13a**, **14b**, **13c**, **14d**, **13e**, **8**, **11** or **13f**, HBTU or HATU, DIEA, DMF; (d) HCl·ACCA-OMe or HCl·vinyl-ACCA-OEt, HBTU, DIEA, DMF; (e) H_2NSO_2R'', HATU, DIEA, DMAP, DBU, DMF.

tion. However, **24** and **25** are still slightly more potent than **23**, indicating that also the sulfonyl oxygens are involved in interactions with the enzyme. According to docking studies of **20** it seems likely that one of the sulfonyl oxygens is H-bonded to the εNHs of Q41 and the other to the backbone NH of G137 (Fig. 2B). Attempts were made to model the acyl sulfonamides constraining the two sulfonyl oxygens in the oxyanion hole as reported for α-ketoacids by Di Marco et al.[35] However, the compounds have a high internal strain in this pose indicating a non-favorable binding mode.

Rancourt et al. have recently reported the bright design of the optimized P1 amino acid vinyl-ACCA, which yielded inhibitors more potent than the ones containing

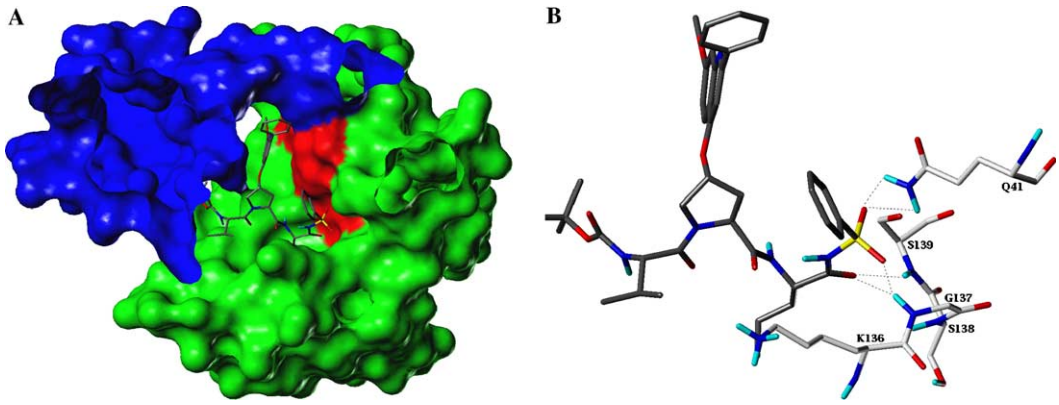


Figure 2. (A) Connolly surface representation of the full-length NS3 protein with docked inhibitor **20** occupying the active site. The helicase domain is depicted in blue, the protease domain in green, and the catalytic triad in red (H57, D81, and S139). (B) Possible H-bonding interactions between the acyl sulfonamide functionality of inhibitor **20** and the full-length NS3 protein. The P1 carbonyl is seen lying in the oxyanion hole (NHs of G137 and S139), the sulfonyl oxygens bind to the oxyanion hole, and the εNHs of Q41 and the phenyl ring pi-stacks with the side chain of Q41. H-bond distances: 2.0–2.7 Å. Atoms are colored according to atom type (oxygen in red, nitrogen in blue, and sulfur in yellow). Only essential hydrogens are displayed for clarity.

the native cysteine in tetrapeptides with a C-terminal carboxylate.[36] On the contrary, Orvieto et al. observed that when using a scaffold similar to the one used herein, the P1 vinyl-ACCA was detrimental to the potencies of their amide-based inhibitors.[37] In addition, we have seen that the potencies of our hexa- and pentapeptide acyl sulfonamide inhibitors were dependent on the P1 side chain.[11] Consequently, we decided to explore the outcome from incorporation of vinyl-ACCA and its unsubstituted analog ACCA in the tripeptide acyl sulfonamide inhibitors for comparison to the linear Nva-analogs (Table 3). These two modifications render inhibitors closely related to the ones disclosed in Bristol-Myers Squibb's patent application.[13] Thus, for inhibitors with a C-terminal carboxylate the vinyl-ACCA (**29**)[27] gave a 25-fold increase in potency as compared to the Nva-based inhibitor (**17**) and a 11-fold increase in potency as compared to the ACCA-based inhibitor (**28**)[13]. However, we were surprised to see the effect of exchanging the C-terminal carboxylic acid for the phenyl acyl sulfonamide in inhibitors comprising the P1 vinyl-ACCA (**29**[27] vs. **31**). This alteration gave a 39-fold increase in the potency, as compared to a 2.5-fold and a sixfold increase for the same substitution in the Nva- (**17** vs. **20**) and the ACCA-based (**28**[13] vs. **30**) inhibitors, respectively. In contrast to the observations made by Orvieto et al. on neutral amide inhibitors,[37] the P1 vinyl-ACCA seems to be optimal for acyl sulfonamide based inhibitors. Modeling studies suggest that the vinyl-ACCA lies more inside the S1 pocket than Nva or ACCA thereby packing its inhibitors more tightly with the enzyme.

Surprisingly, no gain in potency was observed upon combining the effects of the vinyl-ACCA and the benzyl substituent of **21** that was the most potent inhibitor in the Nva series. In fact, the benzyl acyl sulfonamide **32** turned out to be 24 times less potent than the corresponding phenyl substituted inhibitor **31**. This finding might be explained by a different binding mode of the vinyl-ACCA inhibitors compared to the Nva-based inhibitors as discussed above.

The acyl sulfonamide NS3 protease inhibitors are not only more potent than the corresponding carboxylic acids in the enzymatic assay but also in the cell-based replicon assay, as seen from the EC_{50} values (Tables 2 and 3). The benzyl acyl sulfonamide **21** was the only Nva containing inhibitor showing detectable potency (EC_{50} = 4.6 μM). The most potent inhibitor **31**, which has a K_i of 0.76 nM, also displays a low EC_{50} of 40 nM. Compared to the inhibitors containing a C-terminal carboxylic acid, the acyl sulfonamide inhibitors can be structurally modified to enhance the cellular potency.

5. Conclusion

We have performed an investigation of acyl sulfonamides as replacements to the C-terminal carboxylic acid found in product-based protease inhibitors of the HCV full-length NS3. The tetra- and tripeptide acyl sulfonamide inhibitors synthesized were shown to exhibit inhibitory potencies higher than those of the corresponding carboxylic acids in both enzymatic and cell-based assays. Results herein demonstrate that the acidity of the acyl sulfonamide functionality is important for inhibitory potency and that the sulfonyl oxygens of the acyl sulfonamide and the acyl sulfonamide substituent contribute to the inhibitory potencies. Moreover, it has been demonstrated that the positive outcome of the acyl sulfonamide inhibitors is highly dependent on the character of the P1 side chain. Taken together, this study emphasizes the potential of acyl sulfonamides as carboxylic acid replacements in product based NS3 protease inhibitors.

6. Experimental

6.1. Chemistry

Reagents and solvents were obtained commercially and used without further purification. Thin layer chromatography (TLC) was performed using aluminum sheets

precoated with silica gel 60 F_{254} (0.2 mm, E. Merck). Chromatographic spots were visualized using UV-detection and/or spraying with an ethanolic ninhydrin solution (2%) followed by heating. Column chromatography was performed using silica gel 60 (40–63 μm, E. Merck). Analytical RP-HPLC-MS was performed on a Gilson-Finnigan ThermoQuest AQA system equipped with a C18 (Chromolith Performance RP-18e (4.6×100 mm)) or a C4 (Hichrom ACE C4 (5 μm, 4.6×50 mm)) column using a MeCN/H_2O (0.05% HCOOH) gradient with UV (214 and 254 nm) and MS (ESI, 10 eV) detection. HPLC-purity of the tetrapeptides was determined at 214 nm. Preparative RP-HPLC-MS was performed on a Gilson-Finnigan ThermoQuest AQA system equipped with a C8 (Zorbax SB-C8 (5 μm, 21.2×150 mm)) column using a MeCN/H_2O (0.05% HCOOH) or MeCN/H_2O (50 mM NH_4OAc) gradient with UV (214 and 254 nm) and MS (ESI, 10 eV) detection. Preparative RP-HPLC was performed on a system equipped with a C18 (Vydac C18 218TP1022) column using a MeCN/H_2O (0.1% TFA) or a MeCN/H_2O (25 mM NH_4OAc) gradient with UV detection. Elemental analyses were performed by Analytische Laboratorien, Lindlar, Germany. Amino acid analyses were performed at the Department of Biochemistry, Uppsala University, Sweden, on 72 h hydrolyzates with an LKB 4151 alpha plus analyzer using ninhydrin detection. Exact molecular masses were determined on a Micromass Q-Tof2 mass spectrometer equipped with an electrospray ion source. NMR spectra were recorded on a JEOL JNM EX270 spectrometer (^1H at 270.2 MHz, ^{13}C at 67.8 MHz), a Varian Mercury plus spectrometer (^1H at 300.0 MHz, ^{13}C at 75.5 MHz), a Varian Mercury plus spectrometer (^1H at 399.8 MHz) or on a JEOL JNM EX400 spectrometer (^1H at 399.8 MHz, ^{13}C at 100.5 MHz) at ambient temperature. Chemical shifts (δ) are reported in ppm referenced indirectly to TMS via the solvent signals (^1H: $CHCl_3$ δ 7.26, CHD_2OD δ 3.31, DMSO-d_5 δ 2.50; ^{13}C: $CDCl_3$ δ 77.16, CD_3OD δ 49.00, DMSO-d_6 δ 39.50).

6.1.1. General procedure for the preparation of compounds 1–6. Prepared according to the procedures described by Johansson et al.[11] Purification by preparative RP-HPLC-MS or preparative RP-HPLC gave the desired tetrapeptides **1**–**6**.

6.1.2. Suc-Chg-Glu-2-Nal-Nva-OH (1). ^1H NMR (CD_3OD, 400 MHz) δ 7.84–7.72 (m, 4H), 7.44–7.37 (m, 3H), 4.74 (dd, J = 4.6, 9.0 Hz, 1H), 4.30–4.25 (m, 2H), 4.07 (d, J = 6.6 Hz, 1H), 3.44–3.39 (m, 1H), 3.18–3.13 (m, 1H), 2.61–2.50 (m, 4H), 2.25–2.15 (m, 2H), 2.01–1.92 (m, 1H), 1.90–1.75 (m, 2H), 1.75–1.58 (m, 5H), 1.55–1.51 (m, 1H), 1.42–1.29 (m, 3H), 1.22–1.00 (m, 5H), 0.90 (t, J = 7.3 Hz, 3H). HPLC purity (C18 column: 97%, C4 column: 98%). HRMS ($M+H^+$) calcd for $C_{35}H_{47}N_4O_{10}$: 683.3292; found: 683.3301. Amino acid analysis Chg 0.99, Glu 1.00, Nva 1.01.

6.1.3. Suc-Chg-Glu-2-Nal-Nva-NHSO$_2$*i*Pr (2). ^1H NMR (CD_3OD, 400 MHz) δ 7.85–7.82 (m, 1H), 7.79–7.76 (m, 2H), 7.73–7.72 (m, 1H), 7.45–7.33 (m, 3H), 4.73 (dd, J = 4.6, 9.1 Hz, 1H), 4.25 (dd, J = 4.7, 8.5 Hz, 1H), 4.23–4.19 (m, 1H), 4.07 (d, J = 6.2 Hz, 1H), 3.59–3.51 (m, 1H), 3.45–3.39 (m, 1H), 3.20–3.14 (m, 1H), 2.61–2.51 (m, 4H), 2.20–2.14 (m, 2H), 1.90–1.55 (m, 9H), 1.46–1.30 (m, 3H), 1.28 (d, J = 6.9 Hz, 3H), 1.28 (d, J = 6.9 Hz, 3H), 1.23–1.05 (m, 5H), 0.91 (dd, J = 7.3, 7.4 Hz, 3H). HPLC purity (C18 column: 96%, C4 column: 100%). HRMS ($M+H^+$) calcd for $C_{38}H_{54}N_5O_{11}S$: 788.3541; found: 788.3575. Amino acid analysis Chg 0.99, Glu 1.02, Nva 0.99.

6.1.4. Suc-Chg-Glu-2-Nal-Nva-NHSO$_2$ Ph (3). ^1H NMR (CD_3OD, 400 MHz) δ (60:40 mixture of diastereomers at Nva) 7.97–7.93 (m, 2H), 7.80–7.72 (m, 3H), 7.68–7.67 (m, 1H), 7.58–7.47 (m, 3H), 7.43–7.34 (m, 3H), 4.71–4.65 (m, 1H), 4.28–4.22 (m, 2H), 4.11 (d, J = 6.3 Hz, 0.6H), 4.04 (d, J = 6.5 Hz, 0.4H), 3.37–3.35 (m, 1H), 3.12–3.07 (m, 1H), 2.66–2.53 (m, 4H), 2.28–2.16 (m, 2H), 2.00–1.92 (m, 1H), 1.90–1.81 (m, 1H), 1.75–1.58 (m, 6H), 1.55–1.50 (m, 1H), 1.27–1.00 (m, 7H), 0.97–0.88 (m, 1H), 0.84 (t, J = 7.4 Hz, 1.8H), 0.59 (t, J = 7.3 Hz, 1.2H). HPLC purity (C18 column: 96%, C4 column: 100%). HRMS ($M+H^+$) calcd for $C_{41}H_{52}N_5O_{11}S$: 822.3384; found: 822.3347. Amino acid analysis Chg 1.00, Glu 1.02, Nva 0.98.

6.1.5. Suc-Chg-Ile-Cha-Nva-OH (4). ^1H NMR (CD_3OD, 400 MHz) δ 4.46 (dd, J = 5.8, 9.8 Hz, 1H), 4.29–4.25 (m, 1H), 4.22–4.17 (m, 2H), 2.58–2.50 (m, 4H), 1.88–1.52 (m, 16H), 1.42–1.10 (m, 15H), 0.92 (d, J = 6.8 Hz, 3H), 0.92 (t, J = 7.2 Hz, 3H), 0.89 (t, J = 7.4 Hz, 3H). HPLC purity (C18 column: 100%, C4 column: 97%). HRMS ($M+H^+$) calcd for $C_{32}H_{55}N_4O_8$: 623.4020; found: 623.4012. Amino acid analysis Chg 0.98, Ile 1.00, Cha 1.01, Nva 1.00.

6.1.6. Suc-Chg-Ile-Cha-Nva-NHSO$_2$*i*Pr (5). ^1H NMR (CD_3OD, 400 MHz) δ 4.42 (dd, J = 5.5, 9.8 Hz, 1H), 4.24 (dd, J = 5.3, 8.8 Hz, 1H), 4.14 (d, J = 8.0 Hz, 1H), 4.11 (d, J = 7.0 Hz, 1H), 3.72–3.63 (m, 1H), 2.61–2.48 (m, 4H), 1.84–1.91 (m, 1H), 1.80–1.58 (m, 17H), 1.37 (d, J = 6.8 Hz, 3H), 1.36 (d, J = 6.8 Hz, 3H), 1.32–1.10 (m, 13H), 0.94 (t, J = 7.4 Hz, 3H), 0.91 (d, J = 7.0 Hz, 3H), 0.90 (dd, J = 7.2, 7.4 Hz, 3H). HPLC purity (C18 column: 95%, C4 column: 99%). HRMS ($M+H^+$) calcd for $C_{35}H_{62}N_5O_9S$: 728.4268; found: 728.4260. Amino acid analysis Chg 1.01, Ile 1.00, Cha 0.99, Nva 1.00.

6.1.7. Suc-Chg-Ile-Cha-Nva-NHSO$_2$ Ph (6). ^1H NMR (CD_3OD, 400 MHz) δ 7.97–7.93 (m, 2H), 7.59–7.55 (m, 1H), 7.52–7.48 (m, 2H), 4.41–4.33 (m, 1H), 4.23–4.18 (m, 1H), 4.16–4.13 (m, 2H), 2.63–2.52 (m, 4H), 1.92–1.51 (m, 17H), 1.36–1.08 (m, 14H), 0.88 (t, J = 7.3 Hz, 3H), 0.86 (d, J = 6.8 Hz, 3H), 0.85 (t, J = 7.2 Hz, 3H). HPLC purity (C18 column: 96%, C4 column: 98%). HRMS ($M+H^+$) calcd for $C_{38}H_{60}N_5O_9S$: 762.4112; found: 762.4120. Amino acid analysis Chg 0.99, Ile 0.96, Cha 1.03, Nva 1.01.

6.1.8. *tert*-Butyl [(1*S*)-1-benzylcarbamoyl]butylcarbamate (7). To a solution of Boc-L-Nva (0.420 g, 1.93 mmol) in CH_2Cl_2 (50 mL) were added benzylamine (0.32 mL, 2.9 mmol), HBTU (0.880 g, 2.32 mmol), and DIEA (1.35 mL, 7.73 mmol). The suspension was stirred at room temperature for 2 h and thereafter washed with

5% aqueous citric acid (2 × 25 mL), 5% aqueous $NaHCO_3$ (2 × 25 mL), 5% aqueous citric acid (25 mL), and brine (30 mL). The organic layer was dried ($MgSO_4$), filtered, and evaporated. Purification by column chromatography (EtOAc/*i*-hexane 1:1) gave **7** as a white solid (0.531 g, 90%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.32–7.23 (m, 5H), 6.67 (m, 1H), 5.10 (br d, J = 7.4 Hz, 1H), 4.44 (dd, J = 5.8, 15.1 Hz, 1H), 4.39 (dd, J = 5.8, 15.1 Hz, 1H), 4.14–4.08 (m, 1H), 1.86–1.80 (m, 1H), 1.63–1.53 (m, 1H), 1.43–1.30 (m, 2H), 1.40 (s, 9H), 0.92 (t, J = 7.3 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.4, 155.9, 138.1, 128.8, 127.7, 127.6, 80.2, 54.7, 43.5, 34.6, 28.4, 19.1, 13.9. MS (M+H$^+$) 307.1. Anal. Calcd for $C_{17}H_{26}N_2O_3$: C, 66.64; H, 8.55; N, 9.14; found: C, 66.59; H, 8.59; N, 9.14.

6.1.9. (2*S*)-2-Amino-pentanoic acid benzylamide hydrochloride (8). A 5 M solution of HCl in EtOAc (5.4 mL, 27 mmol) was added to a solution of **7** (0.417 g, 1.36 mmol) in EtOAc (3 mL) and the resulting mixture was stirred at room temperature overnight. Coevaporation with MeOH gave **8** as a white solid (0.33 g, 100%). ^{1}H NMR (CD_3OD, 400 MHz) δ 7.36–7.24 (m, 5H), 4.45 (d, J = 14.7 Hz, 1H), 4.39 (d, J = 14.7 Hz, 1H), 3.88 (dd, J = 6.6, 6.7 Hz, 1H), 1.90–1.75 (m, 2H), 1.46–1.36 (m, 2H), 0.97 (dd, J = 7.2, 7.4 Hz, 3H). ^{13}C NMR (CD_3OD, 100 MHz) δ 170.1, 139.4, 129.6, 128.8, 128.5, 54.4, 44.3, 34.8, 19.2, 13.9. MS (M+H$^+$) 207.1. Anal. Calcd for $C_{12}H_{19}ClN_2O \cdot \frac{1}{3}H_2O$: C, 57.94; H, 7.97; N, 11.26; found: C, 57.69; H, 7.88; N, 11.06.

6.1.10. *tert*-Butyl [1-(2-Benzenesulfonyl-acetyl)]butylcarbamate (10). A 1.4 M solution of *n*-BuLi in hexane (2.0 mL, 2.8 mmol) was added dropwise over 30 min to a solution of $MeSO_2Ph$ (0.203 mg, 1.30 mmol) in dry THF (3 mL) at 0 °C under N_2 atmosphere. The mixture was stirred at 0 °C for 40 min and thereafter cooled to −78 °C. A solution of **9** (0.150 g, 0.649 mmol) in dry THF (3 mL) was added dropwise over 20 min and the resulting mixture was slowly allowed to reach −30 °C over 3 h and finally allowed to adopt room temperature over 1 h. The mixture was quenched with saturated aqueous NH_4Cl (6 mL) and extracted with EtOAc (3 × 7 mL). The organic layer was washed with brine (20 mL), dried ($MgSO_4$), filtered, and evaporated. Purification by column chromatography (EtOAc/*i*-hexane 1:3 followed by EtOAc/*i*-hexane 1:2) gave **10** as white crystals (0.196 g, 85%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.92–7.89 (m, 2H), 7.70–7.66 (m, 1H), 7.59–7.55 (m, 2H), 5.14 (br d, J = 8.4 Hz, 1H), 4.47 (d, J = 14.1 Hz, 1H), 4.26 (dt, J = 4.5, 8.4 Hz, 1H), 4.20 (d, J = 14.1 Hz, 1H), 1.87–1.79 (m, 1H), 1.58–1.47 (m, 1H), 1.43 (s, 9H), 1.39–1.29 (m, 2H), 0.91 (t, J = 7.3 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 198.4, 155.7, 139.0, 134.4, 129.4, 128.6, 80.6, 63.4, 60.4, 32.4, 28.4, 18.9, 13.8. MS (M+H$^+$) 356.1. Anal. Calcd for $C_{17}H_{25}NO_5S$: C, 57.44; H, 7.09; N, 3.94; found: C, 57.34; H, 7.03; N, 4.09.

6.1.11. 3-Amino-1-benzenesulfonyl-hexan-2-one hydrochloride (11). A 5 M solution of HCl in EtOAc (0.50 mL, 2.0 mmol) was added to a solution of **10** (0.035 g, 0.099 mmol) in EtOAc (1.5 mL). The resulting mixture was stirred at room temperature for 7 h before the addition of another portion of 5 M HCl in EtOAc (0.50 mL, 2.0 mmol). The mixture was left for 3 days before evaporation, which gave **11** as a white solid (0.029 g, 100%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.44 (m, 3H), 7.96–7.94 (m, 2H), 7.79–7.56 (m, 1H), 7.70–7.66 (m, 2H), 5.12 (s, 2H), 4.16–4.13 (m, 1H), 1.91–1.82 (m, 1H), 1.73–1.64 (m, 1H), 1.36–1.23 (m, 1H), 1.20–1.07 (m, 1H), 0.83 (t, J = 7.2 Hz, 3H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 195.9, 139.4, 134.2, 129.3, 128.1, 61.7, 58.6, 29.8, 17.4, 13.5. MS (M+H$^+$) 256.0. Anal. Calcd for $C_{12}H_{18}ClNO_3S$: C, 49.39; H, 6.22; N, 4.80; found: C, 49.48; H, 6.20; N, 4.83.

6.1.12. General procedure for the preparation of compounds 12a–e. *Method A*: A solution of Boc-L-Nva in dry THF was added to a solution of CDI in dry THF under N_2 atmosphere. The mixture was stirred at room temperature for 1 h before the addition of a solution of sulfonamide in dry THF followed by a solution of DBU in dry THF. The resulting mixture was then stirred at room temperature for 6 h.

Method B: A solution of Boc-L-Nva in dry THF was added to a solution of CDI in dry THF under N_2 atmosphere. The mixture was stirred at room temperature for 1 h before the addition of a solution of sulfonamide in dry THF followed by a solution of DBU in dry THF. The mixture was stirred at room temperature for 1 h and refluxed for 30 min. The resulting mixture was then stirred at room temperature overnight.

Methods A and B: The solvent was reduced to half by evaporation, diluted with CH_2Cl_2 (∼30 mL/mmol Boc-L-Nva), and washed with 5% aqueous citric acid (∼20 mL/mmol Boc-L-Nva), H_2O (∼20 mL/mmol Boc-L-Nva), and brine (∼20 mL/mmol Boc-L-Nva). The organic layer was dried ($MgSO_4$), filtered, and evaporated. Purification by column chromatography or preparative RP-HPLC-MS gave the desired product.

6.1.13. *tert*-Butyl [(1*S*)-1-methanesulfonylaminocarbonyl]butylcarbamate (12a). Compound **12a** was prepared according to method A using Boc-L-Nva (0.800 g, 3.68 mmol), CDI (1.20 g, 7.37 mmol), methanesulfonamide (0.527 g, 5.54 mmol), DBU (0.826 mL, 5.52 mmol), and dry THF (65 mL). Purification by column chromatography (EtOAc/*i*-hexane 2:1) gave **12a** as a white foam (0.866 g, 80%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.70 (br s, 1H), 5.15 (br d, J = 6.9 Hz, 1H), 4.20–4.14 (m, 1H), 3.29 (s, 3H), 1.85–1.76 (m, 1H), 1.66–1.57 (m, 1H), 1.45 (s, 9H), 1.43–1.33 (m, 2H), 0.94 (t, J = 7.3 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.0, 156.4, 81.6, 55.0, 41.5, 33.3, 28.4, 18.9, 13.7. MS (M+H$^+$) 295.1. Anal. Calcd for $C_{11}H_{22}N_2O_5S$: C, 44.88; H, 7.53; N, 9.52; found: C, 45.03; H, 7.49; N, 9.47.

6.1.14. *tert*-Butyl {(1*S*)-1-[propane-2-sulfonylaminocarbonyl]}butylcarbamate (12b). Compound **12b** was prepared according to method B using Boc-L-Nva (0.869 g, 4.00 mmol), CDI (1.30 g, 8.00 mmol), isopropylsulfonamide (0.739 g, 6.00 mmol), prepared from isopropylsulfo-

nyl chloride,[14] DBU (0.898 mL, 6.00 mmol) and dry THF (73 mL). Purification by column chromatography (EtOAc/*i*-hexane 2:1) gave **12b** as a white foam (1.06 g, 82%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.29 (br s, 1H), 5.11 (br d, J = 7.2 Hz, 1H), 4.17–4.12 (m, 1H), 3.79 (h, J = 6.9 Hz, 1H), 1.87–1.74 (m, 1H), 1.68–1.54 (m, 1H), 1.44 (s, 9H), 1.41 (d, J = 6.9 Hz, 3H), 1.40 (d, J = 6.9 Hz, 3H), 1.37–1.21 (m, 2H), 0.93 (t, J = 7.3 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.9, 156.3, 81.3, 55.2, 54.0, 33.3, 28.3, 18.9, 15.94, 15.90, 13.8. MS ($M+H^+$) 323.0. Anal. Calcd for $C_{13}H_{26}N_2O_5S$: C, 48.43; H, 8.13; N, 8.69; found: C, 48.51; H, 8.16; N, 8.74.

6.1.15. *tert*-Butyl[(1*S*)-1-benzenesulfonylaminocarbonyl]butylcarbamate (12c). Compound **12c** was prepared according to method A using Boc-L-Nva (0.602 g, 2.77 mmol), CDI (0.898 g, 5.54 mmol), benzenesulfonamide (0.654 g, 4.16 mmol), DBU (0.620 mL, 4.14 mmol), and dry THF (50 mL). Purification by preparative RP-HPLC-MS (MeCN/H_2O (0.05% HCOOH)) gave **12c** as a white solid (0.554 g, 58%).[11]

6.1.16. *tert*-Butyl[(1*S*)-1-phenylmethanesulfonylaminocarbonyl]butylcarbamate (12d). Compound **12d** was prepared according to method B using Boc-L-Nva (0.869 g, 4.00 mmol), CDI (1.30 g, 8.00 mmol), α-toluenesulfonamide (1.03 g, 6.00 mmol), DBU (0.898 mL, 6.00 mmol), and dry THF (73 mL). Purification by column chromatography (EtOAc/*i*-hexane 1:1) gave **12d** as a white foam (0.858 g, 58%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.18 (br s, 1H), 7.40–7.32 (m, 5H), 4.93 (br d, J = 7.1 Hz, 1H), 4.68–4.60 (m, 2H), 4.11–4.06 (m, 1H), 1.82–1.72 (m, 1H), 1.59–1.48 (m, 1H), 1.47–1.29 (m, 2H), 1.39 (s, 9H), 0.92 (t, J = 7.3 Hz, 3H). ^{13}C NMR ($CDCl_3$, 67.9 MHz) δ 172.2, 156.1, 130.9, 129.3, 129.0, 127.9, 81.3, 59.0, 54.8, 33.4, 28.3, 18.9, 13.7. MS ($M+H^+$) 371.1. Anal. Calcd for $C_{17}H_{26}N_2O_5S$: C, 55.12; H, 7.07; N, 7.56; found: C, 55.25; H, 7.06; N, 7.74.

6.1.17. *tert*-Butyl {(1*S*)-1-[2-phenyl-ethanesulfonylaminocarbonyl]}butylcarbamate (12e). Compound **12e** was prepared according to method A using Boc-L-Nva (0.047 g, 0.22 mmol), CDI (0.070 g, 0.43 mmol), phenethylsulfonamide[38] (0.060 g, 0.32 mmol) prepared from phenethylsulfonyl chloride,[14,39] DBU (0.049 mL, 0.33 mmol), and dry THF (4 mL). Purification by preparative RP-HPLC-MS (MeCN/H_2O (0.05% HCOOH)) gave **12e** as a white solid (0.030 g, 36%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.33 (br s, 1H), 7.32–7.20 (m, 5H), 4.97 (br d, J = 6.9 Hz, 1H), 4.08–4.03 (m, 1H), 3.72–3.68 (m, 2H), 3.14–3.10 (m, 2H), 1.84–1.73 (m, 1H), 1.62–1.51 (m, 1H), 1.45 (s, 9H), 1.44–1.32 (m, 2H), 0.94 (t, J = 7.3 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.8, 156.4, 137.2, 129.0, 128.6, 127.2, 81.5, 55.0, 54.4, 33.1, 29.5, 28.3, 18.9, 13.8. MS ($M+H^+$) 385.1. Anal. Calcd for $C_{18}H_{28}N_2O_5S$: C, 56.23; H, 7.34; N, 7.29; found: C, 56.28; H, 7.50; N, 7.14.

6.1.18. *tert*-Butyl {(1*S*)-1-[benzenesulfonyl-methyl-aminocarbonyl]}butylcarbamate (12f). To a suspension of **12c** (0.030 g, 0.084 mmol) and Cs_2CO_3 (0.041 g, 0.13 mmol) in dry DMF (3 mL) was added MeI (0.011 mL, 0.18 mmol). The resulting mixture was stirred at 65 °C overnight. The reaction was diluted with EtOAc (9 mL) and washed with 5% aqueous $NaHCO_3$ (4 × 5 mL), H_2O (5 mL), and brine (7 mL). The organic layer was dried ($MgSO_4$), filtered, and evaporated to give **12f** as a yellow oil (0.025 g, 81%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.04–8.02 (m, 2H), 7.65–7.61 (m, 1H), 7.56–7.52 (m, 2H), 5.17 (dt, J = 4, 9 Hz, 1H), 5.03 (d, J = 9 Hz, 1H), 3.26 (s, 3H), 1.82–1.74 (m, 1H), 1.55–1.34 (m, 3H), 1.39 (s, 9H), 0.92 (dd, J = 7.1, 7.2 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 174.7, 155.6, 138.5, 134.0, 129.3, 128.0, 80.0, 53.6, 36.0, 33.2, 28.4, 18.9, 13.8. MS ($M+H^+$) 371.1. Anal. Calcd for $C_{17}H_{26}N_2O_5S \cdot \frac{1}{2}H_2O$: C, 54.24; H, 7.14; N, 7.44; found: C, 54.6; H, 7.0; N, 7.0.

6.1.19. *N*-[(2*S*)-2-Amino-pentanoyl]methanesulfonamide hydrochloride (13a). A 5 M solution of HCl in EtOAc (4.5 mL, 23 mmol) was added to a solution of **12a** (0.392 g, 1.33 mmol) in EtOAc (5.5 mL). The mixture was stirred overnight and coevaporated with MeOH to give **13a** as a white solid (0.292 g, 95%). ^{1}H NMR (CD_3OD, 400 MHz) δ 3.95 (dd, J = 5.4, 7.2 Hz, 1H), 3.31 (s, 3H), 1.95–1.80 (m, 2H), 1.53–1.40 (m, 2H), 1.02 (t, J = 7.3 Hz, 3H). ^{13}C NMR (CD_3OD, 100 MHz) δ 170.4, 54.8, 41.6, 34.0, 19.0, 13.9. MS ($M + H^+$) 195.1. Anal. Calcd for $C_6H_{15}ClN_2O_3S$: C, 31.24; H, 6.55; N, 12.14; found: C, 31.34; H, 6.54; N, 12.01.

6.1.20. *N*-[(2*S*)-2-Amino-pentanoyl]propane-2-sulfonamide hydrochloride (13b). A 4 M solution of HCl in 1,4-dioxane (5.5 mL, 22 mmol) was added to **12b** (0.699 g, 2.17 mmol). The mixture was stirred for 3 h and coevaporated with MeOH to give **13b** as a beige solid (0.559 g, 100%). ^{1}H NMR (CD_3OD, 270 MHz) δ 3.97 (dd, J = 5.6, 7.0 Hz, 1H), 3.72 (h, J = 6.9 Hz, 1H), 1.98–1.77 (m, 2H), 1.54–1.36 (m, 2H), 1.40 (d, J = 6.9 Hz, 6H), 1.02 (dd, J = 7.2, 7.4 Hz, 3H). ^{13}C NMR (CD_3OD, 67.9 MHz) δ 170.5, 55.1, 54.9, 34.1, 19.0, 16.5, 15.8, 13.9. MS ($M+H^+$) 223.0. Anal. Calcd for $C_8H_{19}ClN_2O_3S \cdot \frac{1}{2}H_2O$: C, 35.88; H, 7.53; N, 10.46; found: C, 35.95; H, 7.39; N, 10.35.

6.1.21. *N*-[(2*S*)-2-Amino-pentanoyl]benzenesulfonamide hydrochloride (13c). A 5 M solution of HCl in EtOAc (5.0 mL, 25 mmol) was added to a solution of **12c** (0.467 g, 1.31 mmol) in EtOAc (5 mL). The mixture was stirred overnight and coevaporated with MeOH to give **13c** as a white solid (0.371 g, 97%).[11]

6.1.22. *N*-[(2*S*)-2-Amino-pentanoyl]-*C*-phenyl-methanesulfonamide hydrochloride (13d). A 4 M solution of HCl in 1,4-dioxane (8.2 mL, 33 mmol) was added to a solution of **12d** (0.622 g, 1.68 mmol) in 1,4-dioxane (5 mL) and MeOH (3 mL). The resulting mixture was stirred overnight before addition of another portion of 4 M HCl in 1,4-dioxane (1.0 mL, 4.0 mmol). After an additional 4 h, the mixture was coevaporated with MeOH to give **13d** as a white solid (0.504 g, 98%). ^{1}H NMR (CD_3OD, 270 MHz) δ 7.45–7.37 (m, 5H), 4.74 (s, 2H), 3.86 (dd, J = 6.3, 6.4 Hz, 1H), 1.78–1.70 (m, 2H), 1.47–1.31 (m, 2H), 0.96 (dd, J = 7.2, 7.3 Hz, 3H). ^{13}C NMR (DMSO-d_6/CD_3OD 9:1, 67.9 MHz) δ 169.7, 131.1, 129.0, 128.85, 128.83, 58.3, 52.9, 32.7, 17.7, 13.5. MS ($M+H^+$) 271.0. Anal. Calcd for $C_{12}H_{19}ClN_2O_3S$: C, 46.98; H, 6.24; N, 9.13; found: C, 47.01; H, 6.42; N, 9.16.

6.1.23. *N*-[(2*S*)-2-Amino-pentanoyl]-2-phenyl-ethanesulfonamide hydrochloride (13e). A 5 M solution of HCl in EtOAc (1.0 mL, 5.0 mmol) was added to a solution of **12e** (0.032 g, 0.083 mmol) in EtOAc (0.5 mL). The mixture was stirred overnight and coevaporated with MeOH to give **13e** as a white solid (0.025 g, 93%). ^{1}H NMR (CD_3OD, 400 MHz) δ 7.34–7.22 (m, 5H), 3.91 (dd, J = 5.3, 7.4 Hz, 1H), 3.80–3.68 (m, 2H), 3.18–3.06 (m, 2H), 1.89–1.74 (m, 2H), 1.50–1.41 (m, 2H), 1.01 (dd, J = 7.2, 7.4 Hz, 3H). ^{13}C NMR (CD_3OD, 100 MHz) δ 170.4, 138.9, 129.9, 129.5, 128.0, 55.3, 54.8, 34.0, 30.4, 19.0, 13.9. MS ($M+H^+$) 285.1. Anal. Calcd for $C_{13}H_{21}ClN_2O_3S$: C, 48.67; H, 6.60; N, 8.73; found: C, 48.48; H, 6.73; N, 8.63.

6.1.24. *N*-[(2*S*)-2-Amino-pentanoyl]-*N*-methyl-benzenesulfonamide hydrochloride (13f). A 5 M solution of HCl in EtOAc (1.5 mL, 7.5 mmol) was added to a solution of **12f** (0.021 g, 0.057 mmol) in EtOAc (0.5 mL). The mixture was stirred for 7 h before the addition of another portion of 5 M HCl in EtOAc (1.0 mL, 5.0 mmol) and finally left overnight. Evaporation gave **13f** as a white solid (0.016 g, 94%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.72 (br s, 3H), 8.03–8.01 (m, 2H), 7.62–7.52 (m, 3H), 5.22–5.16 (m, 1H), 3.09 (s, 3H), 2.10–1.92 (m, 2H), 1.60–1.48 (m, 2H), 0.92 (t, J = 7.0 Hz, 3H). ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 170.8, 136.9, 134.3, 129.6, 128.1, 54.5, 33.9, 33.7, 18.3, 13.6. MS ($M+H^+$) 271.1. Anal. Calcd for $C_{12}H_{19}ClN_2O_3S \cdot H_2O$: C, 44.37; H, 6.52; N, 8.62; found: C, 44.4; H, 6.4; N, 8.1.

6.1.25. General procedure for the preparation of compounds 14b and 14d. A solution of the deprotected acyl sulfonamide building block in EtOH or EtOH/MeOH was treated with propylene oxide and heated to 50 °C under N_2 atmosphere in a closed reaction tube. Evaporation after 5 h gave the desired product without further purification.

6.1.26. *N*-[(2*S*)-2-Amino-pentanoyl]propane-2-sulfonamide (14b). Compound **13b** (0.510 g, 1.97 mmol) was used according to the general procedure with EtOH (17 mL) and propylene oxide (1.54 mL, 22.0 mmol) to give **14b** (0.435 g, 99%) as a white solid. ^{1}H NMR (CD_3OD, 270 MHz) δ 3.60 (dd, J = 5.4, 7.1 Hz, 1H), 3.55 (h, J = 6.9 Hz, 1H), 1.96–1.71 (m, 2H), 1.55–1.39 (m, 2H), 1.30 (d, J = 6.9 Hz, 6H), 0.98 (dd, J = 7.2, 7.4 Hz, 3H). ^{13}C NMR (CD_3OD, 67.9 MHz) δ 175.6, 56.8, 52.6, 34.9, 19.4, 16.9, 16.8, 14.1. MS ($M+H^+$) 223.0. Anal. Calcd for $C_8H_{18}N_2O_3S$: C, 43.22; H, 8.16; N, 12.60; found: C, 43.30; H, 8.17; N, 12.46.

6.1.27. *N*-[(2*S*)-2-Amino-pentanoyl]-*C*-phenyl-methanesulfonamide (14d). Compound **13d** (0.450 g, 1.47 mmol) was used according to the general procedure with EtOH (10 mL), MeOH (4 mL), and propylene oxide (1.15 mL, 16.4 mmol) to give **14d** (0.383 g, 99%) as a white solid. ^{1}H NMR (CD_3OD, 270 MHz) δ 7.42–7.28 (m, 5H), 4.47 (s, 2H), 3.54 (dd, J = 5.3, 7.2 Hz, 1H), 1.87–1.62 (m, 2H), 1.48–1.29 (m, 2H), 0.94 (dd, J = 7.2, 7.3 Hz, 3H). ^{13}C NMR (CD_3OD, 67.9 MHz) δ 176.0, 132.7, 131.9, 129.2, 128.9, 58.6, 56.8, 34.9, 19.4, 14.1. MS ($M+H^+$) 271.0. Anal. Calcd for $C_{12}H_{18}N_2O_3S$: C, 53.31; H, 6.71; N, 10.36; found: C, 53.15; H, 6.80; N, 10.21.

6.1.28. Compound 16. To a solution of **15** (0.135 g, 0.240 mmol) in dry DMF (5 mL) were added HCl·L-NvaOMe (0.080 g, 0.479 mmol), HBTU (0.110 g, 0.290 mmol), and DIEA (167 μl, 0.958 mmol). The resulting solution was stirred at room temperature for 2.5 h, diluted with EtOAc (25 mL) and washed with 5% aqueous $NaHCO_3$ (10 mL), 35 mM aqueous $NaHSO_4$ (10 mL), H_2O (10 mL), and brine (10 mL). The organic layer was dried ($MgSO_4$), filtered, and evaporated. Purification by column chromatography (EtOAc/*i*-hexane 1:1 followed by EtOAc/*i*-hexane 2:1) gave **16** as a white solid (0.154 g, 95%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.04–8.02 (m, 2H), 7.97 (d, J = 9.2 Hz, 1H), 7.50–7.40 (m, 4H), 7.28 (d, J = 7.9 Hz, 1H), 7.04 (dd, J = 2.6, 9.2 Hz, 1H), 6.99 (s, 1H), 5.38–5.34 (m, 2H), 4.81 (dd, J = 7.0, 7.7 Hz, 1H), 4.53 (dt, J = 5.3, 7.9 Hz, 1H), 4.37 (dm, J = 11.4 Hz, 1H), 4.25 (dd, J = 7.2, 9.2 Hz, 1H), 4.00–3.96 (m, 1H), 3.91 (s, 3H), 3.68 (s, 3H), 2.79 (ddd, J = 5.3, 7.0, 13.9 Hz, 1H), 2.49–2.42 (m, 1H), 2.02–1.93 (m, 1H), 1.78–1.69 (m, 1H), 1.67–1.58 (m, 1H), 1.40–1.27 (m, 2H), 1.34 (s, 9H), 0.96 (d, J = 6.8 Hz, 3H), 0.92 (d, J = 6.8 Hz, 3H), 0.84 (dd, J = 7.2, 7.4 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.7, 172.6, 170.2, 161.7, 160.8, 158.7, 155.8, 150.3, 139.0, 129.7, 128.8, 127.8, 123.1, 118.7, 114.9, 106.5, 98.2, 79.7, 76.5, 58.6, 57.3, 55.6, 52.9, 52.29, 52.28, 34.3, 33.2, 31.4, 28.2, 19.2, 18.5, 17.8, 13.6. MS ($M+H^+$) 677.3. Anal. Calcd for $C_{37}H_{48}N_4O_8$: C, 65.66; H, 7.15; N, 8.28; found: C, 65.39; H, 7.13; N, 8.12.

6.1.29. Compound 17. A solution of LiOH (0.026 g, 1.1 mmol) in H_2O (1.5 mL) was added to a solution of **16** (0.075 g, 0.11 mmol) in THF (4 mL) and MeOH (0.5 mL). The resulting mixture was stirred at room temperature for 6.5 h and neutralized with 1.0 M aqueous HCl. The organic solvents were evaporated and the remaining aqueous phase was acidified to pH 3 using 1.0 M HCl (aq) and extracted with EtOAc (3 × 15 mL). The organic layer was washed with brine (20 mL), dried ($MgSO_4$), and filtered. Evaporation gave **17** as a white solid (0.067 g, 92%). ^{1}H NMR (CD_3OD, 400 MHz) δ 8.10 (d, J = 9.2 Hz, 1H), 8.04–8.02 (m, 2H), 7.57–7.49 (m, 3H), 7.36 (d, J = 2.5 Hz, 1H), 7.21 (s, 1H), 7.09 (dd, J = 2.5, 9.2 Hz, 1H), 5.49–5.47 (m, 1H), 4.75 (dd, J = 7.6, 9.3 Hz, 1H), 4.59 (dm, J = 11.5 Hz, 1H), 4.39 (dd, J = 4.9, 8.8 Hz, 1H), 4.12–4.02 (m, 2H), 3.92 (s, 3H), 2.72 (ddm, J = 7.6, 13.9 Hz, 1H), 2.42 (ddd, J = 4.4, 9.3, 13.9 Hz, 1H), 2.04–1.94 (m, 1H), 1.87–1.78 (m, 1H), 1.74–1.64 (m, 1H), 1.53–1.39 (m, 2H), 1.24 (s, 9H), 1.00 (d, J = 6.8 Hz, 3H), 0.96 (d, J = 6.8 Hz, 3H), 0.93 (dd, J = 7.2, 7.4 Hz, 3H). ^{13}C NMR (CD_3OD, 100 MHz) δ 176.0, 174.0, 173.4, 163.5, 162.8, 160.7, 157.9, 150.9, 140.1, 131.0, 130.0, 129.2, 124.7, 119.6, 116.4, 106.3, 100.3, 80.3, 78.5, 60.2, 59.4, 56.1, 54.5, 53.9, 36.0, 34.9, 31.8, 28.6, 20.0, 19.7, 19.0, 14.0. MS ($M+H^+$) 663.4. Anal. Calcd for $C_{36}H_{46}N_4O_8$: C, 65.24; H, 7.00; N, 8.45; found: C, 65.01; H, 7.08; N, 8.30.

6.1.30. Compound 18. To a solution of **15** (0.100 g, 0.177 mmol) and **13a** (0.061 g, 0.27 mmol) in dry DMF (3.8 mL) were added HBTU (0.081 g, 0.21 mmol) and DIEA (0.124 mL, 0.710 mmol). The mixture was

stirred for 3.5 h at room temperature, diluted with EtOAc (25 mL), and washed with aqueous NaOAc buffer (pH 4, 3 × 9 mL) and brine (13 mL). The organic layer was dried ($MgSO_4$), filtered, and evaporated. Purification by column chromatography ($CHCl_3$/MeOH 96:4 followed by $CHCl_3$/MeOH 95:5) gave **18** as a white solid (0.089 g, 68%). ^{1}H NMR (CD_3OD, 400 MHz) δ 8.10 (d, J = 9.2 Hz, 1H), 8.05–8.02 (m, 2H), 7.58–7.49 (m, 3H), 7.34 (d, J = 2.5 Hz, 1H), 7.21 (s, 1H), 7.09 (dd, J = 2.5, 9.2 Hz, 1H), 5.47–5.45 (m, 1H), 4.74 (dd, J = 7.6, 9.3 Hz, 1H), 4.58 (dm, J = 12.0 Hz, 1H), 4.29 (dd, J = 4.9, 8.9 Hz, 1H), 4.09–4.01 (m, 2H), 3.92 (s, 3H), 3.16 (s, 3H), 2.71 (ddm, J = 7.6, 13.9 Hz, 1H), 2.39 (ddd, J = 4.2, 9.3, 13.9 Hz, 1H), 1.98 (dh, J = 6.7, 8.7 Hz, 1H), 1.82–1.73 (m, 1H), 1.71–1.62 (m, 1H), 1.53–1.37 (m, 2H), 1.24 (s, 9H), 0.99 (d, J = 6.7 Hz, 3H), 0.95 (d, J = 6.7 Hz, 3H), 0.91 (dd, J = 7.2, 7.4 Hz, 3H). ^{13}C NMR (CD_3OD, 100 MHz) δ 175.4, 174.0, 173.6, 163.5, 162.8, 160.7, 157.8, 150.9, 140.1, 131.0, 129.9, 129.1, 124.7, 119.6, 116.3, 106.3, 100.3, 80.3, 78.5, 60.1, 59.4, 56.1, 55.6, 54.5, 41.2, 36.0, 34.9, 31.8, 28.6, 19.9, 19.7, 19.0, 14.1. MS ($M+H^+$) 740.3. Anal. Calcd for $C_{37}H_{49}N_5O_9S \cdot \frac{1}{2}H_2O$: C, 59.34; H, 6.73; N, 9.35; found: C, 59.28; H, 6.57; N, 9.16.

6.1.31. Compound 19. To a solution of **15** (0.080 g, 0.14 mmol) and **14b** (0.048 g, 0.21 mmol) in dry DMF (3 mL) were added HBTU (0.065 g, 0.17 mmol) and DIEA (0.099 mL, 0.57 mmol). The mixture was stirred for 3.5 h at room temperature, diluted with EtOAc (20 mL), and washed with aqueous NaOAc buffer (pH 4, 3 × 7 mL) and brine (10 mL). The organic layer was dried ($MgSO_4$), filtered, and evaporated. Purification by column chromatography (repeated columns, EtOAc/*i*-hexane 2:1 and $CHCl_3$/MeOH 96:4 followed by $CHCl_3$/MeOH 95:5) gave **19** as a white solid (0.020 g, 18%). ^{1}H NMR (CD_3OD, 400 MHz) δ 8.13 (d, J = 9.2 Hz, 1H), 8.07–8.04 (m, 2H), 7.58–7.50 (m, 3H), 7.39 (d, J = 2.5 Hz, 1H), 7.26 (s, 1H), 7.11 (dd, J = 2.5, 9.2 Hz, 1H), 5.53–5.52 (m, 1H), 4.73 (dd, J = 7.7, 9.3 Hz, 1H), 4.61 (dm, J = 11.7 Hz, 1H), 4.28 (dd, J = 5.0, 8.9 Hz, 1H), 4.08–4.04 (m, 2H), 3.95 (s, 3H), 3.64 (h, J = 6.9 Hz, 1H), 2.75 (ddm, J = 7.7, 13.9 Hz, 1H), 2.39 (ddd, J = 4.3, 9.3, 13.9 Hz, 1H), 1.99 (dh, J = 6.7, 8.7 Hz, 1H), 1.82–1.63 (m, 2H), 1.56–1.40 (m, 2H), 1.36 (d, J = 6.9 Hz, 3H), 1.35 (d, J = 6.9 Hz, 3H), 1.25 (s, 9H), 1.00 (d, J = 6.7 Hz, 3H), 0.96 (d, J = 6.7 Hz, 3H), 0.95 (t, J = 7.4 Hz, 3H). ^{13}C NMR (CD_3OD, 100 MHz) δ 174.5, 174.1, 173.7, 163.4, 162.5, 161.2, 157.9, 151.6, 140.8, 130.8, 129.9, 129.1, 124.6, 119.5, 116.5, 106.8, 100.3, 80.3, 78.3, 60.1, 59.5, 56.1, 55.6, 54.6, 54.5, 36.1, 34.7, 31.7, 28.6, 20.0, 19.7, 19.0, 16.6, 15.8, 14.0. MS ($M+H^+$) 768.3. Anal. Calcd for $C_{39}H_{53}N_5O_9S \cdot \frac{1}{2}H_2O$: C, 60.29; H, 7.01; N, 9.01; found: C, 60.48; H, 7.07; N, 8.73.

6.1.32. Compound 20. To a solution of **15** (0.075 g, 0.13 mmol) and **13 c** (0.059 g, 0.20 mmol) in dry DMF (3 mL) were added HBTU (0.061 g, 0.16 mmol) and DIEA (0.093 mL, 0.53 mmol). The mixture was stirred for 3 h at room temperature, diluted with EtOAc (20 mL), and washed with aqueous NaOAc buffer (pH 4, 4 × 7 mL) and brine (7 mL). The organic layer was dried ($MgSO_4$), filtered, and evaporated. Purification by column chromatography ($CHCl_3$/MeOH 96:4 followed by $CHCl_3$/MeOH 95:5) gave **20** as a white solid (0.085 g, 79%). ^{1}H NMR (CD_3OD, 400 MHz) δ 8.07 (d, J = 9.2 Hz, 1H), 8.04–8.02 (m, 2H), 7.96–7.94 (m, 2H), 7.58–7.51 (m, 4H), 7.48–7.44 (m, 2H), 7.34 (d, J = 2.5 Hz, 1H), 7.19 (s, 1H), 7.08 (dd, J = 2.5, 9.2 Hz, 1H), 5.41–5.40 (m, 1H), 4.69 (dd, J = 7.9, 9.2 Hz, 1H), 4.53 (dm, J = 11.6 Hz, 1H), 4.28 (dd, J = 4.9, 8.5 Hz, 1H), 4.04 (d, J = 8.6 Hz, 1H), 4.00 (dd, J = 3.5, 11.6 Hz, 1H), 3.91 (s, 3H), 2.60 (ddm, J = 7.9, 13.9 Hz, 1H), 2.28 (ddd, J = 4.3, 9.2, 13.9 Hz, 1H), 1.96 (dh, J = 6.7, 8.6 Hz, 1H), 1.70–1.61 (m, 1H), 1.58–1.49 (m, 1H), 1.34–1.20 (m, 2H), 1.23 (s, 9H), 0.96 (d, J = 6.7 Hz, 3H), 0.93 (d, J = 6.7 Hz, 3H), 0.80 (dd, J = 7.2, 7.4 Hz, 3H). ^{13}C NMR (CD_3OD, 100 MHz) δ 174.9, 173.9, 173.3, 163.6, 163.0, 160.5, 157.8, 150.4, 142.0, 139.7, 134.0, 131.2, 130.0, 129.7, 129.2, 128.7, 124.8, 119.7, 116.3, 106.0, 100.4, 80.3, 78.6, 60.1, 59.4, 56.2, 55.6, 54.4, 35.9, 35.0, 31.8, 28.6, 19.69, 19.68, 19.0, 14.0. MS ($M+H^+$) 802.4. Anal. Calcd for $C_{42}H_{51}N_5O_9S \cdot H_2O$: C, 61.52; H, 6.52; N, 8.54; found: C, 61.72; H, 6.28; N, 8.41.

6.1.33. Compound 21. To a solution of **15** (0.080 g, 0.14 mmol) and **14d** (0.058 g, 0.21 mmol) in dry DMF (3 mL) were added HBTU (0.065 g, 0.17 mmol) and DIEA (0.099 mL, 0.57 mmol). The mixture was stirred for 3 h at room temperature, diluted with EtOAc (20 mL), and washed with 5% aqueous $NaHCO_3$ (7 mL), 35 mM aqueous $NaHSO_4$ (7 mL), H_2O (7 mL), and brine (7 mL). The organic layer was dried ($MgSO_4$), filtered, and evaporated. Purification by column chromatography ($CHCl_3$/MeOH 95:5) gave **21** as a white solid (0.052 g, 45%). ^{1}H NMR (CD_3OD, 400 MHz) δ 8.13 (d, J = 9.2 Hz, 1H), 8.06–8.03 (m, 2H), 7.58–7.50 (m, 3H), 7.40–7.31 (m, 5H), 7.32 (d, J = 2.5 Hz, 1H), 7.24 (s, 1H), 7.10 (dd, J = 2.5, 9.2 Hz, 1H), 5.50–5.48 (m, 1H), 4.75 (dd, J = 7.6, 9.3 Hz, 1H), 4.63 (d, J = 14.0 Hz, 1H), 4.62–4.58 (m, 1H), 4.57 (d, J = 14.0 Hz, 1H), 4.28 (dd, J = 5.0, 8.7 Hz, 1H), 4.12–4.04 (m, 2H), 3.93 (s, 3H), 2.73 (ddm, J = 7.6, 14.0 Hz, 1H), 2.40 (ddd, J = 4.3, 9.3, 14.0 Hz, 1H), 1.99 (dh, J = 6.7, 8.8 Hz, 1H), 1.69–1.56 (m, 2H), 1.52–1.33 (m, 2H), 1.25 (s, 9H), 1.01 (d, J = 6.7 Hz, 3H), 0.97 (d, J = 6.7 Hz, 3H), 0.88 (t, J = 7.3 Hz, 3H). ^{13}C NMR (CD_3OD, 100 MHz) δ 175.0, 174.0, 173.5, 163.6, 162.9, 160.7, 157.9, 150.7, 140.0, 132.1, 131.1, 130.5, 130.0, 129.71, 129.66, 129.2, 124.8, 119.7, 116.4, 106.2, 100.4, 80.3, 78.6, 60.1, 59.5, 59.2, 56.2, 55.4, 54.5, 36.1, 35.0, 31.8, 28.6, 20.0, 19.7, 19.1, 14.0. MS ($M+H^+$) 816.3. Anal. Calcd for $C_{43}H_{53}N_5O_9S \cdot H_2O$: C, 61.93; H, 6.65; N, 8.40; found: C, 62.12; H, 6.36; N, 8.16.

6.1.34. Compound 22. To a solution of **15** (0.020 g, 0.036 mmol) and **13e** (0.017 g, 0.053 mmol) in dry DMF (0.6 mL) were added HBTU (0.016 g, 0.042 mmol) and DIEA (0.025 mL, 0.14 mmol). The mixture was stirred for 3 h at room temperature, diluted with EtOAc (10 mL) and washed with aqueous NaOAc buffer (pH 4, 4 × 4 mL) and brine (6 mL). The organic layer was dried ($MgSO_4$), filtered, and evaporated. Puri-

fication by column chromatography ($CHCl_3$/MeOH 97:3 followed by $CHCl_3$/MeOH 96:4) gave **22** as a white solid (0.024 g, 83%). ^{1}H NMR (CD_3OD, 400 MHz) δ 8.13 (d, J = 9.2 Hz, 1H), 8.05–8.02 (m, 2H), 7.58–7.50 (m, 3H), 7.38 (d, J = 2.5 Hz, 1H), 7.26–7.13 (m, 6H), 7.11 (dd, J = 2.5, 9.2 Hz, 1H), 5.49–5.46 (m, 1H), 4.74 (dd, J = 7.7, 9.1 Hz, 1H), 4.59 (dm, J = 11.8 Hz, 1H), 4.24 (dd, J = 5.1, 8.8 Hz, 1H), 4.08–4.02 (m, 2H), 3.94 (s, 3H), 3.59 (ddd, J = 5.3, 11.5, 14.1 Hz, 1H), 3.49 (ddd, J = 5.6, 11.2, 14.1 Hz, 1H), 3.12 (ddd, J = 5.3, 11.2, 13.7 Hz, 1H), 3.02 (ddd, J = 5.6, 11.5, 13.7 Hz, 1H), 2.73 (ddm, J = 7.7, 14.0 Hz, 1H), 2.40 (ddd, J = 4.4, 9.1, 14.0 Hz, 1H), 1.97 (dh, J = 6.7, 8.8 Hz, 1H), 1.80–1.62 (m, 2H), 1.56–1.40 (m, 2H), 1.25 (s, 9H), 0.98 (d, J = 6.7 Hz, 3H), 0.95 (d, J = 6.7 Hz, 3H), 0.94 (dd, J = 7.1, 7.4 Hz, 3H). ^{13}C NMR (CD_3OD, 100 MHz) δ 175.8, 174.1, 173.5, 163.5, 162.7, 161.1, 158.0, 151.3, 140.6, 139.5, 130.9, 129.9, 129.7, 129.5, 129.2, 127.8, 124.6, 119.6, 116.4, 106.6, 100.4, 80.3, 78.3, 60.1, 59.5, 56.1, 55.9, 54.9, 54.5, 36.0, 34.7, 31.7, 30.8, 28.6, 20.1, 19.7, 19.1, 14.1. MS ($M+H^+$) 830.3. Anal. Calcd for $C_{44}H_{55}N_5O_9S$: C, 63.67; H, 6.68; N, 8.44; found: C, 63.46; H, 6.50; N, 8.38.

6.1.35. Compound 23. To a solution of **15** (0.050 g, 0.089 mmol) and **8** (0.033 g, 0.14 mmol) in dry DMF (2 mL) were added HBTU (0.040 g, 0.11 mmol) and DIEA (0.062 mL, 0.36 mmol). The mixture was stirred for 3 h at room temperature, diluted with EtOAc (13 mL), and washed with aqueous NaOAc buffer (pH 4, 2 × 5 mL), 5% aqueous $NaHCO_3$ (5 mL), H_2O (5 mL), and brine (5 mL). The organic layer was dried ($MgSO_4$), filtered, and evaporated. Purification by preparative RP-HPLC-MS (MeCN/H_2O (0.05% HCOOH)) gave **23** as a white solid (0.053 g, 79%). ^{1}H NMR (CD_3OD, 400 MHz) δ 8.08 (d, J = 9.2 Hz, 1H), 8.05–8.02 (m, 2H), 7.56–7.47 (m, 3H), 7.36 (d, J = 2.5 Hz, 1H), 7.29–7.24 (m, 4H), 7.22–7.16 (m, 1H), 7.16 (s, 1H), 7.06 (dd, J = 2.5, 9.2 Hz, 1H), 5.45–5.43 (m, 1H), 4.71 (dd, J = 7.5, 9.6 Hz, 1H), 4.57 (dm, J = 11.8 Hz, 1H), 4.38 (d, J = 14.9 Hz, 1H), 4.33 (d, J = 14.9 Hz, 1H), 4.33 (dd, J = 5.5, 8.7 Hz, 1H), 4.06–4.01 (m, 2H), 3.92 (s, 3H), 2.68 (ddm, J = 7.5, 14.1 Hz, 1H), 2.31 (ddd, J = 4.3, 9.6, 14.1 Hz, 1H), 1.96 (dh, J = 6.7, 8.9 Hz, 1H), 1.81–1.64 (m, 2H), 1.50–1.34 (m, 2H), 1.27 (s, 9H), 0.97 (d, J = 6.7 Hz, 3H), 0.95 (d, J = 6.7 Hz, 3H), 0.91 (dd, J = 7.3, 7.4 Hz, 3H). ^{13}C NMR (CD_3OD, 100 MHz) δ 174.3, 174.1, 173.6, 163.1, 161.9, 161.3, 157.9, 152.2, 141.4, 139.8, 130.5, 129.8, 129.5, 129.0, 128.5, 128.2, 124.4, 119.3, 116.4, 107.4, 100.1, 80.4, 78.0, 60.4, 59.7, 56.0, 55.0, 54.5, 44.0, 35.9, 35.1, 31.7, 28.6, 20.1, 19.7, 19.1, 14.0. MS ($M+H^+$) 752.3. Anal. Calcd for $C_{43}H_{53}N_5O_7$: C, 68.69; H, 7.10; N, 9.31; found: C, 68.46; H, 7.20; N, 9.20.

6.1.36. Compound 24. To a solution of **15** (0.025 g, 0.044 mmol) and **11** (0.019 g, 0.065 mmol) in dry DMF (1 mL) were added HATU (0.020 g, 0.053 mmol) and DIEA (0.031 mL, 0.18 mmol). The mixture was stirred for 1 h at room temperature, diluted with EtOAc (10 mL), and washed with aqueous NaOAc buffer (pH 4, 4 × 4 mL) and brine (7 mL). The organic layer was dried ($MgSO_4$), filtered, and evaporated. Purification by column chromatography (EtOAc/*i*-hexane 1:1) gave **24** (80:20 mixture of diastereomers) as a white solid (0.030 g, 86%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ (major diastereomer reported) 8.06–8.03 (m, 2H), 8.01 (d, J = 9.2 Hz, 1H), 7.94–7.86 (m, 2H), 7.65–7.60 (m, 1H), 7.56–7.43 (m, 6H), 7.40 (br d, J = 7.3 Hz, 1H), 7.08 (dd, J = 2.5, 9.2 Hz, 1H), 7.00 (s, 1H), 5.38–5.35 (m, 1H), 5.33 (d, J = 9.4 Hz, 1H), 4.79 (dd, J = 7.5, 7.7 Hz, 1H), 4.50–4.42 (m, 2H), 4.45 (d, J = 14.2 Hz, 1H), 4.27–4.23 (m, 1H), 4.25 (d, J = 14.2 Hz, 1H), 4.00–3.97 (m, 1H), 3.95 (s, 3H), 2.75–2.68 (m, 1H), 2.61–2.55 (m, 1H), 2.02–1.92 (m, 1H), 1.88–1.79 (m, 1H), 1.64–1.48 (m, 1H), 1.37 (s, 9H), 1.35–1.25 (m, 2H), 0.94 (d, J = 6.7 Hz, 3H), 0.91 (d, J = 6.7 Hz, 3H), 0.86 (dd, J = 7.2, 7.4 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (major diastereomer reported) 196.8, 172.9, 171.0, 161.6, 160.3, 159.3, 155.9, 151.3, 140.1, 139.1, 134.3, 129.5, 129.4, 128.9, 128.5, 127.7, 123.1, 118.6, 115.1, 107.3, 98.1, 80.0, 76.2, 63.8, 59.6, 58.9, 57.5, 55.7, 53.1, 33.4, 31.5, 31.4, 28.4, 19.4, 18.8, 17.9, 13.8. MS ($M+H^+$) 801.2. Anal. Calcd for $C_{43}H_{52}N_4O_9S$: C, 64.48; H, 6.54; N, 6.99; found: C, 64.73; H, 6.71; N, 7.07.

6.1.37. Compound 25. To a solution of **15** (0.016 g, 0.028 mmol) and **13f** (0.013 g, 0.042 mmol) in dry DMF (1 mL) were added HBTU (0.013 g, 0.034 mmol) and DIEA (0.020 mL, 0.12 mmol). The mixture was stirred for 3 h at room temperature, diluted with CH_2Cl_2 (15 mL), and washed with 5% aqueous $NaHCO_3$ (3 × 7 mL), H_2O (7 mL) and brine (8 mL). The organic layer was dried ($MgSO_4$), filtered, and evaporated. Purification by column chromatography (EtOAc/*i*-hexane 1:2 followed by EtOAc/*i*-hexane 2:1) gave **25** as a white solid (0.014 g, 61%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.06–8.01 (m, 4H), 8.00 (d, J = 9.2 Hz, 1H), 7.62–7.58 (m, 1H), 7.54–7.44 (m, 5H), 7.43 (d, J = 2.7 Hz, 1H), 7.12 (br d, J = 8.5 Hz, 1H), 7.08 (dd, J = 2.7, 9.2 Hz, 1H), 7.02 (s, 1H), 5.45 (dt, J = 3.8, 8.5 Hz, 1H), 5.38–5.35 (m, 1H), 5.24 (d, J = 9.3 Hz, 1H), 4.76 (dd, J = 6.9, 7.9 Hz, 1H), 4.38 (dm, J = 11.4 Hz, 1H), 4.29 (dd, J = 6.8, 9.3 Hz, 1H), 4.00 (dd, J = 4.7, 11.4 Hz, 1H), 3.95 (s, 3H), 3.22 (s, 3H), 2.76 (ddd, J = 5.1, 6.9, 13.9 Hz, 1H), 2.44 (ddd, J = 3.8, 7.9, 13.9 Hz, 1H), 2.01 (dh, J = 6.7, 6.8 Hz, 1H), 1.87–1.78 (m, 1H), 1.63–1.53 (m, 1H), 1.42–1.25 (m, 2H), 1.38 (s, 9H), 1.00 (d, J = 6.7 Hz, 3H), 0.96 (d, J = 6.7 Hz, 3H), 0.89 (t, J = 7.3 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.5, 172.9, 170.2, 161.5, 160.3, 159.4, 155.9, 151.6, 140.5, 138.3, 134.1, 129.44, 129.40, 128.9, 128.0, 127.7, 123.1, 118.6, 115.1, 107.6, 98.2, 79.9, 76.1, 58.7, 57.4, 55.7, 53.0, 52.9, 35.5, 33.2, 33.1, 31.5, 28.4, 19.5, 18.8, 17.9, 13.7. MS ($M+H^+$) 816.2. Anal. Calcd for $C_{43}H_{53}N_5O_9S$: C, 63.29; H, 6.55; N, 8.58; found: C, 63.11; H, 6.67; N, 8.42.

6.1.38. Compound 26. To a solution of **15** (0.080 g, 0.14 mmol) and the hydrochloride salt of 1-amino-cyclopropanecarboxylic acid methyl ester (0.032 g, 0.21 mmol) in dry DMF (3 mL) were added HBTU (0.064 g, 0.17 mmol) and DIEA (0.099 mL, 0.57 mmol). The mixture was stirred for 2 h at room temperature,

diluted with EtOAc (18 mL), and washed with aqueous NaOAc buffer (pH 4, 2 × 7 mL), 5% aqueous $NaHCO_3$ (7 mL), and brine (10 mL). The organic layer was dried ($MgSO_4$), filtered, and evaporated. Purification by column chromatography (EtOAc/*i*-hexane 2:1) gave **26** as a white solid (0.079 g, 84%). ^{1}H NMR (CD_3OD, 400 MHz) δ 8.06 (d, J = 9.2 Hz, 1H), 8.05–8.02 (m, 2H), 7.56–7.47 (m, 3H), 7.35 (d, J = 2.5 Hz, 1H), 7.19 (s, 1H), 7.05 (dd, J = 2.5, 9.2 Hz, 1H), 5.48–5.47 (m, 1H), 4.60 (dd, J = 7.6, 9.5 Hz, 1H), 4.58 (dm, J = 11.5 Hz, 1H), 4.06–4.02 (m, 2H), 3.92 (s, 3H), 3.67 (s, 3H), 2.72 (ddm, J = 7.6, 14.0 Hz, 1H), 2.44 (ddd, J = 4.2, 9.5, 14.0 Hz, 1H), 1.98 (dh, J = 6.7, 8.9 Hz, 1H), 1.57–1.53 (m, 1H), 1.44–1.39 (m, 1H), 1.24 (s, 9H), 1.23–1.13 (m, 2H), 1.00 (d, J = 6.7 Hz, 3H), 0.96 (d, J = 6.7 Hz, 3H). ^{13}C NMR (CD_3OD, 100 MHz) δ 174.9, 174.2, 174.0, 163.1, 161.9, 161.3, 157.9, 152.2, 141.4, 130.5, 129.8, 129.0, 124.3, 119.3, 116.4, 107.3, 100.0, 80.3, 78.0, 60.3, 59.6, 56.0, 54.4, 52.9, 35.9, 34.3, 31.7, 28.6, 19.7, 19.1, 18.1, 17.3. MS (M+H$^+$) 661.2. Anal. Calcd for $C_{36}H_{44}N_4O_8$: C, 65.44; H, 6.71; N, 8.48; found: C, 65.20; H, 6.89; N, 8.30.

6.1.39. Compound 27. To a solution of **15** (0.080 g, 0.14 mmol) and the hydrochloride salt of (1*R*, 2*S*)-1-amino-2-vinyl-cyclopropanecarboxylic acid ethyl ester (0.041 g, 0.21 mmol) in dry DMF (3 mL) were added HBTU (0.064 g, 0.17 mmol) and DIEA (0.099 mL, 0.57 mmol). The mixture was stirred for 2 h at room temperature, diluted with EtOAc (18 mL), and washed with aqueous NaOAc buffer (pH 4, 2 × 7 mL), 5% aqueous $NaHCO_3$ (7 mL), and brine (10 mL). The organic layer was dried ($MgSO_4$), filtered, and evaporated. Purification by column chromatography (EtOAc/*i*-hexane 2:3) gave **27** as a white solid (0.076 g, 76%). ^{1}H NMR (CD_3OD, 400 MHz) δ (5:1 mixture of rotamers, major rotamer reported) 8.06 (d, J = 9.2 Hz, 1H), 8.05–8.01 (m, 2H), 7.56–7.48 (m, 3H), 7.36 (d, J = 2.5 Hz, 1H), 7.20 (s, 1H), 7.08 (dd, J = 2.5, 9.2 Hz, 1H), 5.76 (ddd, J = 8.7, 10.3, 17.2 Hz, 1H), 5.50–5.49 (m, 1H), 5.27 (dd, J = 1.9, 17.2 Hz, 1H), 5.09 (dd, J = 1.9, 10.3 Hz, 1H), 4.63 (dd, J = 7.7, 9.4 Hz, 1H), 4.55 (dm, J = 11.9 Hz, 1H), 4.14 (dq, J = 7.2, 10.6 Hz, 1H), 4.10 (dq, J = 7.2, 10.6 Hz, 1H), 4.06–4.00 (m, 2H), 3.92 (s, 3H), 2.70 (ddm, J = 7.7, 14.1 Hz, 1H), 2.41 (ddd, J = 4.3, 9.4, 14.1 Hz, 1H), 2.26–2.20 (m, 1H), 1.98 (dh, J = 6.7, 8.8 Hz, 1H), 1.72 (dd, J = 5.2, 8.2 Hz, 1H), 1.44–1.40 (m, 1H), 1.24 (s, 9H), 1.23 (t, J = 7.2 Hz, 3H), 0.98 (d, J = 6.7 Hz, 3H), 0.96 (d, J = 6.7 Hz, 3H). ^{13}C NMR (CD_3OD, 100 MHz) δ (5:1 mixture of rotamers, major rotamer reported) 174.5, 173.9, 171.5, 163.3, 162.3, 160.9, 157.9, 151.5, 140.7, 135.1, 130.7, 129.8, 129.0, 124.4, 119.4, 118.1, 116.3, 106.9, 100.1, 80.4, 78.2, 62.4, 60.5, 59.9, 56.0, 54.3, 40.9, 35.9, 34.8, 31.5, 28.6, 23.2, 19.7, 19.1, 14.6. MS (M+H$^+$) 701.3. Anal. Calcd for $C_{39}H_{48}N_4O_8$: C, 66.84; H, 6.90; N, 7.99; found: C, 66.92; H, 6.55; N, 7.62.

6.1.40. Compound 28. A solution of LiOH (0.038 g, 1.6 mmol) in H_2O (1 mL) was added to a solution of **26** (0.069 g, 0.10 mmol) in THF (3.5 mL) and MeOH (1.5 mL). The resulting suspension was stirred at room temperature overnight and thereafter neutralized with 1.0 M aqueous HCl. The organic solvents were evaporated and the remaining aqueous phase was diluted with H_2O (10 mL), acidified to pH 3 using 1.0 M HCl (aq), and extracted with EtOAc (3 × 35 mL). The organic layer was washed with brine (40 mL), dried ($MgSO_4$), and filtered. Evaporation gave **28** as a white solid (0.065 g, 96%). ^{1}H NMR (CD_3OD, 400 MHz) δ 8.07 (d, J = 9.2 Hz, 1H), 8.04–8.02 (m, 2H), 7.57–7.48 (m, 3H), 7.35 (d, J = 2.5 Hz, 1H), 7.20 (s, 1H), 7.06 (dd, J = 2.5, 9.2 Hz, 1H), 5.49–5.48 (m, 1H), 4.61 (dd, J = 7.6, 9.5 Hz, 1H), 4.58 (dm, J = 11.9 Hz, 1H), 4.05–4.01 (m, 2H), 3.92 (s, 3H), 2.74 (ddm, J = 7.6, 14.1 Hz, 1H), 2.49 (ddd, J = 4.4, 9.5, 14.1 Hz, 1H), 2.02–1.93 (m, 1H), 1.59–1.54 (m, 1H), 1.43–1.39 (m, 1H), 1.23 (s, 9H), 1.22–1.11 (m, 2H), 0.99 (d, J = 6.7 Hz, 3H), 0.96 (d, J = 6.7 Hz, 3H). ^{13}C NMR (CD_3OD, 100 MHz) δ 175.9, 174.7, 174.0, 163.3, 162.3, 161.1, 157.9, 151.6, 140.8, 130.7, 129.8, 129.1, 124.5, 119.4, 116.4, 106.8, 100.2, 80.3, 78.3, 60.4, 59.7, 56.0, 54.4, 35.9, 34.2, 31.6, 28.6, 19.7, 19.1, 18.0, 17.2. MS (M+H$^+$) 647.2. Anal. Calcd for $C_{35}H_{42}N_4O_8 \cdot \frac{1}{2}H_2O$: C, 64.11; H, 6.61; N, 8.54; found: C, 63.93; H, 6.95; N, 8.26.

6.1.41. Compound 29. A solution of LiOH (0.042 g, 1.8 mmol) in H_2O (1.5 mL) was added to a solution of **27** (0.085 g, 0.12 mmol) in THF (4.7 mL) and MeOH (0.7 mL). The resulting suspension was stirred at room temperature overnight and thereafter neutralized with 1.0 M aqueous HCl. The organic solvents were evaporated and the remaining aqueous phase was acidified to pH 3 using 1.0 M HCl (aq) and extracted with EtOAc (3 × 18 mL). The organic layer was washed with brine (25 mL), dried ($MgSO_4$), and filtered. Evaporation gave **29** as a white solid (0.069 g, 85%). ^{1}H NMR (CD_3OD, 400 MHz) δ (7:1 mixture of rotamers, major rotamer reported) 8.09 (d, J = 9.2 Hz, 1H), 8.05–8.03 (m, 2H), 7.57–7.48 (m, 3H), 7.38 (d, J = 2.5 Hz, 1H), 7.23 (s, 1H), 7.09 (dd, J = 2.5, 9.2 Hz, 1H), 5.86 (ddd, J = 9.3, 10.3, 17.1 Hz, 1H), 5.53–5.51 (m, 1H), 5.25 (dd, J = 2.0, 17.1 Hz, 1H), 5.07 (dd, J = 2.0, 10.3 Hz, 1H), 4.62 (dd, J = 7.6, 9.6 Hz, 1H), 4.56 (dm, J = 11.9 Hz, 1H), 4.08–4.01 (m, 2H), 3.94 (s, 3H), 2.72 (ddm, J = 7.6, 14.1 Hz, 1H), 2.47 (ddd, J = 4.5, 9.6, 14.1 Hz, 1H), 2.23–2.16 (m, 1H), 2.03–1.93 (m, 1H), 1.70 (dd, J = 5.0, 8.1 Hz, 1H), 1.44–1.41 (m, 1H), 1.24 (s, 9H), 0.98 (d, J = 6.7 Hz, 3H), 0.96 (d, J = 6.7 Hz, 3H). ^{13}C NMR (CD_3OD, 100 MHz) δ (7:1 mixture of rotamers, major rotamer reported) 174.4, 173.7, 173.6 (detected with HMBC), 163.3, 162.2, 161.2, 158.0, 151.9, 141.1, 136.0, 130.7, 129.8, 129.1, 124.4, 119.4, 117.4, 116.4, 107.1, 100.2, 80.5, 78.2, 60.7, 60.0, 56.0, 54.3, 41.1, 35.8, 34.8, 31.6, 28.6, 23.2, 19.7, 19.1. MS (M+H$^+$) 673.2. Anal. Calcd for $C_{37}H_{44}N_4O_8$: C, 66.05; H, 6.59; N, 8.33; found: C, 65.66; H, 6.81; N, 8.06.

6.1.42. Compound 30. A solution of **28** (0.030 g, 0.046 mmol), HATU (0.021 g, 0.055 mmol), and DIEA (0.032 mL, 0.18 mmol) in dry DMF (1 mL) was stirred for 1 h and 15 min before the addition of a solution of benzenesulfonamide (0.029 g, 0.18 mmol), DMAP (0.023 g, 0.19 mmol), and DBU (0.028 mL, 0.19 mmol) in dry DMF (1 mL). The mixture was stirred at room temperature overnight, diluted with EtOAc (30 mL), and washed with aqueous NaOAc buffer (pH 4, 2 × 10 mL), 5% aque-

ous $NaHCO_3$ (10 mL), and brine (15 mL). The organic layer was dried ($MgSO_4$), filtered, and evaporated. Purification by preparative RP-HPLC-MS (MeCN/H_2O (0.05% HCOOH)) gave **30** as a white solid (0.027 g, 75%). ^{1}H NMR (CD_3OD, 400 MHz) δ 8.08 (d, J = 9.2 Hz, 1H), 8.05–8.03 (m, 2H), 7.99–7.97 (m, 2H), 7.65–7.61 (m, 1H), 7.56–7.48 (m, 5H), 7.37 (d, J = 2.5 Hz, 1H), 7.23 (s, 1H), 7.10 (dd, J = 2.5, 9.2 Hz, 1H), 5.55–5.54 (m, 1H), 4.65 (dm, J = 11.8 Hz, 1H), 4.56 (dd, J = 6.6, 10.8 Hz, 1H), 4.12–4.04 (m, 2H), 3.94 (s, 3H), 2.62 (ddm, J = 6.6, 14.0 Hz, 1H), 2.36 (ddd, J = 3.9, 10.8, 14.0 Hz, 1H), 2.19 (dh, J = 6.7, 9.3 Hz, 1H), 1.49–1.45 (m, 1H), 1.35–1.31 (m, 1H), 1.22 (s, 9H), 1.10–1.02 (m, 2H), 1.00 (d, J = 6.7 Hz, 3H), 0.98 (d, J = 6.7 Hz, 3H). ^{13}C NMR (CD_3OD, 100 MHz) δ 175.1, 174.4, 173.4, 163.4, 162.3, 161.0, 157.9, 151.4, 141.0, 140.6, 134.6, 130.8, 129.9 (two overlapping signals), 129.11, 129.05, 124.4, 119.5, 116.4, 106.8, 100.3, 80.3, 78.4, 61.1, 59.6, 59.5, 56.1, 54.5, 35.4, 31.5, 28.5, 19.9, 19.5, 19.1, 18.8. MS (M+H$^+$) 786.3. Anal. Calcd for $C_{41}H_{47}N_5O_9S$: C, 62.66; H, 6.03; N, 8.91; found: C, 62.47; H, 6.10; N, 8.83.

6.1.43. Compound 31. A solution of **29** (0.040 g, 0.060 mmol), HATU (0.027 g, 0.071 mmol), and DIEA (0.042 mL, 0.24 mmol) in dry DMF (5 mL) was stirred for 1 h before the addition of a solution of benzenesulfonamide (0.038 g, 0.24 mmol), DMAP (0.030 g, 0.24 mmol), and DBU (0.036 mL, 0.24 mmol) in dry DMF (1.5 mL). The mixture was stirred overnight, diluted with EtOAc (60 mL), and washed with aqueous NaOAc buffer (pH 4, 2 × 15 mL), 5% aqueous $NaHCO_3$ (15 mL) and brine (20 mL). The organic layer was dried ($MgSO_4$), filtered, and evaporated. Purification by preparative RP-HPLC-MS (MeCN/H_2O (0.05% HCOOH)) gave **31** as a white solid (0.029 g, 60%). ^{1}H NMR (CD_3OD, 400 MHz) δ (3:1 mixture of rotamers, major rotamer reported) 8.12 (d, J = 9.2 Hz, 1H), 8.07–8.03 (m, 2H), 7.99–7.96 (m, 2H), 7.68–7.62 (m, 1H), 7.58–7.50 (m, 5H), 7.40 (d, J = 2.5 Hz, 1H), 7.27 (s, 1H), 7.12 (dd, J = 2.5, 9.2 Hz, 1H), 5.59–5.56 (m, 1H), 5.52 (ddd, J = 8.9, 10.3, 17.1 Hz, 1H), 5.20 (dd, J = 1.8, 17.1 Hz, 1H), 4.96 (dd, J = 1.8, 10.3 Hz, 1H), 4.68 (dm, J = 11.8 Hz, 1H), 4.56 (dd, J = 6.9, 10.4 Hz, 1H), 4.11 (dm, J = 11.8 Hz, 1H), 4.07–4.02 (m, 1H), 3.96 (s, 3H), 2.65 (ddm, J = 6.9, 14.4 Hz, 1H), 2.37 (ddd, J = 3.9, 10.4, 14.4 Hz, 1H), 2.21–2.10 (m, 2H), 1.72 (dd, J = 5.2, 8.2 Hz, 1H), 1.33 (dd, J = 5.2, 9.4 Hz, 1H), 1.23 (s, 9H), 0.99 (d, J = 6.7 Hz, 3H), 0.98 (d, J = 6.7 Hz, 3H). ^{13}C NMR (CD_3OD, 100 MHz) δ (3:1 mixture of rotamers, major rotamer reported) 174.9, 174.5, 170.4, 163.5, 162.4, 161.1, 158.0, 151.4, 140.9, 140.6, 134.6, 134.0, 130.9, 129.9, 129.8, 129.14, 129.14, 124.5, 119.6, 118.4, 116.4, 106.7, 100.4, 80.3, 78.4, 61.0, 59.6, 56.1, 54.6, 42.5, 35.8, 35.5, 31.5, 28.5, 24.0, 19.9, 19.2. MS (M+H$^+$) 812.2. Anal. Calcd for $C_{43}H_{49}N_5O_9S \cdot H_2O$: C, 62.23; H, 6.19; N, 8.44; found: C, 62.33; H, 6.06; N, 8.42.

6.1.44. Compound 32. A solution of **29** (0.031 g, 0.046 mmol), HATU (0.021 g, 0.055 mmol), and DIEA (0.032 mL, 0.18 mmol) in dry DMF (3 mL) was stirred for 1.5 h before the addition of a solution of α-toluenesulfonamide (0.032 g, 0.18 mmol), DMAP (0.022 g, 0.18 mmol), and DBU (0.028 mL, 0.18 mmol) in dry DMF (1 mL). The mixture was stirred overnight, diluted with EtOAc (50 mL), and washed with aqueous NaOAc buffer (pH 4, 2 × 15 mL), 5% aqueous $NaHCO_3$ (15 mL), and brine (20 mL). The organic layer was dried ($MgSO_4$), filtered, and evaporated. Purification by preparative RP-HPLC-MS (MeCN/H_2O (0.05% HCOOH)) gave **32** as a white solid (0.022 g, 63%). ^{1}H NMR (CD_3OD/$CDCl_3$ 2:1, 400 MHz) δ 8.04 (d, J = 9.2 Hz, 1H), 8.00–7.98 (m, 2H), 7.55–7.47 (m, 3H), 7.38 (d, J = 2.5 Hz, 1H), 7.37–7.30 (m, 5H), 7.14 (s, 1H), 7.07 (dd, J = 2.5, 9.2 Hz, 1H), 6.50 (d, J = 9.0 Hz, 1H), 5.87 (ddd, J = 8.9, 10.3, 17.1 Hz, 1H), 5.53–5.51 (m, 1H), 5.34 (dd, J = 1.7, 17.1 Hz, 1H), 5.20 (dd, J = 1.7, 10.3 Hz, 1H), 4.78 (d, J = 13.9 Hz, 1H), 4.54 (dm, J = 11.9 Hz, 1H), 4.57 (dd, J = 7.0, 10.6 Hz, 1H), 4.46 (d, J = 13.9 Hz, 1H), 4.09 (dd, J = 3.6, 11.9 Hz, 1H), 4.04 (dd, J = 8.8, 9.0 Hz, 1H), 3.95 (s, 3H), 2.61 (ddm, J = 7.0, 14.1 Hz, 1H), 2.36 (ddd, J = 4.2, 10.6, 14.1 Hz, 1H), 2.26–2.19 (m, 1H), 2.08 (dh, J = 6.7, 8.8 Hz, 1H), 1.97 (dd, J = 5.4, 8.3 Hz, 1H), 1.42 (dd, J = 5.4, 9.4 Hz, 1H), 1.26 (s, 9H), 0.93 (d, J = 6.7 Hz, 6H). ^{13}C NMR (CD_3OD/$CDCl_3$ 2:1, 100 MHz) δ 174.5, 173.9, 171.1, 162.8, 161.5, 160.9, 157.4, 151.5, 140.6, 133.9, 131.8, 130.4, 129.6, 129.5, 129.4, 128.8, 128.7, 123.9, 119.2, 118.8, 115.9, 106.8, 99.8, 80.2, 77.7, 60.5, 59.3, 58.8, 55.9, 54.1, 42.1, 35.7, 35.0, 31.2, 28.5, 24.4, 19.6, 18.7. MS (M+H$^+$) 826.2. Anal. Calcd for $C_{44}H_{51}N_5O_9S$: C, 63.98; H, 6.22; N, 8.48; found: C, 63.71; H, 6.45; N, 8.35.

6.2. Enzyme Inhibition

The protease activity of the full-length HCV NS3 protein (protease-helicase/NTPase) was measured using a FRET assay as previously described.[29,30] 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 (KKGSVVIVGRIVLSGK) 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). Inhibitor **25**, suspected to bind irreversibly to the enzyme, was tested by incubation with the enzyme for several different periods of time, from 10 min to 1 h before addition of substrate. The nonlinear regression analysis was made using Grafit 5.0.8 (Erithacus software limited).

6.3. HCV replicon assay

Huh7 cells transfected with pFKI_{389}luc/NS3-3'/ET, constitutively expressing HCV replicons, were used for the evaluation of the anti-HCV effect of our protease inhibitors in cell culture. The cells were maintained in Dulbecco's modified Eagle's media (DMEM) containing 10% FCS and 250 μg/ml G418 (geneticin). The day prior to assay, cells were trypsinized and 15,000 cells were plated in each well in a 96-well plate. The next day, serial dilutions of test compounds in cell culture medium were added to the cells and they were further incubated for

48 h. After three days, the medium was removed and the cells were lysed with 50 μl lysis buffer. The luciferase activity was measured using a Luciferase Assay kit (Biothema, Haninge, Sweden) and a Microlumat Plus (Berthold Technologies). IFN-αA was used as reference inhibitor in each plate.

6.4. Computational methodology

(a) *Ligand preparation.* The ligands were constructed and geometry optimized in SYBYL.[40] All the ligands were assigned Gasteiger-Hückel charges.[41,42] The constructed ligands were locally optimized as follows. The first step in ligand optimization encompassed employing the Powell Method[43] using the conjugate gradient termination criterion of 0.05 kcal/mol Å for 1000 iterations. A distance dependent dielectric of 4 Å was used for all molecular mechanics (MM) calculations in order to take into account the shielding in the protein. The ligands were then further subjected to the BFGS minimization method[44–47] where they were optimized until a conjugate gradient of 0.001 kcal/mol Å was reached.

(b) *Preparation of protein coordinates and definition of the active site.* Reference protein coordinates used for docking were taken from the X-ray structure of bifunctional NS3 protein found in the Protein Databank under the accession code 1CU1 resolved at 2.50 Å.[48] This 631-residue protein consists of the C-terminal helicase domain and the N-terminal protease domain covalently linked to the NS4A cofactor. Although alternative rotameric states exist for a few side chains, we felt that choosing the crystal coordinates was a reasonable choice since the active site is defined by the C-terminal. Nevertheless, there exist a few structural discrepancies in assigning the side chains of an X-ray structure resolved at more than 2.0 Å. Therefore, the structure was subjected to refinement by restrained minimization. The crystallographic waters were removed and hydrogens were added via an all-atom treatment with no atoms having lone pairs. The refinement was done using the BatchMin algorithm of Macromodel v.7.1,[49] employing the AMBER*[50] all-atom force field with a dielectric constant of 78. The extended non-bonded cutoff distances for van der Waals and Coulombic interactions were set to 3.5 Å and 6.0 Å, respectively, while the hydrogen bonding cutoff distance was kept at 4.0 Å. These cutoffs have been shown to give optimal results in terms of speed and accuracy.[51] The protein was minimized with a total of 300 iterations employing the Polak-Ribiere Conjugate Gradient (PRCG) method, and convergence was monitored with the derivative convergence criterion using a cutoff value of 0.05 kJ/Å mol. Because of the large size of 1CU1 (~70 kDa), it was decided to work with only the binding pocket, which was defined by including all the residues that lie within a distance of 9 Å from A621 to T631 (C-terminal) that were later removed before docking the inhibitors. Arginines, lysines, aspartic, and glutamic acids were kept positively and negatively charged, respectively.

(c) *Flexible docking.* Since it is well documented that ligand binding causes conformational changes in the protein, we choose to dock the ligands by taking into account the protein flexibility. FLO (also called QXP) developed by McMartin et al. was employed to dock the database of molecules into the active site of the catalytic core of the enzyme.[52] FLO search algorithms are derived from the method of Monte Carlo perturbation with energy minimization in Cartesian space. It uses the modified version of the AMBER force field. Partial charges were calculated using bond dipole moments. Crucial amino acid residues in the binding pocket were allowed to move freely up to 0.2 Å. Movement larger than 0.2 Å was then penalized by 20.0 kJ/mol/Å^2. The inhibitor along with residue R155 had full conformational freedom.

(d) *Docking protocol.* The ligands were docked into the active site by employing 2000 Monte Carlo perturbation cycles and 10 unique binding poses for each ligand were generated. Each of these poses was further subjected to 20 steps of simulated annealing followed by gradient minimization. Each perturbation cycle involves 400 rapid Monte Carlo search steps followed by energy minimization of the best conformer from the set of 400. The conformations are randomly generated in such a way that each new conformation generated differs from the previous one by a similarity distance of 0.5 Å and has an energy window of not more than 50.0 kJ/mol/Å. The simulated annealing implements the Beeman algorithm and rescales the velocities to give an exact average kinetic temperature at each step.[53] Each of the complexes was submitted to 3 fs of dynamics at 600 K following a 3000 fs equilibration step. The maximum movement of an atom in any single time step was limited to 0.1 Å. Hydrogen vibrations were damped by assigning an atomic weight of 10. This was followed by minimization of each saved pose by the PRCG method.

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