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Hepatitis C virus NS3 protease inhibitors comprising a novel aromatic P_1 moiety

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Abstract—Inhibition of the hepatitis C virus (HCV) NS3 protease has emerged as an attractive approach to defeat the global hepatitis C epidemic. In this work, we present the synthesis and biochemical evaluation of HCV NS3 protease inhibitors comprising a non-natural aromatic P_1 moiety. A series of inhibitors with aminobenzoyl sulfonamides displaying submicromolar potencies in the full-length NS3 protease assay was prepared through a microwave-irradiated, palladium-catalyzed, amidocarbonylation protocol.

1. Introduction

Hepatitis C is a global epidemic with an estimated prevalence of 2%. The disease is caused by the hepatitis C virus (HCV) which is the major cause of chronic liver disease and liver transplantations in the developed world.[1] The current standard treatment with a combination of pegylated interferon and ribavirin has a sustained virological response rate of only ~55%.[2] Moreover, this therapy is associated with high costs and severe adverse effects. Clearly, more efficient and better-tolerated therapies against HCV are highly sought.

The HCV genome consists of a single-stranded, positive sense RNA molecule of approximately 9600 nucleotides. Translation of the viral RNA generates a polyprotein that is proteolytically cleaved into 10 viral proteins of different function: the structural proteins C, E1 and E2, p7, and the non-structural proteins NS2, NS3, NS4A, NS4B, NS5A and NS5B.[3]

The NS3 protein is a bifunctional enzyme with a helicase/NTPase domain and a protease domain. The NS3 protease, in complex with its co-factor NS4A, is a key actor in the processing of the HCV polyprotein.[4] Moreover, it has been suggested that the NS3 protease interferes with cellular mechanisms involved in the host immune response to an HCV infection.[5] Thus, inhibition of the protease is an attractive antiviral approach that will block viral replication as well as potentially restore the host immune response.

The NS3 protease was early recognized as a potential drug target, possibly as a result of the successful use of HIV-1 protease inhibitors, and initial substrate-based structure–activity relationship (SAR) studies identified hexapeptides as potent NS3 protease inhibitors.[6,7] Today, approximately one decade after the first reports on NS3 protease inhibitors, a huge arsenal of preclinical shorter peptide-based inhibitors has emerged.[8–10] Additionally, in the last couple of years we have witnessed the first NS3 protease inhibitors to enter clinical trials: BILN 2061 (ciluprevir), VX-950 (telaprevir) and SCH 503034 (Fig. 1).[11] These three inhibitors have shown powerful antiviral effects in HCV-infected patients and both telaprevir and SCH 503034 are currently being evaluated in phase II trials whereas the clinical evaluation of ciluprevir has been stopped due to cardiac toxicity in animals.[12–15] ITMN-191, a novel inhibitor developed by InterMune, is structurally related to ciluprevir, but one interesting modification is that the cyclopropyl acyl sulfonamide group has replaced the

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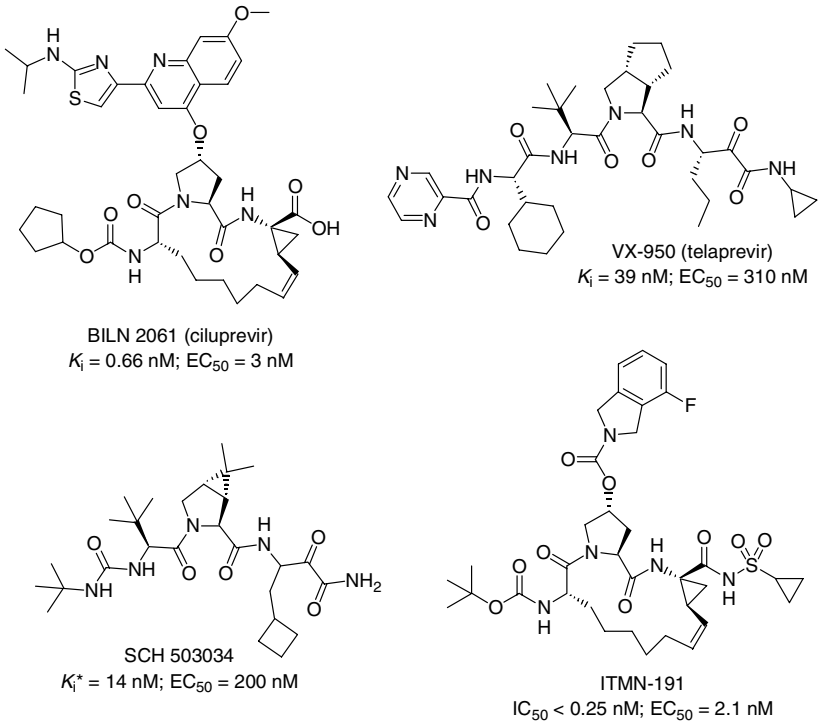


Figure 1. Chemical structures and inhibition data of BILN 2061,[12] VX-950,[17] SCH 503034[18] and ITMN-191.[19,20]

C-terminal carboxylic acid (Fig. 1). Clinical evaluation of ITMN-191 has recently been initiated.[16]

One major concern regarding future HCV therapy in general and thus with NS3 protease inhibitors is the development of viral resistance. HCV strains resistant to ciluprevir, telaprevir and SCH 503034 have been observed in cell studies and more recently in patients treated with telaprevir.[21–24] Consequently, the development of NS3 protease inhibitors comprising novel structural motifs is highly sought in order to combat HCV.

The vast majority of NS3 protease inhibitors developed so far consist of a peptide backbone comprising α-amino acids.[8–10] Although ciluprevir, telaprevir and SCH 503034 are evaluated as oral drugs, the use of peptides as drugs is often associated with poor oral bioavailability and rapid metabolic degradation.[25]

We were interested in examining whether the α-amino acid in P_1 could be substituted with a non-natural aromatic moiety. Such a replacement would not only make more rigid and less peptide-like inhibitors, but also enable us to explore the vital C-terminal end of the inhibitors—interacting with the catalytic machinery of the protease—and the S_1 pocket in a new way. Furthermore, the aryl moiety allows the use of a more diverse range of synthetic methods in analogue preparation.

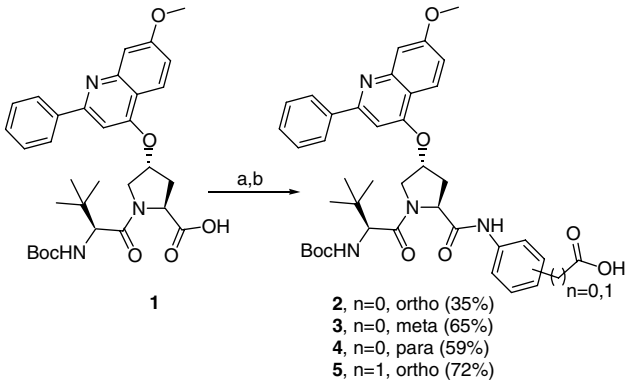
Herein, we present the synthesis and biochemical evaluation of NS3 protease inhibitors comprising a non-natural aromatic P_1 moiety. Aminobenzoic acids and aminobenzoyl sulfonamides, aimed at interacting with the active site of the protease, were investigated as potential P_1 motifs and compounds containing the aminobenzoyl sulfonamide moiety were found to be the most promising. A series of compounds was generated by the use of a palladium-catalyzed amidocarbonylation protocol providing NS3 protease inhibitors displaying a diverse SAR and with submicromolar potencies.

2. Results

2.1. Chemistry

Synthesis of compound **1**, herein used as a starting point for further synthesis, has previously been reported.[26] Compounds **2**–**4**, comprising the aminobenzoic acid moiety, were prepared through amide bond formation between carboxylic acid **1** and the aminobenzoic acid ester using *N*-[(dimethylamino)-1*H*-1,2,3-triazolo-[4,5-*b*]pyridin-1-yl-methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (HATU) as the activating agent in the presence of *N*,*N*-diisopropylethylamine (DIEA) at 45 °C in CH_2Cl_2. Subsequent ester hydrolysis provided the desired carboxylic acids **2**–**4** (Scheme 1). Compound **5**, comprising the 2-aminophenylacetic acid moiety, was prepared according to the same procedure as compounds **2**–**4** (Scheme 1).

Carboxylic acid **1** was also used in the preparation of compounds **6**–**8**, **18** and **19**, comprising a bromoaniline moiety (Scheme 2). Amide bond formation between **1** and 2-, 3- or 4-bromoaniline was enabled by the use of HATU as described above, providing the aryl bromides **6**–**8** in good yields. The coupling between **1** and 2-bromo-6-methylaniline and 2-bromo-5-(trifluoromethyl)aniline required the use of a different activating agent. We found that the use of $POCl_3$ in pyridine as



Scheme 1. Reagents and conditions: (a) 2-aminobenzoic acid methyl ester, 3-aminobenzoic acid ethyl ester, 4-aminobenzoic acid methyl ester or 2-aminophenylacetic acid methyl ester, HATU, DIEA, CH_2Cl_2, 45 °C; (b) LiOH, THF, MeOH, H_2O, rt.

reported by Rijkers and co-workers[27] was very convenient and furnished the aryl bromides **18** and **19** in good yields.

The aryl bromides **6**–**8**, **18** and **19** served as precursors for the synthesis of compounds **9**–**17**, **20** and **21** comprising the acyl sulfonamide functionality (Scheme 2). A palladium-catalyzed amidocarbonylation protocol using microwave irradiation recently presented by our group yielded the desired acyl sulfonamide containing compounds **9**–**17**, **20** and **21** in reasonably good yields.[28] This method employs the thermostable catalytic combination of Herrmann's palladacycle (Fig. 2)[29] and the Fu salt, $[(t\text{-}Bu)_3PH]BF_4$,[30] as well as $Mo(CO)_6$ as a convenient carbon monoxide source.[31]

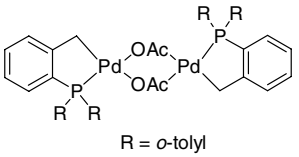
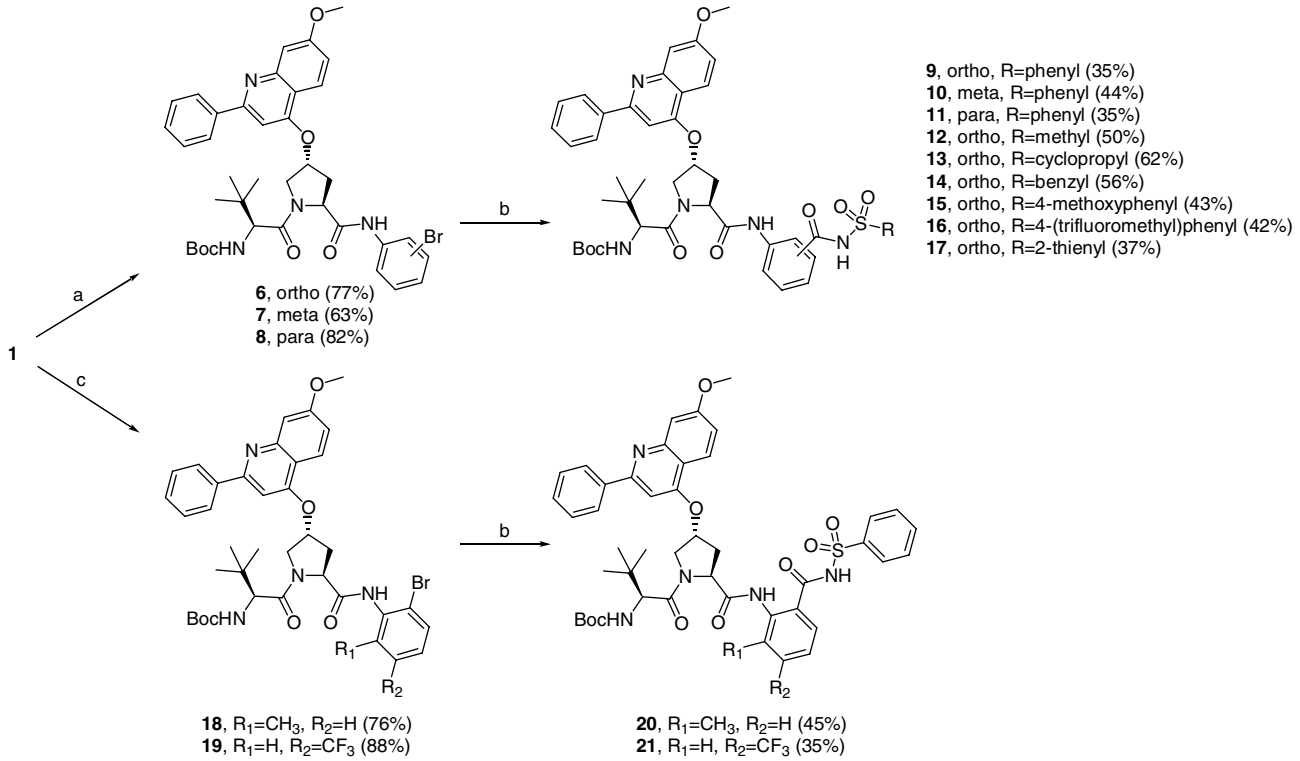


Figure 2. Chemical structure of Herrmann's palladacycle.

2.2. Biochemical evaluation

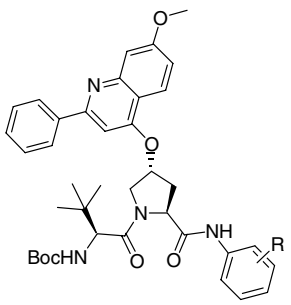
The compounds **1**–**17**, **20** and **21** were biochemically evaluated in a protease activity assay for the full-length NS3 protein (K_i values) and the results are summarized in Tables 1 and 2.[32]

Carboxylic acid **1** lacking the aromatic P_1 moiety is a very weak inhibitor of the NS3 protease with a K_i value of 66 μM (Table 1). Compounds **2**–**4** comprising the aminobenzoic acid moiety display inhibitory potencies of 5.0, 6.8 and 12 μM for the *ortho*, *meta* and *para*-compound, respectively. Introduction of a carbon spacer between the aromatic moiety and the carboxylic acid group as in compound **5** was not beneficial for the potency and increased the K_i value to 17 μM. The aryl bromide precursors **6**–**8** displayed K_i-values of 3.9, 1.4 and 1.8 μM for the *ortho*-, *meta*- and *para*-bromide, respectively. Replacement of the aromatic carboxylic acid



Scheme 2. Reagents and conditions: (a) 2-, 3- or 4-bromoaniline, HATU, DIEA, CH_2Cl_2, 45 °C; (b) Herrmann's palladacycle, $[(t\text{Bu})_3PH]BF_4$, RSO_2NH_2, $Mo(CO)_6$, DBU, 1,4-dioxane, microwave irradiation at 140 °C for 15 min; (c) 2-bromo-6-methylaniline or 2-bromo-5-(trifluoromethyl)aniline, $POCl_3$, pyridine, −15 °C to rt.

Table 1.



Compound	R	Position	$K_i \pm SD$ (μM)[a]
1[b]	—	—	66 ± 30
2	–COOH	ortho	5.0 ± 1.5
3	–COOH	meta	6.8 ± 2.5
4	–COOH	para	12 ± 5
5	$-CH_2COOH$	ortho	17 ± 5
6	–Br	ortho	3.9 ± 0.6
7	–Br	meta	1.4 ± 0.3
8	–Br	para	1.8 ± 0.3
9	$-CONHSO_2Ph$	ortho	0.83 ± 0.11
10	$-CONHSO_2Ph$	meta	0.78 ± 0.15
11	$-CONHSO_2Ph$	para	1.1 ± 0.2

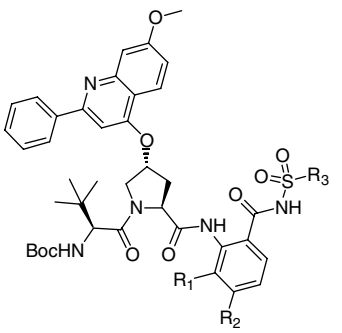
SD, standard deviation.
[a] Determined in a full-length NS3 protein inhibition assay.
[b] See Scheme 1 for structure.

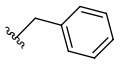
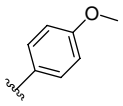
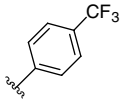
group in compounds **2**–**4** with the corresponding phenyl acyl sulfonamide in compounds **9**–**11** was successful and the inhibitory potencies improved between 6- and 11-fold, rendering inhibitors with submicromolar potencies.

The *ortho* acyl sulfonamide compounds were further explored as HCV NS3 protease inhibitors by variation of the sulfonamide substituent (Table 2). The SAR study included alkyl groups (**12**–**13**), a benzyl group (**14**) and several aryl groups (**15**–**17**). The methyl and cyclopropyl aliphatic substituents used in compounds **12** and **13**, respectively, produced inhibitors with potencies weaker than the initial phenyl-substituted inhibitor **9**. Inhibitor **14**, comprising the benzylic substituent, was slightly more potent than inhibitor **9**, displaying a K_i value of 0.61 μM. The 4-methoxyphenyl-based inhibitor **15** was slightly less potent than **9** but the 4-(trifluoromethyl)phenyl group rendered inhibitor **16** with improved potency compared to **9** and a K_i value of 0.31 μM. The heteroaromatic thiophene-based inhibitor **17** (K_i = 0.92 μM) was equipotent to **9**.

Furthermore, we explored the effect of an additional substituent on the aromatic P_1 moiety, as in compounds **20** and **21** (Table 2). A methyl substituent *ortho* to the aniline nitrogen present in compound **20** resulted in an inhibitor with a K_i value of 3.2 μM. In comparison to the unsubstituted analogue **9**, the *ortho* methyl compound **20** is approximately 4 times weaker in inhibitory potency. On the other hand, the introduction of a trifluoromethyl substituent *para* to the acyl sulfonamide group was well tolerated and provided inhibitor **21** with a K_i value of 0.35 μM. Compound **21** was the only inhibitor displaying effect in a cell-based subgenomic HCV replicon assay with an EC_{50} of 5.2 μM (CC_{50} = 28 μM).[33]

Table 2.



Compound	R_1	R_2	R_3	$K_i \pm SD$ (μM)[a]
9	–H	–H		0.83 ± 0.11
12	–H	–H		5.9 ± 1.1
13	–H	–H		2.0 ± 0.3
14	–H	–H		0.61 ± 0.09
15	–H	–H		1.2 ± 0.2
16	–H	–H		0.31 ± 0.05
17	–H	–H		0.92 ± 0.15
20	$-CH_3$	–H		3.2 ± 0.4
21	–H	$-CF_3$		0.35 ± 0.05

SD, standard deviation.
[a] Determined in a full-length NS3 protein inhibition assay.

3. Discussion

One common structural feature for the vast majority of NS3 protease inhibitors is the presence of a peptide backbone comprising α-amino acids (Fig. 1). In an effort to develop less peptide-like inhibitors, we decided to investigate the replacement of the normal α-amino acid with a non-natural aromatic P_1 moiety. Although a large structural modification like this was expected to produce less efficient inhibitors as compared to those comprising well-optimized α-amino acids in P_1, at least initially, we looked forward to finding a new starting point in a forthcoming drug discovery process. Moreover, this structural modification would enable synthetic methods not possible on non-aromatic P_1 groups.

We envisioned that properly substituted anilines could work well as P_1 moieties and that an acidic substituent

would be favorable for interactions with the active site of the NS3 protease, as utilized in product-based NS3 protease inhibitors such as ciluprevir (Fig. 1). Consequently, compounds **2**–**5** comprising a carboxylic acid were synthesized but failed to produce highly potent inhibitors. Apparently, the carboxylate group is not capable of interacting with the oxyanion hole in an optimal way as it does in inhibitors with P_1 α-amino acids.[34]

We have previously used the acyl sulfonamide group as a potent P_1 C-terminal carboxylic acid replacement and introduction of this group in the aromatic P_1 moiety resulted in submicromolar inhibitors (compounds **9**–**11**).[35–37] Molecular modeling of compound **9** (Fig. 3) suggests that the aromatic carbonyl is not positioned in the oxyanion hole, which is also indicated by the modest potency observed for the carboxylic acid **2**. On the other hand, the sulfone oxygens in **9** have the possibility to hydrogen bond to the side chains of Gln41 and Lys136 in the protease. These hydrogen bonds, in combination with additional interactions between the protease and the sulfonamide substituent as well as electrostatic interactions between Lys136 and the acyl sulfonamide part, could explain the difference in potency between compounds **2** and **9**.

Although no significant difference in potency between the *ortho*, *meta* and *para* compounds was observed, we decided to further explore the *ortho* acyl sulfonamide compound since this analogue bears closer resemblance to an inhibitor with a P_1 α-amino acid. A series of inhibitors with different sulfonamide substituents was prepared (compounds **12**–**17**, Table 2). The new inhibitors displayed a diverse SAR, which was encouraging. The most potent inhibitor **16** with a 4-(trifluoromethyl)phenyl substituent (K_i = 0.31 μM) is 19 times more potent than inhibitor **12** with a methyl substituent (K_i = 5.9 μM) and 16 times more potent than the corresponding carboxylic acid inhibitor **2** (K_i = 5.0 μM). Interestingly, the cyclopropyl substituent in compound **13** did not function as well as was previously seen for inhibitors with (1*R*,2*S*)-1-amino-2-vinyl-cyclopropanecarboxylic acid in the P_1 position.[26,39] This emphasizes that the acyl sulfonamide group in the inhibitors comprising an aromatic P_1 has a different binding mode than it does in inhibitors with a P_1 α-amino acid.

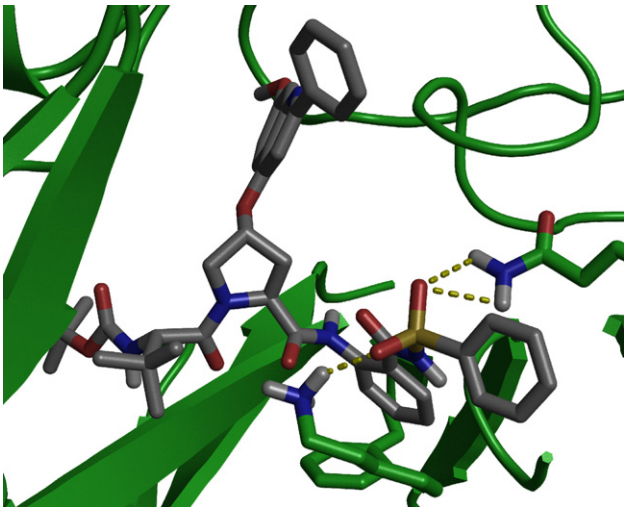


Figure 3. Compound **9** docked in the NS3 protease binding site of the 1CU1 crystal structure.[38] The enzyme is shown in a ribbon structure with residues Gln41, Lys136 and Phe154 displayed as sticks with carbon atoms in green and heteroatoms colored according to atom type (oxygens in red and nitrogens in blue). The hydrogen bond distances shown are between the sulfone oxygens of the inhibitor and the side chains of Lys136 (1.95 Å) and Gln41 (2.47 and 2.67 Å). The aromatic P_1 moiety of **9** is positioned above the aromatic ring of Phe154. All essential ligand hydrogens as well as the hydrogens of the protease thought to participate in hydrogen bonding with the acyl sulfonamide group are displayed. Ligand atoms are colored according to atom type (oxygens in red, nitrogens in blue and sulfur in yellow).

According to molecular modeling studies, it is possible that the aromatic P_1 moiety interacts with the aromatic ring of Phe154, which forms the bottom of the S_1 pocket of the protease (Fig. 3). This is also indicated by the fact that the bromoanilines **6**–**8**, lacking the acidic functionality, are modest inhibitors of the NS3 protease and that compound **1**, lacking the aromatic P_1 moiety, is a very weak inhibitor (Table 1). This encouraged us to introduce an additional substituent on the aromatic P_1 moiety that could interact more favorably with the S_1 pocket. Hence, compound **20**, with a methyl group *ortho* to the aniline nitrogen, was prepared but resulted in an inhibitor with four times lower potency compared to the unsubstituted analogue **9**. On the other hand, the trifluoromethyl group *para* to the acyl sulfonamide group in compound **21** rendered an inhibitor displaying a K_i of 0.35 μM, almost three times more potent than the unsubstituted inhibitor **9**. Compound **21** was the only inhibitor displaying a measurable inhibition in a cell-based HCV replicon assay with an EC_{50} of 5.2 μM.

4. Conclusions

Herein, we present the synthesis and biochemical evaluation of the first account of HCV NS3 protease inhibitors comprising a non-natural aromatic P_1 moiety. The aminobenzoyl sulfonamide fragment was identified as a novel P_1 structural motif. A microwave irradiated, palladium catalyzed, amidocarbonylation protocol enabled the facile preparation of a series of compounds displaying submicromolar potencies in the full-length NS3 assay. Encouragingly, a diverse SAR was found both for the sulfonamide substituent and for the substituent on the aromatic P_1 moiety. This indicates that the inhibitors have two sites that can be utilized in a forthcoming optimization process. Further studies are underway to fully exploit this new P_1 moiety.

5. Experimental

5.1. Chemistry

5.1.1. General methods. TLC was performed using aluminum sheets precoated with silica gel 60 F_{254} (0.2 mm, Merck). Chromatographic spots were visualized using UV-detection and/or ethanolic ninhydrin solution (2%)

followed by heating. Column chromatography was performed using silica gel 60 (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) and MS (ESI) detection. HPLC purity of the compounds was determined using the C18 or the C4 column through integration of the UV-trace at 254 nm. Preparative HPLC–MS was performed on a Gilson-Finnigan ThermoQuest AQA system equipped with a C8 (Sorbax 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. Elemental analyses were performed by Analytische Laboratorien, Lindlar, Germany or by MikroKemi AB, Uppsala, Sweden. NMR spectra were recorded on a Varian Mercury plus spectrometer (^{1}H 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 (^{1}H: $CHCl_3$ δ 7.26, CHD_2OD δ 3.31; ^{13}C: $CDCl_3$ δ 77.16, CD_3OD δ 49.00). Microwave irradiated reactions were carried out in a SmithSyntheSizer™ or in an Initiator™ single-mode microwave cavity producing controlled irradiation at 2450 MHz (Biotage AB, Uppsala, Sweden).

5.1.2. General procedure for the synthesis of compounds 2–5. *Amide bond formation:* a mixture of **1**, aminobenzoic acid ester or 2-aminophenylacetic acid methyl ester, HATU, DIEA and CH_2Cl_2 was stirred in a sealed reaction tube under N_2-atmosphere at 45 °C for 3–26 h. Aqueous workup and/or purification by column chromatography gave the corresponding ester intermediates that were hydrolyzed in the next step without further characterization. *Ester hydrolysis:* a solution of LiOH in H_2O was added to a solution of the ester intermediate in THF and MeOH and the resulting mixture was stirred at room temperature. The reaction mixture was thereafter neutralized with 1.0 M HCl (aq) and the organic solvents were evaporated. The remaining aqueous phase was acidified to pH 4 using 1.0 M HCl (aq) and extracted three times with EtOAc. The combined organic layer was washed with brine, dried ($MgSO_4$), filtered and evaporated. Purification by preparative HPLC–MS or column chromatography gave the desired carboxylic acids **2**–**5**.

5.1.2.1. Compound 2. Prepared according to the general procedure described above. *Amide bond formation:* **1** (40 mg, 0.069 mmol), 2-aminobenzoic acid methyl ester (16 mg, 0.11 mmol), HATU (32 mg, 0.084 mmol), DIEA (45 μL, 0.26 mmol) and CH_2Cl_2 (2 mL). *Reaction time:* 26 h. Evaporation and purification by column chromatography (gradient elution, EtOAc:*i*-hexane 1:2–2:3) gave the ester intermediate as a white solid (22 mg). *Ester hydrolysis:* ester intermediate (21 mg, 0.030 mmol), LiOH (2.8 mg, 0.12 mmol), H_2O (0.41 mL), THF (0.95 mL) and MeOH (0.14 mL). *Reaction time:* overnight. *Extraction:* EtOAc (3 × 5 mL). Purification by preparative HPLC–MS gave compound **2** (16 mg, 35%) as a white solid. ^{1}H NMR ($CDCl_3$): δ (10:1 mixture of rotamers, major rotamer reported) 11.88 (br s, NH), 8.64 (d, J = 8.7 Hz, 1H), 7.96–7.94 (m, 2H), 7.89–7.86 (m, 2H), 7.50 (d, J = 2.0 Hz, 1H), 7.45 (ddd, J = 1.6, 7.6, 8.7 Hz, 1H), 7.40–7.38 (m, 3H), 7.01–6.96 (m, 2H), 6.80 (s, 1H), 5.30 (d, J = 9.8 Hz, NH), 5.30–5.29 (m, 1H), 4.83 (dd, J = 7.9, 9.1 Hz, 1H), 4.59 (dm, J = 11.6 Hz, 1H), 4.39 (d, J = 9.8 Hz, 1H), 4.14 (dm, J = 11.6 Hz, 1H), 3.88 (s, 3H), 2.66 (ddm, J = 7.9, 13.8 Hz, 1H), 2.39 (ddd, J = 4.3, 9.1, 13.8 Hz, 1H), 1.37 (s, 9H), 1.09 (s, 9H). ^{13}C NMR ($CDCl_3$): δ (10:1 mixture of rotamers, major rotamer reported) 172.2, 171.1, 169.5, 162.1, 160.9, 159.1, 156.2, 149.8, 141.4, 138.8, 134.1, 131.6, 129.8, 128.8, 128.1, 123.4, 122.8, 120.2, 119.0, 116.6, 115.2, 105.9, 98.7, 80.1, 76.7, 60.9, 59.1, 55.8, 54.5, 35.6, 35.0, 28.4, 26.6. MS $[M+H]^+$ 697.5. *HPLC purity:* C18 column 99%, C4 column >99%. Anal. Calcd for $C_{39}H_{44}N_4O_8 \cdot 1/2H_2O$: C, 66.37; H, 6.43; N, 7.94. Found: C, 66.58; H, 6.57; N, 8.06.

5.1.2.2. Compound 3. Prepared according to the general procedure described above. *Amide bond formation:* **1** (40 mg, 0.069 mmol), 3-aminobenzoic acid ethyl ester (17 mg, 0.10 mmol), HATU (32 mg, 0.084 mmol), DIEA (45 μL, 0.26 mmol) and CH_2Cl_2 (2 mL). *Reaction time:* 3 h. Evaporation and purification by column chromatography (EtOAc:*i*-hexane 1:2) gave the ester intermediate as a white solid (46 mg). *Ester hydrolysis:* ester intermediate (25 mg, 0.034 mmol), LiOH (3.6 mg, 0.15 mmol), H_2O (0.41 mL), THF (0.95 mL) and MeOH (0.14 mL). *Reaction time:* overnight. *Extraction:* EtOAc (3 × 5 mL). Purification by preparative HPLC–MS gave compound **3** (17 mg, 65%) as a white solid. ^{1}H NMR ($CDCl_3$): δ (10:1 mixture of rotamers, major rotamer reported) 9.46 (br s, NH), 8.11 (dm, J = 8.2 Hz, 1H), 8.08–8.03 (m, 4H), 7.78 (dm, J = 7.8 Hz, 1H), 7.51–7.42 (m, 4H), 7.38 (dd, J = 7.8, 8.2 Hz, 1H), 7.05 (dd, J = 2.5, 9.1 Hz, 1H), 7.03 (s, 1H), 5.66 (d, J = 9.4 Hz, NH), 5.43 (m, 1H), 5.04 (t, J = 7.4 Hz, 1H), 4.56 (dm, J = 11.6 Hz, 1H), 4.41 (d, J = 9.4 Hz, 1H), 4.09 (dd, J = 4.9, 11.6 Hz, 1H), 3.93 (s, 3H), 3.01–2.95 (m, 1H), 2.61–2.55 (m, 1H), 1.42 (s, 9H), 1.02 (s, 9H). ^{13}C NMR ($CDCl_3$): δ (10:1 mixture of rotamers, major rotamer reported) 173.5, 169.4, 168.6, 161.6, 160.4, 159.3, 156.2, 140.1, 138.2, 131.0, 129.5, 129.3, 128.9, 127.8, 125.8, 124.5, 123.3, 121.4, 118.7, 115.2, 107.2, 104.9, 98.2, 80.1, 76.3, 59.9, 59.5, 55.7, 54.4, 35.5, 33.1, 28.4, 26.6. MS $[M+H]^+$ 697.5. *HPLC purity:* C18 column >99%, C4 column >99%. Anal. Calcd for $C_{39}H_{44}N_4O_8 \cdot 1/2H_2O$: C, 66.37; H, 6.43; N, 7.94. Found: C, 66.12; H, 6.54; N, 7.79.

5.1.2.3. Compound 4. Prepared according to the general procedure described above. *Amide bond formation:* **1** (40 mg, 0.069 mmol), 4-aminobenzoic acid methyl ester (16 mg, 0.11 mmol), HATU (32 mg, 0.084 mmol), DIEA (45 μL, 0.26 mmol) and CH_2Cl_2 (2 mL). *Reaction time:* 3.5 h. Evaporation and purification by column chromatography (EtOAc:*i*-hexane 1:2) gave the ester intermediate as a white solid (40 mg). *Ester hydrolysis:* Ester intermediate (38 mg, 0.053 mmol), LiOH (5.0 mg, 0.21 mmol), H_2O (0.41 mL), THF (0.95 mL) and MeOH (0.14 mL). *Reaction time:* overnight. *Extraction:* EtOAc (3 × 5 mL). Purification by preparative HPLC–MS gave

compound **4** (27 mg, 59%) as a white solid. ^{1}H NMR ($CDCl_3$): δ (10:1 mixture of rotamers, major rotamer reported) 9.94 (br s, NH), 8.06–8.03 (m, 2H), 7.85–7.83 (m, 2H), 7.77 (d, J = 9.1 Hz, 1H), 7.53–7.46 (m, 5H), 7.33 (d, J = 2.3 Hz, 1H), 6.97 (s, 1H), 6.92 (dd, J = 2.3, 9.1 Hz, 1H), 5.39 (m, 1H), 5.31 (d, J = 9.5 Hz, NH), 5.10 (t, J = 7.7 Hz, 1H), 4.52–4.48 (m, 2H), 4.19–4.15 (m, 1H), 3.81 (s, 3H), 2.92–2.86 (m, 1H), 2.58–2.52 (m, 1H), 1.23 (s, 9H), 1.09 (s, 9H). ^{13}C NMR ($CDCl_3$): δ (10:1 mixture of rotamers, major rotamer reported) 173.1, 170.8, 169.1, 161.4, 160.2, 159.1, 155.6, 151.0, 142.7, 140.1, 131.1, 129.5, 128.8, 127.9, 125.1, 122.4, 118.8, 118.7, 114.7, 107.0, 97.9, 80.1, 76.1, 60.2, 59.1, 55.5, 54.1, 36.3, 33.8, 28.3, 26.6. MS $[M+H]^+$ 697.6. *HPLC purity:* C18 column >99%, C4 column >99%. Anal. Calcd for $C_{39}H_{44}N_4O_8 \cdot 1/2H_2O$: C, 66.37; H, 6.43; N, 7.94. Found: C, 66.68; H, 6.42; N, 7.82.

5.1.2.4. Compound 5. Prepared according to the general procedure described above. *Amide bond formation:* **1** (60 mg, 0.10 mmol), 2-aminophenylacetic acid methyl ester[40] (26 mg, 0.16 mmol), HATU (47 mg, 0.12 mmol), DIEA (67 μL, 0.38 mmol) and CH_2Cl_2 (2 mL). *Reaction time:* 15 h. The mixture was diluted with EtOAc (15 mL) and washed with aqueous NaOAc buffer (pH 4, 2 × 8 mL), 5% aqueous $NaHCO_3$ (8 mL) and brine (8 mL). The organic layer was dried ($MgSO_4$), filtered and evaporated. Purification by column chromatography (EtOAc:*i*-hexane 2:3) gave the ester intermediate as a white solid (60 mg). *Ester hydrolysis:* ester intermediate (60 mg, 0.083 mmol), LiOH (30 mg, 1.3 mmol), H_2O (1.1 mL), THF (2.4 mL) and MeOH (0.3 mL). *Reaction time:* 1 h. *Extraction:* EtOAc (2 × 9 mL). Purification by column chromatography (gradient elution: CH_2Cl_2:MeOH:HCOOH 96:4:0.2–94:6:0.2) gave compound **5** (53 mg, 72%) as a white solid. ^{1}H NMR (CD_3OD): δ (10:1 mixture of rotamers, major rotamer reported) 8.15 (d, J = 9.2 Hz, 1H), 8.05–8.03 (m, 2H), 7.59–7.53 (m, 3H), 7.45 (dd, J = 1.5, 7.9 Hz, 1H), 7.39 (d, J = 2.5 Hz, 1H), 7.29–7.23 (m, 2H), 7.28 (s, 1H), 7.19–7.15 (m, 1H), 7.11 (dd, J = 2.5, 9.2 Hz, 1H), 6.51 (d, J = 8.9 Hz, NH), 5.57–5.56 (m, 1H), 4.84 (dd, J = 7.4, 10.0 Hz, 1H), 4.60 (dm, J = 12.0 Hz, 1H), 4.27–4.25 (m, 1H), 4.07 (dd, J = 3.5, 12.0 Hz, 1H), 3.93 (s, 3H), 3.78 (d, J = 15.6 Hz, 1H), 3.58 (d, J = 15.6 Hz, 1H), 2.81 (ddm, J = 7.4, 14.1 Hz, 1H), 2.51 (ddd, J = 4.2, 10.0 14.1 Hz, 1H), 1.28 (s, 9H), 1.04 (s, 9H). ^{13}C NMR (CD_3OD): δ (10:1 mixture of rotamers, major rotamer reported) 176.0, 173.5, 172.5, 163.8, 163.2, 160.5, 158.0, 150.2, 139.5, 137.1, 131.9, 131.7, 131.3, 130.0, 129.2, 128.6, 127.5, 127.0, 124.8, 119.9, 116.4, 105.8, 100.5, 80.4, 78.9, 61.1, 60.7, 56.2, 55.3, 39.4, 36.1, 35.9, 28.6, 27.0. MS $[M+H]^+$ 711.3. *HPLC purity:* C18 column >99%, C4 column >99%. Anal. Calcd for $C_{40}H_{46}N_4O_8 \cdot 1/2H_2O$: C, 66.74; H, 6.58; N, 7.78. Found: C, 67.05; H, 6.56; N, 7.38.

5.1.3. General procedure for the synthesis of compounds 6–8. A mixture of **1**, bromoaniline, HATU, DIEA and CH_2Cl_2 was stirred in a sealed reaction tube under N_2-atmosphere at 45 °C for 3–28 h. Evaporation and purification by column chromatography gave the desired compounds **6–8**.

5.1.3.1. Compound 6. Prepared according to the general procedure described above using: **1** (150 mg, 0.260 mmol), 2-bromoaniline (42 μL, 0.385 mmol), HATU (120 mg, 0.316 mmol), DIEA (167 μL, 0.958 mmol) and CH_2Cl_2 (5 mL). *Reaction time:* 28 h. Repeated purification by column chromatography (gradient elution, EtOAc:*i*-hexane 1:2–2:3) gave compound **6** (146 mg, 77%) as a white solid. ^{1}H NMR (CD_3OD): δ (10:1 mixture of rotamers, major rotamer reported) 8.09 (d, J = 9.2 Hz, 1H), 8.05–8.02 (m, 2H), 7.74 (dd, J = 1.6, 8.1 Hz, 1H), 7.57 (dd, J = 1.5, 8.1 Hz, 1H), 7.54–7.47 (m, 3H), 7.37 (d, J = 2.6 Hz, 1H), 7.32–7.28 (m, 1H), 7.17 (s, 1H), 7.09–7.02 (m, 2H), 6.45 (d, J = 8.9 Hz, NH), 5.45–5.44 (m, 1H), 4.95 (dd, J = 8.0, 9.1 Hz, 1H), 4.55 (dm, J = 11.9 Hz, 1H), 4.30–4.27 (m, 1H), 4.01 (dm, J = 11.9 Hz, 1H), 3.92 (s, 3H), 2.75 (ddm, J = 8.0, 14.0 Hz, 1H), 2.51 (ddd, J = 4.2, 9.1, 14.0 Hz, 1H), 1.30 (s, 9H), 1.04 (s, 9H). ^{13}C NMR ($CDCl_3$): δ (10:1 mixture of rotamers, major rotamer reported) 172.9, 168.5, 161.6, 160.4, 159.4, 156.0, 151.7, 140.5, 136.1, 132.8, 129.5, 129.0, 128.4, 127.8, 125.8, 123.3, 122.4, 118.7, 115.3, 113.9, 107.7, 98.2, 80.1, 76.3, 60.0, 58.9, 55.8, 54.0, 35.8, 32.7, 28.5, 26.6. MS $[M+H]^+$ 731.2, 733.2. *HPLC purity:* C18 column >99%, C4 column >99%. Anal. Calcd for $C_{38}H_{43}BrN_4O_6$: C, 62.38; H, 5.92; N, 7.66. Found: C, 62.07; H, 6.11; N, 7.50.

5.1.3.2. Compound 7. Prepared according to the general procedure described above using: **1** (50 mg, 0.087 mmol), 3-bromoaniline (14 μL, 0.13 mmol), HATU (41 mg, 0.11 mmol), DIEA (56 μL, 0.32 mmol) and CH_2Cl_2 (2.5 mL). *Reaction time:* 3 h. Purification by column chromatography (EtOAc:*i*-hexane 1:2) gave compound **7** (40 mg, 63%) as a white solid. ^{1}H NMR (CD_3OD): δ (10:1 mixture of rotamers, major rotamer reported) 8.08 (d, J = 9.1 Hz, 1H), 8.05–8.02 (m, 2H), 7.90–7.89 (m, 1H), 7.55–7.43 (m, 4H), 7.37 (d, J = 2.5 Hz, 1H), 7.20–7.16 (m, 3H), 7.04 (dd, J = 2.5, 9.1 Hz, 1H), 6.50 (d, J = 8.9 Hz, NH), 5.50–5.49 (m, 1H), 4.77 (dd, J = 7.5, 10.0 Hz, 1H), 4.58 (dm, J = 11.7 Hz, 1H), 4.30–4.27 (m, 1H), 4.07 (dm, J = 11.7 Hz, 1H), 3.92 (s, 3H), 2.74 (ddm, J = 7.5, 14.0 Hz, 1H), 2.41 (ddd, J = 3.9, 10.0, 14.0 Hz, 1H), 1.28 (s, 9H), 1.06 (s, 9H). ^{13}C NMR (CD_3OD:$CDCl_3$ 1:1): δ (10:1 mixture of rotamers, major rotamer reported) 173.5, 168.0, 161.5, 160.3, 159.3, 155.8, 151.6, 140.4, 139.2, 130.4, 129.5, 129.0, 127.7, 127.5, 123.1, 122.9, 118.6, 118.3, 115.2, 107.6, 104.9, 98.1, 80.2, 76.0, 59.3, 59.0, 55.6, 54.0, 35.5, 31.9, 28.4, 26.6. MS $[M+H]^+$ 731.2, 733.2. *HPLC purity:* C18 column >99%, C4 column >99%. Anal. Calcd for $C_{38}H_{43}BrN_4O_6$: C, 62.38; H, 5.92; N, 7.66. Found: C, 62.22; H, 6.04; N, 7.57.

5.1.3.3. Compound 8. Prepared according to the general procedure described above using: **1** (50 mg, 0.087 mmol), 4-bromoaniline (22 mg, 0.13 mmol), HATU (41 mg, 0.11 mmol), DIEA (56 μL, 0.32 mmol) and CH_2Cl_2 (2.5 mL). *Reaction time:* 4 h. Purification by column chromatography (EtOAc:*i*-hexane 3:7) gave compound **8** (52 mg, 82%) as a white solid.^{1}H NMR (CD_3OD): δ (10:1 mixture of rotamers, major rotamer

reported) 8.08 (d, J = 9.1 Hz, 1H), 8.05–8.02 (m, 2H), 7.54–7.47 (m, 5H), 7.39–7.37 (m, 3H), 7.19 (s, 1H), 7.04 (dd, J = 2.5, 9.1 Hz, 1H), 6.48 (d, J = 8.9 Hz, NH), 5.49–5.47 (m, 1H), 4.77 (dd, J = 7.4, 10.0 Hz, 1H), 4.58 (dm, J = 11.6 Hz, 1H), 4.29–4.27 (m, 1H), 4.05 (dm, J = 11.6 Hz, 1H), 3.92 (s, 3H), 2.73 (ddm, J = 7.4, 14.0 Hz, 1H), 2.40 (ddd, J = 4.0, 10.0, 14.0 Hz, 1H), 1.28 (s, 9H), 1.05 (s, 9H). ^{13}C NMR ($CDCl_3$): δ (10:1 mixture of rotamers, major rotamer reported) 173.4, 168.1, 161.5, 160.2, 159.3, 155.8, 151.6, 140.4, 137.0, 132.1, 129.4, 128.9, 127.7, 123.1, 121.3, 118.6, 117.1, 115.1, 107.5, 98.1, 80.2, 76.0, 59.4, 59.0, 55.6, 54.0, 35.6, 32.0, 28.4, 26.5. MS $[M+H]^+$ 731.2, 733.2. *HPLC purity:* C18 column >99%, C4 column >99%. Anal. Calcd for $C_{38}H_{43}BrN_4O_6 \cdot 1/2H_2O$: C, 61.62; H, 5.99; N, 7.56. Found: C, 61.83; H, 6.12; N, 7.54.

5.1.4. General procedure for the synthesis of compounds 9–17, 20 and 21. A microwave process vial was charged with Herrmann's palladacycle (trans-di(μ-acetato)bis[*o*-(di-*o*-tolylphosphino)benzyl]dipalladium(II)), $[(t\text{-Bu})_3\text{PH}]BF_4$, aryl bromide **6**, **7**, **8**, **18** or **19**, the appropriate sulfonamide and $Mo(CO)_6$. 1,4-Dioxane followed by 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU) was added and the process vial was thereafter sealed and rigorously stirred before it was exposed to microwave irradiation for 15 min at 140 °C. The process vial was cooled to room temperature before it was diluted with CH_2Cl_2 and filtered through a plug of cotton. Purification by column chromatography and/or preparative HPLC–MS gave the desired compounds **9**–**17**, **20** and **21**.

5.1.4.1. Compound 9. Prepared according to the general procedure described above using: Herrmann's palladacycle (2.5 mg, 0.0027 mmol), $[(t\text{-Bu})_3\text{PH}]BF_4$ (1.6 mg, 0.0055 mmol), **6** (40 mg, 0.055 mmol), benzenesulfonamide (45 mg, 0.29 mmol), $Mo(CO)_6$ (16 mg, 0.061 mmol), 1,4-dioxane (0.3 mL) and DBU (24 μL, 0.16 mmol). Purification by column chromatography (gradient elution, CH_2Cl_2:MeOH 97:3–95:5) followed by preparative HPLC–MS gave compound **9** (16 mg, 35%) as a white solid. ^{1}H NMR (CD_3OD): δ (10:1 mixture of rotamers, major rotamer reported) 8.33 (d, J = 9.2 Hz, 1H), 8.22 (dd, J = 1.0, 8.3 Hz, 1H), 8.07–8.04 (m, 2H), 7.96–7.94 (m, 2H), 7.90 (dd, J = 1.6, 8.0 Hz, 1H), 7.64–7.58 (m, 3H), 7.48 (s, 1H), 7.44–7.35 (m, 5H), 7.23 (dd, J = 2.5, 9.2 Hz, 1H), 7.07–7.02 (m, 1H), 5.71–5.69 (m, 1H), 4.75 (dd, J = 7.4, 10.1 Hz, 1H), 4.66 (dm, J = 12.0 Hz, 1H), 4.23 (s, 1H), 4.18 (dm, J = 12.0 Hz, 1H), 3.97 (s, 3H), 2.93 (ddm, J = 7.4, 14.0 Hz, 1H), 2.46 (ddd, J = 4.0, 10.0, 14.0 Hz, 1H), 1.25 (s, 9H), 1.03 (s, 9H). ^{13}C NMR (CD_3OD:$CDCl_3$ 1:1): δ (10:1 mixture of rotamers, major rotamer reported) 172.7, 171.7, 170.3, 164.6, 164.3, 158.8, 157.1, 146.6, 143.2, 139.4, 135.9, 132.7, 132.3, 131.9, 130.9, 129.8, 129.7, 128.9 (two signals), 125.1, 123.9, 123.7, 121.4, 120.4, 115.6, 102.8, 100.7, 80.2, 79.3, 61.6, 59.8, 56.3, 54.6, 35.6, 35.5, 28.5, 26.7. MS $[M+H]^+$ 836.2. *HPLC purity:* C18 column >99%, C4 column >99%. Anal. Calcd for $C_{45}H_{49}N_5O_9S \cdot H_2O$: C, 63.29; H, 6.02; N, 8.20. Found: C, 63.40; H, 6.10; N, 8.01.

5.1.4.2. Compound 10. Prepared according to the general procedure described above using: Herrmann's palladacycle (1.5 mg, 0.0016 mmol), $[(t\text{Bu})_3\text{PH}]BF_4$ (0.94 mg, 0.0032 mmol), **7** (24 mg, 0.033 mmol), benzenesulfonamide (15 mg, 0.095 mmol), $Mo(CO)_6$ (8.6 mg, 0.033 mmol), 1,4-dioxane (0.5 mL) and DBU (15 μL, 0.10 mmol). Purification by column chromatography (CH_2Cl_2:MeOH 96:4) gave compound **10** (12 mg, 44%) as a white solid. ^{1}H NMR (CD_3OD): δ (10:1 mixture of rotamers, major rotamer reported) 8.18 (d, J = 9.2 Hz, 1H), 8.07–8.03 (m, 4H), 8.00–7.99 (m, 1H), 7.74 (ddd, J = 0.9, 2.2, 8.1 Hz, 1H), 7.63–7.51 (m, 7H), 7.42 (d, J = 2.4 Hz, 1H), 7.36–7.32 (m, 2H), 7.14 (dd, J = 2.4, 9.2 Hz, 1H), 6.51 (d, J = 8.9 Hz, NH), 5.64–5.62 (m, 1H), 4.81 (dd, J = 7.2, 10.0 Hz, 1H), 4.64 (dm, J = 12.0 Hz, 1H), 4.27–4.25 (m, 1H), 4.10 (dm, J = 12.0 Hz, 1H), 3.97 (s, 3H), 2.82 (ddm, J = 7.2, 13.9 Hz, 1H), 2.46 (ddd, J = 4.0, 10.0, 13.9 Hz, 1H), 1.27 (s, 9H), 1.05 (s, 9H). ^{13}C NMR (CD_3OD): δ (10:1 mixture of rotamers, major rotamer reported) 173.5, 171.9, 169.5, 163.9, 163.4, 160.7, 158.1, 150.3, 142.3, 139.9, 139.5, 136.0, 134.1, 131.3, 130.0, 129.8, 129.2, 129.0, 125.1 (two signals), 124.9, 120.9, 119.9, 116.5, 105.8, 100.6, 80.5, 79.0, 61.3, 60.9, 56.2, 55.4, 36.2, 35.9, 28.7, 28.6, 27.0. MS $[M+H]^+$ 836.3. *HPLC purity:* C18 column >99%, C4 column >99%. Anal. Calcd for $C_{45}H_{49}N_5O_9S \cdot 1/2H_2O$: C, 63.96; H, 5.96; N, 8.29. Found: C, 63.64; H, 6.13; N, 8.08.

5.1.4.3. Compound 11. Prepared according to the general procedure described above using: Herrmann's palladacycle (2.7 mg, 0.0029 mmol), $[(t\text{Bu})_3\text{PH}]BF_4$ (1.5 mg, 0.0052 mmol), **8** (38 mg, 0.052 mmol), benzenesulfonamide (29 mg, 0.18 mmol), $Mo(CO)_6$ (14 mg, 0.053 mmol), 1,4-dioxane (0.6 mL) and DBU (23 μL, 0.15 mmol). Purification by column chromatography (CH_2Cl_2:MeOH 96:4) gave compound **11** (15 mg, 35%) as a white solid. ^{1}H NMR (CD_3OD): δ (10:1 mixture of rotamers, major rotamer reported) 8.17 (d, J = 9.2 Hz, 1H), 8.07–8.04 (m, 4H), 7.81–7.79 (m, 2H), 7.67–7.60 (m, 3H), 7.57–7.52 (m, 5H), 7.42 (d, J = 2.5 Hz, 1H), 7.33 (s, 1H), 7.12 (dd, J = 2.5, 9.2 Hz, 1H), 6.49 (d, J = 8.9 Hz, NH), 5.63–5.61 (m, 1H), 4.81 (dd, J = 7.3, 9.9 Hz, 1H), 4.64 (dm, J = 12.1 Hz, 1H), 4.27–4.25 (m, 1H), 4.10 (dm, J = 12.1 Hz, 1H), 3.96 (s, 3H), 2.80 (ddm, J = 7.3, 14.0 Hz, 1H), 2.45 (ddd, J = 4.0, 9.9, 14.0 Hz, 1H), 1.27 (s, 9H), 1.05 (s, 9H). ^{13}C NMR (CD_3OD): δ (10:1 mixture of rotamers, major rotamer reported) 173.5, 172.0, 168.6, 163.8, 163.1, 160.9, 158.0, 150.7, 144.1, 142.2, 134.2, 131.2, 130.5, 130.0, 129.8, 129.6, 129.2, 129.1, 124.8, 120.1 (two signals), 119.8, 116.5, 106.1, 100.5, 80.5, 78.8, 61.4, 60.8, 56.2, 55.4, 36.1, 35.9, 28.6, 27.0. MS $[M+H]^+$ 836.3. *HPLC purity:* C18 column >99%, C4 column >99%. Anal. Calcd for $C_{45}H_{49}N_5O_9S \cdot H_2O$: C, 63.29; H, 6.02; N, 8.20. Found: C, 62.98; H, 5.98; N, 7.95.

5.1.4.4. Compound 12. Prepared according to the general procedure described above using: Herrmann's palladacycle (2.6 mg, 0.0028 mmol), $[(t\text{Bu})_3\text{PH}]BF_4$ (1.6 mg, 0.0055 mmol), **6** (40 mg, 0.055 mmol), methanesulfonamide (26 mg, 0.27 mmol), $Mo(CO)_6$ (14 mg, 0.053 mmol), 1,4-dioxane (0.3 mL) and DBU (25 μL,

0.17 mmol). Purification by column chromatography (gradient elution, CH_2Cl_2:MeOH 97:3 to CH_2Cl_2:MeOH:HCOOH 96.9:3:0.1) gave compound **12** (21 mg, 50%) as a white solid. ^{1}H NMR (CD_3OD): δ (10:1 mixture of rotamers, major rotamer reported) 8.31 (d, J = 9.2 Hz, 1H), 8.20 (dd, J = 1.0, 8.4 Hz, 1H), 8.08–8.05 (m, 2H), 7.93 (dd, J = 1.6, 7.9 Hz, 1H), 7.65–7.62 (m, 3H), 7.47 (s, 1H), 7.44–7.42 (m, 2H), 7.25 (dd, J = 2.5, 9.2 Hz, 1H), 7.13–7.09 (m, 1H), 6.51 (d, J = 8.9 Hz, NH), 5.71–5.70 (m, 1H), 4.80 (dd, J = 7.5, 10.2 Hz, 1H), 4.67 (dm, J = 12.3 Hz, 1H), 4.25–4.22 (m, 2H), 4.00 (s, 3H), 3.17 (s, 3H), 2.96 (ddm, J = 7.5, 14.3 Hz, 1H), 2.58 (ddd, J = 4.0, 10.2, 14.3 Hz, 1H), 1.23 (s, 9H), 1.04 (s, 9H). ^{13}C NMR (CD_3OD): δ (10:1 mixture of rotamers, major rotamer reported) 173.6, 172.8, 171.4, 165.8, 165.2, 159.4, 158.1, 146.8, 139.8, 136.5, 133.2, 132.6, 131.3, 130.4, 129.6, 126.0, 125.6, 124.4, 122.3, 121.0, 116.4, 103.0, 101.6, 80.4, 80.3, 62.3, 60.9, 56.6, 55.2, 41.2, 36.2, 35.9, 28.6, 27.0. MS $[M+H]^+$ 774.3. *HPLC purity:* C18 column >99%, C4 column >99%. Anal. Calcd for $C_{40}H_{47}N_5O_9S{\cdot}1/2H_2O$: C, 61.36; H, 6.18; N, 8.95. Found: C, 61.22; H, 6.41; N, 8.70.

5.1.4.5. Compound 13. Prepared according to the general procedure described above using: Herrmann's palladacycle (2.9 mg, 0.0031 mmol), $[(t\text{Bu})_3\text{PH}]BF_4$ (1.6 mg, 0.0055 mmol), **6** (40 mg, 0.055 mmol), cyclopropylsulfonamide (33 mg, 0.27 mmol), $Mo(CO)_6$ (15 mg, 0.057 mmol), 1,4-dioxane (0.3 mL) and DBU (25 μL, 0.17 mmol). Purification by column chromatography (gradient elution, CH_2Cl_2:MeOH 98:2–95:5) followed by preparative HPLC–MS gave compound **13** (27 mg, 62%) as a white solid. ^{1}H NMR (CD_3OD): δ (10:1 mixture of rotamers, major rotamer reported) 8.26 (d, J = 9.2 Hz, 1H), 8.22 (dd, J = 1.0, 8.2 Hz, 1H), 8.07–8.05 (m, 2H), 7.89 (dd, J = 1.6, 7.9 Hz, 1H), 7.62–7.59 (m, 3H), 7.42–7.40 (m, 3H), 7.20 (dd, J = 2.4, 9.2 Hz, 1H), 7.12–7.08 (m, 1H), 6.51 (d, J = 8.9 Hz, NH), 5.68–5.67 (m, 1H), 4.77 (dd, J = 7.6, 10.1 Hz, 1H), 4.64 (dm, J = 12.1 Hz, 1H), 4.25–4.23 (m, 1H), 4.20 (dm, J = 12.1 Hz, 1H), 3.98 (s, 3H), 3.05–2.98 (m, 1H), 2.92 (ddm, J = 7.6, 14.0 Hz, 1H), 2.55 (ddd, J = 4.1, 10.1, 14.0 Hz, 1H), 1.24 (s, 9H), 1.12–1.08 (m, 2H), 1.04 (s, 9H), 0.95–0.90 (m, 2H). ^{13}C NMR (CD_3OD): δ (10:1 mixture of rotamers, major rotamer reported) 173.5, 172.2, 171.2, 165.2, 164.9, 159.4, 158.0, 147.3, 139.8, 136.9, 133.2, 132.3, 131.1, 130.3, 129.6, 125.7, 125.2, 124.4, 122.2, 120.7, 116.3, 103.4, 101.4, 80.3, 80.0, 62.2, 60.8, 56.5, 55.1, 36.2, 35.8, 31.5, 28.6, 27.0, 5.9. MS $[M + H]^+$ 800.3. *HPLC purity:* C18 column >99%, C4 column >99%. Anal. Calcd for $C_{42}H_{49}N_5O_9S{\cdot}H_2O$: C, 61.67; H, 6.28; N, 8.56. Found: C, 61.49; H, 6.35; N, 8.40.

5.1.4.6. Compound 14. Prepared according to the general procedure described above using: Herrmann's palladacycle (2.6 mg, 0.0028 mmol), $[(t\text{Bu})_3\text{PH}]BF_4$ (1.6 mg, 0.0055 mmol), **6** (40 mg, 0.055 mmol), α-toluenesulfonamide (47 mg, 0.27 mmol), $Mo(CO)_6$ (14 mg, 0.053 mmol), 1,4-dioxane (0.3 mL) and DBU (25 μL, 0.17 mmol). Purification by column chromatography (CH_2Cl_2:MeOH 95:5) followed by preparative HPLC–MS gave compound **14** (26 mg, 56%) as a white solid. ^{1}H NMR (CD_3OD): δ (10:1 mixture of rotamers, major rotamer reported) 8.31 (d, J = 9.2 Hz, 1H), 8.22 (dm, J = 8.0 Hz, 1H), 8.08–8.05 (m, 2H), 7.91 (dm, J = 7.9 Hz, 1H), 7.64–7.62 (m, 3H), 7.43–7.38 (m, 3H), 7.31–7.28 (m, 2H), 7.24 (dd, J = 2.5, 9.2 Hz, 1H), 7.22–7.18 (m, 3H), 7.10–7.06 (m, 1H), 6.48 (d, J = 8.8 Hz, NH), 5.60–5.58 (m, 1H), 4.68–4.58 (m, 4H), 4.23–4.18 (m, 2H), 3.99 (s, 3H), 2.71 (ddm, J = 7.5, 14.1 Hz, 1H), 2.32–2.25 (m, 1H), 1.23 (s, 9H), 1.03 (s, 9H). ^{13}C NMR (CD_3OD): δ (10:1 mixture of rotamers, major rotamer reported) 173.5, 173.0, 171.1, 166.0, 165.3, 159.1, 157.9, 146.2, 139.9, 135.9, 133.0, 132.7, 132.3, 131.9, 131.3, 130.4, 129.7, 129.4, 129.1, 126.1, 125.4, 124.2, 122.0, 121.0, 116.2, 102.5, 101.7, 80.4, 80.3, 62.2, 60.8, 58.8, 56.6, 55.0, 36.0, 35.8, 28.5, 27.0. MS $[M + H]^+$ 850.2. *HPLC purity:* C18 column >99%, C4 column >99%. Anal. Calcd for $C_{46}H_{51}N_5O_9S{\cdot}H_2O$: C, 63.65; H, 6.15; N, 8.07. Found: C, 63.44; H, 6.15; N, 7.90.

5.1.4.7. Compound 15. Prepared according to the general procedure described above using: Herrmann's palladacycle (2.2 mg, 0.0023 mmol), $[(t\text{Bu})_3\text{PH}]BF_4$ (1.4 mg, 0.0048 mmol), **6** (35 mg, 0.048 mmol), 4-methoxybenzenesulfonamide (45 mg, 0.24 mmol), $Mo(CO)_6$ (13 mg, 0.049 mmol), 1,4-dioxane (0.3 mL) and DBU (21 μL, 0.14 mmol). Filtration of the crude product through a short plug of silica gel (CH_2Cl_2:MeOH 95:5), purification by column chromatography (gradient elution, CH_2Cl_2:MeOH 97:3–95:5) followed by preparative HPLC–MS gave compound **15** (18 mg, 43%) as a white solid. ^{1}H NMR (CD_3OD): δ (10:1 mixture of rotamers, major rotamer reported) 8.27 (d, J = 9.3 Hz, 1H), 8.19 (dm, J = 8.2 Hz, 1H), 8.06–8.04 (m, 2H), 7.90–7.86 (m, 3H), 7.59–7.54 (m, 3H), 7.44 (s, 1H), 7.40–7.36 (m, 1H), 7.39 (d, J = 2.6 Hz, 1H), 7.19 (dd, J = 2.6, 9.3 Hz, 1H), 7.06 (ddm, J = 7.5, 7.7 Hz, 1H), 6.92–6.89 (m, 2H), 6.55 (d, J = 8.6 Hz, NH), 5.66–5.64 (m, 1H), 4.75 (dd, J = 7.7, 10.1 Hz, 1H), 4.64 (dm, J = 12.1 Hz, 1H), 4.23–4.21 (m, 1H), 4.15 (ddm, J = 3.2, 12.1 Hz, 1H), 3.95 (s, 3H), 3.74 (s, 3H), 2.90 (ddm, J = 7.7, 14.0 Hz, 1H), 2.45 (ddd, J = 4.1, 10.1, 14.0 Hz, 1H), 1.24 (s, 9H), 1.00 (s, 9H). ^{13}C NMR (CD_3OD): δ (10:1 mixture of rotamers, major rotamer reported) 172.7, 170.5, 170.4, 163.9, 163.4, 159.3, 157.0, 147.7, 139.3, 137.0, 133.7, 133.2, 131.5, 131.2, 130.6, 130.4, 129.7, 128.8, 124.8, 123.9, 123.1, 121.8, 120.1, 115.6, 114.2, 103.7, 100.4, 80.3, 78.7, 61.4, 59.7, 56.2, 55.9, 54.5, 35.6, 35.4, 28.5, 26.7. MS $[M+H]^+$ 866.3. *HPLC purity:* C18 column >99%, C4 column >99%. Anal. Calcd for $C_{46}H_{51}N_5O_{10}S{\cdot}H_2O$: C, 62.50; H, 6.04; N, 7.92. Found: C, 62.50; H, 6.00; N, 7.75.

5.1.4.8. Compound 16. Prepared according to the general procedure described above using: Herrmann's palladacycle (2.2 mg, 0.0023 mmol), $[(t\text{Bu})_3\text{PH}]BF_4$ (1.4 mg, 0.0048 mmol), **6** (35 mg, 0.048 mmol), 4-(trifluoromethyl)benzenesulfonamide (54 mg, 0.24 mmol), $Mo(CO)_6$ (13 mg, 0.049 mmol), 1,4-dioxane (0.3 mL) and DBU (21 μL, 0.14 mmol). The crude product was diluted in a total of 15 mL CH_2Cl_2 and thereafter washed with aqueous NaOAc buffer (pH 4, 3 × 10 mL)

and brine (10 mL). The organic layer was dried ($MgSO_4$), filtered and evaporated. Purification by column chromatography (EtOAc:*i*-hexane:MeOH 48:48:4) gave compound **16** (18 mg, 42%) as a white solid. ^{1}H NMR (CD_3OD): δ (10:1 mixture of rotamers, major rotamer reported) 8.38 (ddm, J = 1.1, 8.4 Hz, 1H), 8.17 (d, J = 9.2 Hz, 1H), 8.13–8.11 (m, 2H), 8.08–8.02 (m, 3H), 7.72–7.69 (m, 2H), 7.55–7.47 (m, 3H), 7.41–7.35 (m, 2H), 7.26 (s, 1H), 7.09 (dd, J = 2.6, 9.2 Hz, 1H), 7.05–7.00 (m, 1H), 6.48 (d, J = 9.0 Hz, NH), 5.57–5.55 (m, 1H), 4.78 (dd, J = 7.8, 9.9 Hz, 1H), 4.60 (dm, J = 11.9 Hz, 1H), 4.29–4.23 (m, 2H), 3.94 (s, 3H), 2.90 (ddm, J = 7.8, 14.1 Hz, 1H), 2.55 (ddd, J = 3.8, 9.9, 14.1 Hz, 1H), 1.22 (s, 9H), 0.99 (s, 9H). ^{13}C NMR (CD_3OD): δ (10:1 mixture of rotamers, major rotamer reported) 174.4, 173.6, 171.5, 163.4, 162.5, 161.2, 157.9, 151.6, 149.1, 140.9, 140.7, 133.7 (q, $^2J_{CF}$ = 32 Hz), 132.9, 132.1, 130.7, 129.8, 129.2, 128.9, 126.5 (q, $^3J_{CF}$ = 4 Hz), 125.2, 125.2 (q, $^1J_{CF}$ = 271 Hz), 124.8, 123.8, 121.4, 119.5, 116.5, 106.8, 100.3, 80.5, 78.5, 62.8, 60.5, 56.0, 55.2, 36.4, 36.0, 28.5, 26.9. MS $[M+H]^+$ 904.2. *HPLC purity:* C18 column >99%, C4 column >99%. Anal. Calcd for $C_{46}H_{48}F_3N_5O_9S{\cdot}3H_2O$: C, 57.67; H, 5.68; N, 7.31. Found: C, 57.02; H, 5.30; N, 6.91.

5.1.4.9. Compound 17. Prepared according to the general procedure described above using: Herrmann's palladacycle (2.6 mg, 0.0028 mmol), $[(t\mathrm{Bu})_3\mathrm{PH}]BF_4$ (1.6 mg, 0.0055 mmol), **6** (40 mg, 0.055 mmol), 2-thiophenesulfonamide (45 mg, 0.28 mmol), $Mo(CO)_6$ (14 mg, 0.053 mmol), 1,4-dioxane (0.3 mL) and DBU (25 μL, 0.17 mmol). The crude product was diluted in a total of 15 mL CH_2Cl_2 and thereafter washed with aqueous NaOAc buffer (pH 4, 3 × 10 mL) and brine (10 mL). The organic layer was dried ($MgSO_4$), filtered and evaporated. Repeated purification by column chromatography (gradient elution, EtOAc:*i*-hexane:MeOH 48:48:4–47.5:47.5:5 followed by CH_2Cl_2:MeOH 97:3–96:4) gave compound **17** (17 mg, 37%) as a white solid. ^{1}H NMR (CD_3OD): δ (10:1 mixture of rotamers, major rotamer reported) 8.36 (dd, J = 1.3, 8.4 Hz, 1H), 8.16 (d, J = 9.2 Hz, 1H), 8.09 (dd, J = 1.6, 7.9 Hz, 1H), 8.07–8.03 (m, 2H), 7.63 (dd, J = 1.4, 3.7 Hz, 1H), 7.55–7.47 (m, 3H), 7.40–7.36 (m, 2H), 7.37 (d, J = 2.5 Hz, 1H), 7.21 (s, 1H), 7.09 (dd, J = 2.5, 9.2 Hz, 1H), 7.02 (ddd, J = 1.3, 7.4, 7.9 Hz, 1H), 6.87 (dd, J = 3.7, 4.9 Hz, 1H), 5.51–5.49 (m, 1H), 4.73 (dd, J = 8.0, 9.8 Hz, 1H), 4.58 (dm, J = 11.7 Hz, 1H), 4.29 (s, 1H), 4.25 (dm, J = 11.7 Hz, 1H), 3.93 (s, 3H), 2.81 (ddm, J = 8.0, 14.1 Hz, 1H), 2.51 (ddd, J = 4.1, 9.8, 14.1 Hz, 1H), 1.25 (s, 9H), 0.98 (s, 9H). ^{13}C NMR (CD_3OD): δ (10:1 mixture of rotamers, major rotamer reported) 174.4, 173.5, 171.5, 163.3, 162.3, 161.3, 157.9, 151.9, 146.3, 141.1, 140.5, 133.0, 132.2, 131.7, 131.2, 130.7, 129.8, 129.2, 127.5, 125.3, 124.8, 123.9, 121.5, 119.4, 116.5, 107.0, 100.2, 80.5, 78.4, 62.7, 60.5, 56.0, 55.3, 36.3, 36.1, 28.6, 26.9. MS $[M+H]^+$ 842.2. *HPLC purity:* C18 column >99%, C4 column >99%. Anal. Calcd for $C_{43}H_{47}N_5O_9S_2{\cdot}3H_2O$: C, 57.64; H, 5.96; N, 7.82. Found: C, 57.99; H, 5.19; N, 7.47.

5.1.4.10. Compound 20. Prepared according to the general procedure described above using: Herrmann's palladacycle (2.2 mg, 0.0023 mmol), $[(t\mathrm{Bu})_3\mathrm{PH}]BF_4$ (1.4 mg, 0.0048 mmol), **18** (35 mg, 0.047 mmol), benzenesulfonamide (37 mg, 0.24 mmol), $Mo(CO)_6$ (13 mg, 0.049 mmol), 1,4-dioxane (0.3 mL) and DBU (21 μL, 0.14 mmol). The crude product was diluted in a total of 15 mL CH_2Cl_2 and thereafter washed with aqueous NaOAc buffer (pH 4, 3 × 10 mL) and brine (10 mL). The organic layer was dried ($MgSO_4$), filtered and evaporated. Repeated purification by column chromatography (EtOAc:*i*-hexane:MeOH 48:48:4 followed by CH_2Cl_2:MeOH 97:3) gave compound **20** (18 mg, 45%) as a white solid. ^{1}H NMR (CD_3OD): δ (9:1 mixture of rotamers, major rotamer reported) 8.14 (d, J = 9.2 Hz, 1H), 8.06–8.04 (m, 2H), 7.91–7.88 (m, 2H), 7.58–7.50 (m, 4H), 7.40 (d, J = 2.5 Hz, 1H), 7.39–7.34 (m, 3H), 7.22 (dm, J = 7.6 Hz, 1H), 7.12 (s, 1H), 7.07 (dd, J = 2.5, 9.2 Hz, 1H), 7.00 (t, J = 7.6 Hz, 1H), 5.41–5.39 (m, 1H), 4.76 (t, J = 8.1 Hz, 1H), 4.47 (dm, J = 12.0 Hz, 1H), 4.36 (s, 1H), 4.15 (dm, J = 12.0 Hz, 1H), 3.96 (s, 3H), 2.38–2.24 (m, 2H), 2.07 (s, 3H), 1.30 (s, 9H), 1.00 (s, 9H). ^{13}C NMR (CD_3OD): δ (9:1 mixture of rotamers, major rotamer reported) 175.4, 173.7, 171.8, 163.3, 162.3, 161.2, 157.8, 152.0, 144.3, 141.2, 137.2, 135.1, 134.6, 134.2, 133.0, 130.7, 129.9, 129.6, 129.2, 128.7, 127.7, 127.2, 124.8, 119.5, 116.4, 107.2, 100.2, 80.8, 78.1, 61.0, 60.5, 56.0, 55.4, 36.5, 36.0, 28.6, 27.0, 18.8. MS $[M + H]^+$ 850.2. *HPLC purity:* C18 column 98%, C4 column 98%. Anal. Calcd for $C_{46}H_{51}N_5O_9S{\cdot}2H_2O$: C, 62.36; H, 6.26; N, 7.90. Found: C, 62.22; H, 6.10; N, 7.34.

5.1.4.11. Compound 21. Prepared according to the general procedure described above using: Herrmann's palladacycle (2.2 mg, 0.0023 mmol), $[(t\mathrm{Bu})_3\mathrm{PH}]BF_4$ (1.4 mg, 0.0048 mmol), **19** (38 mg, 0.048 mmol), benzenesulfonamide (37 mg, 0.24 mmol), $Mo(CO)_6$ (13 mg, 0.049 mmol), 1,4-dioxane (0.3 mL) and DBU (21 μL, 0.14 mmol). The crude product was diluted in a total of 15 mL CH_2Cl_2 and thereafter washed with aqueous NaOAc buffer (pH 4, 3 × 10 mL) and brine (10 mL). The organic layer was dried ($MgSO_4$), filtered and evaporated. Purification by column chromatography (EtOAc:*i*-hexane:MeOH 48:48:4) followed by preparative HPLC–MS gave compound **21** (15 mg, 35%) as a white solid. ^{1}H NMR (CD_3OD:$CDCl_3$ 19:1): δ (9:1 mixture of rotamers, major rotamer reported) 8.62 (m, 1H), 8.41 (d, J = 9.3 Hz, 1H), 8.07–8.04 (m, 3H), 7.96–7.93 (m, 2H), 7.69–7.62 (m, 3H), 7.61 (s, 1H), 7.50–7.44 (m, 2H), 7.43–7.38 (m, 2H), 7.31 (dd, J = 2.4, 9.3 Hz, 1H), 7.31–7.28 (m, 1H), 5.82–5.81 (m, 1H), 4.79 (dd, J = 7.4, 10.4 Hz, 1H), 4.73 (dm, J = 12.3 Hz, 1H), 4.22 (s, 1H), 4.22 (dm, J = 12.3 Hz, 1H), 4.01 (s, 3H), 3.04 (ddm, J = 7.4, 14.2 Hz, 1H), 2.53 (ddd, J = 3.8, 10.4, 14.2 Hz, 1H), 1.26 (s, 9H), 1.05 (s, 9H). ^{13}C NMR (CD_3OD:$CDCl_3$ 19:1): δ (9:1 mixture of rotamers, major rotamer reported) 173.6, 171.0, 170.8, 167.5, 166.2, 158.2, 158.0, 144.1, 143.9, 140.5, 134.1 (q, $^2J_{CF}$ = 32 Hz), 133.7, 133.5, 132.7, 132.2, 130.6, 129.8, 129.3, 128.4, 128.3, 126.6, 125.0 (q, $^1J_{CF}$ = 272 Hz), 121.7, 120.1 (q, $^3J_{CF}$ = 4 Hz), 118.3 (q, $^3J_{CF}$ = 4 Hz), 116.2, 102.4, 100.6, 81.2, 80.3, 62.1, 60.8, 56.8, 55.0, 36.1, 35.7, 28.6, 27.0. MS $[M+H]^+$ 904.2. *HPLC purity:* C18 column 98%, C4 column >99%. Anal. Calcd for

$C_{46}H_{48}F_3N_5O_9S{\cdot}11/2H_2O$: C, 59.34; H, 5.52; N, 7.52. Found: C, 59.61; H, 5.48; N, 6.97.

5.1.5. General procedure for the synthesis of compounds 18 and 19. A mixture of **1**, pyridine and substituted 2-bromoaniline was cooled to −15 °C whereafter $POCl_3$ was added dropwise over approximately 1 min. The resulting mixture was stirred at −15 °C for 45 min and thereafter at room temperature for 1 h. The mixture was cooled to −15 °C and another portion of $POCl_3$ was added. The mixture was stirred at −15 °C for 30 min and then allowed to reach room temperature. H_2O was added and the mixture was thereafter extracted repeatedly with EtOAc. The combined organic layer was washed with brine, dried ($MgSO_4$), filtered and evaporated. Purification by column chromatography (EtOAc:*i*-hexane 2:3) gave the desired compounds **18** and **19**.

5.1.5.1. Compound 18. Prepared according to the general procedure described above using: **1** (45 mg, 0.078 mmol), pyridine (0.25 mL), 2-bromo-6-methylaniline (10 μL, 0.079 mmol) and $POCl_3$ (8 μL + 4 μL, 0.088 mmol + 0.044 mmol). Compound **18** (44 mg, 76%) was obtained as a white solid. ^{1}H NMR (CD_3OD): δ 8.12 (d, J = 9.2 Hz, 1H), 8.07–8.05 (m, 2H), 7.56–7.47 (m, 3H), 7.43 (dm, J = 7.8 Hz, 1H), 7.38 (d, J = 2.5 Hz, 1H), 7.23 (s, 1H), 7.21 (dm, J = 7.8 Hz, 1H), 7.08 (t, J = 7.8 Hz, 1H), 7.06 (dd, J = 2.5, 9.2 Hz, 1H), 6.39 (d, J = 8.9 Hz, NH), 5.52–5.50 (m, 1H), 4.93 (dd, J = 7.6, 9.8 Hz, 1H), 4.58 (dm, J = 11.7 Hz, 1H), 4.27–4.25 (m, 1H), 4.04 (dm, J = 11.7 Hz, 1H), 3.93 (s, 3H), 2.83 (ddm, J = 7.6, 14.0 Hz, 1H), 2.51 (ddd, J = 4.1, 9.8, 14.0 Hz, 1H), 2.32 (s, 3H), 1.30 (s, 9H), 1.04 (s, 9H). ^{13}C NMR (CD_3OD): δ 173.4, 172.6, 163.1, 162.0, 161.3, 157.9, 152.2, 141.3, 140.5, 135.4, 131.4, 130.8, 130.6, 129.9, 129.8, 129.0, 124.4, 123.9, 119.3, 116.5, 107.3, 100.0, 80.5, 78.2, 60.7, 60.4, 56.0, 55.3, 36.2, 36.0, 28.6, 26.9, 19.3. MS $[M + H]^+$ 745.2, 747.2. *HPLC purity:* C18 column >99%, C4 column >99%. Anal. Calcd for $C_{39}H_{45}BrN_4O_6$: C, 62.82; H, 6.08; N, 7.51. Found: C, 62.98; H, 6.32; N, 6.88.

5.1.5.2. Compound 19. Prepared according to the general procedure described above using: **1** (45 mg, 0.078 mmol), pyridine (0.25 mL), 2-bromo-5-(trifluoromethyl)aniline (11 μL, 0.077 mmol) and $POCl_3$ (8 μL + 4 μL, 0.088 mmol + 0.044 mmol). Compound **19** (55 mg, 88%) was obtained as a white solid. ^{1}H NMR (CD_3OD): δ (10:1 mixture of rotamers, major rotamer reported) 8.19 (d, J = 2.3 Hz, 1H), 8.10 (d, J = 9.1 Hz, 1H), 8.05–8.03 (m, 2H), 7.77 (d, J = 8.4 Hz, 1H), 7.54–7.47 (m, 3H), 7.38 (d, J = 2.4 Hz, 1H), 7.35 (dd, J = 2.3, 8.4 Hz, 1H), 7.19 (s, 1H), 7.04 (dd, J = 2.4, 9.1 Hz, 1H), 6.50 (d, J = 9.0 Hz, NH), 5.49–5.47 (m, 1H), 4.97 (dd, J = 7.7, 9.5 Hz, 1H), 4.58 (dm, J = 11.8 Hz, 1H), 4.30–4.27 (m, 1H), 4.01 (ddm, J = 3.5, 11.8 Hz, 1H), 3.92 (s, 3H), 2.77 (ddm, J = 7.7, 14.0 Hz, 1H), 2.53 (ddd, J = 4.2, 9.5, 14.0 Hz, 1H), 1.30 (s, 9H), 1.04 (s, 9H). ^{13}C NMR (CD_3OD): δ (10:1 mixture of rotamers, major rotamer reported) 173.6, 172.3, 163.1, 161.9, 161.2, 157.9, 152.2, 141.3, 138.1, 134.9, 131.3 (q, $^2J_{CF}$ = 33 Hz), 130.6, 129.8, 129.0, 125.1 (q, $^1J_{CF}$ = 271 Hz), 124.4, 124.1 (q, $^3J_{CF}$ = 4 Hz), 123.3 (q, $^3J_{CF}$ = 4 Hz), 121.9, 119.3, 116.5, 107.4, 99.9, 80.5, 78.1, 60.8, 60.7, 56.0, 55.3, 36.0, 35.6, 28.6, 26.9. MS $[M+H]^+$ 799.2, 801.2. *HPLC purity:* C18 column >99%, C4 column >99%. Anal. Calcd for $C_{39}H_{42}BrF_3N_4O_6$: C, 58.58; H, 5.29; N, 7.01. Found: C, 59.14; H, 5.57; N, 6.42.

5.2. Enzyme inhibition

The protease activity of the full-length HCV NS3 protein (protease-helicase/NTPase) was measured using a FRET-assay as previously described.[32,41] In short, 1 nM enzyme was incubated for 10 minutes 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 (KKGSVVIVGRIV LSGK) 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). The non-linear regression analysis was made using Grafit 5.0.8 (Erithacus Software Limited).

5.3. Computational methodology

The FLO+ docking suite[42] was used for all flexible docking calculations. FLO+ was used partially due to the fact that it allows for protein flexibility, which is a more accurate representation of protein-ligand interactions.[43] The active site used for docking was developed using the NS3 protease/helicase crystal structure (PDB code 1CU1). Following constrained minimization, the active site was formed by extracting all residues within 9 Å of the last eleven residues of the C-terminus. This active site has been used previously[36,37,39] and greater detail regarding its derivation has been published previously.[36]

The docking protocol used herein relied on conformational analysis (limited Monte Carlo perturbation) followed by simulated annealing. To retain the conformation obtained using Monte Carlo, an energy penalty of 20 kJ/(molÅ^2) was applied in the simulated annealing step when the similarity distance between two sequential conformations differed by more than 0.2 Å. The FLO+ software suite allows control over the flexibility of particular residues in the active site and Arg155 and Lys136 were allowed full conformational freedom without energy penalty. However, movement of all other active site residues by more than 0.2 Å was penalized by 20 kJ/(molÅ^2). Docking in the NS3 protease/helicase is complicated by the shallow and featureless nature of the protein. Therefore, it was necessary to add several zero-order bonds to hold the ligand in the general vicinity of the catalytic region in the active site.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2007.12.041.

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