JOC
The Journal of Organic Chemistry

VOLUME 66, NUMBER 14 JULY 13, 2001

Articles

Solid-Phase Synthesis of Peptidomimetic Inhibitors for the Hepatitis C Virus NS3 Protease

Marc-André Poupart, Dale R. Cameron, Catherine Chabot, Elise Ghiro, Nathalie Goudreau, Sylvie Goulet, Martin Poirier, and Youla S. Tsantrizos*

Department of Chemistry, Boehringer Ingelheim (Canada) Ltd., Quebec H7S 2G5, Canada

ytsantrizos@lav.boehringer-ingelheim.com

Received February 13, 2001

The NS3 serine protease enzyme of the hepatitis C virus (HCV) is essential for viral replication. Short peptides mimicking the N-terminal substrate cleavage products of the NS3 protease are known to act as weak inhibitors of the enzyme and have been used as templates for the design of peptidomimetic inhibitors. Automated solid-phase synthesis of a small library of compounds based on such a peptidomimetic scaffold has led to the identification of potent and highly selective inhibitors of the NS3 protease enzyme.

Introduction

Hepatitis C virus (HCV) infection is an important cause of chronic hepatitis, cirrhosis of the liver, and hepatocellular carcinoma in humans. An alarmingly large portion of the world population (an estimated 170 million people) are currently infected with HCV, and of those infected, approximately 20% and 4% are likely to develop cirrhosis of the liver and liver cancer (respectively) within the next decade.[1] Unfortunately, current treatments with interferon α-2b alone or in combination with ribavirin are only effective in a limited number of cases.[2] Thus, given the prevalence of hepatitis C viral infections, the development of highly effective antiviral agents is currently the focus of intensive research.

Since the discovery and cloning of the HCV genome, its ~9.6 kb positive-sense single-stranded RNA has been shown to contain a single open reading frame (ORF) encoding a polyprotein of ~3011 amino acids.[3] The ORF is flanked at the 5′-end by a nontranslated region that serves as the internal ribosome entry site (IRES) required for internal initiation of translation and at the 3′-end by a highly conserved sequence essential for genome replication. The N-terminal region of the translation product of the ORF is comprised of three structural proteins, C, E1, and E2, whereas the remaining polyprotein contains six nonstructural proteins, the NS2, NS3, NS4A, NS4B, NS5A, and NS5B proteins. The three structural proteins are released by cleavage of the polyprotein by host enzymes, whereas the nonstructural proteins are cleaved by the NS2 and NS3 virally encoded proteases. Recently, the NS3 protease was shown to be essential for viral replication in vivo, thus validating this chymotrypsin-like enzyme as an important target for drug design.[4]

In vivo, the NS3 protein exists as a heterodimer with the NS4A polypeptide. The latter is a 54-residue polypep-

* To whom correspondence should be addressed. Tel: (450) 682-4640. Fax: (450) 682-4189.

(1) (a) Di Bisceglie, A. M. *Lancet* **1998**, *351*, 351. (b) Brown, J. L. *Lancet* **1998**, *351*, 78.

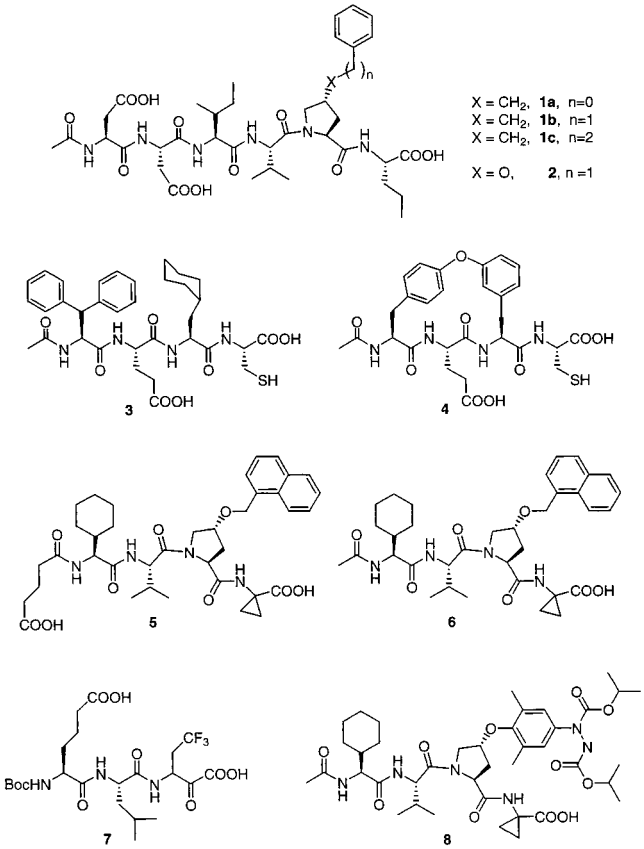
(2) Reichard, O.; Norkrans, G.; Frydén, A.; Braconier, J.-H.; Sönnerborg, A.; Weiland, O. *Lancet* **1998**, *351*, 83.

(3) Choo, Q.-L.; Kuo, G.; Weiner, A. J.; Overby, L. R.; Bradley, D. W.; Houghton, M. *Science* **1989**, *244*, 359.

(4) Kolykhalov, A. A.; Mihalik, K.; Feinstone, S. M.; Rice, C. M. *J. Virol.* **2000**, *74*, 2046.

10.1021/jo010164d CCC: $20.00
Published on Web 06/16/2001

Chart 1



tide that interacts with the protease domain of NS3 via a hydrophobic 12-residue sequence in the center of the NS4A. The NS4A cofactor is absolutely essential for cleavage at the NS4A−NS4B and NS4B−NS5A junctions, and it enhances the activity of the NS3 at all other sites.[5] Recently, peptides corresponding to N-terminal cleavage products of the NS3 substrates were found to be competitive inhibitors of this protease.[6] Sequence optimization of these peptides has led to the identification of analogues that can be used as leads toward the development of antiviral therapeutics. The structure−activity relationship of peptidomimetics, such as compounds **1**−**7** (Chart 1), was recently reported by our coworkers, as well as others.[7] The interactions between these types of inhibitors and the NS3 protease domain have been investigated by NMR and molecular modeling,[8] as well as by crystallography.[9]

In this paper, we present the solid-phase synthesis of a small library of HCV protease inhibitors based on the backbone of peptide **6**. The library design of these compounds was guided by computational studies and was focused on the optimization of the 4-hydroxyproline moiety at the P2 position.[10] Computational analysis of compounds from a subset of this library revealed a correlation between the electrostatic potential (ESP) of the aromatic moieties at P2 and the potency of these inhibitors; this observation was helpful in the selection of the library building blocks.

Results and Discussion

Recently, we reported that the attachment of an aromatic substituent to the C4 of the proline unit via a short linker, as in compounds **1a**−**c**, **2**, **5**, and **6**, leads to a significant increase in the potency of the inhibitors in each corresponding series.[11] Among the derivatives of compound **1**, those having longer methylene linkers between the proline backbone and the aromatic ring were found to exhibit enhanced activity (IC_{50} values of **1a**, **1b**, and **1c** are 60, 10, and 3 μM, respectively),[11] suggesting the existence of a lipophilic pocket at a distance from the binding site of the backbone. To further explore this pocket and optimize the interactions with our inhibitors, a synthetic protocol was established to produce a focused library of tetrapeptides having structural diversity at the aromatic substituent linked to the proline moiety.

In recent years, combinatorial or parallel solid-phase synthesis has become an important tool for the rapid optimization of chemical leads in drug discovery programs. Numerous synthetic methodologies have been adapted to solid-phase synthesis, including cross-coupling reactions under Mitsunobu[12] and Suzuki conditions.[13] During our investigations into novel HCV protease inhibitors, we utilized both of these reactions to prepare a library of inhibitors with structural diversity at the aromatic substituent of the 4-hydroxyproline moiety (Table 1).

(5) Bartenschlager, R. *J. Viral Hepat.* **1999**, *6*, 165.

(6) (a) Llinàs-Brunet, M.; Bailey, M.; Fazal, G.; Goulet, S.; Halmos, T.; LaPlante, S.; Maurice, R.; Poirier, M.; Poupart, M.-A.; Thibeault, D.; Wernic, D.; Lamarre, D. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1713. (b) Steinkühler, C.; Biasiol, G.; Brunetti, M.; Urbani, A.; Koch, U.; Cortese, R.; Pessi, A.; De Francesco, R. *Biochemistry* **1998**, *37*, 8899.

(7) (a) Llinàs-Brunet, M.; Bailey, M.; Déziel, R.; Fazal, G.; Gorys, V.; Goulet, S.; Halmos, T.; Maurice, R.; Poirier, M.; Poupart, M.-A.; Rancourt, J.; Thibeault, D.; Wernic, D.; Lamarre, D. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2719. (b) Ingallinella, P.; Altamura, S.; Bianchi, E.; Taliani, M.; Ingenito, R.; Cortese, R.; De Francesco, R.; Steinkühler, C.; Pessi, A. *Biochemistry* **1998**, *37*, 8906.

(8) (a) LaPlante, S. R.; Cameron, D. R.; Aubry, N.; Lefebvre, S.; Kukolj, G.; Maurice, R.; Thibeault, D.; Lamarre, D.; Llinàs-Brunet, M. *J. Biol. Chem.* **1999**, *274*, 18618. (b) Cicero, D. O.; Barbato, G.; Koch, U.; Ingallinella, P.; Bianchi, E.; Nardi, M. C.; Steinkühler, C.; Cortese, R.; Matassa, V.; De Francesco, R.; Pessi, A.; Bazzo, R. *J. Mol. Biol.* **1999**, *289*, 385. **(c)** Barbato, G.; Cicero, D. O.; Cordier, F.; Narjes, F.; Gerlach, B.; Sambucini, S.; Grzesiek, S.; Matassa, V. G.; De Francesco, R.; Pessi, A.; Bazzo, R *EMBO J.* **2000**, *19*, 1195. (d) LaPlante, S. R.; Aubry, N.; Bonneau, P. R.; Kukolj, G.; Lamarre, D.; Lefebvre, S.; Li, H.; Llinàs-Brunet, M.; Plouffe C.; Cameron, D. R. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2271.

(9) Di Marco, S.; Rizzi, M.; Volpari, C.; Walsh, M. A.; Narjes, F.; Colarusso, S.; De Francesco, R.; Matassa, V. G.; Sollazzo, M. *J. Biol. Chem.* **2000**, *275*, 7152.

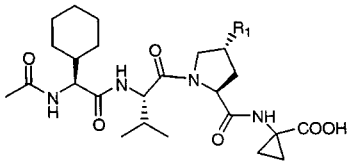
(10) Terminology of protease specificity: (a) Schechter, I.; Berger, A. *Biochem. Biophys. Res. Commun.* **1967**, *27*, 157. (b) Berger, A.; Schechter, I. *Philos. Trans. R. Soc. London* **1970**, B257, 249.

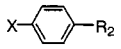
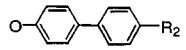
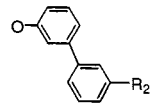
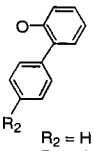
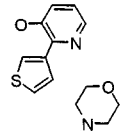
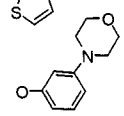
(11) Llinàs-Brunet, M.; Bailey, M.; Fazal, G.; Ghiro, E.; Gorys, V.; Goulet, S.; Halmos, T.; Maurice, R.; Poirier, M.; Poupart, M.-A.; Rancourt, J.; Thibeault, D.; Wernic, D.; Lamarre, D. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2267.

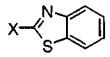
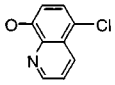
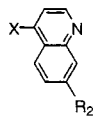
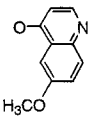
(12) (a) Rano, A. T.; Chapman, K. T. *Tetrahedron Lett.* **1995**, *36*, 3789. (b) Krchnák, V.; Flegelová, Z.; Weichsel, A. S.; Lebl, M. *Tetrahedron Lett.* **1995**, *36*, 6193. (c) Devraj, R.; Cushman, M. *J. Org. Chem.* **1996**, *61*, 9368. (d) Boeijen, A.; Kruijtzer, J. A. W.; Liskamp, R. M. J. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2375. (e) Greenlee, M. L.; Laub, J. B.; Balkovec, J. M.; Hammond, M. L.; Hammond, G. G.; Pompliano, D. L.; Epstein-Toney, J. H. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2549. (f) Kung, P.-P.; Swayze, E. *Tetrahedron Lett.* **1999**, *40*, 5651. (g) Chaturvedi, S.; Otteson, K.; Bergot, J. *Tetrahedron Lett.* **1999**, *40*, 8205. (h) Johnson, M. G.; Bronson, D. D.; Gillespie, J. E.; Gifford-Moore, D. S.; Kalter, K.; Lynch, M. P.; McCowan, J. R.; Redick, C. C.; Sall, D. J.; Smith, G. F.; Foglesong, R. J. *Tetrahedron* **1999**, *55*, 11641. (i) Barber, A. M.; Hardcastle, I. R.; Rowlands, M. G.; Nutley, B. P.; Marriott, J. H.; Jarman, M. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 623. (j) Zaragoza, F.; Stephensen, H. *Tetrahedron Lett.* **2000**, *41*, 1841. (k) Barrero, A. F.; Alvarez-Manzaneda, E. J.; Chahboun, R. *Tetrahedron Lett.* **2000**, *41*, 1959.

(13) (a) Frenette, R.; Friesen, R. W. *Tetrahedron Lett.* **1994**, *35*, 9177. (b) Guiles, J. W.; Johnson, S. G.; Murray, W. V. *J. Org. Chem.* **1996**, *61*, 5169. (c) Larhed, M.; Lindeberg, G.; Hallberg, A. *Tetrahedron Lett.* **1996**, *37*, 8219. (d) Piettre, S. R.; Baltzer, S. *Tetrahedron Lett.* **1997**, *38*, 1197. (e) Jang, S.-B. *Tetrahedron Lett.* **1997**, *38*, 1793. (f) Han, Y.; Giroux, A.; Lépine, C.; Laliberté, F.; Huang, Z.; Perrier, H.; Bayly, C. I.; Young, R. N. *Tetrahedron* **1999**, *55*, 11669.

Table 1. Inhibitors of the Hepatitis C NS3 Protease Prepared by Solid-Phase Synthesis



Compound	R_1	% Product[a] (in rxn mixture)	IC_{50}[b] (μM)
10	OH	60%	>1000
			
11	X = O, R_2 = H	45%	80
12	X = O, R_2 = F	50%	100
13	X = O, R_2 = Cl	45%	50
14	X = O, R_2 = Br	67%	25
15	X = O, R_2 = I	74%	10
16	X = S, R_2 = Br	67%	40
17		53%	50
18		82%	25
			
19	R = H	30%	35
20	R = OCH_3	23%	35
			
21	R_2 = H	23%	~6
22	R_2 = NO_2	58%	
23	R_2 = $NHCOCH_3$	70%	
			
24	R_2 = H	44%	>200
25	R_2 = OCH_3	52%	
26	R_2 = COOH	10%	
27		40%	>200
28		23%	32

Compound	R_1	% Product[a] (in rxn mixture)	IC_{50}[b] (μM)
			
29	X = O	60%	~20
30	X = S	77%	
			
31	X = N, Y = CH	26%	170
32	X = CH, Y = N	16%	190
33		21%	250
34		50%	100
35		41%	8
36		23%	4
			
37	X = O, R_2 = H	75%	5
38	X = O, R_2 = CF_3	57%	6
39	X = S, R_2 = CF_3	61%	9
40	X = O, R_2 = Cl	39%	2
41	X = O, R_2 = OCH_3	nd	0.8
42		nd	16

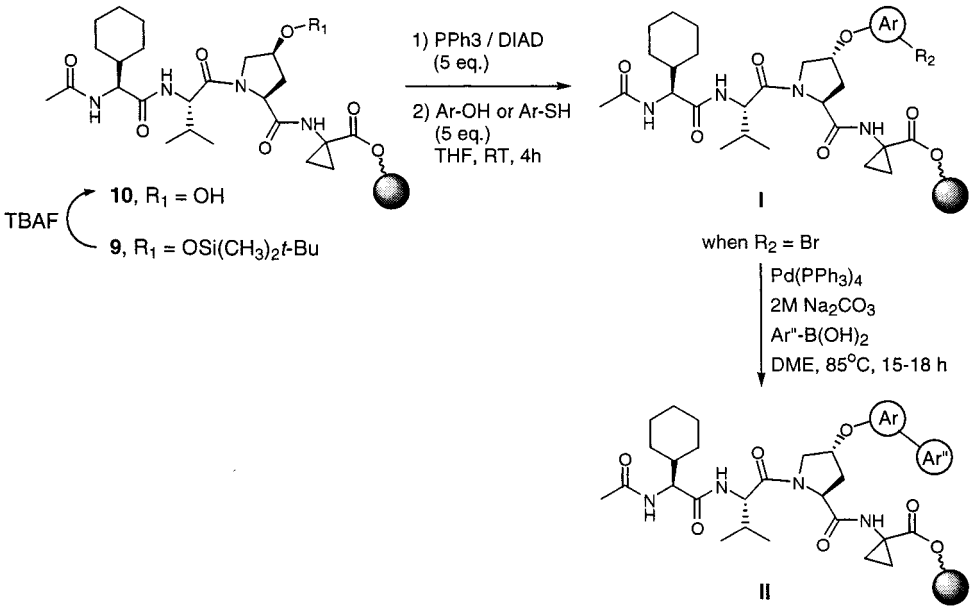
[a] As determined by analytical reversed-phase HPLC of the crude product (λ = 220 nm); nd = not determined. [b] IC_{50} values are the average of multiple tesings of the pure compounds (homogeneity >85%).

Automated peptide chemistry was used to synthesize the polymer-bound tetrapeptide scaffold **10**, having the *S* absolute stereochemistry at the C4 of proline, as the TBDMS protected polymer-bound silyl derivative **9** (Scheme 1).[14] Each coupling step was carried out using 2 equiv of Fmoc-protected amino acid, HOBt, and TBTU in the presence of 4 equiv of base. Upon completion of all the peptide elongation steps, the N-terminus was capped with acetic acid and the TBDMS group was removed with tetrabutylammonium fluoride. The resin-bound peptide was subsequently dried under vacuum, and small aliquots (120 mg) were loaded into the 96-well block of an Advanced ChemTech 396 synthesizer for production of the Mitsunobu library (Table 1).

The polymer was first washed with anhydrous CH_2Cl_2 and THF to ensure proper swelling of the resin before the reagents were added. Aliquots of anhydrous THF solutions containing Ph_3P, diisopropyl azodicarboxylate (DIAD), and each of the building blocks were added sequentially to the reaction wells, and the reaction block was shaken vigorously for 4 h (4 × 1 h), with a 10 min delay period after each hour. A variety of commercially available building blocks were used including phenols (e.g., **11**−**15**, **17**−**18**), thiophenols (e.g., **16**), hydroxy- and

(14) Commercially available *cis*-hydroxy proline was N-protected as the Fmoc derivative and then persilylated with *tert*-butyldimethylsilyl chloride in the presence of imidazole/DMAP. *In situ* hydrolysis of the silyl-ester moiety was achieved during workup with $Na_2S_2O_5$ to obtain the 4-*tert*-butyldimethylsilyloxy proline building block required for the solid-phase synthesis of the polymer-bound tetrapeptide; Perich, J. W.; Reynolds, E. C. *Synlett* **1991**, 577.

Scheme 1. Library Synthesis on Solid Support



mercaptobenzothiazoles (e.g., **29** and **30**), hydroxypyridines (**31**, **32**), hydroxyquinolines (e.g., **37**, **38**, **40**−**42**), hydroxyisoquinolines (e.g., **33**−**36**), and mercaptoquinolines (e.g., **39**) as shown in Table 1.

At the end of the reaction period, each polymer-bound product (Scheme 1, peptide **I**) was washed thoroughly with different solvents, dried under vacuum, and treated with 40% trifluoroacetic acid in CH_2Cl_2 to cleave the final peptide from the resin. Analysis of the crude products by reversed-phase HPLC provided an estimate of the purity and yield for the overall synthesis of each compound (Table 1). In most cases, the desired product represented the main component of the crude material with some unreacted tetrapeptide **10** as the major contaminant. Interestingly, a diisopropyl azodicarboxylate adduct was occasionally detected by LC MS as a minor side product; however, in a few exceptional cases the DIAD adduct was the only isolated product of the reaction (e.g., compound **8** was the only product formed with 2,6-dimethylphenol). Although these azodicarboxylate adducts are unusual as side products of the Mitsunobu reaction, their formation by electrophilic aromatic substitution has been previously reported.[15]

The structural diversity of our library was further enriched by the creation of biaryl systems (e.g., peptides **20**−**27**) via a Suzuki cross-coupling reaction between commercially available phenyl and thiophene boronic acids and the bromophenoxy derivatives **14**, **17**, and **18**. The successful synthesis of the polymer-bound precursor peptides **14**, **17**, and **18** was monitored by magic-angle spinning ^{1}H NMR using a small aliquot of resin (typically a 40 μL volume of pre-swollen resin in CD_2Cl_2).[16] All of the Suzuki reactions were carried out in small, screw-cap pressure vials equipped with a magnetic stir bar. Each aliquot of the polymer-bound peptides **I** was suspended in degassed DME. The desired boronic acid building block, aqueous Na_2CO_3 and catalytic amounts of $Pd(Ph_3P)_4$ were then added, and the reaction was stirred gently for 15−18 h at 85 °C to give the tetrapeptides of general structure **II** (Scheme 1). Finally, the products were cleaved from the solid support using 45% TFA in CH_2Cl_2 as previously reported.

All of the final compounds were purified by preparative C18 reversed-phase HPLC (to >85% purity), and their structural identity was confirmed by ^{1}H NMR and MS before their activity was evaluated in our enzymatic assay. In the absence of any NMR or X-ray data pertaining to the binding interactions between the aromatic substituent of the 4-hydroxyproline moiety and the NS3 protease, the main goal of this library was to provide means by which we could probe the size of the P2 binding pocket, as well as gain some insight into the types of interactions involved between the aromatic ring of our inhibitors and the enzyme. For example, building blocks bearing electron-donating and -withdrawing groups were specifically chosen in order to modulate the electrostatic potential of the aromatic system.

Initially, an interesting trend in the enzymatic activity was observed with the tetrapeptide analogues having a halogen para to the phenoxy substituent (Table 1). A 10-fold increase in activity was observed in going from a *p*-fluoro analogue **12** to a *p*-iodo analogue **15**, prompting us to speculate on the plausible electrostatic interactions between the aromatic moiety and the binding pocket of the NS3 protease active site. We observed that the differences in the calculated electrostatic potentials of the model compounds (Figure 1), as well as the polarizability difference of the halides, correlated well with the trend observed in their enzymatic activity. A similar correlation was observed between the electrostatic potentials of the aromatic system, the ability to induce a dipole, and the increase in enzymatic activity of the quinoline analogues **37**−**41** (Figure 1). This may suggest that, in addition to hydrophobic interactions, a dipole/quadrupole interaction between the aromatic system and the P2 binding pocket may also contribute to the activity of these compounds.[17] It should also be noted that these compounds are highly selective inhibitors of the HCV NS3 protease as demonstrated by their inability to inhibit other serine protease

(15) (a) Huisgen, R.; Jakob, F.; Siegel, W.; Cadus, A. *Ann.* **1954**, *590*, 1. (b) Carlin, R. B.; Moores, M. S. *J. Am. Chem. Soc.* **1962**, *84*, 4107.
(16) Keifer, P. A. *J. Org. Chem.* **1996**, *61*, 1558.
(17) Dougherty, D. A. *Science* **1996**, *271*, 163.

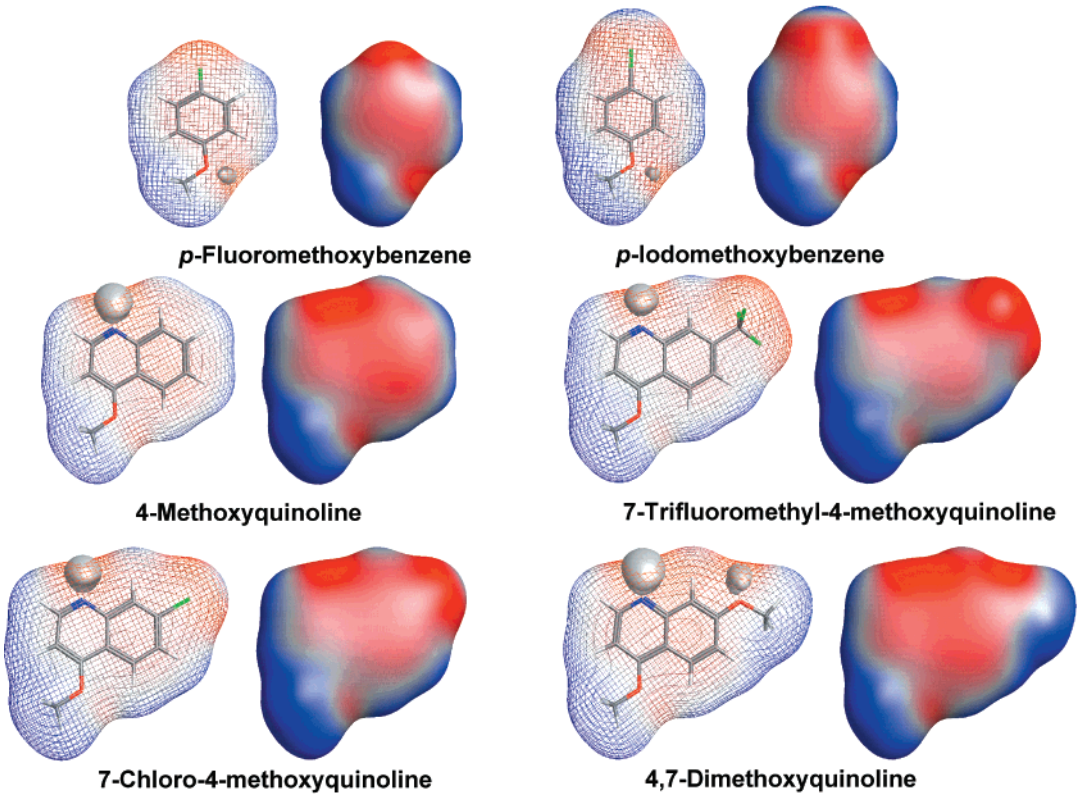


Figure 1. Electrostatic potentials (ESP) of model compounds mapped onto the solid molecular surface. Red indicates negative potential and blue indicates positive potential. The gray ESP isosurface shown in the meshed surface version of each fragment was plotted at 5 kcal/mol. ESP calculated at the RHF//6-31G** level.

enzymes such as the human leucocyte elastase or the bovine pancreatic α-chymotrypsin (e.g., for compounds **37** and **41**, IC_{50} > 150 μM for both enzymes).

Understanding the structure−activity relationships between a chemical probe and its biological target is often a very challenging task in medicinal chemistry. This is primarily due to the fact that even a minor structural modification of a compound may lead to the introduction of a large number of variable factors that cannot be easily identified or quantified. Thus, the structural modifications introduced in the tetrapeptide NS3 protease inhibitors described would be expected to exert rigidification effects on the backbone of the tetrapeptide, modulate the lipophilicity and desolvation energy of the inhibitors, and alter the polarizability of the electron density in the aromatic system. The latter effect will inevitably modulate the ability of the aromatic moiety to participate in dipole−quadrupole, π-stacking, or H-bond interactions, as well as affect the pK_a of the quinoline and isoquinoline analogues. Thus, a simple correlation between electrostatic potential and activity cannot be proposed, given the number of factors that vary between any two compounds. Nonetheless, the observations that were based on the library of NS3 protease inhibitors described in Table 1 are intriguing and worthy of further investigation.

Summary

We have developed an efficient protocol for the solid-phase synthesis of peptidomimetic compounds involving Mitsunobu and Suzuki cross-coupling reactions on a highly functionalized scaffold. Our protocol is amenable to the production of a much larger number of compounds from commercially available building blocks, thus expanding the structural diversity of our inhibitors. From the small library of tetrapetides produced, a number of interesting new leads were identified, enriching our medicinal chemistry efforts toward the discovery of an effective antiviral agent for the treatment of hepatitis C infections.

Experimental Section

General Methods. ^{1}H NMR spectra were obtained at 27 °C on a Bruker AMX400 spectrometer, and the chemical shifts are given in ppm, referenced to the internal deuterated solvent. The ^{1}H NMR (500 MHz) spectra of the resin-bound peptides **9** and **10** were acquired by magic-angle spinning using a Varian Nanoprobe. Reagents and solvents, including anhydrous THF and CH_2Cl_2, were purchased from Aldrich Chemical Co. or VWR Scientific of Canada. The Wang resin was purchased from Novabiochem (loading capacity 0.79 mmol/g). The peptide backbone (resin-bound peptide **9**) was assembled on an ACT90 peptide synthesizer following a protocol appropriate for each of the amino acids used. After coupling of the first amino acid unit, the remaining unreacted hydroxyl moieties of the resin were capped with benzoyl chloride in the presence of Et_3N. The loading efficiency of the first amino acid was evaluated by quantitative analysis of its picric acid salt;[18] an average coupling yield of ∼50% (∼0.35 mmol/g) was obtained. The completion of all subsequent peptide-bond coupling reactions was monitored by the Kaiser test.[19] Preparative C18 reversed-phase HPLC was carried out on a Whatman Partisil 10 ODS-3 column using a linear gradient from 5% aqueous CH_3CN (containing 0.06% TFA) to 100% CH_3CN (containing 0.06% TFA).

(18) Gisin, B. F. *Anal. Chim. Acta* **1972**, 58.
(19) Sarin, V. K.; Kent, S. B. H.; Tam, J. P.; Merrifield, R. B. *Anal. Biochem.* **1981**, *117*, 147.

Synthesis of the Resin-Bound Peptide 10. The Wang resin (6.33 g of dry powder, 0.79 mmol/g loading capacity) was placed in a reaction vessel of the automated peptide synthesizer and washed thoroughly with MeOH, 2-propanol, CH_2Cl_2, and DMF. The resin was allowed to swell in CH_2Cl_2 (30 mL) and then as a solution of Fmoc-protected 1-aminocyclopropylcarboxylic acid (ACCA, 4.85 g, 15 mmol, 3 equiv) in anhydrous DMF (20 mL) was added, followed by DCC (15 mL of 1 M solution in CH_2Cl_2, 3 equiv) and DMAP (190 mg, ~1.5 mmol, ~2 equiv). The reaction mixture was allowed to shake for 15 h at rt. The resin-bound ACCA was washed with CH_2Cl_2 (3 × 30 mL), DMF (3 × 30 mL), 2-propanol (3 × 30 mL), and once again with CH_2Cl_2 (3 × 30 mL) and DMF (3 × 30 mL). A solution of benzoyl chloride (4.8 mL, ~5 equiv, in 30 mL of DMF) and Et_3N (5.7 mL, ~5 equiv) was added, and the mixture was shaken for 1 h at rt to ensure capping of any unreacted sites on the resin. After that period, the resin was once again washed thoroughly following the same protocol as previously reported.

A small amount of resin (100 mg) was then removed, washed with MeOH, and dried under high vacuum. This sample was divided into three aliquots (exact mass of each aliquot determined), treated twice (a 5 and 25 min period) with freshly prepared 25% piperidine solution in DMF (2 mL), and washed thoroughly with CH_2Cl_2 (3 × 2 mL), ethanol (3 × 2 mL), CH_2Cl_2, and a solution of picric acid (2 mL 0.05% solution in CH_2Cl_2). A fresh batch of picric acid solution was added (2 mL), and resin-bound amino acid was allowed to form a complex over a period of 5 min. The resin was washed once again thoroughly and treated with 5% DIPEA solution in CH_2Cl_2, and the mole content of its sample was measured by quantitative analysis of the picric acid absorption at 358 nm.

The following protocol was used for all subsequent steps of peptide elongation: (a) The Fmoc protecting group was removed by treating the resin twice with 25% piperidine in DMF (2 × 30 mL) for a period of 5 and 20 min while shaking.[20] (b) The resin was washed thoroughly with CH_2Cl_2 (3 × 30 mL), DMF (3 × 30 mL), 2-propanol (3 × 30 mL), and once again with CH_2Cl_2 (3 × 30 mL) and DMF (3 × 30 mL). A fresh aliquot of anhydrous DMF was added (30 mL) and then the Fmoc protected amino acid[21] (2 equiv), HOBt (2 equiv), TBTU (2 equiv), and DIPEA (4 equiv); the mole ratio was based on the loading of 0.33 mmol/g of resin determined after coupling of the fist amino acid. The mixture was shaken for 2 h at rt, and the completion of the reaction was determined using the Kaiser test. After coupling and hydrolysis of the Fmoc protecting group of the last amino acid unit (2-cyclohexylglycine), the resin-bound peptide was capped by acetylation with acetic acid (2 equiv) under the same conditions as the couplings of all amino acid units. Finally, hydrolysis of the TBDMS silyl ether was achieved by treating the resin-bound peptide **9** with a solution of *n*-Bu_4NF (2 equiv) in THF (30 mL) for 2.5 h. Complete deprotection of the 4-hydroxyproline moiety was confirmed by comparing the magic-angle spinning ^{1}H NMR spectra of resin-bound peptide **9** with that of the resin-bound peptide **10**. Aliquots of resin-bound peptides **9** and **10** were used (typically a 40 μL volume of pre-swollen resin in CD_2Cl_2) to acquire their respective ^{1}H NMR spectra at 500 MHz using a Varian Nanoprobe.

Synthesis of Mitsunobu Library. The polymer-bound peptide **10** (0.33 mmol of peptide per gram of Wang resin) was dried under high vacuum in a desiccator over P_2O_5. A 96-well block of an Advanced ChemTech Model 396 synthesizer was furnished with aliquots of resin (120 mg, 0.04 mmol peptide per well), and each sample was washed for 5 min with anhydrous CH_2Cl_2 (5 × 1200 μL) and then with anhydrous THF (5 × 1500 μL). Anhydrous THF (200 μL) was added to each sample, and the synthesizer was temporarily stopped to allow the manual addition of reagents. Ph_3P (5 equiv in 400 μL of anhydrous THF) and diisopropyl azodicarboxylate (DIAD, 5 equiv in 250 μL of anhydrous THF) were added to each sample before the addition of a phenol, thiophenol, and hydroxyquinoline reagent (5 equiv, 0.2 mmol, dissolved in 7500 μL of anhydrous THF).[22] After the addition of all reagents, the mixtures were shaken for a total of 4 h, with a 10 min delay after each hour. Each resin-bound product was washed with THF (2 × 1500 μL), DMF (4 × 1500 μL), 2-propanol (4 × 1500 μL), CH_2Cl_2 (4 × 1500 μL), and finally methanol (2 × 1500 μL). All samples were dried under vacuum and then treated with 40% TFA in CH_2Cl_2 for 1 h in order to cleave the peptide product from the Wang resin. All products were purified by preparative HPLC on a reversed-phase C18 column to >85% purity and their structural identity was confirmed by ^{1}H NMR and MS. The ^{1}H NMR and MS data for a subset of specific compounds from our library (Table 1) is given below:

Synthesis of Suzuki Library on Solid Support. All reactions were carried out in 16 × 100 mm, high-pressure screw-cap test tubes with Teflon caps, equipped with small magnetic stirring bars. For each reaction, a degassed suspension of the polymer-bound peptide **14**, **17**, or **18** (100 mg of Wang resin with 0.033 mmol of polymer-bound peptide) was first added to the test tube, followed by the addition of DME (2 mL), $Pd(Ph_3P)_4$ (~3 mg, 0.05 equiv), Na_2CO_3 (70 μL of a 2 M solution in H_2O, 2.5 equiv), and a reagent (2−3 equiv depending on the solubility of the reagent) from the library of aromatic boronic acid reagents. The test tubes were flushed with nitrogen gas, sealed, and placed in an oil bath at 80 °C. All of the reactions were stirred gently and allowed to proceed for 15−18 h. Each resin-bound peptide product (Scheme 1, general structure **II**) was subsequently transferred into a plastic filtration tube, washed with DME/H_2O (1:1, 5 × 2 mL), DME (5 × 2 mL), methanol (5 × 2 mL), CH_3CN (5 × 2 mL), and CH_2Cl_2 (5 × 2 mL), and dried under high vacuum. Each product was cleaved from the resin by treating the sample with 45% TFA in CH_2Cl_2 (1 mL) for 1 h. All products were purified to >85% purity by preparative HPLC on a reversed-phase C18 column, using a solvent linear gradient from 5% aqueous CH_3CN to 100% CH_3CN. ^{1}H NMR and MS data of all pure products confirmed their structural identity as indicated. ^{1}H NMR and MS data for a subset of specific compounds from this library are given below:

Compound 10. ^{1}H NMR (DMSO, 400 MHz) major rotamer (mixture of 8.5:1.5 ratio) δ: 0.81 (d, J = 6.7 Hz, 3H, Val-Me), 0.86 (d, J = 6.7 Hz, 3H, Val-Me), 0.83−1.0 (m, 4H, ACCA-2Hβ syn to the acid moiety, Chg-2Hγ), 1.05−1.15 (m, 3H, Chg-2Hδ and 1Hϵ), 1.20−1.30 (m, 1H, ACCA-Hβ syn to the NH), 1.32−1.37 (m, 1H, ACCA-Hβ syn to the NH), 1.5−1.7 (m, 6H, Chg-Hβ, 2Hγ, 2Hδ, 1Hϵ), 1.85 (s, 3H, Ac), 1.9−2.0 (m, 3H, Val-Hβ and Pro-2Hβ), 3.54 (dd, J = 10.5, 1.5 Hz, 1H, Pro-Hδ), 3.65 (dd, J = 10.5, 4.5 Hz, 1H, Pro-Hδ), 4.19 (t, J = 8.2 Hz, 1H, Chg-Hα), 4.26 (t, J = 8.0 Hz, 1H, Pro-Hα), 4.32−4.36 (m, 2H, Val-Hα and OH), 5.04 (m, 1H, Pro-Hγ), 7.67 (d, J = 8.6 Hz, 1H, Val-NH), 7.83 (d, J = 8.9 Hz, 1H, Chg-NH), 8.67 (s, 1H, ACCA-NH). The chemical shift assignments were confirmed by the COSY NMR data of compound **10**. HRFAB$^+$ MS m/z: 495.28080 $(M + H)^+$, calcd mass for $C_{24}H_{39}O_7N_4$ = 495.28186.

Compound 11. ^{1}H NMR (DMSO, 400 MHz) major rotamer (mixture of ~9:1 ratio) δ: 0.83 (d, J = 6.7 Hz, 3H), 0.89 (d, J = 6.7 Hz, 3H), 0.79−0.97 (m, 4H), 1.05−1.15 (m, 3H), 1.20−1.28 (m, 2H), 1.35−1.40 (m, 1H), 1.45−1.70 (m, 5H), 1.83 (s, 3H), 1.94−2.00 (m, 1H), 2.13−2.22 (m, 1H), 2.25−2.35 (m, 1H), 2.84−2.94 (m, 1H), 3.91−3.99 (m, 1H), 4.18−4.22 (dt, J = 8.6 Hz, 1H), 4.28−4.32 (m, 2H), 5.07 (bs, 1H), 6.89−6.98 (m, 3H), 7.3 (dd, J = 8.6 Hz, 2H), 7.81 (d, J = 8.6 Hz, 1H), 7.83 (d, J = 8.6 Hz, 1H), 8.43 (s, 1H). HRFAB$^+$ MS m/z: 571.31410 $(M + H)^+$, calcd mass for $C_{30}H_{43}O_7N_4$ = 571.31317.

(20) A modified protocol, 50% piperidine in DMF for a 5 min reaction period, was used for the hydrolysis of the Fmoc protecting group from the proline moiety to avoid any potential diketopiperazine formation.

(21) For the purpose of the library production, the hydroxyl moiety of the *cis* 4-hydroxyproline building block was protected as the TBDMS silyl ether **ref 13**. For the synthesis of the reference compound **10** (Table 1), the commercially available *tert*-butyl ether of the Fmoc-protected *trans*-4-hydroxyproline was used, since the *tert*-butyl ether substituent gets hydrolyzed during cleavage of the peptide from the Wang resin with TFA.

(22) In cases where this building block was insoluble in THF, a small volume of DMF was used to dissolve the compound without any noticeable detrimental effect to the outcome of the Mitsunobu reaction.

Compound 12−15. As expected, the ^{1}H NMR spectra of compounds **12**−**15** were almost identical with some minor differences in chemical shifts and coupling constants. The ^{1}H NMR data of compound **12** are shown below as a representative example.

^{1}H NMR (DMSO, 400 MHz) major rotamer of compound **12** (mixture of ∼9:1 ratio) δ: 0.83 (d, J = 6.7 Hz, 3H), 0.89 (d, J = 6.7 Hz, 3H), 0.79−0.98 (m, 4H), 1.05−1.15 (m, 3H), 1.20−1.28 (m, 2H), 1.33−1.40 (m, 1H), 1.45−1.70 (m, 5H), 1.83 (s, 3H), 1.94−2.00 (m, 1H), 2.11−2.17 (m, 1H), 2.24−2.35 (m, 1H), 2.84−2.94 (m, 1H), 3.89 (dd, J = 11.1, 4.1 Hz, 1H), 3.85 (bd, J = 11.8 Hz, 1H), 4.18−4.22 (m, 1H), 4.26−4.32 (m, 1H), 5.03 (bs, 1H), 6.95 (dd, J = 9.2 & 4.5 Hz, 2H), 7.13 (dd, J = 8.9 Hz, 2H), 7.81 (d, J = 8.6 Hz, 1H), 7.83 (d, J = 8.6 Hz, 1H), 8.43 (s, 1H). HRFAB$^+$ MS m/z: 589.30200 (M + H)$^+$, calcd mass for $C_{30}H_{42}O_7N_4F$ = 589.30377.

Compound 13. HRFAB$^+$ MS m/z: 605.27490 (M + H)$^+$, calcd mass for $C_{30}H_{42}O_7N_4{}^{35}Cl$ = 605.27423.

Compound 14. HRFAB$^+$ MS m/z: 649.22480 (M + H)$^+$, calcd mass for $C_{30}H_{42}O_7N_4{}^{79}Br$ = 649.22369.

Compound 15. HRFAB$^+$ MS m/z: 697.21100 (M + H)$^+$, calcd mass for $C_{30}H_{42}O_7N_4I$ = 697.20984.

Compound 16. ^{1}H NMR (DMSO, 400 MHz) δ: 0.83 (d, J = 6.7 Hz, 3H), 0.87 (d, J = 6.7 Hz, 3H), 0.79−0.97 (m, 4H), 1.04−1.18 (m, 3H), 1.20−1.28 (m, 2H), 1.32−1.38 (m, 1H), 1.48−1.69 (m, 7H), 1.84 (s, 3H), 1.90−2.05 (m, 2H), 3.77−3.82 (m, 1H), 4.01−4.07 (m, 1H), 4.16−4.25 (m, 2H), 4.31−4.34 (dd, J = 7.0 Hz, 1H), 7.33 (d, J = 8.5 Hz, 2H), 7.56 (d, J = 8.5 Hz, 2H), 7.82 (d, J = 8.9 Hz, 1H), 7.92 (d, J = 8.6 Hz, 1H), 8.40 (s, 1H). ES$^+$ MS m/z: 687.3 & 689.3 (M + Na)$^+$. ES$^-$ MS m/z: 663.3, 665.3 (M − H)$^-$.

Compound 17. ^{1}H NMR (DMSO, 400 MHz) major rotamer of compound **17** (mixture of ∼9:1 ratio) δ: 0.83 (d, J = 6.7 Hz, 3H), 0.89 (d, J = 6.7 Hz, 3H), 0.79−1.0 (m, 4H), 1.2−1.15 (m, 3H), 1.21−1.29 (m, 1H), 1.33−1.39 (m, 1H), 1.45−1.68 (m, 6H), 1.83 (s, 3H), 1.92−2.00 (m, 1H), 2.10−2.18 (m, 1H), 2.25−2.33 (m, 1H), 3.88−3.92 (dd, J = 11.4, 4.1 Hz, 1H), 4.04 (bd, J = 11.4 Hz, 1H), 4.18−4.22 [appt (dd), J = 7 Hz, 1H], 4.26−4.30 [appt (2 overlapping dd), J = 8 Hz, 2H], 5.12 (bs, 1H), 6.94−6.96 (dd, J = 8.3 & 1.6 Hz, 1H), 7.13−7.17 (m, 2H), 7.24−7.28 [appt (dd), J = 7.8 Hz, 1H), 7.80 (d, J = 8.6 Hz, 1H), 7.86 (d, J = 8.6 Hz, 1H), 8.42 (s, 1H). HRFAB$^+$ MS m/z: 649.22160 (M + H)$^+$, calcd mass for $C_{30}H_{42}O_7N_4{}^{79}Br$ = 649.22369.

Compound 18. ^{1}H NMR (DMSO, 400 MHz) major rotamer of compound **18** (mixture of ∼4:1 ratio) δ: 0.83 (d, J = 6.7 Hz, 3H), 0.89 (d, J = 6.7 Hz, 3H), 0.85−1.0 (m, 4H), 1.12−1.14 (m, 2H), 1.21−1.28 (m, 1H), 1.33−1.39 (m, 1H), 1.45−1.68 (m, 6H), 1.83 (s, 3H), 1.90−1.98 (m, 1H), 2.12−2.20 (m, 1H), 2.25−2.35 (m, 1H), 3.91−3.95 (dd, J = 11.5, 4.1 Hz, 1H), 4.04 (bd, J = 11.1 Hz, 1H), 4.15−4.25 (m, 2H], 4.30−4.40 (m, 2H), 5.15 (bs, 1H), 6.93−6.96 [app dt (ddd), J = 8.3, 1.3 Hz, 1H], 7.18 (dd, J = 8.3 & 1.3 Hz, 1H), 7.34−7.38 [app dt (ddd), J = 8.3, 1.6 Hz, 1H], 7.56−7.59 (dd, J = 7.9, 1.6 Hz, 1H), 7.79 (d, J = 8.6 Hz, 1H), 7.81 (d, J = 8.3 Hz, 1H), 8.47 (s, 1H). HRFAB$^+$ MS m/z: 649.22290 (M + H)$^+$, calcd mass for $C_{30}H_{42}O_7N_4$-^{79}Br = 649.22369.

Compound 19. ^{1}H NMR (DMSO, 400 MHz) major rotamer of compound **19** (mixture of ∼4:1 ratio) δ: 0.85 (d, J = 6.7 Hz, 3H), 0.92 (d, J = 6.7 Hz, 3H), 0.85−1.0 (m, 4H), 1.02−1.20 (m, 3H), 1.21−1.28 (m, 1H), 1.35−1.41 (m, 1H), 1.47−1.72 (m, 6H), 1.83 (s, 3H), 1.90−2.05 (m, 1H), 2.15−2.25 (m, 1H), 2.28−2.38 (m, 1H), 3.95−4.05 (m, 2H), 4.15−4.25 (m, 1H), 4.28−4.38 (m, 2H], 5.14 (bs, 1H), 7.02 (d, J = 8.9 Hz, 2H), 7.31 [app t (dd), J = 7.3 Hz, 1H), 7.44 [app t (dd), J = 7.6 Hz, 1H], 7.82 (d, J = 9.9 Hz, 1H), 7.84 (d, J = 8.9 Hz, 1H), 8.44 (s, 1H). HRFAB$^+$ MS m/z: 647.34290 (M + H)$^+$, calcd mass for $C_{36}H_{46}O_7N_4$ = 647.34448.

Compound 20. ^{1}H NMR (DMSO, 400 MHz) major rotamer of compound **20** (mixture of ∼7:3 ratio) δ: 0.84 (d, J = 6.7 Hz, 3H), 0.90 (d, J = 6.7 Hz, 3H), 0.88−1.0 (m, 4H), 1.02−1.16 (m, 3H), 1.21−1.28 (m, 1H), 1.35−1.40 (m, 1H), 1.47−1.69 (m, 6H), 1.83 (s, 3H), 1.90−2.02 (m, 1H), 2.15−2.25 (m, 1H), 2.28−2.35 (m, 1H), 3.78 (s, 3H), 3.92−3.97 (dd, J = 11.1 & 3.8 Hz, 1H), 4.00 (bd, J = 11.6 Hz, 1H), 4.19−4.23 (dd, J = 7.0 Hz, 1H), 4.29−4.34 (m, 2H), 5.11 (bs, 1H), 6.99 (dd, J = 8.9 & 3.8 Hz, 4H), 7.54 (d, J = 8.3 Hz, 4H), 7.82 (d, J = 8.6 Hz, 1H), 7.84 (d, J = 8.6 Hz, 1H), 8.44 (s, 1H). ES$^+$ MS m/z: 699.3 (M + Na)$^+$. ES$^-$ MS m/z: 675.3 (M − H)$^-$.

Compound 21. HRFAB$^+$ MS m/z: 647.34620 (M + H)$^+$, calcd mass for $C_{36}H_{46}O_7N_4$ = 647.34448.

Compound 22. ^{1}H NMR (DMSO, 400 MHz) major rotamer of compound **22** (mixture of ∼7:3 ratio) δ: 0.84 (d, J = 6.7 Hz, 3H, CH_3-Val), 0.90 (d, J = 6.7 Hz, 3H, CH_3-Val), 0.80−0.89 (m, 2H, 1Hγ + 1Hδ-Chg), 0.89−1.14 (m, 5H, 2Hβ-ACCA + 1Hγ-Chg + 1Hδ-Chg + 1Hϵ-Chg), 1.21−1.28 (m, 1Hβ-ACCA), 1.33−1.40 (m, 1Hβ-ACCA), 1.45−1.67 (m, 6H, 2Hg + 2Hd + 1He + 1Hb- Chg), 1.83 (s, 3H, CH_3CO), 1.95−2.01 (m, 1H, Hβ-Val), 2.15−2.22 (m, 1H, Hβ-Pro), 2.32−2.37 (m, 1H, Hβ-Pro), 3.93−3.97 (dd, J = 11.1, 3.5 Hz, 1Hδ-Pro), 4.07 (bd, J = 11.1 Hz, 1Hδ-Pro), 4.17−4.21 (dd, J = 7.0 Hz, 1Hα-Chg), 4.30−4.34 (2 overlapping dd, 2H, Hα-Pro + Hα-Val), 5.24 (bs, 1H, Hγ-Pro), 7.04 (dd, J = 8.0, 2.2 Hz, 1H, H2-Ar), 7.30 (bs, 1H, H10-Ar), 7.38 (bd, J = 8.0 Hz, 1H, H4-Ar), 7.44−7.48 (dd, J = 8.0 Hz, 1H, H3-Ar), 7.74−7.78 (dd, J = 8.0 Hz, 1H, H8-Ar), 7.80 (d, J = 9.2 Hz, 1H, NH-Chg), 7.83 (d, J = 7.6 Hz, 1H, H9-Ar), 8.16 (bd, J = 7.6 Hz, 1H, NH-Val), 8.22−8.24 (dd, J = 8.2, 1.7 Hz, 1H, H7-Ar), 8.42 (d, J = 1.7 Hz, 1H, H5-Ar), 8.44 (s, 1H, NH-ACCA). The complete chemical shift assignment and structural identity were confirmed by the ^{1}H, COSY and ROESY NMR data acquired in phosphate buffer (pH = 6.5) with presaturation of H_2O. ES$^+$ MS m/z: 714.4 (M + Na)$^+$. ES$^-$ MS m/z: 690.4 (M − H)$^-$.

Compound 23. ^{1}H NMR (DMSO, 400 MHz) major rotamer of compound **23** (mixture of ∼9:1 ratio) δ: 0.84 (d, J = 6.7 Hz, 3H), 0.90 (d, J = 6.7 Hz, 3H), 0.80−0.94 (m, 5H), 1.02−1.15 (m, 2H), 1.21−1.28 (m, 1H), 1.33−1.40 (m, 1H), 1.45−1.67 (m, 6H), 1.83 (s, 3H), 1.95−2.00 (m, 1H), 2.06 (s, 3H), 2.13−2.20 (m, 1H), 2.30−2.36 (m, 1H), 3.92−3.96 (dd, J = 11.5, 4.1 Hz, 1H), 4.06 (bd, J = 11.1 Hz, 1H), 4.18−4.22 (dd, J = 7.7 Hz, 1H), 4.30−4.34 (2 overlapping dd, J = 8.2 Hz, 2H), 5.17 (bs, 1H), 6.95 (dd, J = 8.5, 2.2 Hz, 1H), 7.08 (bs, 1H), 7.19 (bd, J = 7.6 Hz, 1H), 7.31 (bd, J = 7.6 Hz, 1H), 7.36−7.42 (m, 2H), 7.56 (d, J = 8.0 Hz, 1H), 7.79 (d, J = 8.9 Hz, 1H), 7.86 (d, J = 8.0 Hz, 1H), 7.87 (s, 1H), 8.43 (s, 1H) 10.00 (s, 1H). HRFAB$^+$ MS m/z: 704.36320 (M + H)$^+$, calcd mass for $C_{38}H_{50}O_8N_5$ = 704.36597.

Compound 24. ^{1}H NMR (DMSO, 400 MHz) major rotamer of compound **24** (mixture of ∼9:1 ratio) δ: 0.84 (d, J = 6.7 Hz, 3H), 0.90 (d, J = 6.7 Hz, 3H), 0.80−1.08 (m, 7H), 1.20−1.28 (m, 1H), 1.32−1.38 (m, 1H), 1.44−1.67 (m, 6H), 1.83 (s, 3H), 1.93−2.07 (m, 1H), 2.08−2.18 (m, 1H), 2.19−2.28 (m, 1H), 3.89 (bs, 2H), 4.13−4.28 (m, 2H), 4.32−4.22 (dd, J = 7.0 Hz, 1H), 5.09 (bs, 1H), 7.05−7.08 (dd, J = 7.1 Hz, 1H), 7.15 (bd, J = 8.0 Hz, 1H), 7.25−7.45 (m, 7H), 7.70 (d, J = 8.6 Hz, 1H), 7.82 (d, J = 8.9 Hz, 1H), 8.42 (s, 1H). HRFAB$^+$ MS m/z: 647.34560 (M + H)$^+$, calcd mass for $C_{36}H_{47}O_7N_4$ = 647.34448.

Compound 25. ^{1}H NMR (DMSO, 400 MHz) major rotamer of compound **25** (mixture of ∼4:1 ratio) δ: 0.82 (d, J = 6.7 Hz, 3H), 0.88 (d, J = 6.7 Hz, 3H), 0.80−1.08 (m, 7H), 1.22−1.27 (m, 1H), 1.32−1.38 (m, 1H), 1.43−1.70 (m, 6H), 1.82 (s, 3H), 1.95−2.00 (m, 1H), 2.13−2.25 (m, 2H), 3.78 (s, 3H), 3.90 (bs, 2H), 4.15−4.19 (dd, J = 7.9 Hz, 1H), 4.25−4.34 (m, 2H), 5.04 (bs, 1H), 6.90 (d, J = 8.6 Hz, 2H), 7.15 (bd, J = 8.0 Hz, 1H), 7.03−7.06 (dd, J = 7.3 Hz, 1H), 7.10 (d, J = 8.3 Hz, 1H), 7.27−7.31 (m, 2H), 7.35 (d, J = 8.9 Hz, 2H), 7.73 (d, J = 8.3 Hz, 1H), 7.78 (d, J = 8.9 Hz, 1H), 8.43 (s, 1H). ES$^+$ MS m/z: 699.3 (M + Na)$^+$. ES$^-$ MS m/z: 675.4 (M − H)$^-$.

Compound 26. ^{1}H NMR (DMSO, 400 MHz) major rotamer of compound **26** (mixture of ∼4:1 ratio) δ: 0.83 (d, J = 6.7 Hz, 3H), 0.89 (d, J = 6.7 Hz, 3H), 0.80−1.17 (m, 8H), 1.22−1.28 (m, 1H), 1.34−1.38 (m, 1H), 1.40−1.70 (m, 6H), 1.79 (s, 3H), 1.92−1.99 (m, 1H), 2.13−2.20 (m, 1H), 3.90−3.98 (m, 2H), 4.12−4.18 (m, 1H), 4.24−4.31 (m, 2H), 5.15 (bs, 1H), 7.07−7.11 (m, 1H), 7.18 (bd, J = 8.3 Hz, 1H), 7.35−7.40 (m, 2H), 7.52 (d, J = 8.6 Hz, 2H), 7.69 (d, J = 8.9 Hz, 1H), 7.81 (d, J = 8.3 Hz, 1H), 7.92 (d, J = 8.6 Hz, 2H), 8.44 (s, 1H). ES$^+$ MS m/z: 713 (M + Na)$^+$. ES$^-$ MS m/z: 689 (M − H)$^-$.

Compound 27. ^{1}H NMR (DMSO, 400 MHz) major rotamer of compound **27** (mixture of ∼4:1 ratio) δ: 0.99 (d, J = 6.7 Hz, 3H), 1.06 (d, J = 6.7 Hz, 3H), 0.92−1.26 (m, 7H), 1.33−1.39

(m, 1H), 1.46−1.52 (m, 1H), 1.56−1.79 (m, 6H), 1.96 (s, 3H), 2.08−2.13 (m, 1H), 2.28−2.34 (m, 1H), 2.46−2.51 (m, 1H), 4.10−4.13 (dd, J = 11.4, 3.8 Hz, 1H), 4.28−4.37 (m, 2H), 4.41−4.46 (m, 2H), 5.45 (bs, 1H), 7.45−7.48 (dd, J = 8.5, 4.7 Hz, 1H), 7.63−7.66 (m, 1H), 7.77 (d, J = 8.2 Hz, 1H), 7.85 (d, J = 8.8 Hz, 1H), 7.91−7.94 (m, 2H), 8.24−8.25 (m 1H), 8.37 (d, J = 3.5 Hz, 1H), 8.57 (s, 1H). ES$^+$ MS m/z: 676.3 (M + Na)$^+$, 654.3 (M + H)$^+$. ES$^-$ MS m/z: 652.3 (M − H)$^-$.

Compound 28. ES$^+$ MS m/z: 656.4 (M + H)$^+$. ES$^-$ MS m/z: 654.4 (M − H)$^-$.

Compound 29. ^{1}H NMR (DMSO, 400 MHz) major rotamer of compound **29** δ: 0.85 (d, J = 6.7 Hz, 3H), 0.90 (d, J = 6.7 Hz, 3H), 0.80−0.98 (m, 4H), 1.05−1.18 (m, 3H), 1.22−1.23−1.28 (m, 1H), 1.34−1.41 (m, 1H), 1.45−1.71 (m, 6H), 1.81 (s, 3H), 1.82−1.84 (m, 1H), 1.93−2.00 (m, 1H), 2.24−2.33 (m, 1H), 3.99−4.03 (dd, J = 12.0, 4.2 Hz, 1H), 4.18−4.28 (m, 3H), 4.31−4.36 (dd, J = 8.3 Hz, 1H), 5.76 (bs, 1H), 7.27−7.31 (ddd, J = 8.0 & 1.0 Hz, 1H), 7.39−7.43 (ddd, J = 8.3, 1.3 Hz, 1H), 7.71 (d, J = 7.6 Hz, 1H), 7.77 (d, J = 8.9 Hz, 1H), 7.91 (d, J = 8.0 Hz, 1H), 7.94 (d, J = 8.6 Hz, 1H), 8.47 (s, 1H). ES$^+$ MS m/z: 628.3 (M + H)$^+$, 650.3 (M + Na)$^+$. ES$^-$ MS m/z: 626.3 (M − H)$^-$.

Compound 30. ^{1}H NMR (DMSO, 400 MHz) major rotamer of compound **30** (mixture of ~5:1 ratio) δ: 0.86 (d, J = 6.7 Hz, 3H), 0.90 (d, J = 6.7 Hz, 3H), 0.80−1.02 (m, 4H), 1.05−1.18 (m, 3H), 1.21−1.28 (m, 1H), 1.35−1.41 (m, 1H), 1.50−1.71 (m, 6H), 1.82 (s, 3H), 1.93−1.99 (m, 1H), 2.33−2.40 (m, 1H), 2.44−2.50 (m, 1H), 4.05−4.08 (dd, J = 10.9, 4.0 Hz, 1H), 4.19−4.28 (m, 3H), 4.37−4.41 (dd, J = 7.1 Hz, 1H), 4.58−4.62 (m, 1H), 7.38−7.41 (dd, J = 7.7 Hz, 1H), 7.47−7.51 (ddd, J = 8.3, 1.0 Hz, 1H), 7.71 (d, J = 8.6 Hz, 1H), 7.91 (d, J = 8.3 Hz, 1H), 8.00 (d, J = 8.6 Hz, 1H), 8.04 (d, J = 8.0 Hz, 1H), 8.48 (s, 1H). ES$^+$ MS m/z: 666.4 (M + Na)$^+$. ES$^-$ MS m/z: 642.3 (M − H)$^-$.

Compound 31. ^{1}H NMR (DMSO, 400 MHz) major rotamer of compound **31** (mixture of ~9:1 ratio) δ: 0.83 (d, J = 6.7 Hz, 3H), 0.89 (d, J = 6.7 Hz, 3H), 0.79−1.02 (m, 4H), 1.04−1.18 (m, 3H), 1.24−1.29 (m, 1H), 1.35−1.40 (m, 1H), 1.43−1.71 (m, 6H), 1.83 (s, 3H), 1.95−2.01 (m, 1H), 2.25−2.32 (m, 1H), 2.38−2.43 (m, 1H), 4.00−4.01 (dd, J = 11.7, 3.8 Hz, 1H), 4.11−4.15 (dd, J = 7.8 Hz, 1H), 4.19−4.24 (m, 2H), 4.32−4.36 (dd, J = 8.2 Hz, 1H), 5.47 (bs, 1H), 7.45 (d, J = 6.5 Hz, 2H), 7.74 (d, J = 8.8, 1H), 7.82 (d, J = 8.1 Hz, 1H), 8.47 (s, 1H), 8.72 (d, J = 7.0 Hz, 2H). HRFAB$^+$ MS m/z: 572.30630 (M + H)$^+$, calcd mass for $C_{29}H_{42}O_7N_5$ = 572.30841.

Compound 32. ^{1}H NMR (DMSO, 400 MHz) major rotamer of compound **32** (mixture of ~9:1 ratio) δ: 0.83 (d, J = 6.7 Hz, 3H), 0.89 (d, J = 6.7 Hz, 3H), 0.79−1.02 (m, 4H), 1.03−1.17 (m, 3H), 1.23−1.28 (m, 1H), 1.34−1.39 (m, 1H), 1.45−1.71 (m, 6H), 1.83 (s, 3H), 1.94−2.01 (m, 1H), 2.15−2.22 (m, 1H), 2.32−2.35 (m, 1H), 3.92−3.96 (dd, J = 11.8, 4.1 Hz, 1H), 4.08 (d, J = 11.8 Hz, 1H), 4.15−4.19 (dd, J = 7.8 Hz, 1H), 4.24−4.29 (dd, J = 8.4 Hz, 1H), 4.30−4.34 (dd, J = 8.3 Hz, 1H), 5.20 (bs, 1H), 7.45−7.48 (dd, J = 8.3, 4.6 Hz, 1H), 7.54 (bd, J = 7.3 Hz, 1H), 7.79 (d, J = 8.6 Hz, 1H), 7.84 (d, J = 8.6 Hz, 1H), 8.27 (d, J = 4.1 Hz, 1H), 8.34 (bs, 1H), 8.44 (s, 1H). HRFAB$^+$ MS m/z: 572.30740 (M + H)$^+$, calcd mass for $C_{29}H_{42}O_7N_5$ = 572.30841.

Compound 33. ^{1}H NMR (DMSO, 400 MHz) major rotamer of compound **33** (mixture of ~9:1 ratio) δ: 0.84 (d, J = 6.7 Hz, 3H), 0.89 (d, J = 6.7 Hz, 3H), 0.82−1.17 (m, 7H), 1.23−1.25 (m, 1H), 1.27−1.40 (m, 1H), 1.42−1.70 (m, 6H), 1.83 (s, 3H), 1.98−2.03 (m, 1H), 2.25−2.34 (m, 1H), 2.41−2.45 (m, 1H), 3.90−4.20 (m, 3H), 4.30−4.34 (dd, J = 7.8 Hz, 1H), 4.48−4.52 (dd, J = 7.8 Hz, 1H), 5.37 (bs, 1H), 7.31−7.36 (m, 1H), 7.58−7.68 (m, 3H), 7.79 (d, J = 8.3 Hz, 2H), 8.48 (bs, 2H), 8.93 (bs, 1H). HRFAB$^+$ MS m/z: 622.32600 (M + H)$^+$, calcd mass for $C_{33}H_{44}O_7N_5$ = 622.32410

Compound 34. ^{1}H NMR (DMSO, 400 MHz) major rotamer of compound **34** (mixture of ~9:1 ratio) δ: 0.84 (d, J = 6.7 Hz, 3H), 0.89 (d, J = 6.7 Hz, 3H), 0.82−1.04 (m, 6H), 1.10−1.28 (m, 3H), 1.32−1.70 (m, 6H), 1.81 (s, 3H), 1.94−2.03 (m, 1H), 2.23−2.34 (m, 1H), 2.42−2.55 (m, 1H), 4.44−4.48 (dd, J = 11.1, 4.5 Hz, 1H), 4.18−4.28 (m, 3H), 4.38−4.44 (dd, J = 8.1 Hz, 1H), 5.79 (bs, 1H), 7.41 (d, J = 5.7 Hz, 1H), 7.58−7.62 (dd, J = 7.7 Hz, 1H), 7.75 (d, J = 9.2 Hz, 1H), 7.78−7.80 (dd, J = 7.0, 1.0 Hz, 1H), 7.90 (d, J = 8.3 Hz, 1H), 7.95 (d, J = 8.6 Hz, 1H), 8.01 (d, J = 5.7 Hz, 1H), 8.12 (d, J = 8.3 Hz, 1H), 8.43 (s, 1H). HRFAB$^+$ MS m/z: 622.32160 (M + H)$^+$, calcd mass for $C_{33}H_{44}O_7N_5$= 622.32410.

Compound 35. ^{1}H NMR (DMSO, 400 MHz) major rotamer of compound **35** (mixture of ~4:1 ratio) δ: 0.86 (d, J = 6.7 Hz, 3H), 0.92 (d, J = 6.7 Hz, 3H), 0.82−1.04 (m, 6H), 1.05−1.28 (m, 3H), 1.35−1.70 (m, 6H), 1.82 (s, 3H), 1.94−2.03 (m, 1H), 2.23−2.34 (m, 1H), 2.40−2.55 (m, 1H), 4.03−4.08 (dd, J = 11.1, 4.5, 1H), 4.18−4.28 (m, 3H), 4.37−4.43 (dd, J = 8.0 Hz, 1H), 5.79 (bs, 1H), 7.41 (d, J = 5.7 Hz, 1H), 7.58−7.62 (dd, J = 7.7 Hz, 1H), 7.75 (d, J = 9.2 Hz, 1H), 7.78−7.80 (dd, J = 7.0, 1.0 Hz, 1H), 7.90 (d, J = 8.3 Hz, 1H), 7.95 (d, J = 8.6 Hz, 1H), 8.02 (d, J = 5.7 Hz, 1H), 8.12 (d, J = 8.3 Hz, 1H), 8.43 (s, 1H). HRFAB$^+$ MS m/z: 622.32160 (M + H)$^+$, calcd mass for $C_{33}H_{44}O_7N_5$ = 622.32410.

Compound 36. ^{1}H NMR (DMSO, 400 MHz) major rotamer of compound **36** (mixture of ~5:1 ratio) δ: 0.88 (d, J = 6.7 Hz, 3H), 0.92 (d, J = 6.7 Hz, 3H), 0.82−1.14 (m, 7H), 1.23−1.28 (m, 1H), 1.35−1.38 (m, 1H), 1.42−1.70 (m, 6H), 1.83 (s, 3H), 1.94−2.03 (m, 1H), 2.23−2.28 (m, 1H), 2.40−2.55 (m, 1H), 3.99−4.03 (dd, J = 11.4, 4.1 Hz, 1H), 4.17−4.21 (dd, J = 7.8 Hz, 1H), 4.26−4.30 (dd, J = 8.5 Hz, 1H), 4.34−4.40 (m, 2H), 5.43 (s, 1H),7.75 (d, J = 8.9 Hz, 1H), 7.79−7.88 (m, 2H), 7.94 (d, J = 8.3 Hz, 1H), 8.14 (d, J = 8.3 Hz, 1H), 8.21 (d, J = 8.3 Hz, 1H), 8.30 (s, 1H), 8.42 (s, 1H), 9.13 (s, 1H). ES$^+$ MS m/z: 622.3 (M + H)$^+$, 644.3 (M + Na)$^+$. ES$^-$ MS m/z: 620.3 (M − H)$^-$.

Compound 37. ^{1}H NMR (DMSO, 400 MHz) major rotamer of compound **37** (mixture of ~6:1 ratio) δ: 0.89 (d, J = 6.7 Hz, 3H), 0.93 (d, J = 6.7 Hz, 3H), 0.75−1.20 (m, 7H), 1.22−1.32 (m, 1H), 1.32−1.43 (m, 1H), 1.43−1.70 (m, 6H), 1.82 (s, 3H), 1.90−2.02 (m, 1H), 2.31−2.39 (m, 1H), 2.5−2.59 (m, 1H), 4.05 (bd, J = 7.9 Hz, 1H), 4.13−4.21 (m, 2H), 4.38−4.42 (dd, J = 8.5 Hz, 1H), 4.52 (d, J = 12.1 Hz, 1H), 5.68 (s, 1H), 7.56 (d, J = 6.4 Hz, 1H), 7.67 (d, J = 8.6 Hz, 1H), 7.79−7.83 (dd, J = 7.6 Hz, 1H), 7.98 (d, J = 8.0 Hz, 1H), 8.05−8.09 (dd, J = 7.3 Hz, 1H), 8.14 (d, J = 8.3 Hz, 1H), 8.31 (d, J = 8.3 Hz, 1H), 8.44 (s, 1H), 9.13 (d, J = 6.4 Hz, 1H). HRFAB$^+$ MS m/z: 622.32660 (M + H)$^+$, calcd mass for $C_{33}H_{44}O_7N_5$ = 622.32410.

Compound 38. ^{1}H NMR (DMSO, 400 MHz) major rotamer of compound **38** (mixture of ~6:1 ratio) δ: 0.89 (d, J = 6.7 Hz, 3H), 0.92 (d, J = 6.7 Hz, 3H), 0.77−1.15 (m, 7H), 1.22−1.28 (m, 1H), 1.32−1.40 (m, 1H), 1.42−1.65 (m, 6H), 1.82 (s, 3H), 1.92−1.99 (m, 1H), 2.26−2.35 (m, 1H), 2.5−2.58 (m, 1H), 4.00−4.03 (dd, J = 11.8, 3.8 Hz, 1H), 4.13−4.17 (dd, J = 8.1 Hz, 1H), 4.20−4.24 (dd, J = 8.4 Hz, 1H), 4.36−4.40 (dd, J = 8.3 Hz, 1H), 4.45 (d, J = 11.8 Hz, 1H), 5.51 (s, 1H), 7.35 (d, J = 5.4 Hz, 1H), 7.70 (d, J = 8.9 Hz, 1H), 7.84−7.86 (dd, J = 8.9, 1.3 Hz, 1H), 7.96 (d, J = 8.3 Hz, 1H), 8.33 (s, 1H), 8.39 (d, J = 8.9 Hz, 1H), 8.43 (s, 1H), 8.98 (d, J = 5.4 Hz, 1H). HRFAB$^+$ MS m/z: 690.30950 (M + H)$^+$, calcd mass for $C_{34}H_{43}O_7N_5F_3$ = 690.31146.

Compound 39. ^{1}H NMR (DMSO, 400 MHz) major rotamer of compound **39** (mixture of ~6:1 ratio) δ: 0.85 (d, J = 6.7 Hz, 3H), 0.91 (d, J = 6.7 Hz, 3H), 0.80−1.02 (m, 5H), 1.02−1.13 (m, 2H), 1.22−1.29 (m, 1H), 1.35−1.41 (m, 1H), 1.44−1.68 (m, 6H), 1.83 (s, 3H), 1.95−2.01 (m, 1H), 2.22−2.29 (m, 1H), ~2.5 (m, 1H, observed by COSY), 4.04−4.09 (dd, J = 11.8, 3.8 Hz, 1H), 4.17−4.27 (m, 3H), 4.39−4.43 (dd, J = 7.2 Hz, 1H), 4.46−4.48 (dd, J = 5.0 Hz, 1H), 7.68 (d, J = 4.8 Hz, 1H), 7.76 (d, J = 8.9 Hz, 1H), 7.89−7.92 (dd, J = 8.9, 1.9 Hz, 1H), 7.95 (d, J = 8.3 Hz, 1H), 8.25 (d, J = 8.9 Hz, 1H), 8.37 (s, 1H), 8.44 (s, 1H), 8.93 (d, J = 4.8 Hz, 1H). HRFAB$^+$ MS m/z: 706.28660 (M + H)$^+$, calcd mass for $C_{34}H_{43}O_6N_5F_3S$ = 706.28864.

Compound 40. ^{1}H NMR (DMSO, 400 MHz) major rotamer of compound **40** (mixture of ~10:1 ratio) δ: 0.88 (d, J = 6.7 Hz, 3H), 0.92 (d, J = 6.7 Hz, 3H), 0.80−1.14 (m, 7H), 1.22−1.29 (m, 1H), 1.34−1.42 (m, 1H), 1.44−1.68 (m, 6H), 1.83 (s, 3H), 1.92−2.00 (m, 1H), 2.26−2.34 (m, 1H), ~2.5 (m, 1H), 3.98−4.01 (dd, J = 11.8, 3.5 Hz, 1H), 4.12−4.16 (dd, J = 7.9 Hz, 1H), 4.18−4.22 (dd, J = 8.3 Hz, 1H), 4.36−4.40 (dd, J = 8.4 Hz, 1H), 4.45 (d, J = 11.8 Hz, 1H), 5.53 (s, 1H), 7.32 (d, J = 5.7 Hz, 1H), 7.69 (bd, J = 8.9 Hz, 2H), 7.95 (d, J = 8.0 Hz, 1H), 8.07 (d, J = 1.9 Hz, 1H), 8.24 (d, J = 9.2 Hz, 1H), 8.43 (s,

1H), 8.94 (d, J = 5.7 Hz, 1H). HRFAB$^+$ MS m/z: 656.28710 $(M + H)^+$, calcd mass for $C_{33}H_{43}O_7N_5{}^{35}Cl$ = 656.28510.

Compound 41. ^{1}H NMR (DMSO, 400 MHz) major rotamer of compound **41** (mixture of ~8:1 ratio) δ: 0.89 (d, J = 6.7 Hz, 3H), 0.93 (d, J = 6.7 Hz, 3H), 0.80−1.14 (m, 7H), 1.22−1.29 (m, 1H), 1.34−1.42 (m, 1H), 1.44−1.68 (m, 6H), 1.83 (s, 3H), 1.92−1.99 (m, 1H), 2.31−2.39 (m, 1H), 2.50−2.59 (m, 1H), 3.99−4.02 [overlapping dd (1H) and s (−OCH_3) 3H], 4.10−4.14 (dd, J = 7.9 Hz, 1H), 4.16−4.20 (dd, J = 8.3 Hz, 1H), 4.37−4.41 (dd, J = 8.4 Hz, 1H), 4.54 (d, J = 12.1 Hz, 1H), 5.69 (s, 1H), 7.47−7.52 (m, 3H), 7.66 (d, J = 8.6 Hz, 1H), 7.95 (d, J = 7.6 Hz, 1H), 8.27 (d, J = 9.9 Hz, 1H), 8.44 (s, 1H), 9.08 (d, J = 6.7 Hz, 1H). HRFAB$^+$ MS m/z: 652.33540 $(M + H)^+$, calcd mass for $C_{34}H_{46}O_8N_5$ = 652.33466.

Compound 42. ^{1}H NMR (DMSO, 400 MHz) major rotamer of compound **42** (mixture of ~3:1 ratio) δ: 0.88 (d, J = 6.7 Hz, 3H), 0.93 (d, J = 6.7 Hz, 3H), 0.80−1.14 (m, 7H), 1.05−1.15 (m, 1H), 1.22−1.68 (5 overlapping m, 7H), 1.81 (s, 3H), 1.93−2.02 (m, 1H), 2.32−2.42 (m, 1H), 2.53−2.62 (m, 1H), 3.92−3.95 [overlapping d (1H) and s (−OCH_3) 3H], 4.05−4.09 (m, 1H), 4.19−4.23 (dd, J = 7.8 Hz, 1H), 4.40−4.47 (m, 2H), 5.65 (bs, 1H), 7.51−7.55 (m, 3H), 7.71−7.75 (dd, J = 9.5, 2.9 Hz, 1H), 7.80 (d, J = 8.3 Hz, 1H), 8.07 (d, J = 9.5 Hz, 1H), 8.45 (s, 1H), 8.99 (d, J = 6.4 Hz, 1H). HRFAB$^+$ MS m/z: 652.33610 $(M + H)^+$, calcd mass for $C_{34}H_{46}O_8N_5$ = 652.33466.

Compound 43. ^{1}H NMR (DMSO, 400 MHz) major rotamer (mixture of ~9:1 ratio) δ: 0.80 (d, J = 6.4 Hz, 3H), 0.85 (d, J = 6.4 Hz, 3H), 0.84−1.1 (m, 6H), 1.19 (d, J = 4.4 Hz, 6H), 1.21 (d, J = 4.4 Hz, 6H), 1.22−1.28 (m, 2H), 1.32−1.37 (m, 1H), 1.48−1.63 (m, 7H), 1.82 (s, 3H), 1.90−1.96 (m, 1H), 2.00−2.08 (m, 1H), 2.12 (s, 6H), 2.25−2.32 (m, 1H), 3.72−3.75 (dd, J = 10.8, 3.2 Hz, 1H), 3.90 (bd, J = 11.4 Hz, 1H), 4.20−4.24 (dd, J = 8.3 Hz, 1H), 4.38−4.42 (m, 2H), 4.71 (bs, 1H), 4.77−4.86 (m, 2H), 6.97 (s, 2H), 7.84 (d, J = 8.3 Hz, 1H), 7.87 (d, J = 8.9 Hz, 1H), 8.51 (s, 1H), 9.81 (s, 1H). ES$^+$ MS m/z: 801.4 $(M + H)^+$, 823.4 $(M + Na)^+$. ES$^-$ MS m/z: 799.4 $(M - H)^+$.

Acknowledgment. We wish to thank Ms. Diane Thibeault and Mr. Roger Maurice for all the enzymatic assays with NS3 protease, as well as Dr. Pierre Bonneau and Ms. Céline Plouffe for the specificity assays against human leucocyte elastase and bovine pancreatic α-chymotrypsin. We are also grateful to Dr. Pierre Lavallée for many valuable discussions on this project and for proof reading the manuscript.

Supporting Information Available: Copies of ^{1}H, COSY, ^{13}C, HMQC, and HMBC NMR data for key compounds **10** and **41**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO010164D