# Novel Peptide Mimetics Based on *N*-protected Amino Acids Derived from Isomannide as Potential Inhibitors of NS3 Serine Protease of Hepatitis C Virus

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**Abstract:** Hepatitis C virus (HCV) is among the most important flaviviruses. It has a serine protease which is important for viral replication and this enzyme constitutes a suitable target for new anti-retroviral drugs. Herein we disclose a series of amide and ester peptide mimetic inhibitors of serine proteases, all of them obtained via coupling reactions of isomannide derivatives with *N*-protected amino acids. The arginine derivative **19** showed 45% of inhibition of NS3/4A serine protease at 100 μM and molecular modeling studies had shown that **19** interacted with the active site of this enzyme.

**Keywords:** HCV, isomannide, peptide mimetic, serine protease.

## INTRODUCTION

The most important flavivirues known are the Hepatitis C virus (HCV), the West Nile virus (WNV) and the Dengue virus (DENV). More than 170 million people worldwide are affected by HCV and this infection is associated with liver cirrhosis and hepatocellular carcinoma [1, 2]. The current therapeutic for HCV based on alpha interferon and the nucleoside analog ribavirin is effective in only 50% of the patients and is limited by the adverse effects of both agents [3].

This flavivirus presents a non structural NS3 serine protease (NS3 pro) which has in its active site a characteristic catalytic triad His, Asp and Ser [4-6]. The NS3 pro activity is essential for viral replication and maturation of infectious virions, representing an attractive target for the development of chemotherapeutic approaches for the treatment of flaviviruses [7, 8]. In fact, new inhibitors of HCV NS3 protease are in clinical trials [9]. Among them, the clinical candidate BILN-2061 was shown to be effective in reducing HCV viral load but was abandoned due to cardiotoxicity in animal studies [10].

Peptides interact with NS3 pro and have received high interest due to their activity and specificity. However, there are some limitations associated with peptide drugs, the greatest one being the low bioavailability upon oral administration [11]. The drugs can undergo a pre-systemic enzymatic degradation and, moreover, the gastrointestinal tract provides an additional barrier. If a peptide drug manages to pass these barriers, enzymatic digestion in the blood stream will occur, resulting in a very low half-life [12]. Studies on the development of peptide mimetic inhibitors have demonstrated the importance of the peptide approach in drug research in order to overcome the low bioavailability of peptide drugs [13-15]. Indeed, in the last decade the development of peptide mimetic-based inhibitors of NS3 pro with activity against both HCV and Dengue virus have enjoyed a surge in popularity [16-26].

Compounds possessing a fused-bicycle structure, such as Darunavir (TCM-114) [27] and VX-950 [28], represent an effort toward the design and development of new drugs. On the other hand, as the diversity of carbohydrate-based templates has been and can be further exploited in the preparation of carbohydrate-based peptide mimetics [29-31], we envisaged the isomannide (**1**) rigid scaffold as an important moiety in the structure of compounds that can be used as protease inhibitors.

Isomannide itself is a kind of U-shaped structure so forcing a kind of $\beta$-sheet structure in a given compound. Inversion of configuration on the hydroxy group leads to a kind of W-shaped conformation forcing a *quasi* linear structure. These easy structural manipulations are of great importance in designing new compounds, in particular peptide mimetic compounds [27].

We have employed **1** as the core unity of the peptide mimetic compounds, rationalizing its use as a structural analog of cyclic rigid dipeptides and a $C_2$ symmetry providing a rigid scaffold [32-34]. In this context we have

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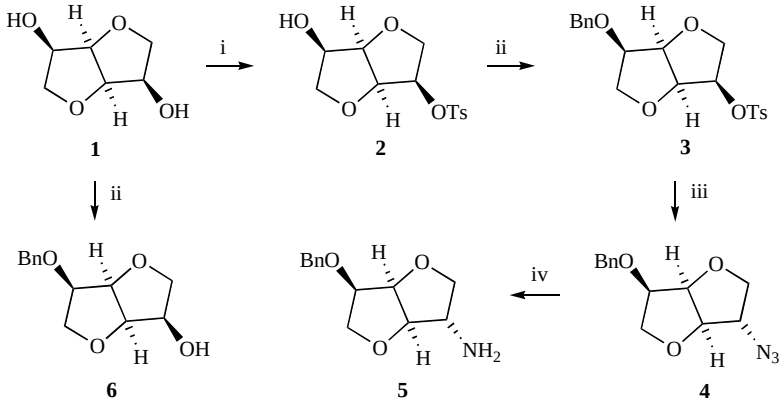
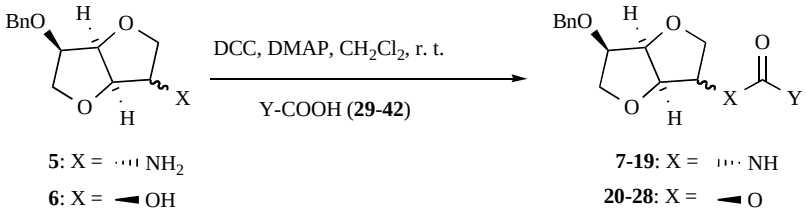
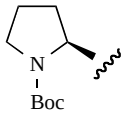
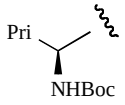
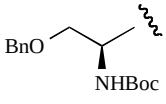
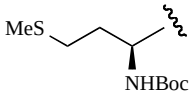
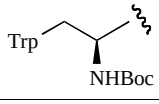
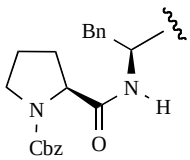
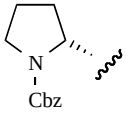


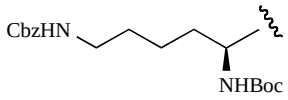
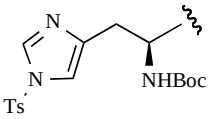
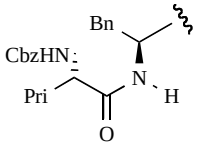
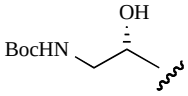
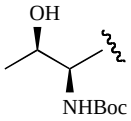
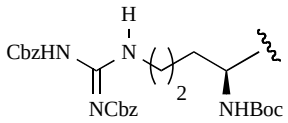
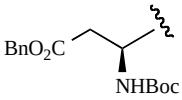

**Scheme 1. i**) TsCl, pyridine, $CH_2Cl_2$, r. t., overnight, 40%. **ii**) BnCl, TBAB, 50% NaOH, $CH_2Cl_2$, r. t., overnight, 75% (for **2**) or 45% (for **6**). **iii**) $NaN_3$, $[bmim]^+[BF4]^-$, r. t., overnight, 73%. iv) $H_2$ (2.75 bar), 5% Pd-C, EtOH, 5 h, 100%.

**Table 1. Structures and Yields of Amides (7-19) and Esters (20-28)**




| Y-COOH (29-42) | Y | # | Yield (%) | # | Yield (%) |
|---|---|---|---|---|---|
| *N*-Boc- L-Pro (**29**) |  | **7** | 31 | **20** | 50 |
| *N*-Boc-L-Val (**30**) |  | **8** | 40 | **21** | 60 |
| *N*-Boc-O-Benzyl-L-Ser (**31**) |  | **9** | 46 | **22** | 53 |
| *N*-Boc-L-Met (**32**) |  | **10** | 63 | **23** | 75 |
| *N*-Boc-L-Trp (**33**) |  | **11** | 67 | **24** | 75 |
| *N*-Cbz-Pro-Phe (**34**) |  | **12** | 97 | **25** | 46 |
| *N*-Cbz-D-Pro (**35**) |  | **13** | 63 | **26** | 63 |

**(Table 1). Contd…..**

| Y-COOH (29-42) | Y | # | Yield (%) | # | Yield (%) |
|---|---|---|---|---|---|
| *N*- -Boc-*N* -Cbz-L-Lys (**36**) |  | **14** | 64 | **27** | 52 |
| *N*-Boc imTosyl-L-His (**37**) |  | **15** | 63 | | |
| *N*-Cbz-Val-Phe (**38**) |  | **16** | 80 | | |
| *N*-Boc-L-IsoSer (**39**) |  | **17** | 50 | | |
| *N*-Boc-L-Thr (**40**) |  | **18** | 56 | | |
| *N*-α-Boc-*N*-ω-di-Cbz-L-Arg (**41**) |  | **19** | 88 | | |
| *N*-Boc-L-Asp-benzyl ester (**42**) |  | | | **28** | |

found that some synthetic peptide mimetics derived from **1** presented activity against HCV [32, 33] and appears to interact with the desired target as shown by computational studies [34, 35]. Indeed, the docking of diester-based peptide mimetics derived from **1** into the active site of NS3 showed that one oxygen atom of the bicycle group at P1' position interacts with a Gly residue via hydrogen bond. In spite of their cytotoxicities, in diamide-based peptide mimetic series derived from **1** the amino acids residues at position P1 interacts via hydrogen bond with Ser135 in the catalytic site of NS3 [34, 35]. In order to direct our research program toward new peptide mimetic antiviral compounds, we present a series of *N*-protected amino acids derived from **1**. The biological data on the inhibition of NS3 as well as the modeling studies on the interaction of the best active compound **19** with this enzyme are also shown.

## RESULTS AND DISCUSSION

### Chemistry

The first step of our synthesis is the transformation of the readily available isomannide (**1**) into its monotosyl derivative **2** using tosyl chloride in pyridine (Scheme **1**). Compound **2** was alkylated with benzyl chloride in 50% aqueous NaOH under phase-transfer conditions affording the *O*-benzylated product **3** [35-37]. The $S_N2$-type reaction of **3** with sodium azide performed in $[bmim]^+[BF_4]^-$ yielded the azido derivative **4** with inversion of configuration [38]. Reduction of **4** with $H_2$/Pd-C gave the amino derivative **5**, the starting material for the production of the amide derivatives [39]. Monobenzylate isomannide **6**, the starting material for formation of the ester derivatives, was obtained from **1** [37].

Among the peptide-coupling reagents, DCC has attracted attention because it shows at least reasonable performance, besides being relatively cheap and the reactional by products are generally insoluble in most organic solvents [40, 41]. So we decided to use DCC reagent for coupling reactions of amine derivative **5** and monobenzylate isomannide **6** with different commercially available *N*-protected amino acids **29**-**42** (Table **1**). Using a standard protocol it was made possible to reach the corresponding amides **7**-**19** and esters **20**-**28** in moderate to good yields.

### Biology

#### *Inhibitory effect of peptide mimetics (in vitro)*

In our preliminary studies we identified a lead peptide mimetic compound as inhibitor of HCV-1b replicon in Huh-7 cell culture [32]. The scaffold of this lead compound was used to design the new peptide mimetics **7**-**19** and **20**-**28**,

which were diluted in DMSO. The standard inhibitor Ac-DE-Dif-E-Cha-C (AnaSpec, CA, USA) was diluted in water.

The inhibitory effect of peptide mimetic compounds **7**-**19** and **20**-**28** on substrate cleavage by NS3/4A protease was investigated. In Fig. (**1**), the percentage of substrate cleavage in the presence of **7**-**19** and **20**-**28** as well as of the standard inhibitor was determined as residual activity using the control sample in the absence of inhibitor compounds as reference. The arginine derivative **19** was shown to inhibit 45% of the NS3/4A protease activity at 100 μM, whereas other compounds such as **16**, **17** and **18** showed only ~15-40% inhibition at the same concentration.

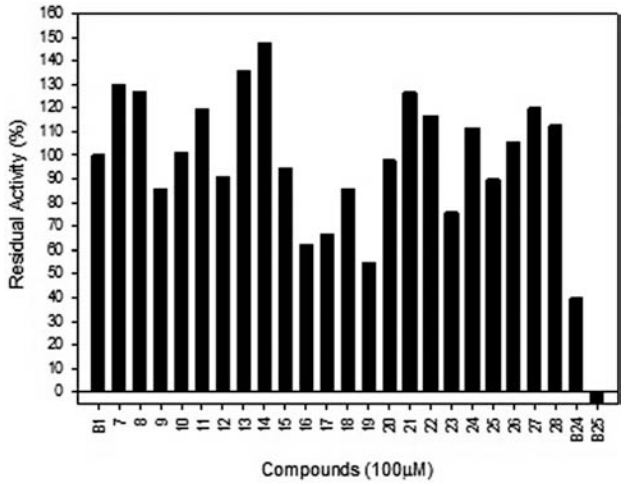



**Fig. (1).** Inhibitory effect of NS3/4A protease. Bar 1 (B1): no inhibitor control. Bars 2 to 23: compounds 7 to 28. Bar 24 (B24): Ac-DE-Dif-Cha-E-C inhibitor. Bar 25 (B25): without inhibitor and NS3/4A protease.

To measure the dose-response activity of **19**, the proteolytic activity assay was carried out at different inhibitor concentrations using the protocol described in the Experimental section. Inhibitory studies of the NS3/4A protease were performed using different concentrations of compound **19** (Fig. **2A**). The results showed that the compound **19** is unable to inhibit 100% of the proteolytic activity at that high concentration gave a maximum inhibition of 50% of initial activity. Interestingly, compound **19** at a low concentration yielded 2.5 fold increase in the proteolytic activity of NS3/4A protease enzyme. This activation effect at low concentration was already observed with certain protease inhibitors recently described in the literature [42]. To confirm whether the NS3/4A protease enzyme (commercially available) could be fully inhibited, the dose-response of enzyme was evaluated using the Ac-DE-Dif-Cha-E-C inhibitor (AnaSpec, CA, USA). Various concentrations of the inhibitor were tested in four replicates each, and the inhibitory effect was observed in the range of concentrations tested ($IC_{50}$ = 0.8 ± 0.3 μM; Fig. **2B**).

## Molecular Modeling

In order to investigate the binding mode of the most active peptide mimetic compound **19**, we docked it into NS3/4A serine protease using Autodock 4.0 program running on a Windows based PC [43]. The 3D structure of the ligand was built and optimized with the semi-empirical AM1 Hamiltonian with the molecular modeling program Spartan'06 (Wavefunction, Inc.). NS3/4A serine protease crystal structure was obtained from Protein Data Bank (PDB ID: 2OC0) [44].

Briefly the compound **19** was embedded in the NS3/4A protease active site represented by the cubic grid box of 60x60x60Å³ with a spacing of 0.375Å. Docking studies were carried out using the empirical free energy function and the Lamarckian genetic algorithm applying a standard protocol, with an initial population of 150 randomly placed individuals and a maximum number of $2.5 \times 10^6$ energy evaluations. A total of 50 independent docking runs were carried out for the most active compound **19**. To evaluate the accuracy of AutoDock 4.0 as an appropriate docking tool for the present

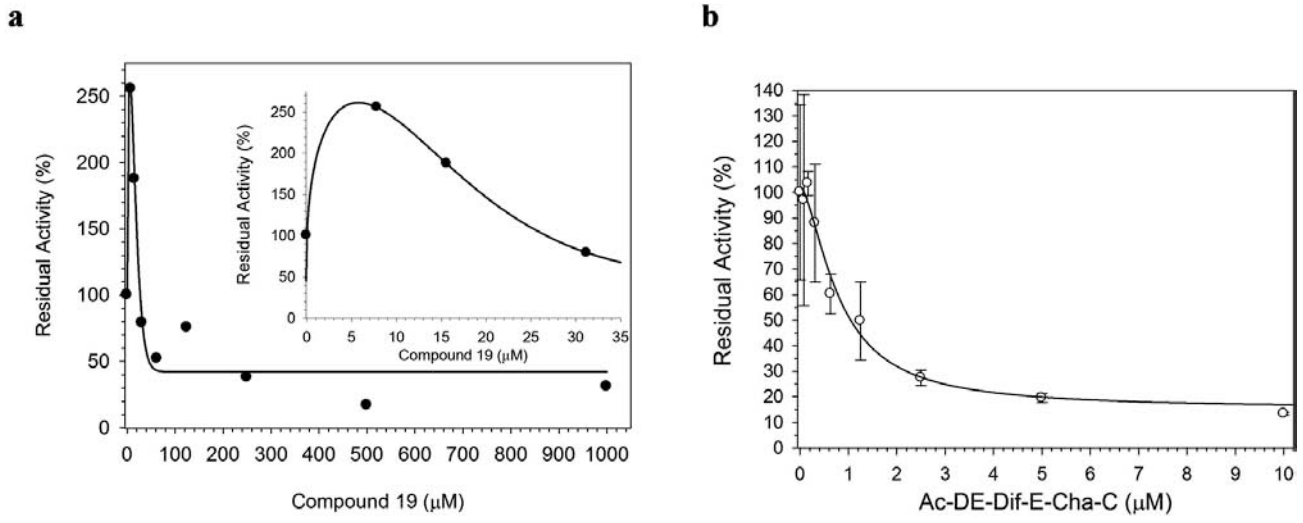



**Fig. (2).** Inhibition of the proteolytic activity by the compound **19** (**a**) Inhibitory activity of **19** against the NS3/4A protease. *Inset*, it is showing the residual activity of NS3/4A protease at low concentration (0.78, 15.6 and 31.2μM) of **19**, and (**b**) inhibitory activity of Ac-DE-Dif-E-Cha-C against the NS3/4A protease. Each point represents the mean of four replicates independent in different concentrations of inhibitors (0.08, 0.16, 0.31, 0.63, 1.25, 2.5, 5.0, and 10.0μM).

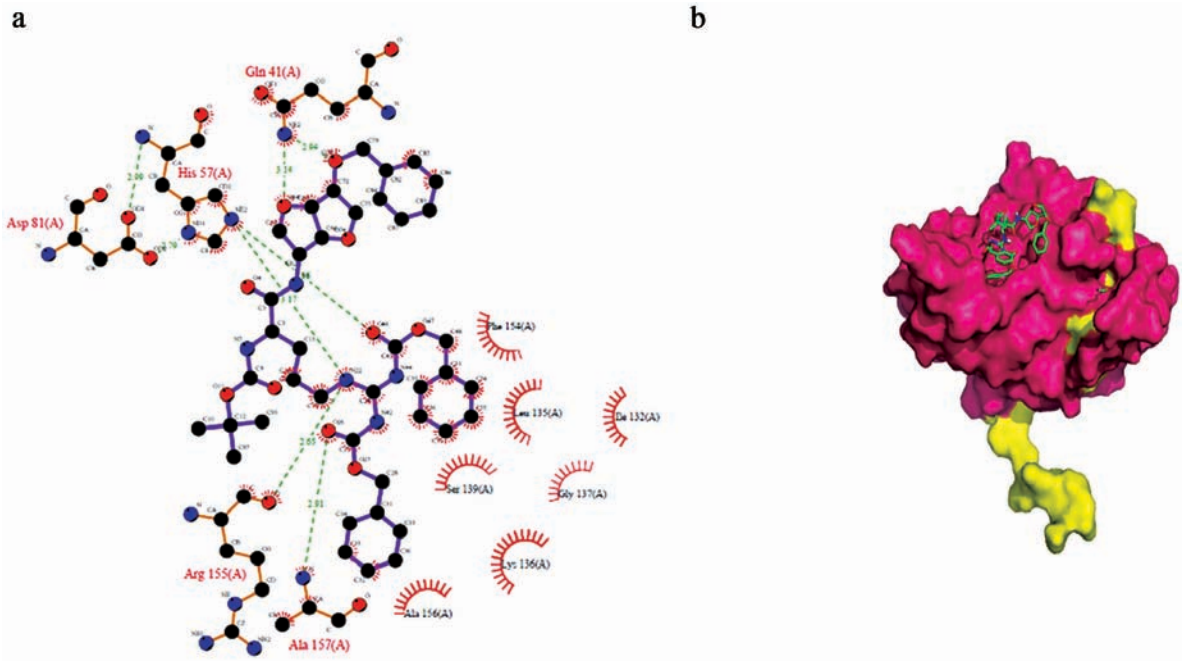



**Fig. (3).** (**a**) Schematic figure of the important interactions between compound **19** and NS3/4A protease, including hydrogen bonds and hydrophobic interactions with specific residues. (**b**) Compound **19** (in green) bound to NS3/4A protease (NS3 is colored in pink and NS4A cofactor is colored in yellow).

work, we performed a re-docking of the native co-crystallized ligand (SCh491762) into NS3/4A protease binding site.

In order to analyze the binding mode of compound **19**, our most active peptide mimetic inhibitor, docking studies were performed (Fig. **3**). Docking of **19** revealed that this compound is placed in S1 pocket, a hydrophobic surface consisting of Ile132, Leu135, Lys136, Gly137, Ser138, Ser139, Phe154 and Ala157 residues [44]. In fact, the docking complex of **19**-NS3/4A protease showed that the arginine moiety interacts with Ile132, Leu135, Lys136, Gly137, Ser139 and Phe154 residues from the hydrophobic S1 pocket of NS3/4A and also with Gln41, His57, Ala156 residues. Additionally, six hydrogen bonds were observed in the complex with Gln41, His57, Arg155 and Ala157 with distances between 2.65Å and 3.17Å. These six hydrogen bonds are obtained from these amino acid residues with the arginine portion of the molecule as well as the isomannide ring. This moiety shows also a hydrogen bond with Gln41. These interactions may contribute to the stabilization and maintenance of **19** into NS3/4A protease active site.

## CONCLUSIONS

New isomannide-based peptide mimetics **7-28** possessing amide and ester groups were easily prepared in high purities employing a general and suitable protocol for larger library of compounds that can be potentially useful against Hepatitis C or other flaviviruses. The most active compound of the series, the arginine derivative **19**, has shown some inhibitory effect on HCV NS3/4A serine protease at 100μM. The docking studies showed a good interaction of arginine and isomannide moieties of **19** into the active site of NS3 serine protease.

## EXPERIMENTAL

### General Procedures

All solvents were purchased as reagent grade, dried using standard conditions and stored over molecular sieves. Purification of products was carried out using silica gel flash chromatography (Whatman 60, 230-400 mesh). NMR analyses were carried out on a Varian Unity Plus-300 spectrometer. Melting points were obtained on a Thomas Hoover capillary melting point apparatus and are uncorrected. High-resolution mass spectra (HRMS) were performed on a Waters Micromass Q-Tof Micromass spectrometer equipped with a lock spray source. The IR spectra were obtained on a Perkin-Elmer spectrometer model Spectrum One in liquid film or KBr pellets. Optical rotation measurements were determined on a Perkin-Elmer 341 LC polarimeter.

### *1,4:3,6-dianhydro-mono(4-methylbenzenesulfonate)-D-mannitol (2)*

To a solution of **1** (1.0 g, 6.85 mmol) and pyridine (1.10 mL) in $CH_2Cl_2$ (10 mL) was added TsCl (1.49 g, 7.81 mmol) at 0 $^oC$. The mixture was stirred at r. t. overnight and diluted with $CH_2Cl_2$ (20 mL). The solution was washed with $H_2O$, 1M HCl, brine and dried ($Na_2SO_4$). The product was recrystallized (EtOAc-*i*-PrOH) affording a white solid (0.822 g, 40%), mp 103-104°C. IR (KBr) $\nu_{max}$ ($cm^{-1}$): 3526, 2933, 2866, 1596, 1359, 1189, 1173, 1050, 1019, 818, 663. $^1H$ NMR (300 MHz, $CDCl_3$): 7.84 (d, J = 8.1Hz, 2H), 7.34 (d, J = 8.1Hz, 2H), 4.91 (dd, J = 6.6Hz, J = 5.5Hz, 1H), 4.42 (t, J = 5.1Hz, 1H), 4.49 (t, J = 4.8Hz, 1H), 4.29-4.25 (m, 1H), 4.04-3.95 (m, 2H), 3.79 (t, J = 7.8Hz, 1H), 3.55 (dd, J = 7.2Hz, J = 1.8Hz, 1H), 2.46 (s, 3H).

***1,4:3,6-dianhydro-5-O-(phenylmethyl)-2-(4-methylbenzen-esulfonate)-D-mannitol (3)***

To a mixture of **2** (10 g, 33.3 mmol), aq 50% KOH and TBAB (0.322 g, 1 mmol) in $CH_2Cl_2$ (80 mL) was added BnCl (6.54 g, 38.2 mmol). After stirring overnight, the mixture was diluted with water (10 mL) and the organic layer was separated. The aqueous layer was extracted with $CH_2Cl_2$ and the combined organic extracts were dried ($Na_2SO_4$). The product was purified by column chromatography on $SiO_2$ (EtOAc-hexane 5/ 95) affording a colorless oil (9.75 g, 75%) $[\alpha]_D^{20}$ +98 (*c* 0.1, DMSO). IR (film) $\nu_{max}$ ($cm^{-1}$): 3063, 2977, 2950, 2879, 1598, 1454, 1366, 1190, 1178, 1141, 1027, 853. $^1$H NMR (300 MHz, $CDCl_3$): 7.81 (d, J = 8.4Hz, 2H), 7.35-7.32 (m, 7H), 4.91-4.89 (m, 1H), 4.69 (d, J = 12.0Hz, 1H), 4.50 (d, J = 12.0Hz, 1H), 4.47 (t, J = 2.1Hz, 2H), 4.04-3.79 (m, 4H), 3.62 (t, J = 8.4Hz, 1H), 2.44 (s, 3H).

***1,4:3,6-dianhydro-2-azido-2-deoxy-5-O-(phenylmethyl)-D-glucitol (4)***

A mixture of 3 (1.86 g, 4.77 mmol), $[bmim]^+[BF_4]^-$ (previously dried under vacuum at 90 °C) (4.8 mL, 23.85 mmol) and $NaN_3$ (0.93 g, 14.3 mmol) was heated at 120 °C overnight. Water was added and the aqueous layer was extracted with ethyl ether. The product was purified by flash column chromatography on $SiO_2$ (EtOAc-hexane 5/ 95) to give a pale yellow oil (0.908 g, 73%) $[\alpha]_D^{20}$ +92 (*c* 0.1, DMSO). IR (film) $\nu_{max}$ ($cm^{-1}$): 3063, 3031, 2946, 2878, 2102, 1455, 1320, 1256, 1135, 1100, 1083, 1021, 739. $^1$H NMR (300 MHz, $CDCl_3$): 7.36-7.26 (m, 5H), 4.75 (d, J = 11.7Hz, 1H), 4.66 (t, J = 4.5Hz, 1H), 4.54 (d, J = 11.7Hz, 1H), 4.48 (d, J = 4.2Hz, 1H), 4.15-4.00 (m, 4H), 3.86 (dd, J = 6.3Hz, J = 2.7Hz, 1H), 3.66 (t, J = 7.8Hz, 1H). $^{13}$C NMR (75.4 MHz, $CDCl_3$): 137.4, 128.3, 127.8, 127.7, 86.4, 80.5, 78.8, 72.6, 72.4, 70.7, 66.2. HRMS (FAB): Calcd. for $C_{13}H_{15}N_3O_3Na$ $[M + H^+]$: 284.1011. Found: 284.1025.

***1,4:3,6-dianhydro-2-amino-2-deoxy-5-O-(phenylmethyl)-D-glucitol (5)***

To a solution of azide 4 (0.870 g, 3.33 mmol) in EtOH (25 mL) was added 5% Pd-C (0.33 mmol) and the mixture was hydrogenated under 2.75 bar for 5 hours. The mixture was filtered under XAD-4 and washed with EtOH affording the product as a colorless oil (0.783 g, 100%) $[\alpha]_D^{20}$ +104 (*c* 0.1, DMSO). IR (KBr) $\nu_{max}$ ($cm^{-1}$): 3360, 2876, 1605, 1455, 1369, 1209, 1065, 1017, 751, 700. $^1$H NMR (300 MHz, $CDCl_3$): 7.37-7.35 (m, 5H), 4.76 (d, J = 11.4Hz, 1H), 4.68 (t, J = 4.2Hz, 1H), 4.54 (d, J = 12.0Hz, 1H), 4.27 (d, J = 4.2Hz, 1H), 4.06-4.02 (m, 1H), 3.85 (dd, J = 6.3Hz, J = 2.4Hz, 1H), 3.76 (d, J = 9.3Hz, 1H), 3.64 (t, J = 7.8Hz, 1H), 3.53 (d, J = 4.2Hz, 1H), 3.45 (s, 1H). $^{13}$C NMR (75.4 MHz, $CDCl_3$): 137.9, 128.6, 128.0, 127.8, 90.0, 80.1, 79.4, 76.3, 72.6, 70.5, 58.8. HRMS (FAB): Calcd. for $C_{13}H_{17}NO_3Na$ $[M + H^+]$: 236.1287. Found: 236.1284.

***1,4:3,6-dianhydro-5-O-(phenylmethyl)-2-hydroxy-D-mannitol (6)***

To a mixture of **1** (10 g, 68.5 mmol), aq 50% KOH and TBAB (0.622 g, 2.057 mmol) in $CH_2Cl_2$ (160 mL) was added BnCl (13.45 g, 78.6 mmol). After stirring overnight, the mixture was diluted with water (22 mL) and the organic layer was separated. The aqueous layer was extracted with $CH_2Cl_2$ and the combined organic extracts were dried ($Na_2SO_4$). The product was purified by column chromatography on $SiO_2$ (EtOAc-hexane 1/ 4) affording a white solid (7.27 g, 45%), mp 93ºC. IR (KBr) $\nu_{max}$ ($cm^{-1}$): 3459, 3404, 2920, 2865, 1459, 1419, 1329, 1266, 1200, 1118, 1068, 1021, 820. $^1$H NMR (300 MHz, $CDCl_3$): 7.36-7.27 (m, 5H), 4.79 (d, J = 12.0Hz, 1H), 4.58 (d, J = 12.0Hz, 1H), 4.57-4.53 (m, 1H), 4.48 (t, J = 5.1 Hz, 1H), 4.27 (br, 1H), 4.11-3.95 (m, 3H), 3.76-3.68 (m, 2H), 2.82 (br, 1H). $^{13}$C NMR (75.4 MHz, $CDCl_3$, ppm): 137.5, 128.4, 127.9, 127.8, 81.7, 80.6, 78.9, 74.7, 72.5, 72.2, 71.3.

**General Procedure for Amide (7-19) and Ester (20-28) Compounds**

To a solution of the amino derivative **5** or the benzyl-isomannide **6** (1.27 mmol) in $CH_2Cl_2$ (10 mL) at 0ºC was added the appropriate *N*-protected amino acid (1.27 mmol), DCC (1.27 mmol) and DMAP (0.12 mmol). The mixture was stirred for 12 hours at room temperature after which it was filtered and evaporated under reduced pressure. The product was purified either by recrystallization or by flash chromatography on silica gel using hexane and ethyl acetate mixture as eluent.

***1,4:3,6-dianhydro-5-O-(benzyl)-2-N-(N-t-butoxycarbonyl-L-proline)-D-glucitol (7)***

1,4:3,6-dianhydro-2-amino-2-deoxy-5-O-(phenylmethyl)-D-glucitol (**5**) (0.300 g, 1.27 mmol) and *N*-Boc- L-Pro (**29**) (0.273 g, 1.27 mmol) were allowed to react as described above. The pure product was obtained by flash chromatography on $SiO_2$ (EtOAc-hexane 3/ 7). The reaction produced a colorless oil (0.172 g, 31%). $[\alpha]_D^{20}$ +91 (*c* 0.1, DMSO). IR (film) $\nu_{max}$ ($cm^{-1}$): 3055, 2986, 1684, 1604, 1423, 1159, 987, 896, 741. $^1$H NMR (300 MHz): 7.40-7.25 (m, 5H), 4.77-4.74 (m, 1H), 4.66 (t, J = 4.8 Hz, 1H), 4.57 (d, J = 11.4 Hz, 1H), 4.41-4.40 (m, 1H), 4.25-4.24 (m, 1H), 4.16-4.12 (m, 2H), 4.10-4.01 (m, 1H), 3.87-3.82 (m, 1H), 2.21-1.92 (m, 2H), 1.92-1.86 (m, 2H), 1.41 (s, 9H). $^{13}$C NMR (75.4 MHz, $CDCl_3$): 175.7, 155.9, 139.4, 129.4, 129.0, 128.8, 88.4, 81.9, 81.4, 80.7, 74.0, 73.5, 71.7, 61.4, 58.5, 32.6, 28.7, 25.4, 24.7. HRMS (FAB): Calcd. for $C_{23}H_{32}N_2O_6Na$ $[M^+]$: 455.2158. Found: 455.2164.

***1,4:3,6-dianhydro-5-O-(benzyl)-2-N-(N-t-butoxycarbonyl-L-valine)-D-glucitol (8)***

1,4:3,6-dianhydro-2-amino-2-deoxy-5-O-(phenylmethyl)-D-glucitol (**5**) (0.300 g, 1.27 mmol) and *N*-Boc-L-Val (**30**) (0.276 g, 1.27 mmol) were allowed to react as described above. The pure product was obtained by recrystallization in ethyl ether. The reaction produced a white solid (0.223 g, 40%), mp 102-103ºC. $[\alpha]_D^{20}$ +67 (*c* 0.1, DMSO). IR (KBr) $\nu_{max}$ ($cm^{-1}$): 3335, 2968, 2934, 2879, 1687, 1654, 1529, 1461, 1368, 1299, 1250, 1169, 1092, 1052, 1018, 738. $^1$H NMR (300 MHz, $CDCl_3$): 7.38-7.24 (m, 5H), 6.23 (br, 1H), 5.01 (br, 1H), 4.76 (d, J = 11.7Hz, 1H), 4.60-4.53 (m, 2H), 4.42 (q, J = 3.9Hz, 2H), 4.08-4.02 (m, 2H), 3.88-3.81 (m, 3H), 3.69-3.63 (m, 1H), 2.15-2.09 (m, 1H), 1.44 (s, 9H), 0.95 (d, J = 6.9Hz, 3H), 0.91 (d, J = 6.9 Hz, 3H). $^{13}$C NMR (75.4 MHz, $CDCl_3$): 171.4, 155.9, 137.6, 128.4, 127.9, 127.8, 86.9, 80.3, 80.2, 80.0, 79.0, 72.5, 70.8, 59.9, 56.8, 30.6, 28.2, 19.3, 17.8.

HRMS (FAB): Calcd. for $C_{23}H_{34}N_2O_6Na$ [$M^+$]: 457.22315. Found: 457.2324.

***1,4:3,6-dianhydro-5-O-(benzyl)-2-N-(N-t-butoxycarbonyl-O-benzyl-L-serine)-D-glucitol (9)***

1,4:3,6-dianhydro-2-amino-2-deoxy-5-O-(phenylmethyl)-D-glucitol (**5**) (0.300 g, 1.27 mmol) and *N*-Boc-O-Benzyl- L-Ser (**31**) (0,374 g, 1.27 mmol) were allowed to react as described above. The pure product was obtained by recrystallization in ethyl ether. The reaction produced a white solid (0.302 g, 46%), mp 116-117ºC. $[\alpha]_D^{20}$ +57 (*c* 0.1, DMSO). IR (KBr) $\nu_{max}$ ($cm^{-1}$): 3289, 2968, 2974, 2873, 1690, 1650, 1553, 1511, 1299, 1310, 1243, 1168, 1133, 1049, 864, 742. $^1$H NMR (300 MHz, $CDCl_3$): 7.35-7.27 (m, 10H), 4.73 (d, J = 12.0 Hz, 1H), 4.54-4.45 (m, 3H), 4.40-4.38 (m, 2H), 4.32-4.31 (m, 1H), 4.23-4.21 (m, 1H), 4.05-3.95 (m, 2H), 3.91-3.81 (m, 3H), 3.67-3.57 (m, 1H), 3.64 (dd, J = 7.5, 8.7 Hz, 1H), 3.61-3.54 (m, 1H), 1.44 (s, 9H). $^{13}$C NMR (75.4 MHz, $CDCl_3$): 169.9, 155.7, 137.6, 137.3, 128.5, 128.4, 128.0, 127.9, 127.8, 86.8, 80.4, 80.1, 79.1, 73.5, 73.2, 72.4, 70.7, 69.8, 56.8, 51.9, 28.2. HRMS (FAB): Calcd. for $C_{28}H_{36}N_2O_7Na$ [$M^+$]: 535.2420. Found: 535.2400.

***1,4:3,6-dianhydro-5-O-(benzyl)-2-N-(N-t-butoxycarbonyl-L-methionine)-D-glucitol (10)***

1,4:3,6-dianhydro-2-amino-2-deoxy-5-O-(phenylmethyl)-D-glucitol (**5**) (0.300 g, 1.27 mmol) and *N*-Boc-L-Met (**32**) (0.316 g, 1.27 mmol) were allowed to react as described above. The pure product was obtained by flash chromatography on $SiO_2$ (EtOAc-hexane 3/ 7). The reaction produced a white solid (0.377 g, 63%), mp 91-93ºC. $[\alpha]_D^{20}$ +34 (*c* 0.1, $CH_2Cl_2$). IR (KBr) $\nu_{max}$ ($cm^{-1}$): 3297, 2974, 2931, 2873, 1660, 1650, 1530, 1452, 1368, 1249, 1168, 1092, 860, 742. $^1$H NMR (300 MHz, $CDCl_3$): 7.35-7.30 (m, 5H), 6.55 (br, 1H), 5.16 (br, 1H), 4.77 (d, J = 12.0 Hz, 1H), 4.59-4.52 (m, 2H), 4.42-4.38 (m, 2H), 4.24-4.17 (m, 1H), 4.08-4.02 (m 2H), 3.87-3.82 (m, 2H), 3.67 (dd, J = 7.5Hz, J = 9.0 Hz, 1H), 2.55-2.52 (m, 2H), 2.10 (s, 3H), 2.06-2.03 (m, 2H), 1.44 (s, 9H). $^{13}$C NMR (75.4 MHz, $CDCl_3$): 171.3, 155.6, 137.6, 128.4, 127.8, 127.8, 86.9, 80.4, 80.2, 73.2, 72.4, 70.8, 56.8, 53.4, 31.2, 30.2, 28.2, 15.2. HRMS (FAB): Calcd. for $C_{23}H_{32}N_2O_6NaS$ [$M^+$]: 489.2035. Found: 489.2038.

***1,4:3,6-dianhydro-5-O-(benzyl)-2-N-(N-t-butoxycarbonyl-L-triptophan)-D-glucitol (11)***

1,4:3,6-dianhydro-2-amino-2-deoxy-5-O-(phenylmethyl)-D-glucitol (**5**) (0.300 g, 1.27 mmol) and *N*-Boc-L-Trp (**33**) (0.386 g, 1.27 mmol) were allowed to react as described above. The pure product was obtained byg flash chromatography on $SiO_2$ (EtOAc-hexane 1/ 1). The reaction produced a white solid (0.448 g, 67%), mp 161-162ºC. $[\alpha]_D^{20}$ +44 (*c* 0.1, $CH_2Cl_2$). IR (KBr) $\nu_{max}$ ($cm^{-1}$): 3321, 2974, 2933, 2881, 1685, 1646, 1523, 1455, 1366, 1250, 1169, 1095, 1018, 863, 743. $^1$H NMR (300 MHz, $CDCl_3$): 8.24 (br, 1H), 7.64 (d, J = 7.8 Hz, 1H), 7.36-7.29 (m, 6H), 7.19-7.07 (m, 3H), 5.70 (br, 1H), 5.29 (br, 1H), 4.48 (q, J = 12.0 Hz, 1H), 4.39-4.36 (m, 1H), 4.23-4.21 (m, 1H), 3.84-3.65 (m, 5H), 3.55-3.49 (m 3H), 3.29 (dd, J = 14.4Hz, J = 5.7Hz, 1H), 3.10 (dd, J = 14.4Hz, J = 8.4Hz, 1H), 1.44 (s, 9H). $^{13}$C NMR (75.4 MHz, $CDCl_3$): 171.4, 155.4, 137.6, 136.1, 128.4, 127.9, 127.7, 127.3, 123.2, 122.2, 119.7, 118.9, 111.2, 110.6, 86.3, 80.1, 79.6, 79.0, 72.6, 72.3, 70.4, 56.4, 55.6, 28.6, 28.3. HRMS (FAB): Calcd. for $C_{29}H_{35}N_3O_6Na$ [$M^+$]: 544.2424. Found: 544.2408.

***1,4:3,6-dianhydro-5-O-(benzyl)-2-N-(N-carbobenzyloxy-L-proline-L-phenylalanine) D-glucitol (12)***

1,4:3,6-dianhydro-2-amino-2-deoxy-5-O-(phenylmethyl)-D-glucitol (**5**) (0.300 g, 1.27 mmol) and *N*-Cbz-Pro-Phe (**34**) (0.503 g, 1.27 mmol) were allowed to react as described above. The pure product was obtained byg flash chromatography on $SiO_2$ (EtOAc-hexane 1/ 1). The reaction produced a white solid (0.761 g, 97%), mp 141-143ºC. $[\alpha]_D^{20}$ -15 (*c* 0.1, $CH_2Cl_2$). IR (KBr) $\nu_{max}$ ($cm^{-1}$): 3279, 3069, 2949, 2878, 1704, 1649, 1554, 1448, 1415, 1355, 1247, 1093, 1023, 914, 743. $^1$H NMR (300 MHz, $CDCl_3$): 7.27-7.02 (m, 15H), 6.61 (br, 1H), 6.29 (br, 1H), 5.06-4.90 (m, 2H), 4.66 (d, J = 12.0Hz, 2H), 4.45 (d, J = 12.0Hz, 2H), 4.31-4.27 (m, 2H), 4.18-4.15 (m, 1H), 3.99-3.91 (m, 2H), 3.78-3.72 (m, 2H), 3.56 (t, J = 8.1Hz, 1H), 3.44-3.30 (m, 3H), 3.10-3.03 (m, 1H), 1.93-1.60 (m, 4H). $^{13}$C NMR (75.4 MHz, $CDCl_3$): 171.2, 170.2, 155.9, 137.6, 136.4, 135.9, 129.0, 128.6, 128.5, 128.3, 128.2, 127.7, 127.0, 86.8, 80.1, 79.0, 73.4, 72.3, 70.4, 67.5, 61.2, 57.0, 53.4, 47.0, 37.3, 29.3, 24.3. HRMS (FAB): Calcd. for $C_{35}H_{39}N_3O_7Na$ [$M^+$]: 636.2686. Found: 636.2697.

***1,4:3,6-dianhydro-5-O-(benzyl)-2-N-(N-carbobenzyloxy-D-proline)-D-glucitol (13).***

1,4:3,6-dianhydro-2-amino-2-deoxy-5-O-(phenylmethyl)-D-glucitol (**5**) (0.300 g, 1.27 mmol) and *N*-Cbz-D-Pro (**35**) (0.316 g, 1.27 mmol) were allowed to react as described above. The pure product was obtained by flash chromatography on $SiO_2$ (EtOAc-hexane 1/ 1). The reaction produced a white solid (0.377 g, 63%), mp. 89-90ºC. $[\alpha]_D^{20}$ +94 (*c* 0.1, $CH_2Cl_2$). IR (KBr) $\nu_{max}$ ($cm^{-1}$): 3345, 3034, 2974, 2926, 2873, 1679, 1531, 1451, 1421, 1356, 1253, 1208, 1122, 1091, 952, 866, 758. $^1$H NMR (300 MHz, $CDCl_3$): 7.36-7.26 (m, 6H), 5.15-5.13 (m, 2H), 4.75 (d, J = 11.7Hz, 1H), 4.56 (d, J = 12.0Hz, 1H), 4.30-4.26 (m, 2H), 4.00-3.91 (m, 2H), 3.85-3.80 (m, 2H), 3.66-3.62 (m, 1H), 3.61-3.44 (m, 4H), 2.34-1.85 (m, 4H). $^{13}$C NMR (75 MHz, $CDCl_3$): 171.1, 156.5. 137.6, 136.4, 128.5, 128.4, 128.1, 127.8, 86.9, 80.1, 78.9, 73.4, 72.4, 70.6, 67.3, 60.2, 56.8, 47.0, 27.9, 24.6. HRMS (FAB): Calcd. for $C_{26}H_{30}N_2O_6Na$ [$M^+$]: 489.2002. Found: 489.2011.

***1,4:3,6-dianhydro-5-O-(benzyl)-2-N-(Nα-t-butoxycarbonyl-Nε-carbobenzyloxy-L-lysine)-D-glucitol (14)***

1,4:3,6-dianhydro-2-amino-2-deoxy-5-O-(phenylmethyl)-D-glucitol (**5**) (0.300 g, 1.27 mmol) and *N*-α-Boc-*N*ε-Cbz-L-Lys (**36**) (0.483 g, 1.27 mmol) were allowed to react as described above. The pure product was obtained by flash chromatography on $SiO_2$ (EtOAc-hexane 3/ 7). The reaction produced a white solid (0.490 g, 64%), mp. 62-63ºC. $[\alpha]_D^{20}$ +23 (*c* 0.1, $CH_2Cl_2$). IR (KBr) $\nu_{max}$ ($cm^{-1}$): 3360, 2972, 2937, 2879, 1694, 1659, 1459, 1366, 1254, 1168, 1087, 1015, 908, 749. $^1$H NMR (300 MHz, $CDCl_3$): 7.34-7.30 (m, 10H), 6.55 (br, 1H), 5.29 (s, 2H), 5.09-5.02 (m, 1H), 4.75 (d, J = 11.7Hz, 1H), 4.58 (t, J = 4.5Hz, 1H), 4.51 (d, 1H, *J* = 12Hz), 4.41-4.36 (m, 2H), 4.07-4.00 (m, 2H), 3.86-3.81 (m, 2H), 3.65 (dd, J = 8.7Hz, J = 7.5Hz, 1H), 3.17 (q, J = 6.0 Hz, 2H), 1.84-1.82 (m, 2H), 1.64-1.50 (m, 2H), 1.43 (s, 9H), 1.39-1.34 (m, 2H). $^{13}$C NMR (75.4 MHz, $CDCl_3$): 172.0, 156.6,

155.8, 137.6, 136.5, 128.4, 128.3, 128.0, 127.9, 127.8, 86.9, 80.2, 80.1, 79.0, 73.2, 72.4, 70.7, 66.6, 56.8, 54.3, 40.1, 31.5, 29.4, 28.2, 22.4. HRMS (FAB): Calcd. for $C_{32}H_{43}N_3O_8Na$ [$M^+$]: 620.2948. Found: 620.2958.

***1,4:3,6-dianhydro-5-O-(benzyl)-2-N-($N_\alpha$-t-butoxycarbonyl-N -tosil-L-histidine)-D-glucitol (15)***

1,4:3,6-dianhydro-2-amino-2-deoxy-5-O-(phenylmethyl)-D-glucitol (**5**) (0.300 g, 1.27 mmol) and *N*-Boc imTosyl-L-His (**37**) (0.519 g, 1.27 mmol) were allowed to react as described above. The pure product was obtained by flash chromatography on $SiO_2$ (EtOAc-hexane 2/ 3). The reaction produced a white solid (0.505 g, 63%), mp. 91-93ºC. $[\alpha]_D^{20}$ +55 (*c* 0.1, $CH_2Cl_2$). IR (KBr) $\nu_{max}$ ($cm^{-1}$): 3290, 3073, 2974, 2934, 2879, 1661, 1527, 1376, 1300, 1248, 1174, 1088, 814, 744. $^1$H NMR (300 MHz, $CDCl_3$): 7.93 (s, 1H), 7.81 (d, J = 8.4Hz, 2H), 7.35-7.29 (m, 7H), 7.08 (s, 1H), 6.77 (br, 1H), 5.87 (br, 1H), 4.71 (d, J = 11.7Hz, 1H), 4.55 (d, J = 11.7Hz, 1H), 4.46 (t, J = 4.5Hz, 1H), 4.34-4.23 (m, 3H), 4.04 (q, J = 6.9Hz, 1H), 3.92-3.80 (m, 2H), 3.65-3.59 (m, 2H), 3.04 (dd, J = 14.7Hz, J = 5.1Hz, 1H), 2.85 (dd, J = 14.7Hz, J = 6.3Hz, 1H), 2.42 (s, 3H), 1.42 (s, 9H). $^{13}$C NMR (75.4 MHz, $CDCl_3$): 170.8, 155.4, 146.4, 140.4, 137.6, 134.5, 130.4, 128.3, 127.8, 127.3, 114.9, 86.7, 81.7, 80.1, 79.1, 72.9, 72.4, 70.7, 56.6, 53.9, 30.4, 28.2, 21.6. HRMS (FAB): Calcd. for $C_{31}H_{38}N_4O_8NaS$ [$M^+$]: 649.2308. Found 649.2321.

***1,4:3,6-dianhydro-5-O-(benzyl)-2-N-(N-carbobenzyloxy-valine-phenylalanine)-D-glucitol (16)***

1,4:3,6-dianhydro-2-amino-2-deoxy-5-O-(phenylmethyl)-D-glucitol (**5**) (0.300 g, 1.27 mmol) and *N*-Cbz-Val-Phe (**38**) (0.505 g, 1.27 mmol) were allowed to react as described above. The pure product was obtained by flash chromatography on $SiO_2$ (EtOAc-hexane 3/ 7). The reaction produced a white solid (0.630 g, 80%), mp. 81-83ºC. $[\alpha]_D^{20}$ +26 (*c* 0.1, $CH_2Cl_2$). IR (KBr) $\nu_{max}$ ($cm^{-1}$): 3290, 3066, 2962, 1708, 1646, 1544, 1393, 1241, 1132, 1091, 1023, 914, 741. $^1$H NMR (300 MHz, $CDCl_3$): 7.33-7.12 (m, 15H), 6.77 (br, 1H), 5.59-5.57 (m, 1H), 5.12-5.01 (m, 2H), 4.80 (q, J = 7.8Hz, 1H), 4.65 (dd, J = 12.0, J = 4.5Hz, 1H), 4.45-4.38 (m, 1H), 4.32-4.27 (m, 2H), 4.01-3.80 (m, 3H), 3.77-3.70 (m, 2H), 3.66-3.57 (m, 1H), 3.10-2.98 (m, 2H), 2.11-1.95 (m, 1H), 0.91 (d, J = 6.6Hz, 3H), 0.84 (d, J = 6.6Hz, 3H). $^{13}$C NMR (75.4 MHz, $CDCl_3$): 171.6, 170.5, 156.6, 137.8, 136.3, 136.2, 129.4, 129.3, 128.5, 128.4, 128.3, 128.1, 127.7, 126.9, 126.7, 87.0, 80.3, 78.9, 72.9, 72.2, 70.8, 67.1, 60.7, 56.6, 53.7, 39.2, 31.2, 19.3, 18.1. HRMS (FAB): Calcd. for $C_{35}H_{41}N_7O_7Na$ [$M^+$]: 638.2842. Found: 638.2862.

***1,4:3,6-dianhydro-5-O-(benzyl)-2-N-(N-t-butoxycarbonyl-L-isoserine)-D-glucitol (17)***

1,4:3,6-dianhydro-2-amino-2-deoxy-5-O-(phenylmethyl)-D-glucitol (**5**) (0.300 g, 1.27 mmol) and *N*-Boc-L-IsoSer (**39**) (0.260 g, 1.27 mmol) were allowed to react as described above. The pure product was obtained by flash chromatography on $SiO_2$ (EtOAc-hexane 1/ 4). The reaction produced a white solid (0.271 g, 50%), mp. 133-134ºC. $[\alpha]_D^{20}$ +4.0 (*c* 0.1, $CH_2Cl_2$). IR (KBr) $\nu_{max}$ ($cm^{-1}$): 3398, 3315, 2975, 2936, 2889, 1708, 1640, 1547, 1515, 1399, 1325, 1256, 1171, 1110, 1043, 882, 731. $^1$H NMR (300 MHz, $CDCl_3$): 7.36-7.18 (m, 5H), 5.37 (br, 1H), 5.20 (br, 1H), 4.77 (d, J = 12.0Hz, 1H), 4.64 (t, J = 4.5Hz, 1H), 4.53 (d, J = 12.0Hz, 1H), 4.43-4.41 (m, 2H), 4.17 (br, 1H), 4.11-4.02 (m, 2H), 3.88-3.81 (m, 2H), 3.70-3.64 (m, 1H), 3.61-3.44 (m, 2H), 1.74 (br, 1H), 1.43 (s, 9H). $^{13}$C NMR (75.4 MHz, $CDCl_3$): 171.8, 158.9, 137.6, 128.4, 127.8, 87.0, 80.7, 80.3, 79.0, 73.6, 73.4, 72.4, 70.7, 56.5, 44.7, 28.2. HRMS (FAB): Calcd. for $C_{21}H_{30}N_2O_7Na$ [$M^+$]: 445.1951. Found: 445.1947.

***1,4:3,6-dianhydro-5-O-(benzyl)-2-N-(N-t-butoxycarbonyl-L-threonine)-D-glucitol (18)***

1,4:3,6-dianhydro-2-amino-2-deoxy-5-O-(phenylmethyl)-D-glucitol (**5**) (0.300 g, 1.27 mmol) and *N*-Boc-L-Thr (**40**) (0.262 g, 1.27 mmol) were allowed to react as described above. The pure product was obtained by flash chromatography on $SiO_2$ (EtOAc-hexane 3/ 7). The reaction produced a colorless oil (0.305 g, 56%). $[\alpha]_D^{20}$ +6 (*c* 0.1, $CH_2Cl_2$). IR (film) $\nu_{max}$ ($cm^{-1}$): 3944, 3755, 3691, 3056, 2987, 1682, 1604, 1424, 1265, 1159, 986, 896, 742. $^1$H NMR (300 MHz, $CDCl_3$): 7.35-7.29 (m, 5H), 6.92 (br, 1H), 5.51 (br, 1H), 4.73 (d, J = 12.0Hz, 1H), 4.63-4.61 (m, 1H), 4.56 (d, J = 12.0Hz, 1H), 4.43-4.35 (m, 3H), 4.08-4.04 (m, 2H), 4.00 (dd, J = 8.1Hz, J = 2.1 Hz, 1H), 3.88-3.81 (m, 2H), 3.67 (dd, J = 9.0Hz, J = 7.5 Hz, 1H), 1.46 (s, 9H), 1.17 (d, J = 6.3Hz, 3H). $^{13}$C NMR (75.4 MHz, $CDCl_3$): 171.2, 156.6, 137.6, 128.4, 127.9, 127.8, 86.9, 80.5, 80.3, 78.9, 73.2, 72.4, 70.5, 66.6, 58.2, 56.9, 28.2, 18.3. HRMS (FAB): Calcd. for $C_{22}H_{32}N_2O_7Na$ [$M^+$]: 459.2107. Found: 459.2085.

***1,4:3,6-dianhydro-5-O-(benzyl)-2-N-(Nα-t-butoxycarbonyl-Nδ,Nω-di-carbobenzyl oxy-L-arginine)-D-glucitol (19)***

1,4:3,6-dianhydro-2-amino-2-deoxy-5-O-(phenylmethyl)-D-glucitol (**5**) (0.300 g, 1.27 mmol) and *N*-α-Boc-*N*ω-di-Cbz-L-Arg (**41**) (0.670 g, 1.27 mmol) were allowed to react as described above. The pure product was obtained by flash chromatography on $SiO_2$ (EtOAc-hexane 3/ 7). The reaction produced a white solid (0.838 g, 88%), mp. 81-83ºC. $[\alpha]_D^{20}$ +34 (*c* 0.1, $CH_2Cl_2$). IR (KBr) $\nu_{max}$ ($cm^{-1}$): 3387, 3328, 2929, 2857, 1717, 1656, 1615, 1510, 1451, 1376, 1252, 1093, 744. $^1$H NMR (300 MHz, $CDCl_3$): 9.42 (br, 1H), 7.43-7.29 (m, 15H), 6.68 (br, 1H), 5.64 (br, 1H), 5.29 (s, 2H), 5.24 (s, 2H), 5.19-5.10 (m, 2H), 4.73 (d, J = 12.0Hz, 1H), 4.52 (d, J = 12.0Hz, 1H), 4.33-4.20 (m, 3H), 3.96-3.90 (m, 2H), 3.78 (dd, J = 9.0, J = 7.5 Hz, 1H), 3.63-3.57 (m, 1H), 1.94-1.70 (m, 2H), 1.68-1.58 (m, 4H), 1.43 (s, 9H). $^{13}$C NMR (75.4 MHz, $CDCl_3$): 171.8, 163.4, 160.7, 155.8, 155.6, 137.7, 136.6, 134.5, 128.8, 128.7, 128.5, 128.4, 128.3, 127.9, 127.8, 127.7, 86.9, 80.0, 79.9, 78.9, 72.9, 72.3, 70.4, 68.9, 67.0, 56.9, 49.0, 43.9, 28.4, 25.5, 24.7. HRMS (FAB): Calcd. for $C_{40}H_{49}N_5O_{10}Na$ [$M^+$]: 782.3377. Found: 782.3405.

***1,4:3,6-dianhydro-5-O-(benzyl)-2-O-(N-t-butoxycarbonyl-L-proline)-D-mannitol (20)***

1,4:3,6-dianhydro-5-O-(phenylmethyl)-2-hydroxy-D-mannitol (**6**) (0.300 g, 1.27 mmol) and the *N*-Boc- L-Pro (**29**) (0.273 g, 1.27 mmol) were allowed to react as described above. The pure product was obtained by flash chromatography on $SiO_2$ (EtOAc-hexane 1/ 4). The reaction produced a colorless oil (0.278 g, 50%). $[\alpha]_D^{20}$ +91 (*c* 0.1, DMSO). IR (film) $\nu_{max}$ ($cm^{-1}$): 2974, 2880, 1746, 1697, 1454, 1398, 1261, 1128, 1090, 1028, 885, 819. $^1$H NMR (300 MHz, $CDCl_3$): 7.30-7.21 (m, 5H), 5.06-4.95 (m, 1H), 4.76-4.68 (m, 2H), 4.57 (s, 1H), 4.53-4.46 (m, 1H), 4.39-4.30 (m,

1H), 4.07-3.99 (m, 1H), 3.95-3.84 (m, 2H), 3.51-3.35 (m, 2H), 2.23-2.19 (m, 2H), 2.04-1.86 (m, 2H), 1.40 (s, 9H). $^{13}$C NMR (75.4 MHz, $CDCl_3$): 172.5, 153.7, 137.5, 128.4, 127.9, 80.6, 80.4, 79.9, 78.8, 74.3, 72.5, 70.8, 70.5, 59.0, 46.5, 30.9, 28.3, 24.2. HRMS (FAB): Calcd. for $C_{23}H_{31}NO_7Na$ [$M^+$]: 456.1998. Found: 456.2009.

***1,4:3,6-dianhydro-5-O-(benzyl)-2-O-(N-t-butoxycarbonyl-L-valine)-D-mannitol (21)***

1,4:3,6-dianhydro-5-O-(phenylmethyl)-2-hydroxy-D-mannitol (**6**) (0.300 g, 1.27 mmol) and the *N*-Boc-L-Val (**30**) (0.276 g, 1.27 mmol) were allowed to react as described above. The pure product was obtained by flash chromatography on $SiO_2$ (EtOAc-hexane 3/ 7). The reaction produced a colorless oil (0.335 g, 60%). $[\alpha]_D^{20}$ +107 (*c* 0.1, DMSO). IR (film) $\nu_{max}$ ($cm^{-1}$): 3350, 2970, 2880, 1713, 1502, 1461, 1367, 1251, 1161, 1090, 1023, 866, 740. $^1$H NMR (300 MHz, $CDCl_3$): 7.40-7.25 (m, 5H), 5.14 (q, J = 6.0Hz, 1H), 5.01-4.98 (m, 1H), 4.76-4.68 (m, 2H), 4.58 (s, 1H), 4.49 (t, J = 4.8Hz, 1H), 4.31-4.26 (m, 1H), 4.13-4.00 (m, 2H), 3.95-3.90 (m, 2H), 3.62 (t, J = 8.5 Hz, 1H), 1.44 (s, 9H), 0.98 (d, J = 6.9Hz, 3H), 0.90 (d, J = 6.9Hz, 3H). $^{13}$C NMR (75.4 MHz, $CDCl_3$): 171.7, 155.5, 137.5, 128.4, 127.9, 80.5, 80.3, 79.7, 78.7, 74.8, 72.5, 71.0, 70.5, 58.5, 31.2, 28.3, 18.9, 17.4. HRMS (FAB): Calcd. for $C_{23}H_{33}NO_7Na$ [$M^+$]: 458.2155. Found: 458.2163.

***1,4:3,6-dianhydro-5-O-(benzyl)-2-O-(N-t-butoxycarbonyl-O-benzyl-L-serine)-D-mannitol (22)***

1,4:3,6-dianhydro-5-O-(phenylmethyl)-2-hydroxy-D-mannitol (**6**) (0.300 g, 1.27 mmol) and the *N*-Boc-O-Benzyl-L-Ser (**31**) (0.374 g, 1.27 mmol) were allowed to react as described above. The pure product was obtained by flash chromatography on $SiO_2$ (EtOAc-hexane 1/ 4). The reaction produced a colorless oil (0.348 g, 53%). $[\alpha]_D^{20}$ +92 (*c* 0.1, DMSO). IR (film) $\nu_{max}$ ($cm^{-1}$): 3328, 2974, 2928, 2876, 1749, 1713, 1499, 1363, 1245, 1166, 1098, 1028, 859, 742. $^1$H NMR (300 MHz, $CDCl_3$): 7.36-7.27 (m, 10H), 5.43-5.40 (m, 1H) 5.13 (q, J = 5.7Hz, 1H), 4.74-4.70 (m, 2H), 4.57-4.43 (m, 4H), 4.04-3.88 (m, 5H), 3.86-3.60 (m, 1H), 3.51-3.46 (m, 1H), 1.44 (s, 9H). $^{13}$C NMR (75.4 MHz, $CDCl_3$): 170.1, 155.3, 137.5, 137.4, 128.4, 128.3, 127.9, 127.8, 127.7, 127.6, 80.5, 80.3, 79.9, 78.7, 75.1, 73.2, 72.5, 71.0, 70.4, 69.8, 53.9, 28.3. HRMS (FAB): Calcd. for $C_{28}H_{35}NO_8Na$ [$M^+$]: 536.2260. Found: 536.2278.

***1,4:3,6-dianhydro-5-O-(benzyl)-2-O-(N-t-butoxycarbonyl-L-methionine)-D-mannitol (23)***

1,4:3,6-dianhydro-5-O-(phenylmethyl)-2-hydroxy-D-mannitol (**6**) (0.300 g, 1.27 mmol) and the *N*-Boc-L-Met (**32**) (0.316 g, 1.27 mmol) were allowed to react as described above. The pure product was obtained by flash chromatography on $SiO_2$ (EtOAc-hexane 1/ 4). The reaction produced a colorless oil (0.449 g, 75%). $[\alpha]_D^{20}$ +116 (*c* 0.1, $CH_2Cl_2$). IR (film) $\nu_{max}$ ($cm^{-1}$): 3339, 2974, 2923, 2876, 1710, 1509, 1450, 1366, 1253, 1166, 1069, 1028, 864, 743. $^1$H NMR (300 MHz, $CDCl_3$): 7.36-7.32 (m, 5H), 5.13 (q, J = 5.7Hz, 2H), 4.76-4.69 (m, 2H), 4.59 (s, 1H), 4.49-4.46 (m, 1H), 4.07-4.04 (m, 3H), 3.95-3.90 (m, 1H), 3.60 (t, J = 8.5Hz, 1H), 2.57-2.51 (m, 2H), 2.10-2.05 (m, 2H), 2.08 (s, 3H), 1.44 (s, 9H). $^{13}$C NMR (75.4 MHz, $CDCl_3$): 171.5, 155.1, 137.4, 128.4, 127.9, 127.8, 80.5, 80.3, 79.9, 78.6, 75.0, 72.5, 71.1, 70.4, 52.8, 32.0, 29.7, 28.2, 15.4. HRMS (FAB): Calcd. for $C_{22}H_{33}NO_7NaS$ [$M^+$]: 490.1875. Found: 490.1895.

***1,4:3,6-dianhydro-5-O-(benzyl)-2-O-(N-t-butoxycarbonyl-L-triptophan)-D-mannitol (24)***

1,4:3,6-dianhydro-5-O-(phenylmethyl)-2-hydroxy-D-mannitol (**6**) (0.300 g, 1.27 mmol) and the *N*-Boc-L-Trp (**33**) (0.386 g, 1.27 mmol) were allowed to react as described above. The pure product was obtained by flash chromatography on $SiO_2$ (EtOAc-hexane 15/ 85). The reaction produced a colorless oil (0.500 g, 75%). $[\alpha]_D^{20}$ +114 (*c* 0.1, $CH_2Cl_2$). IR (film) $\nu_{max}$ ($cm^{-1}$): 3344, 2974, 2933, 2876, 1740, 1706, 1500, 1455, 1366, 1247, 1166, 1068, 1023, 858, 743. $^1$H NMR (300 MHz, $CDCl_3$): 7.56 (d, J = 8.1Hz, 1H), 7.35-7.25 (m, 7H), 7.17-7.02 (m, 2H), 4.99 (q, J = 5.7Hz, 1H), 4.75-4.46 (m, 5H), 4.13-4.01 (m, 1H), 3.90-3.85 (m, 2H), 3.80-3.67 (m, 1H), 3.58 (t, J = 8.4Hz, 1H), 3.31-3.9 (m, 2H), 1.42 (s, 9H). $^{13}$C NMR (75.4 MHz, $CDCl_3$): 171.7, 155.0, 137.5, 135.9, 128.4, 127.9, 127.6, 127.5, 122.9, 121.9, 119.4, 118.6, 111.1, 110.0, 81.7, 80.6, 80.5, 80.2, 79.7, 78.6, 74.9, 72.5, 60.3, 54.3, 28.2. HRMS (FAB): Calcd. for $C_{29}H_{34}N_2O_7Na$ [$M^+$]: 545.2264. Found: 545.2283.

***1,4:3,6-diahydro-5-O-(benzyl)-2-O-(N-carbobenzyloxy-L-proline-L-phenylalanine)-D-mannitol (25)***

1,4:3,6-dianhydro-5-O-(phenylmethyl)-2-hydroxy-D-mannitol (**6**) (0.300 g, 1.27 mmol) and the *N*-Cbz-Pro-Phe (**34**) (0.503 g, 1.27 mmol) were allowed to react as described above. The pure product was obtained by flash chromatography on $SiO_2$ (EtOAc-hexane 3/ 7). The reaction produced a colorless oil (0.407 g, 46%). $[\alpha]_D^{20}$ +53 (*c* 0.1, $CH_2Cl_2$). IR (film) $\nu_{max}$ ($cm^{-1}$): 3413, 3316, 3060, 2952, 2881, 1744, 1694, 1524, 1448, 1416, 1355, 1200, 1122, 1029, 918, 738. $^1$H NMR (300 MHz, $CDCl_3$): 7.40-7.00 (m, 15H), 5.12-5.09 (m, 2H), 4.90 (q, J = 7.2Hz, 1H), 4.75-4.72 (m, 2H), 4.54-4.46 (m, 2H), 4.35-4.29 (m, 1H), 4.07-3.90 (m, 4H), 3.70-3.60 (m, 1H), 3.49-3.38 (m, 3H), 3.20-3.02 (m, 2H), 1.90-1.65 (m, 4H). $^{13}$C NMR (75.4 MHz, $CDCl_3$): 171.0, 170.7, 155.9, 137.4, 136.3, 135.9, 129.2, 128.4, 127.8, 126.9, 126.8, 80.3, 80.1, 78.6, 74.9, 72.4, 70.9, 70.7, 70.4, 67.2, 60.1, 53.0, 46.7, 37.7, 27.7, 23.3. HRMS (FAB): Calcd. for $C_{35}H_{38}N_2O_8Na$ [$M^+$]: 637.2526. Found: 637.2542.

***1,4:3,6-dianhydro-5-O-(benzyl)-2-O-(N-carbobenzyloxy-D-proline)-D-mannitol (26)***

1,4:3,6-dianhydro-5-O-(phenylmethyl)-2-hydroxy-D-mannitol (**6**) (0.300 g, 1.27 mmol) and the *N*-Cbz-D-Pro (**35**) (0.316 g, 1.27 mmol) were allowed to react as described above. The pure product was obtained by flash chromatography on $SiO_2$ (EtOAc-hexane 3/ 7). The reaction produced a white solid (0.377 g, 63%), mp. 71-72ºC. $[\alpha]_D^{20}$ +158 (*c* 0.1, $CH_2Cl_2$). IR (KBr) $\nu_{max}$ ($cm^{-1}$): 3405, 2964, 2869, 1739, 1708, 1465, 1411, 1351, 1197, 1124, 1071, 880, 746. $^1$H NMR (300 MHz, $CDCl_3$): 7.36-7.29 (m, 10H), 5.18-5.07 (m, 1H), 4.98 (q, J = 5.7Hz, 1H), 4.79-4.64 (m, 2H), 4.54 (dd, J = 11.7Hz, J = 2.4 Hz, 1H), 4.49-4.42 (m, 1H), 4.38 (dd, J = 8.4Hz, J = 3.9 Hz, 1H), 4.06-3.89 (m, 2H), 3.88-3.80 (m, 2H), 3.70-3.45 (m, 4H), 2.19-1.96 (m, 2H), 1.94-1.68 (m, 2H). $^{13}$C NMR (75.4 MHz, $CDCl_3$): 172.3, 160.1, 137.5, 136.6, 128.5, 128.4, 128.3, 127.9, 127.8, 127.7,

80.6, 80.2, 78.6, 74.5, 72.5, 71.4, 70.1, 66.9, 58.8, 46.4, 30.8, 23.4. HRMS (FAB): Calcd. for $C_{26}H_{29}NO_7Na$ [$M^+$]: 490.1842. Found: 490.1824.

***1,4:3,6-dianhidro-5-O-(benzyl)-2-O-(Nα-t-butoxycarbonyl-Nε-carbobenzyloxy-L-lisine)-D-mannitol (27)***

1,4:3,6-dianhydro-5-O-(phenylmethyl)-2-hydroxy-D-mannitol (**6**) (0.300 g, 1.27 mmol) and the *N*-α-Boc-*Nε*-Cbz-L-Lys (**36**) (0.483 g, 1.27 mmol) were allowed to react as described above. The pure product was obtained by flash chromatography on $SiO_2$ (EtOAc-hexane 1/ 4). The reaction produced a colorless oil (0.398 g, 52%). $[\alpha]_D^{20}$ +79 (*c* 0.1, $CH_2Cl_2$). IR (film) $\nu_{max}$ ($cm^{-1}$): 3344, 3033, 2938, 2874, 1710, 1522, 1455, 1366, 1255, 1165, 1091, 1024, 859, 737. $^1$H NMR (300 MHz, $CDCl_3$): 7.35-7.27 (m, 10H), 5.15-5.08 (m, 2H), 4.89 (br, 1H), 4.74-4.68 (m, 2H), 4.57 (d, J = 11.7Hz, 1H), 4.47-4.44 (m, 1H), 4.32 (br, 1H), 4.03-3.89 (m, 4H), 3.60 (t, J = 8.4Hz, 1H), 3.18 (q, J = 6.3Hz, 2H), 1.90-1.81 (m, 2H), 1.66-1.52 (m, 2H), 1.50 (s, 9H), 1.48-1.42 (m, 2H). $^{13}$C NMR (75.4 MHz, $CDCl_3$): 171.9, 156.4, 155.3, 137.5, 136.5, 128.5, 128.4, 128.0, 127.9, 127.8, 127.7, 80.5, 80.2, 79.8, 78.6, 74.8, 72.5, 71.0, 70.4, 66.5, 53.3, 40.5, 32.2, 29.9, 28.2, 22.2. HRMS (FAB): Calcd. for $C_{32}H_{42}N_2O_9Na$ [$M^+$]: 621.2788. Found: 621.2784.

***1,4:3,6-dianhydro-5-O-(benzyl)-2-O-(N-t-butoxycarbonyl-L-aspartic acid benzyl ester)-D-mannitol (28)***

1,4:3,6-dianhydro-5-O-(phenylmethyl)-2-hydroxy-D-mannitol (**6**) (0.300 g, 1.27 mmol) and the *N*-Boc-L-Asp-benzyl ester (**42**) (0.410 g, 1.27 mmol) were allowed to react as described above. The pure product was obtained by flash chromatography on $SiO_2$ (EtOAc-hexane 1/ 4). The reaction produced a colorless oil (0.415 g, 60%). $[\alpha]_D^{20}$ +92 (*c* 0.1, $CH_2Cl_2$). IR (film) $\nu_{max}$ ($cm^{-1}$): 3361, 2975, 2881, 1732, 1501, 1457, 1362, 1212, 1158, 1089, 1065, 1028, 859, 744. $^1$H NMR (300 MHz, $CDCl_3$): 7.36-7.31 (m, 10H), 5.51 (d, J = 8.1Hz, 1H), 5.11-5.06 (m, 3H), 4.75 (d, J = 12.0Hz, 1H), 4.70-4.61 (m, 2H), 4.57 (d, J = 11.7Hz, 1H), 4.53 (t, J = 4.8Hz, 1H), 4.05-3.99 (m, 1H), 3.93-3.81 (m, 3H), 3.56 (t, J = 8.4Hz, 1H), 3.02 (dd, J = 17.0Hz, J = 4.5Hz, 1H), 2.93 (dd, J = 17.0Hz, J = 4.5Hz, 1H), 1.43 (s, 9H). $^{13}$C NMR (75 MHz, $CDCl_3$): 170.0, 170.4, 155.2, 137.5, 135.4, 128.5, 128.4, 128.3, 128.2, 127.9, 127.8, 80.5, 80.2, 80.0, 78.6, 75.2, 72.5, 70.9, 70.3, 66.7, 49.8, 36.7, 28.2. HRMS (FAB): Calcd. for $C_{29}H_{35}NO_7Na$ [$M^+$]: 564.2210. Found: 564.2190.

**Inhibition Measurements *In Vitro***

SensoLyte®520 HCV Protease Assay Kt *Fluorimetric* (AnaSpec, CA, USA) was used for the evaluation of the inhibitory activities of the peptide mimetic compounds against HCV NS3/4A protease. The assay was carried out according to the manufacturer's protocol. The compound screening assay was performed in 96-well black plate, and in each well 10ng NS3/4A protease (AnaSpec, CA, USA) were pre-incubated with 100μM peptide mimetic compounds for 10 min at 25°C, followed by the addition of 5-FAM/QXL™ 520-FRET peptide substrate and incubation for 60 min at 25°C. The fluorescent signal substrate cleavage was monitored at excitation and emission wavelengths at 490nm and 520nm, respectively, by SpectraMax M2$^e$ (Molecular Devices). The initial reaction velocities ($V_i$) were determined from progress curves using the linear regression method. The SigmaPlot v.10.0 software was used to calculate the kinetics data.

## CONFLICT OF INTEREST

Declared none.

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