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Pseudo-peptides derived from isomannide as potential inhibitors of serine proteases*

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Summary. Hepatitis C, Dengue and West Nile virus are among of the most important flaviviruses that share one important serine protease enzyme. Serine proteases belong to the most studied class of proteolytic enzymes, and are a primary target in the drug development field. In this paper, we describe the synthesis and preliminary molecular modeling studies of a novel class of *N-t*-Boc amino acid amides derived of isomannide as potential serine proteases inhibitors.

Keywords: Flaviviruses – Serine protease – Isomannide

Introduction

The family *Flaviviridae* comprises more than 60 viruses, many of which are important human pathogens. Among the most important flaviviruses are the Hepatitis C virus (HCV), the West Nile virus (WN) and the Dengue virus.

All flaviviruses have a positive-sense non-segmented RNA genome that encodes a single long polyprotein, processed to yield three structural proteins (C, prM, and E), and seven nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) (Leung, 2001). A single virus-encoded protease comprising 180 amino acids of NS3 (NS3pro) is responsible for cleavage both in *cis* and in *trans* to generate viral proteins that are essential for viral replication and maturation of infectious virions. The presence of a trypsin-like serine protease within the N-terminal one-third of the flavivirus NS3 protein was first proposed by Bazan and Fletterick (1989, 1990) and Gorbalenya and co-workers (1989). Their analysis of virus sequence alignments revealed that both the structural motifs and the characteristic catalytic triad (His^{51}, Asp^{75}, and Ser^{135}) of mammalian serine proteases were conserved in all flaviviruses. As NS3pro activity is essential for viral replication, it represents a suitable target for the development of chemotherapeutic approaches in the treatment of flaviviruses. As part of our antiviral program for flaviviruses, we describe, in this paper, the synthesis and preliminary molecular modeling study of a series of *N-t*-Boc amino acid amides of isomannide, designed as a potential inhibitors of the catalytic triad of serine proteases. Isomannide was chosen as the center of these pseudo peptides since it has proved to be a good dipeptide rigid scaffold (Bencsik, 2003; Dietrich, 2003) and its C_2 symmetry, according to our previous results (Muri, 2004). The need of a Lys or Arg residue previously observed (Muri, 2004) was fulfilled in the present work.

Material and methods

Computational methods

Computer graphics, structural manipulations, energy minimization, and docking calculations were carried out with a Silicon Graphics O2 Workstation (CPU MIPS R10000, processor speed 150 MHz, and main memory 128MB), using the Insight II 97.0 (Discover) software package, under the operating system IRIX 6.3. Energy minimization and docking calculations were carried out with the Discover 2.9.7 (Costi, 2001) program and Docking module (Kuntz, 1982), respectively, available within Insight II, using the molecular mechanics CVFF force field. The enzyme-ligand reference structure is the Dengue virus NS3-serine-protease complexed with Mungbean Bowman-Birk inhibitor (MbBBI) (PDB code: 1DF9) (Berman, 2000; Murthy, 2000).

* Dedicated to Professor Lucia Mendonça-Previato by occasion of her 2004 L'Oreal/UNESCO for Women in Science Award

Experimental

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). Routine NMR analyses were carried out on a Bruker Advance DPX-400. Melting points were obtained on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Mass spectral measurements were made on a Waters 2690 mass spectrometer. Analytical results are within ±0.40% of the theoretical values and were determined by QTI, Whitehouse, NJ.

1,4:3,6-Dianhydro-2,5-di-O-p-tosyl-D-mannitol (**3**)

A solution of *p*-toluenesulphonyl chloride (27.36 mmol, 5.2 g) in pyridine (40 mL) was added dropwise to a solution of isomannide (13.68 mmol, 2.0 g) in dry pyridine (24 mL) and stirred at r.t. The reaction mixture was stirred at room temperature for additional 5 h, and, then, heated at 100°C for 1 h. The mixture was cooled and poured on ice-cold 2 N HCl. The product was extracted with ethyl acetate, dried and filtered. The crude product was recrystallized from MeOH to give the product **3** as a white solid (3.72 g) in 60% yield. ^{1}H NMR δ ($CDCl_3$, 400 MHz): 7.80 (d, 4H, $J = 8.1$ Hz), 7.32 (d, 4H, $J = 8.1$ Hz), 4.75–4.90 (m, 2H), 4.40–4.50 (m, 2H), 3.94 (dd, 2H, $J = 6.6$ Hz, $J = 9.5$ Hz), 3.72 (dd, 2H, $J = 7.6$ Hz, $J = 9.5$ Hz), 2.41 (s, 6H). ^{13}C NMR: 130.3 (–CH), 128.3 (–CH), 80.3 (–CH), 78.4 (–CH), 70.5 ($–CH_2$), 22.0 ($–CH_3$). $\alpha_D{}^{29} = +94.2$ (c, 1.0) $CHCl_3$, mp 92–93°C.

1,4:3,6-Dianhydro-2,5-diazido-2,5-dideoxy-L-iditol (**4**)

NaN_3 (6.85 mmol, 0.445 g) was added to a solution of ditosylate **3** (2.20 mmol, 1.0 g) in dry DMF (20 mL), and the mixture was stirred for 2 h at 120°C. The mixture was cooled, filtered, and the filtrate evaporated, and the residue was mixed with $CHCl_3$ (200 mL). The undissolved salts were filtered off, and the filtrate was washed in water, dried and evaporated. The crude diazide was purified by column chromatography to give the product as a pale-yellow liquid (0.258 g) in 60% yield. ^{1}H NMR δ ($CDCl_3$, 400 MHz): 4.64 (s, 2H, –CH), 4.00–3.85 (m, 6H, $–CH_2$/–CH). ^{13}C NMR: 86.0 (–CH), 71.8 ($–CH_2$), 65.7 (–CH). ES MS m/z 195 (M − 1). $\alpha_D{}^{29} = +98.0$ (c, 1.0) $CHCl_3$.

1,4:3,6-Dianhydro-2,5-diamino-2,5-dideoxy-L-iditol (**5**)

A mixture of diazide **4** (1.27 mmol, 0.25 g) and 10% Pd/C (0.127 mmol, 0.140 g) in EtOH (10 mL) was hydrogenated at 40 psi. After 12 h, the catalyst was removed by filtration, washed with EtOH and the solvent evaporated giving **5** as hygroscopic solid in 93% yield. ^{1}H NMR δ ($CDCl_3$, 400 MHz): 4.43 (s, 2H, –CH), 3.90 (dd, 2H, $J = 4.4$ Hz, $J = 9.1$ Hz), 3.66 (dd, 2H, $J = 1.9$ Hz, $J = 9.1$ Hz); 3.50 (dd, 2H, $J = 1.9$ Hz, $J = 4.4$ Hz), 1.43 (broad s, 4H, $–NH_2$). ^{13}C NMR: 89.0 (–CH), 75.0 ($–CH_2$), 58.0 (–CH). ES MS m/z 143 (M − 1). $\alpha_D{}^{27} = +42.1$ (c, 1.0) $CHCl_3$, mp 64–65°C.

Procedure for N-tert-butoxycarbonyl protection (Muri, 2004)

N-tert-butoxycarbonyl-L-lysine (**6a**)

L-lysine (1.0 g, 6.84 mmol) was dissolved in 10% aq $NaHCO_3$ (50 mL). Di-*tert*-butyldicarbonate (20.5 mmol, 4.34 g) in THF (8 mL) was added to the reaction mixture in two portions and stirred overnight. The solvent was evaporated; the pH of the mixture reaction was adjusted to 3.5, using 10% HCl, and extracted with ethyl acetate. The organic phases were washed in brine and dried. The solvent was evaporated and the product 6a was obtained as an oil in 88% yield. ^{1}H NMR δ ($CDCl_3$, 400 MHz): 8.02 (broad s, 1H, –NH), 6.23 (broad s, 1H, –NH), 4.31–4.11 (m, 1H, –CH), 3.13–3.06 (m, 2H, $–CH_2$), 2.00–1.73 (m, 4H, $–CH_2$), 1.45 (s, 9H, $–CH_3$), 1.43 (s, 9H, $–CH_3$), 1.28–1.26 (m, 2H, $–CH_2$). $\alpha_D{}^{27} = +11.5$ (c, 1.0) $CHCl_3$.

General procedure for formation of amides **7a**–**7g**

EDC (2.66 mmol, 0.510 g), HOBt (2.66 mmol, 0.331 g) and N-methyl morpholine (3.31 mmol, 0.36 mL) were added to a solution of Boc-protected amino acid (2.65 mmol) in THF (10 mL). After 15 min of stirring, the amine (1.33 mmol) was added and the reaction was again stirred overnight. The solvent was evaporated and the oil was diluted in $CHCl_3$, washed with 0.1 N HCl, water, 0.5 N $NaHCO_3$, brine, dried over Na_2SO_4 and concentrated in vacuum. Purification by flash chromatography gave the corresponding products **7a**–**7g**. All the amide products were white solids.

(3S,6S)-Bis-N-(N-tert-butoxycarbonyl-L-lysine)-1,4-dioxabicyclo[3.3.0]octane (**7a**)

^{1}H NMR δ ($CDCl_3$, 400 MHz): 4.67 (broad s, 2H, –CH), 4.49 (s, 2H, –CH), 4.37 (broad s, 2H, –CH), 3.96–3.93 (m, 4H, $–CH_2$), 3.10–3.04 (m, 4H, $–CH_2$), 1.89–1.60 (m, 8H, $–CH_2$), 1.42 (s, 36H, $–CH_3$), 1.30–1.26 (m, 4H, $–CH_2$). ^{13}C NMR: 172.7 (–C=O), 156.3 (–C=O), 155.8 (–C=O), 86.3 (–CH), 79.83 (–Cq), 79.1 (–Cq), 72.1 ($–CH_2$), 56.3 (–CH), 54.2 (–CH), 39.9 ($–CH_2$), 32.1 ($–CH_2$), 29.5 ($–CH_2$), 28.4 ($–CH_3$), 28.3 ($–CH_3$), 22.6 ($–CH_2$). ES MS m/z 799 (M − 1). Anal. Calcd. for $C_{38}H_{68}N_6O_{12}$: C, 56.98; H, 8.56; N, 10.49. Found: C, 56.65; H, 8.75; N, 10.11. $\alpha_D{}^{26} = -3.6$ (c, 1.0) $CHCl_3$, mp = 85–86°C, 39% yield.

(3S,6S)-Bis-N-(N-tert-butoxycarbonyl-L-valine)-1,4-dioxabicyclo[3.3.0]octane (**7b**)

^{1}H NMR δ (MeOD, 400 MHz): 4.50 (s, 2H, –CH), 4.30–4.28 (m, 2H, –CH), 4.00–3.96 (m, 2H, –CH), 3.84–3.81 (m, 4H, $–CH_2$), 1.97–1.95 (m, 2H, –CH), 1.45 (s, 18H, $–CH_3$), 0.95 (d, 12H, $J = 6.4$ Hz, $–CH_3$). ^{13}C NMR: 173.0 (–C=O), 156.4 (–C=O), 86.1 (–CH), 79.1 (–Cq), 71.3 ($–CH_2$), 59.9 (–CH), 56.5 (–CH), 30.8 (–CH), 27.3 ($–CH_3$), 18.3 ($–CH_3$), 17.5 ($–CH_3$). ES MS m/z 541 (M − 1). Anal. Calcd. for $C_{26}H_{46}N_4O_8$: C, 57.55; H, 8.54; N, 10.32. Found: C, 57.31; H, 8.81; N, 10.51. $\alpha_D{}^{26} = -16.7$ (c, 1.0) $CHCl_3$, mp 198–199°C, 37% yield.

(3S,6S)-Bis-N-(N-tert-butoxycarbonyl-L-phenylalanine)-1,4-dioxabicyclo[3.3.0]octane (**7c**)

^{1}H NMR δ (DMSO, 400 MHz): 8.16 (broad s, 2H, –NH), 7.24–7.15 (m, 10H, –CH), 6.87 (broad s, 2H, –NH), 4.22–4.10 (m, 4H, $–CH_2$), 4.08–4.00 (m, 2H, –CH), 3.83–3.80 (m, 2H, –CH), 3.64–3.62 (m, 2H, –CH), 2.88–2.72 (m, 4H, $–CH_2$), 1.29 (s, 18H, $–CH_3$). ^{13}C NMR: 172.1 (–C=O), 155.6 (–C=O), 138.2 (–Cq), 129.6 (–CH), 128.5 (–CH), 126.7 (–CH), 86.3 (–CH), 78.6 (–Cq), 71.8 ($–CH_2$), 56.3 (–CH), 56.0 (–CH), 38.4 ($–CH_2$), 28.5 ($–CH_3$). ES MS m/z 639 (M + 1). Anal. Calcd. for $C_{34}H_{46}N_4O_8$: C, 63.93; H, 7.26; N, 8.77. Found: C, 63.57; H, 7.33; N, 8.97. $\alpha_D{}^{26} = +35.5$ (c, 1.0) DMF, mp = 203–204°C, 41% yield.

(3S,6S)-Bis-N-(N-1-bis[-tert-butoxycarbonyl]-L-histidine)-1,4-dioxabicyclo[3.3.0]octane (**7d**)

^{1}H NMR δ ($CDCl_3$, 400 MHz): δ 8.00 (s, 2H, –CH), 7.17 (s, 2H, –CH), 4.41 (s, 2H, –CH), 4.33 (s, 2H, –CH), 3.89–3.85 (m, 2H, $–CH_2$), 3.63–3.60 (m, 2H, $–CH_2$), 3.04–3.00 (m, 2H, –CH), 2.90–2.86 (m, 4H, $–CH_2$), 1.58 (s, 18H, $–CH_3$), 1.40 (s, 18H, $–CH_3$). ^{13}C NMR: 171.3 (–C=O), 156.0 (–C=O), 146.7 (–C=O), 138.9 (Cq), 136.7 (–CH), 114.7 (–CH), 86.1 (–CH), 85.7 (–Cq), 79.9 (–Cq), 72.5 ($–CH_2$), 56.2 (–CH), 54.0 (–CH), 30.7 ($–CH_2$), 28.2 ($–CH_3$), 27.8 ($–CH_3$). ES MS m/z 817 (M − 1). Anal. Calcd. for $C_{38}H_{58}N_8O_{12}$: C, 55.73; H, 7.14; N, 13.68. Found: C, 55.53; H, 7.18; N, 13.42. $\alpha_D{}^{26} = +46.8$ (c, 1.0) $CHCl_3$, mp = 115–116°C, 43% yield.

(3S,6S)-Bis-N-(N-tert-butoxycarbonyl-L-proline)-1,4-dioxabicyclo[3.3.0]octane (**7e**)

^{1}H NMR δ ($CDCl_3$, 400 MHz): 4.49–4.36 (m, 4H, $-CH_2$), 4.22 (s, 2H, –CH), 3.92 (s, 2H, –CH), 3.70–3.67 (m, 2H, –CH), 3.40–3.33 (m, 4H, $-CH_2$), 2.00–1.86 (m, 4H, $-CH_2$), 1.68–1.60 (m, 4H, $-CH_2$), 1.45 (s, 18H, $-CH_3$). ^{13}C NMR: 171.9 (–C=O), 155.9 (–C=O), 86.2 (–CH), 80.6 (–Cq), 72.3 ($-CH_2$), 60.3 (–CH), 56.2 (–CH), 47.1 ($-CH_2$), 28.3 ($-CH_3$), 22.6 ($-CH_2$), 21.0 ($-CH_2$). ES MS m/z 539 (M − 1). Anal. Calcd. for $C_{26}H_{42}N_4O_8$: C, 57.98; H, 7.86; N, 10.40. Found: C, 57.75; H, 8.16; N, 10.31. $\alpha_D^{26} = -54.7$ (c, 1.0) $CHCl_3$, mp 87–88°C, 40% yield.

(3S,6S)-Bis-N-(N-tert-butoxycarbonyl-L-glutamic acid 5-methyl ester)-1,4-dioxabicyclo[3.3.0]octane (**7f**)

^{1}H NMR δ ($CDCl_3$, 400 MHz): δ 4.53 (broad s, 2H, –CH), 4.40 (s, 2H, –CH), 4.15–4.10 (m, 2H, –CH), 3.99–3.97 (m, 2H, $-CH_2$), 3.70 (s, 3H, $-CH_3$), 2.53–2.39 (m, 4H, $-CH_2$), 1.93–1.87 (m, 4H, $-CH_2$), 1.44 (s, 18H, $-CH_3$). ^{13}C NMR: δ 173.8 (–C=O), 171.7 (–C=O), 156.2 (–C=O), 86.3 (–CH), 80.1 (–Cq), 72.2 ($-CH_2$), 56.4 (–CH), 53.5 (–CH), 51.9 ($-OCH_3$), 30.2 ($-CH_2$), 28.3 ($-CH_3$), 27.9 ($-CH_2$). ES MS m/z 631 (M + 1). Anal. Calcd. for $C_{28}H_{46}N_4O_{12}$: C, 53.32; H, 7.35; N, 8.88. Found: C, 53.02; H, 7.65; N, 8.86, mp 69–70°C, 40% yield.

(3S,6S)-Bis-N-(L-1-tert-butoxycarbonylpyroglutamic acid)-1,4-dioxabicyclo[3.3.0]octane (**7g**)

^{1}H NMR δ (DMSO, 200 MHz): 8.53 (broad s, 2H, –NH), 4.48–4.36 (m, 2H, $-CH_2$), 4.13–4.06 (m, 2H, $-CH_2$), 3.88–3.85 (m, 2H, –CH), 3.67–3.62 (m, 2H, –CH), 3.17–3.15 (m, 2H, –CH), 2.40–2.10 (m, 4H, $-CH_2$), 1.73–1.69 (m, 4H, $-CH_2$), 1.37 (s, 18H, $-CH_3$). ^{13}C NMR: 174.1 (–C=O), 171.7 (C=O), 149.3 (–C=O), 86.4 (–CH), 82.2 (–Cq), 72.0 ($-CH_2$), 59.5 (–CH), 56.4 (–CH), 31.5 ($-CH_2$), 28.0 ($-CH_3$), 22.2 ($-CH_2$). ES MS m/z 565 (M − 1). Anal. Calcd. for $C_{26}H_{38}N_4O_{10}$: C, 55.11; H, 6.76; N, 9.89. Found: C, 55.34; H, 6.65; N, 9.58. $\alpha_D^{29} = -13.3$ (c, 1.0) $CHCl_3$, mp 164–165°C, 38% yield.

(3S,6S)-Bis-N-(L-proline)-1,4-dioxabicyclo[3.3.0]octane (**8**)

HCl (2.89 mmol, 1.45 mL, 2M sol. in ether) was added under stirring to a solution of *N*-Boc-proline amide (0.725 mmol, 0.39 g) in CH_2Cl_2 (6 mL). The mixture was once more stirred at r.t. for 4 h, then, the precipitate was filtered off and purified by flash chromatography giving the free amine (0.204 g) in 83% yield. ^{1}H NMR δ (MeOD, 400 MHz): 4.56 (broad s, 2H, –NH), 4.35–4.28 (m, 2H, –CH), 4.24–4.18 (m, 2H, $-CH_2$), 4.03–3.96 (m, 2H, $-CH_2$), 3.82–3.75 (m, 2H, –CH), 3.40–3.32 (m, 2H, –CH), 2.50–2.40 (m, 4H, $-CH_2$), 2.07–1.95 (m, 8H, $-CH_2$). ^{13}C NMR: δ 168.4 (–C=O), 86.4 (–CH), 71.7 ($-CH_2$), 59.72 (–CH), 56.8 (–CH), 46.2 ($-CH_2$), 29.9 ($-CH_2$), 23.8 ($-CH_2$). ES MS m/z 339 (M + 1). Anal. Calcd. for $C_{16}H_{26}N_4O_4$: C, 56.79; H, 7.74; N, 16.56. Found: C, 56.48; H, 7.65; N, 16.23. $\alpha_D^{26} = -1.3$ (c, 0.26) EtOH, mp 185–186°C.

(3S,6S)-Bis-N,O-(N-tert-butoxycarbonyl-L-proline-N-tert-butoxycarbonyl-L-phenylalanine)-1,4-dioxabicyclo[3.3.0]octane (**9**)

EDC (0.171 g, 0.885 mmol), HOBt (0.885 mmol, 0.111 g) and N-methyl morpholine (0.12 mL, 1.10 mmol) were added to a solution of Boc-protected L-phenylalanine (0.885 mmol, 0.234 g) in dry DMF (5 mL). After 15 min of stirring, the proline amide (0.44 mmol, 0.151 g) in DMF (3 mL) was added and the reaction was once again stirred overnight. The solvent was evaporated and the oil was diluted in $CHCl_3$, washed with 0.1 N HCl, water, 0.5 N $NaHCO_3$, brine, dried over Na_2SO_4 and concentrated in vacuum. Purification by flash chromatography gave the product as a yellow pale solid (0.184 g) in 50% yield. ^{1}H NMR δ ($CDCl_3$, 400 MHz): 7.35–7.15 (m, 10H, –CH), 4.72–4.57 (m, 4H, $-CH_2$), 4.45–4.44 (m, 2H, –CH), 4.34–4.25 (m, 2H, –CH), 4.00–3.93 (m, 2H, –CH), 3.70–3.67 (m, 2H, –CH), 3.60–3.43 (m, 4H, $-CH_2$), 3.03–2.86 (m, 4H, $-CH_2$), 2.25–2.16 (m, 4H, $-CH_2$), 1.90–1.84 (m, 4H, $-CH_2$), 1.38 (s, 18H, $-CH_3$). ^{13}C NMR: δ 174.9 (–C=O), 172.9 (–C=O), 155.2 (C=O), 136.1 (–Cq), 129.3 (–CH), 128.5 (–CH), 126.9 (–CH), 86.2 (–CH), 79.8 (–Cq), 72.5 ($-CH_2$), 60.0 (–CH), 56.5 (–CH), 53.5 (–CH), 47.4 ($-CH_2$), 39.0 ($-CH_2$), 28.3 ($-CH_3$), 27.0 ($-CH_2$), 25.1 ($-CH_2$). ES MS m/z 833 (M + 1). Anal. Calcd. for $C_{44}H_{60}N_6O_{10}$: C, 63.44; H, 7.26; N, 10.09. Found: C, 63.10; H, 7.15; N, 9.84. $\alpha_D^{26} = -24.5$ (c, 1.0) $CHCl_3$, mp = 68–69°C.

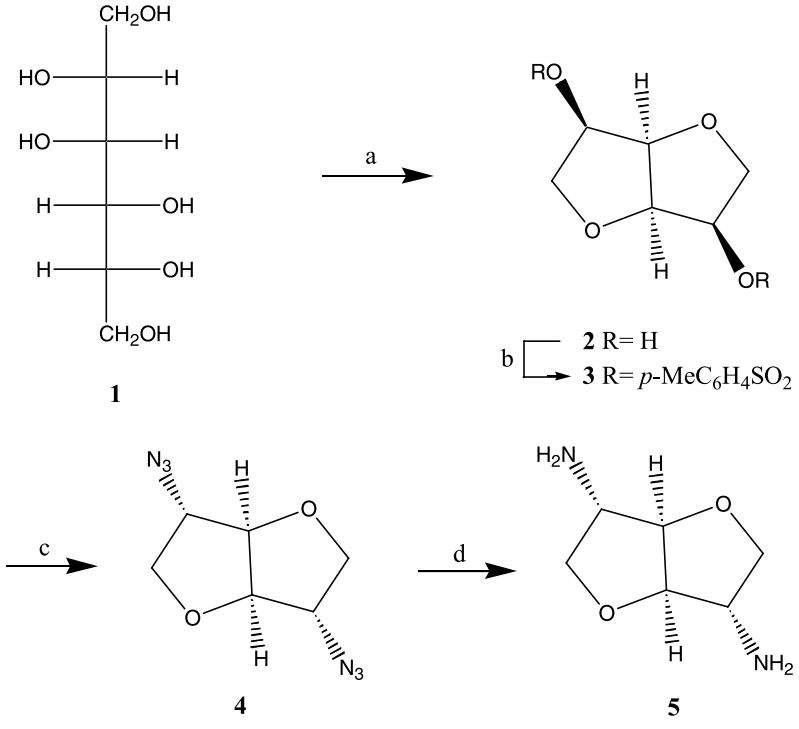
Results and discussion

Chemistry

Ready dehydration of naturally occurring D-mannitol (**1**) provided the symmetrical molecule, 1,4:3,6-dianhydro-D-mannitol (**2**) (Wiggins, 1945). Due to the reactivity of sulfonate groups and their ready replacement by various nucleophilic reagents, bis-sulfonated 1,4:3,6-dianhydro-D-mannitol derivatives would be useful precursors of other symmetrically substituted 1,4:3,6-dianhydro-D-mannitol compounds (Marr, 1997). The conversion of **2** to the ditosylate **3** with p-toluene-sulfonyl chloride in pyridine was reported (Hockett, 1946). 2,5-Di-*O*-tosyl-D-mannitol (**3**) treated with sodium azide in DMF for 2 h at 120°C gave diazido-L-iditiol derivative **4** (Kuszmann, 1980). The tosyl groups can be readily replaced (with inversion of configuration) by azide, thus yielding the diazido compound. ^{1}H-NMR data for compound **4** showed protons 3 and 4 as a sharp singlet, in accordance with the symmetry of the molecule and the lack of coupling with protons 2 and 5. The six others protons appeared as an overlapped multiplet (Kuszmann, 1980).

The reduction of diazido derivative **4** with hydrogen over palladium on carbon gave the 2,5-diamino-1,4:3,6-dianhydro-D-mannitol (**5**) in 93% yield (Scheme 1) (Archibald, 1989). This diamine was previously prepared in low yields from the direct reaction of the ditosylate with ammonia (Montgomery, 1946).

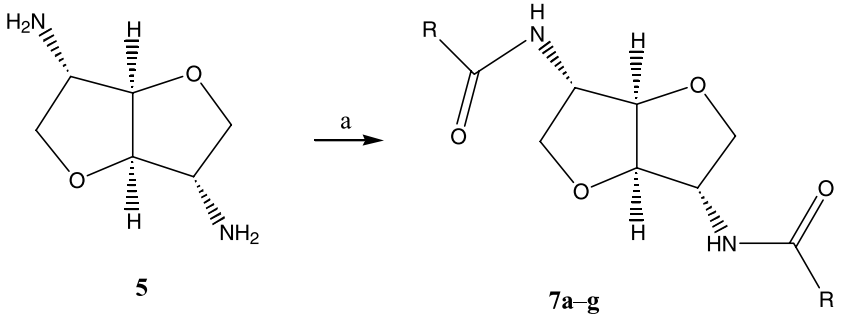
Initially, the L-amino acids were protected with di-*tert*-butyl-dicarbonate (Boc) in accordance with the literature (Muri, 2004), as shown in Table 1 (Benoiton, 1993; Ye, 1992; Gotebiowski, 1987; Barcelo, 1986; Le Nguyen, 1985; Keller, 1985; Ookawa, 1987; Feng, 1999; El Marini, 1992; Li, 1995; Yoshifuji, 1986). The key step in this synthesis is the coupling reaction between the amine **5** and the L-protected amino acids **6a**–**g**, using a carbodiimide reagent, resulting in amide-bond formation (Scheme 2). The carbodiimide-mediated amide formation has been extensively studied employing peptide-coupling additives,



Scheme 1. a. $HOAc/H_2SO_4$; b. TsCl, Py, 7 h, r.t.; c. NaN_3, DMF, 120°C, 2 h; d. H_2, Pd/C, EtOH, 12 h

Table 1. Boc-Amino acids prepared by acylation with Di-*t*-butyl dicarbonate

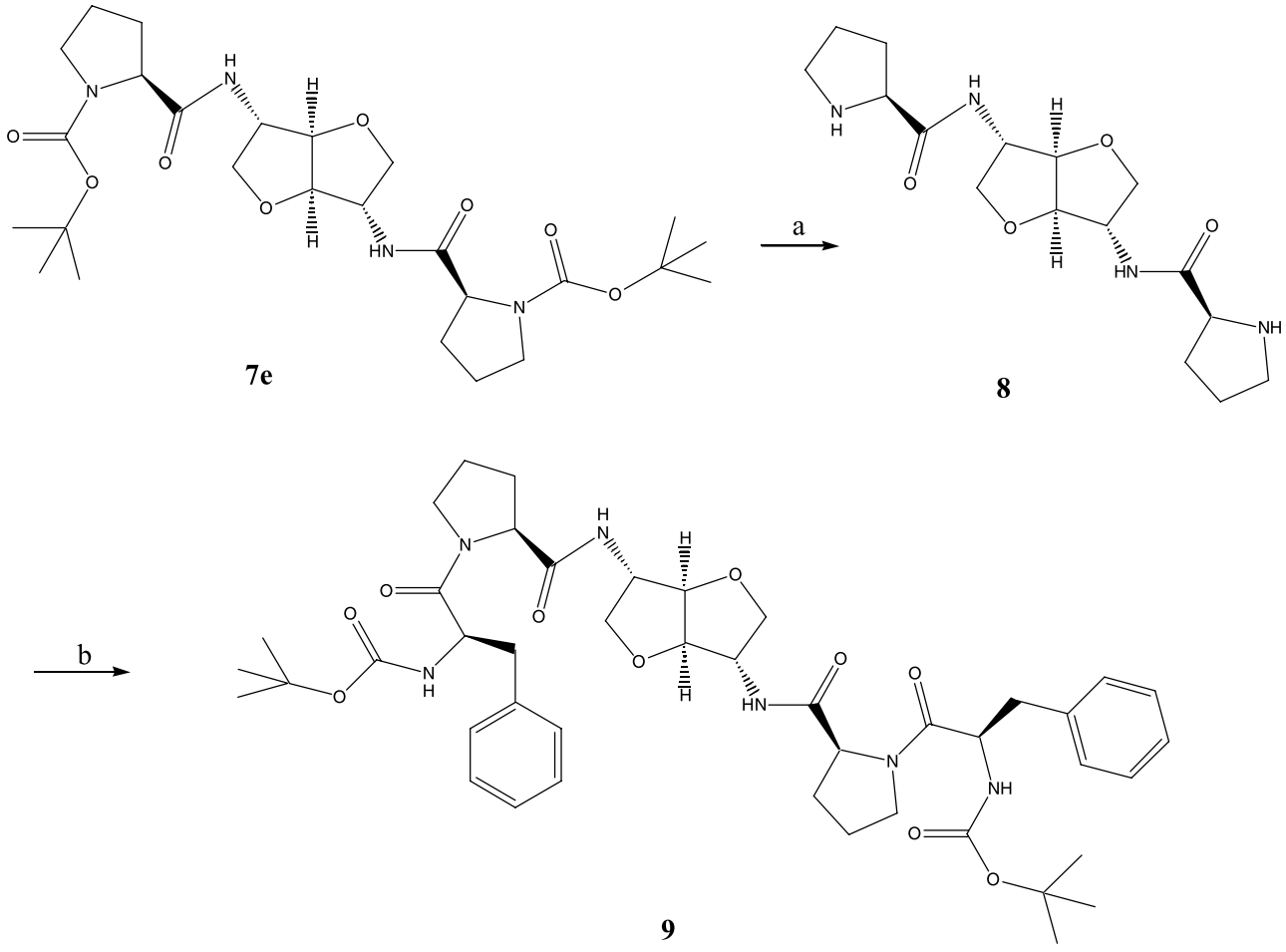
N°	Boc-Amino acids	Conditions	mp, °C	α_D^{25} (c, 1.0)
6a	Lys-Boc	$NaHCO_3$, THF/H_2O	–	+11.5 ($CHCl_3$)
6b	Val-Boc	NaOH, *t*BuOH/H_2O	75–76	+12.9 ($CHCl_3$)
6c	Phe-Boc	NaOH, *t*BuOH/H_2O	68–69	+23.2 (EtOH)
6d	His-Boc	Et_3N, dioxane/H_2O	165–166	+95.5 ($CHCl_3$)
6e	Pro-Boc	NaOH, *t*BuOH/H_2O	120–122	−85.5 ($CHCl_3$)
6f	OMeGlu-Boc	$NaHCO_3$, dioxane/H_2O	75–76	+8.4 ($CHCl_3$)
6g	PyroGlu-Boc	NaHMDS, THF	111–112	−21.3 ($CHCl_3$)



(**7a**) R=Lys-Boc, (**7b**) R=Val-Boc, (**7c**) R=Phe-Boc, (**7d**) R=His-Boc, (**7e**) R=Pro-Boc, (**7f**) R=OMeGlu-Boc, (**7g**) R=PyroGlu-Boc

Scheme 2. a EDC, HOBt, NMM, THF, r.t., overnight

such as 1-hydroxy-benzotriazole (HOBt), 1-hydroxy-7-azabenzotriazole (HOAt) and 2-hydroxypyridine N-oxide (HOPO) (Hanessian, 2000; Woods, 2002; Shi, 2003). Condensation of **5** with L-amino acids **6a**–**g**, in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), HOBt and *N*-methyl morpholine, led to the expected amides, **7a**–**g**. Amide **7e** was deprotected in acid conditions affording the free amine **8** (Hogg, 1995; Katritzky, 2002). After deprotection, **8** and **6c** were coupled to produce dimer **9** (Scheme 3).



Scheme 3. **a** 2 M HCl solution in ether, CH_2Cl_2, r.t., 4 h; **b** EDC, HOBt, NMM, DMF, **6c**, r.t., overnight

Molecular modeling

The deprotected Lys-amide derivative **10**, obtained from the treatment of Boc-amide **7a** under acidic conditions, was docked to the active site of the NS3 protease (Fig. 1a). The docked complex shows three hydrogen bond interactions between **10** and the NS3 protease. The Lys-carbonyl group from Lys-amide compound **10** makes two hydrogen bonds, one with the backbone NH group of Gly^{133} (2.13 Å) and the other with the OH group of Ser^{135} (1.68 Å) (catalytic residue). The third hydrogen bond

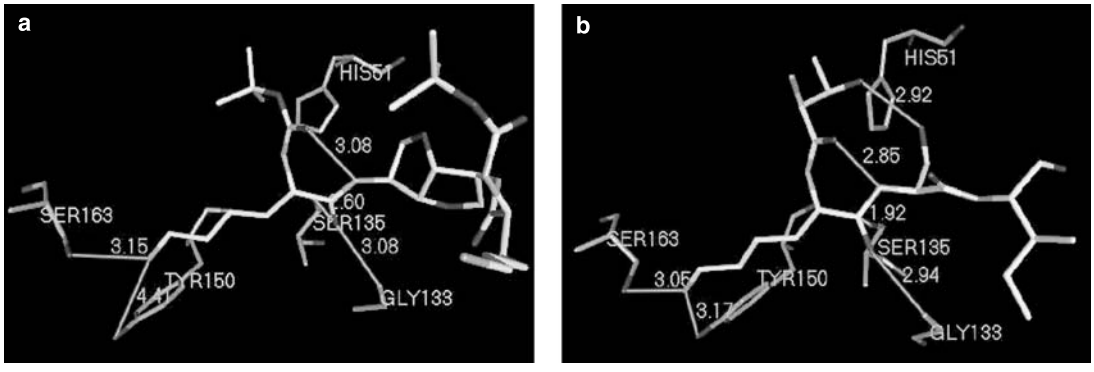


Fig. 1. Close view of the Lys-amide compound **10** (**a**) and of the MbBBI (residues P2′-P1′-P1-P2) crystallographic structure (**b**) docked to the NS3-protease active site (His^{51}, Ser^{135}, and residues making hydrogen bond interactions). The carbon atoms of the MbBBI-tetrapeptide fragment and Lys-amide compound **10** are lighter than the main chain. The hydrogen atoms were omitted for clarity. The measure distances are between heteroatoms

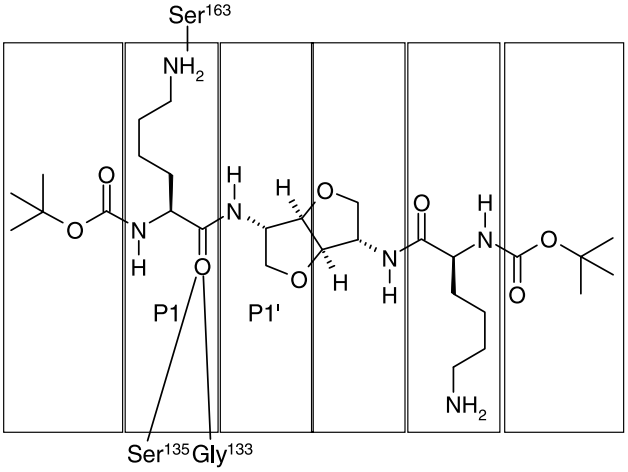


Fig. 2. Schematic hydrogen bonding representation between compound **10** and the residues of NS3-protease after docking calculations

corresponds to the P1 position (Fig. 2): i.e., the Lys-NH_2 group from Lys-amide compound **10** and the hydroxyl group of Ser^{163} (2.15 Å).

There are three residues, namely, Ile^{36}, Leu^{115}, and Pro^{132}, potentially capable of providing specific van der Waals interaction, therefore stabilizing the complex between NS3 protease and compound **10**. In addition, the catalytic His^{51} makes nonspecific interactions as well as the following residues: Gln^{35}, Ser^{131}, Thr^{134}, Tyr^{150}, Gly^{151}, Asn^{152}, and Gly^{153}. The Lys-NH_2 group from **10** is able to reach the Tyr^{150} residue located at the bottom of the cleft at position S1.

It is clear that the putative ligand, the Lys-amide compound **10**, is well accommodated in the active site of the NS3 protease, and shares a similar binding mode when compared with the crystallographic structure of the MbBBI (Murthy, 2000) (Fig. 1b). It should be pointed out that compound **10** has a basic residue (Lys) at position P1 and, should, therefore, be a characteristic substrate for this class of enzyme. In fact, this protease has trypsin-like selectivity: it cleaves the viral polyprotein at four junctions where the residues spanning the scissile bond are Arg-Ser, Arg-Ala, Lys-Ser, and Arg-Gly.

Figure 1a shows a close view of the structure of Lys-amide compound **10** docked to the NS3-protease active site. Figure 1b shows a close view of the crystallographic structure of MbBBI in the NS3-protease active site. They show: a) residues P2′-P1′-P1-P2 (Thr^{519}-Lys^{520}-Ser^{521}-Ile^{522}) of MbBBI; and b) the protein catalytic residues (His^{51} and Ser^{135}) and the protein residues making hydrogen bond interactions with the inhibitor (MbBBI) and with the putative ligand, compound **10**.

Conclusions

As part of our antiviral program (Peçanha, 2003; Muri, 2004), we described here the synthesis of a new series of *N-t*-Boc amino acid amides of isomannide, designed as potential inhibitors of serine proteases. So as to have a model to validate the proposed model of NS3 protease docked to our compounds, a preliminary molecular modeling study was developed using a peptide inhibitor (MbBBI) from the literature and with deprotected Lys-amide derivative **10**, which showed desired interactions with the target. Therefore, the compounds described in the present paper can be seen as potentially active against virus-encoded serine proteases NS3 (NS3pro). Evaluation of these compounds is underway.

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