Amino Acids (2004) 27: 153–159
DOI 10.1007/s00726-004-0121-5

Amino Acids
Printed in Austria

# *N-t*-Boc-amino acid esters of isomannide Potential inhibitors of serine proteases

**E. M. F. Muri**[1], **M. Gomes Jr.**[1], **J. S. Costa**[1], **F. L. Alencar**[1], **A. Sales Jr.**[1], **M. L. Bastos**[1], **R. Hernandez-Valdes**[2], **M. G. Albuquerque**[1], **E. F. F. da Cunha**[1], **R. B. Alencastro**[1], **J. S. Williamson**[3], and **O. A. C. Antunes**[1]

[1] Instituto de Química, UFRJ, Cidade Universitária, CT Bloco A, Rio de Janeiro, Brazil
[2] Núcleo de Pesquisas de Produtos Naturais, UFRJ, Cidade Universitária, CCS Bloco H, Rio de Janeiro, Brazil
[3] Faser Hall, School of Pharmacy, Department of Medicinal Chemistry, University of Mississippi, Mississippi, U.S.A.

Received February 18, 2004
Accepted July 9, 2004
Published online September 15, 2004; 

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

**Keywords:** Isomannide derivatives – Serine protease – Dengue virus

## Introduction

The family *Flaviviridae* comprises more than 60 viruses, many of which are important human pathogens. Hepatitis C virus (HCV), West Nile virus (WN) and Dengue virus are among the most important flaviviruses. HCV is the major cause of blood-borne chronic hepatitis, with nearly 200 million infected people worldwide (Houghton et al., 1991). Chronic HCV infection is associated with liver cirrhosis and hepatocellular carcinoma (Bruix et al., 1989). Current therapeutic regimens based on alpha interferon and the nucleoside analog ribavirin are only partially effective and are limited by the adverse effects of both agents (Wright et al., 2001).

For many years, WN has been recognized as one of the most widely distributed flaviviruses, with a geographic range which includes Africa, Australia, Europe, the Middle East and West Asia. In 1999, WN first established itself in the U.S. In acute severe illness, WN virus can cause hepatitis, meningitis, and encephalitis, leading to paralysis and coma (Hayes, 1989; Pletnev et al., 2002).

Dengue viruses infect up to 20 million people annually, and are a significative cause of morbidity and mortality in tropical and sub-tropical regions throughout the world (World Health Organization, 1996). There are four serotypes (types 1 to 4) that cause widespread human diseases such as dengue fever, dengue hemorrhagic fever and dengue shock syndrome (Yusof, 2000). There is currently no approved vaccine or effective antiviral therapy for these flaviviruses.

All flaviviruses have a positive-sense nonsegmented 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 et al., 2001). A single virus-encoded protease comprising 180 amino acids of NS3 (NS3pro) is responsible for the cleavage of both in *cis* and in *trans* that generates 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 (Gorbalenya et al., 1989). Their analysis of virus sequence alignments revealed that structural motifs as well as 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 for 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 esters of isomannide, designed as a potential inhibitors of the catalytic triad of serine proteases. Isomannide was designed as the center of the peptide mimetic compounds due to its structural analogy with cyclic rigid dipeptides (Bencsik et al., 2003; Dietrich and Lubell, 2003) and its $C_2$ symmetry, thus providing a rigid scaffold for the peptide mimetic compounds described 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 128 MB) 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 program (Costi and Ferrari, 2001) and Docking module (Kuntz et al., 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 Mung-bean Bowman-Birk inhibitor (MbBBI) (PDB code: 1DF9) (Berman et al., 2000; Murthy et al., 2000).

*Chemistry.* All solvents were purchased as a reagent grade, dried using standard conditions, and stored over molecular sieves. Purification of products was accomplished using silica gel flash chromatography (Whatman 60, 230–400 mesh). Routine NMR analyses were accomplished on a Bruker Advance DPX-400. Melting points were obtained on Thomas-Hoover capillary melting point apparatus and are uncorrected. Mass spectral measurements were made on a Waters 2690 mass spectrometer. Analytical results are within $\pm 0.40\%$ of the theoretical values and were determined by QTI, Whitehouse, NJ.s.

*1,4:3,6-Dianhydro-D-mannitol* **(2)**. D-mannitol (25 g), HOAc (37 mL) and $H_2SO_4$ conc. (5 mL) were mixed in a round bottom flask. The mixture was refluxed for 24 h and basified until pH 10 with $Na_2CO_3$ saturated solution. The mixture was again refluxed for 48 h. Then, it was concentrated in vacuum until a precipitate was formed. Water was added and the solution was extracted with ethyl acetate (continuous extraction/24 h). The organic phases were washed with brine and dried. The solvent was evaporated and the product was obtained as an oil which was distillated under vacuum to afford the product as a white solid in 40% yield.

### *General procedure for N-tert-Butoxycarbonyl protection*

*N-tert-Butoxycarbonyl-L-alanine* **(3)**. The procedure is similar to the general procedure for **7**. $^1$H NMR $\delta$ ($CDCl_3$, 400 MHz): 9.38 (br s, 1H, –OH), 5.13 (br s, 1H, –NH), 4.31–4.15 (m, 1H, –CH), 1.43 (s, 9H, $-CH_3$), 1.41 (s, 3H, $-CH_3$). mp 77–78°C. $\alpha_D{}^{25} = -24.5$ (*c*, 2.0) AcOH.

*N-tert-Butoxycarbonyl-L-valine* **(4)**. The procedure is similar to the general procedure for **7**. $^1$H NMR $\delta$ ($CDCl_3$, 400 MHz): 10.25 (br s, 1H, –OH), 5.12 (br s, 1H, –NH), 4.21–4.10 (m, 1H, –CH), 2.16–2.00 (m, 1H, –CH), 1.42 (s, 9H, $-CH_3$), 0.97–0.91 (m, 6H, $-CH_3$). mp 75–76°C. $\alpha_D{}^{25} = +12.9$ (*c*, 1.0) $CHCl_3$.

*N-tert-Butoxycarbonyl-L-phenylalanine* **(5)**. The procedure is similar to the general procedure for **7**. $^1$H NMR $\delta$ ($CDCl_3$, 400 MHz): (1:2 mixture of rotamers) 8.10 (br s, 1H, –NH), 7.27–7.19 (m, 5H, –CH), 6.21 (br s, 1H, –NH), 4.98 (br s, 1H, –NH), 4.60 (m, 1H, –CH), 3.17–2.90 (m, 2H, $-CH_2$), 1.40 (s, 6H, $-CH_3$), 1.30 (s, 3H, $-CH_3$). mp 68–69°C. $\alpha_D{}^{27} = +23.2$ (*c*, 1.0) EtOH.

*N-1-Bis[-tert-Butoxycarbonyl]-L-histidine* **(6)**. To a suspension of L-histidine (6.44 mmol, 1 g) and trietylamine (19.33 mmol, 2.7 mL) in water (3.2 mL), a solution of di-*t*-butyldicarbonate (16.1 mol, 3.54 g) in dioxane (3.2 mL) was slowly added. After the mixture had been stirred overnight at room temperature, water (6 mL) was added and the aqueous phase was washed with diethyl ether ($3 \times 50$ mL) and ethyl acetate ($3 \times 50$ mL). The total volume was increased to 200 mL with ethyl acetate and the solution acidified with 10% chloridric acid until it reached pH 3. The aqueous phase was poured off and extracted with ethyl acetate (100 mL). The organic phases were washed with brine and dried. The solvent was evaporated and the product was obtained (1.65 g). $^1$H NMR $\delta$ ($CDCl_3$, 400 MHz): 8.15 (s, 1H, –CH), 7.19 (s, 1H, –CH), 5.47 (br s, 1H, –NH), 4.48 (s, 1H, –CH), 3.20–3.08 (m, 2H, $-CH_2$), 1.59 (s, 9H, $-CH_3$), 1.46 (s, 9H, $-CH_3$). mp 165–166°C. $\alpha_D{}^{27} = +95.5$ (*c*, 1.0) $CHCl_3$.

*N-tert-Butoxycarbonyl-L-proline* **(7)**. L-Proline (17.39 mmol, 2 g) and sodium hydroxide (20.1 mmol, 0.807 g) were dissolved in *t*-butanol-water mixture (30 mL, 1:1, v/v). To this solution, di-*t*-butyldicarbonate (21.1 mmol, 4.36 g) was added in one portion. The reaction mixture was stirred at rt overnight. The aqueous layer was treated with aqueous acetic acid (2 mL in 11 mL $H_2O$) and, then, extracted with ethyl acetate ($3 \times 70$ mL). The combined extracts were washed with water and dried. The crude product was recrystallised from chloroform-hexane at 0°C to afford the compound (2.27 g). $^1$H NMR $\delta$ ($CDCl_3$, 400 MHz): 9.24 (br s, 1H, –OH), 4.27–4.20 (m, 1H, –CH), 3.50–3.48 (m, 2H, $-CH_2$), 2.12–1.84 (m, 4H, $-CH_2$), 1.43 (s, 9H, $-CH_3$). mp 120–122°C. $\alpha_D{}^{27} = -85.5$ (*c*, 1.0) $CHCl_3$.

*N-tert-Butoxycarbonyl-L-glutamic acid 5-methyl ester* **(8)**. To a stirred solution of L-glutamic acid 5-methyl ester (6.20 mmol, 1 g) in water (18 mL) $NaHCO_3$ was added (13.6 mmol, 1.14 g) at 0°C, followed by di-*t*-butyl dicarbonate (6.57 mmol, 1.42 g) in dioxane (18 mL). The reaction mixture was stirred at rt overnight. The solution was extracted with ethyl ether ($3 \times 20$ mL). The aqueous phase was acidified with 10% chloridric acid until it reached pH 3. The product was extracted by ethyl acetate and the organic phase washed with brine and dried. The solvent was evaporated and the product was obtained (1.56 g). $^1$H NMR $\delta$ ($CDCl_3$, 400 MHz): 8.74 (br s, 1H, –NH), 4.33–4.22 (m, 1H, –CH), 3.70 (s, 3H, $-CH_3$), 2.48–2.03 (m, 4H, $-CH_2$), 1.43 (s, 9H, $-CH_3$). mp 75–76°C. $\alpha_D{}^{27} = +8.4$ (*c*, 1.0) $CHCl_3$.

*O-Benzyl-L-Pyroglutamic acid* **(9a)**. To a solution of L-Pyroglutamic acid (1 g, 7.74 mmol) in benzene (20 mL), benzyl alcohol (1.12 mL, 10.83 mmol), and *p*-TsOH (cat.) were added. The mixture was refluxed in a Dean-Stark apparatus overnight. Ethyl acetate was added to the organic layer and washed with 5% $NaHCO_3$ ($3 \times 50$ mL). The solvent was evaporated under vacuum to obtain 1.66 g of the crude product. Purification by column chromatography gives the product (0.9 g) as oil in 51% yield. $^1$H NMR $\delta$ ($CDCl_3$, 400 MHz): 7.35–7.33 (m, 5H, –CH), 6.76 (br s, 1H, –NH), 5.17 (s, 2H, $-CH_2$), 4.27–4.24 (m, 1H, –CH), 2.48–2.25 (m, 4H, $-CH_2$). $^{13}$C NMR: 178.2 (–C=O), 172.0 (C=O), 135.1 (–Cq), 128.7, 128.6, 128.3 (–CH), 67.2 ($-CH_2$), 55.6 (–CH), 29.3 ($-CH_2$), 24.7 ($-CH_2$). ESMS m/z 218 (M − 1). $\alpha_D{}^{27} = -1.6$ (*c*, 1.0) $CHCl_3$.

*O-Benzyl-N-tert-Butoxycarbonyl-L-Pyroglutamic acid* **(9b)**. To a solution of O-Benzyl-Pyroglutamic acid (**9a**) (5.07 mmol, 1.17 g) in dry THF (25 mL), at −78°C, a NaHMDS 1M solution in THF (5.31 mmol, 5.31 mL) was added. The mixture was stirred for 20 min. A solution of di-*t*-butyldicarbonate (7.6 mmol, 1.66 g) in THF (10 mL) was added, and the mixture was allowed to stir for 20 min more at low temperature. The solvent was evaporated, ethyl acetate was added and the organic phase washed with saturated ammonium chloride. The solvent was evaporated

under vacuum to obtain 1.86 g of the crude product. Purification by column chromatography gives the product (1.36 g) in 81.4% yield. $^1$H NMR $\delta$ ($CDCl_3$, 400 MHz): 7.38–7.32 (m, 5H, –CH), 5.20 (s, 2H, $-CH_2$), 4.65 (dd, $J = 2.8$ Hz, $J = 9.6$ Hz, 1H, –CH), 2.65–2.35 (m, 4H, $-CH_2$), 1.40 (s, 9H, $-CH_3$). $^{13}$C NMR: 173.3 (–C=O), 171.1 (C=O), 149.2 (–C=O), 135.0 (–Cq), 128.7, 128.6, 128.5 (–CH), 83.6 (–Cq), 67.3 ($-CH_2$), 58.9 (–CH), 31.1 ($-CH_2$), 27.8 ($-CH_3$), 21.5 ($-CH_2$). ESMS m/z 318 (M − 1). $\alpha_D^{26} = -34.8$ (*c*, 1.0) $CH_2Cl_2$, $\alpha_D^{lit} = -31.9$ (*c*, 1.0) $CH_2Cl_2$.

*L-N-tert-Butoxycarbonylpyroglutamic acid* **(9)**. A solution of *O*-Benzyl-*N-tert*-Butoxycarbonyl-L-Pyroglutamic acid (**9b**) (4.23 mmol, 1.35 g) in ethyl acetate (30 mL) and 10% Pd/C (0.310 g) was vigorously shaken under an atmosphere of $H_2$ (40 psi) for 1 h. The catalyst was filtered off and the solution was concentrated affording the crude product. Purification by column chromatography gives the product (0.872 g) in 90% yield. $^1$H NMR $\delta$ ($CDCl_3$, 400 MHz): 8.11 (br s, 1H), 4.66–4.63 (m, 1H, –CH), 2.70–2.36 (m, 4H, $-CH_2$), 1.49 (s, 9H, $-CH_3$). $^{13}$C NMR: 175.8 (–C=O), 174.0 (–C=O), 149.3 (–C=O), 84.0 (–Cq), 58.7 (–CH), 31.1 ($-CH_2$), 27.8 ($-CH_3$), 21.4 ($-CH_2$). ESMS m/z 228 (M − 1). mp 111–112°C. $\alpha_D^{29} = -21.3$ (*c*, 1.0) $CHCl_3$.

*General procedure for DCC-mediated condensation:* To a solution of isommanide (2 mmol) and the corresponding protected amino acid (4 mmol) in dry $CH_2Cl_2$ (10 mL) DCC (4 mmol, 0.605 g) was added at 0°C. The mixture was stirred at room temperature overnight, and the solvent was evaporated. Purification by column chromatography gives the corresponding products in around 40% yield.

*(3R,6R)-bis-O-(N-tert-Butoxycarbonyl-L-alanine)-1,4-dioxabicyclo[3.3.0] octane* **(10a)**. $^1$H NMR $\delta$ ($CDCl_3$, 400 MHz): 5.10 (br s, 2H, –CH), 4.69 (s, 2H, –CH), 4.37 (br s, 2H, –CH), 4.03–4.01 (m, 2H, $-CH_2$), 3.81–3.77 (m, 2H, $-CH_2$), 1.43 (s, 18H, $-CH_3$), 1.39 (d, 6H, $J = 7.18$ Hz, $-CH_3$). $^{13}$C NMR: 173.0 (–C=O), 155.3 (–C=O), 80.6 (–CH), 80.2 (–Cq), 74.6 (–CH), 70.8 ($-CH_2$), 49.6 (–CH), 28.7 ($-CH_3$), 19.0 ($-CH_3$). ESMS m/z 489 (M + 1). IR (KBr) $\nu$ $cm^{-1}$: 3347, 2979, 2888, 1747, 1683, 1517, 1303, 1252, 1160, 1074, 978. Anal. Calcd. for $C_{22}H_{36}N_2O_{10}$: C, 54.09; H, 7.43; N, 5.73. Found: C, 53.96; H, 7.56; N, 5.36. $\alpha_D^{27} = +89.0$ (*c*, 1.0) $CHCl_3$, mp = 105–106°C.

*(3R,6R)-bis-O-(N-tert-Butoxycarbonyl-L-phenylalanine)-1,4-dioxabicyclo[3.3.0]octane* **(10b)**. $^1$H NMR $\delta$ ($CDCl_3$, 400 MHz): 7.30–7.13 (m, 10H, –CH), 5.04–5.01 (m, 2H, –CH), 4.65 (s, 2H, –CH), 4.18 (m, 2H, –CH), 3.94–3.90 (m, 2H, $-CH_2$), 3.64–3.62 (m, 2H, $-CH_2$), 3.10 (m, 4H, $-CH_2$), 1.41 (s, 18H, $-CH_3$). $^{13}$C NMR: 171.7 (–C=O), 155.3 (–C=O), 136.3 (–Cq), 129.7 (–CH), 128.9 (–CH), 127.4 (–CH), 80.6 (–CH), 80.3 (–Cq), 74.6 (–CH), 70.5 ($-CH_2$), 54.8 (–CH), 38.8 ($-CH_2$), 28.7 ($-CH_3$). ESMS m/z 641 (M + 1). IR (KBr) $\nu$ $cm^{-1}$: 2976, 2931, 1750, 1710, 1624, 1500, 1451, 1365, 1247, 1163, 1055. Anal. Calcd. for $C_{34}H_{44}N_2O_{10}$: C, 63.74; H, 6.92; N, 4.37. Found: C, 63.39; H, 7.01; N, 4.22. $\alpha_D^{27} = +105.3$ (*c*, 1.0) $CHCl_3$, mp = 55–56°C.

*(3R,6R)-bis-O-(N-tert-Butoxycarbonyl-L-valine)-1,4-dioxabicyclo[3.3.0] octane* **(10c)**. $^1$H NMR $\delta$ ($CDCl_3$, 400 MHz): 5.04–5.00 (m, 2H, –CH), 4.66 (s, 2H, –CH), 4.25 (br s, 2H, –CH), 3.99–3.95 (m, 2H, $-CH_2$), 3.76–3.72 (m, 2H, $-CH_2$), 2.15–1.98 (m, 2H, –CH), 1.44 (s, 18H, $-CH_3$), 0.90 (dd, 12H, $J = 6.7$ Hz, $J = 28.3$ Hz, $-CH_3$). $^{13}$C NMR: 172.1 (–C=O), 155.9 (–C=O), 80.6 (–CH), 80.1 (–Cq), 74.6 (–CH), 70.7 ($-CH_2$), 58.9 (–CH), 31.6 (–CH), 28.7 ($-CH_3$), 19.3 ($-CH_3$), 17.9 ($-CH_3$). ESMS m/z 545 (M + 1). IR ($CH_2Cl_2$) $\nu$ $cm^{-1}$: 2970, 2932, 1742, 1710, 1504, 1365, 1308, 1246, 1162, 1095, 1017. Anal. Calcd. for $C_{26}H_{44}N_2O_{10}$: C, 57.34; H, 8.14; N, 5.14. Found: C, 57.33; H, 8.20; N, 5.42. $\alpha_D^{27} = +85.1$ (*c*, 1.0) $CHCl_3$. The product is a colorless oil.

*(3R,6R)-bis-O-(N-tert-Butoxycarbonyl-L-proline)-1,4-dioxabicyclo[3.3.0] octane* **(10d)**. $^1$H NMR $\delta$ ($CDCl_3$, 400 MHz): 5.12–5.10 (m, 2H, –CH), 4.68–4.67 (s, 2H, –CH), 4.28–4.27 (m, 2H, –CH), 4.08–4.07 (m, 2H, $-CH_2$), 3.94–3.92 (m, 2H, $-CH_2$), 3.54–3.45 (m, 4H, $-CH_2$), 2.21–1.83 (m, 8H, $-CH_2$), 1.43 (s, 18H, $-CH_3$). $^{13}$C NMR: 172.8 (–C=O), 154.2 (–C=O), 82.1 (–CH), 80.6 (–Cq), 74.6 (–CH), 71.1 ($-CH_2$), 59.2 (–CH), 46.9 ($-CH_2$), 28.8 ($-CH_3$), 25.3 ($-CH_2$), 23.8 ($-CH_2$). ESMS m/z 539 (M − 1). Anal. Calcd. for $C_{26}H_{40}N_2O_{10}$: C, 57.76; H, 7.46; N, 5.18. Found: C, 57.40; H, 7.08; N, 4.89. $\alpha_D^{27} = +16.3$ (*c*, 1.0) $CHCl_3$. The product is colorless oil.

*(3R,6R)-bis-O-(N-1-Bis[-tert-Butoxycarbonyl]-L-histidine)-1,4-dioxabicyclo[3.3.0]octane* **(10e)**. $^1$H NMR $\delta$ ($CDCl_3$, 400 MHz): $\delta$ 7.96 (s, 2H, –CH), 7.15 (s, 2H, –CH), 5.68–5.66 (m, 2H, –CH), 5.08 (s, 2H, –CH), 4.65 (s, 2H, –CH), 3.99–3.98 (m, 2H, $-CH_2$), 3.80–3.75 (m, 2H, $-CH_2$), 3.08 (br s, 4H, $-CH_2$), 1.59 (s, 18H, $-CH_3$), 1.42 (s, 18H, $-CH_3$). $^{13}$C NMR: 171.6 (–C=O), 155.6 (–C=O), 147.2 (–C=O), 138.8 (Cq), 137.2 (–CH), 115.1 (–CH), 85.9 (–Cq), 80.6 (–CH), 80.1 (–Cq), 74.7 (–CH), 70.6 ($-CH_2$), 53.5 (–CH), 30.6 ($-CH_2$), 28.6 ($-CH_3$), 28.2 ($-CH_3$). ESMS m/z 821 (M + 1). IR (KBr) $\nu$ $cm^{-1}$: 3388, 2979, 2932, 1755, 1712, 1490, 1391, 1289, 1254, 1159, 1055, 1011, 843. Anal. Calcd. for $C_{38}H_{56}N_6O_{14}$: C, 55.60; H, 6.88; N, 10.24. Found: C, 55.25; H, 7.08; N, 10.01. $\alpha_D^{21} = +72.2$ (*c*, 1.0) $CHCl_3$, mp = 74–75°C.

*(3R,6R)-bis-O-(N-tert-Butoxycarbonyl-L-glutamic acid 5-methyl ester)-1,4-dioxabicyclo[3.3.0]octane* **(10f)**. $^1$H NMR $\delta$ ($CDCl_3$, 400 MHz): $\delta$ 5.15–5.07 (m, 2H, –CH), 4.70 (s, 2H, –CH), 4.40 (br s, 2H, –CH), 4.03 (m, 2H, $-CH_2$), 3.81 (m, 2H, $-CH_2$), 3.67 (s, 6H, $-OCH_3$), 2.46–2.36 (m, 4H, $-CH_2$), 2.21–2.18 (m, 2H, $-CH_2$), 2.02–1.95 (m, 2H, $-CH_2$), 1.43 (s, 18H, $-CH_3$). $^{13}$C NMR: $\delta$ 173.5 (–C=O), 171.8 (–C=O), 155.6 (–C=O), 80.5 (–CH), 80.2 (–Cq), 74.6 (–CH), 70.7 ($-CH_2$), 53.2 (–CH), 52.0 ($-OCH_3$), 30.2 ($-CH_2$), 28.5 ($-CH_3$), 27.8 ($-CH_2$). ESMS *m/z* 633 (M + 1). IR ($CH_2Cl_2$) $\nu$ $cm^{-1}$: 3349, 2977, 2931, 1738, 1713, 1632, 1583, 1515, 1445, 1366, 1251, 1166. Anal. Calcd. for $C_{28}H_{44}N_2O_{14}$: C, 51.65; H, 6.67; N, 4.63. Found: C, 52.01; H, 6.98; N, 4.66. $\alpha_D^{21} = +91.9$ (*c*, 1.0) $CHCl_3$. The product is a colorless oil.

*(3R,6R)-bis-O-(N-tert-Butoxycarbonyl-L-pyroglutamic acid)-1,4-dioxabicyclo[3.3.0]octane* **(10g)**. $^1$H NMR $\delta$ ($CDCl_3$, 400 MHz): 5.13–5.11 (m, 2H, –CH), 4.74–4.73 (m, 2H, –CH), 4.68–4.64 (m, 2H, –CH), 4.03–3.98 (m, 2H, $-CH_2$), 3.76–3.74 (m, 2H, $-CH_2$), 2.66–2.34 (m, 8H, $-CH_2$), 1.48 (s, 18H, $-CH_3$). $^{13}$C NMR: 173.5 (–C=O), 170.6 (C=O), 149.2 (–C=O), 83.8 (–Cq), 80.2 (–CH), 74.4 (–CH), 70.1 ($-CH_2$), 58.9 (–CH), 31.1 ($-CH_2$), 27.8 ($-CH_3$), 21.8 ($-CH_2$). ESMS m/z 569 (M + 1). Anal. Calcd. for $C_{26}H_{36}N_2O_{12}$: C, 53.45; H, 6.38; N, 4.93. Found: C, 53.16; H, 6.40; N, 5.25. $\alpha_D^{27} = +21.3$ (*c*, 1.0) $CHCl_3$.

## Results and discussion

### *Chemistry*

The start material for the synthesis of the isomannide esters was D-mannitol (**1**). Ready dehydration of naturally occurring **1** provided the symmetrical molecule, 1,4:3,6-dianhydro-D-mannitol, the isomannide (**2**) (Wiggins, 1945). Its structure was resolved by X-ray diffraction analysis to assure that no isomerization took place in the acidic cyclization step. Ester formation proved to be very difficult to achieve. Initial attempts to obtain the isomannide esters with the Boc-protected L-amino acids, **3**–**9** (described in Table 1), in the presence of *p*-toluenesulfonic acid in benzene were unsuccessful (Gomurashvili, 2000). The L-amino acids were protected according to literature (Benoiton et al., 1993; Ye and McKervey, 1992; Gołębiowski et al., 1987; Barcelo et al., 1986; Le Nguyen et al., 1985; Keller et al., 1985; Ookawa and Soai, 1987; Feng and Edstrom, 1999; El Marini et al., 1992; Li et al., 1995; Yoshifuji et al., 1986). In view of these results, other amino acid esterification

**Table 1.** Boc-amino acids prepared by acylation with Di-*t*-Butyl dicarbonate

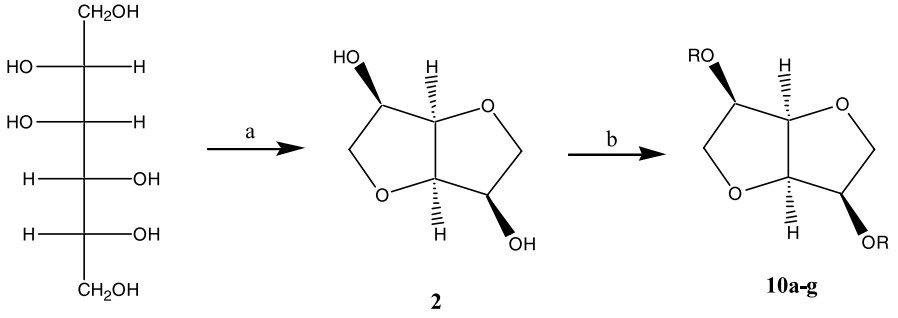
| No. | Boc-amino acids | Conditions | mp, °C | $\alpha_D^{25}$ |
|---|---|---|---|---|
| **3** | Boc-Ala | NaOH, *t*BuOH/$H_2O$ | 77–78 | −24.5 (AcOH, *c* 2.0) |
| **4** | Boc-Val | NaOH, *t*BuOH/$H_2O$ | 75–76 | +12.9 ($CHCl_3$, *c* 1.0) |
| **5** | Boc-Phe | NaOH, *t*BuOH/$H_2O$ | 68–69 | +23.2 (EtOH, *c* 1.0) |
| **6** | Boc-His | $Et_3N$, dioxane/$H_2O$ | 165–166 | +95.5 ($CHCl_3$, *c* 1.0) |
| **7** | Boc-Pro | NaOH, *t*BuOH/$H_2O$ | 120–122 | −85.5 ($CHCl_3$, *c* 1.0) |
| **8** | Boc-OMeGlu | $NaHCO_3$, dioxane/$H_2O$ | 75–76 | +8.4 ($CHCl_3$, *c* 1.0) |
| **9** | Boc-PyroGlu | NaHMDS, THF | 111–112 | −21.3 ($CHCl_3$, *c* 1.0) |

methods were attempted, i.e., as the activation of the carboxylic acid by formation of a mixed anhydride with isobutyl chloroformate (Kim et al., 1985; Muri et al., 2000), and the treatment of amino acids with *N,N*-bis-(2-oxooxazolidin-3-yl)phosphorodiamidic chloride (BOP-Cl) in pyridine (Herranz et al., 1991). Unfortunately, these methodologies led to negative results. The best result one was achieved by the *N,N′*-dicyclohexylcarbodiimide (DCC)-mediated condensation of Boc-protected amino acids with isomannide (Benoiton et al., 1993; Barany and Albericio, 1995). In this manner, compounds **10a**–**g** were obtained free of racemization (Scheme 1). The structural assignment of all the esters of amino acids was based on analytical and spectroscopic data.

### *Molecular modeling*

The Mung-bean Bowman-Birk inhibitor (MbBBI) fragment (nine residues from $Cys^{518}$ to $Cys^{526}$), (Murthy, 2000), was docked into the active site of the Dengue virus NS3 protease showing six hydrogen bond interactions between the NS3 protease and the MbBBI fragment (Fig. 1A/B). The observed hydrogen bonds are between the following pairs of residues, $Gln^{35\cdots}Gln^{525}$ (2.17 Å), $His^{51\cdots}Thr^{519}$ (2.41 Å), $Gly^{133\cdots}Lys^{520}$ (2.06 Å), $Ser^{135\cdots}Lys^{520}$ (1.77 Å), $Asn^{152\cdots}Lys^{520}$ (1.91 Å), and $Gly^{153\cdots}Cys^{518}$ (2.41 Å), from NS3 protease and MbBBI fragment, respectively. It is to be noted that, as in the crystallographic structure of NS3-MbBBI complex, the most extensive interactions occur at the P1 site, where the $Lys^{520}$ makes three hydrogen bond interactions. Although these interactions do not present the same pattern as in the crystallographic structure, they are closer to the same residues. The residues around (5 Å), the oxygen atom of the $Lys^{520}$ carbonyl group, are $Pro^{132}$, $Gly^{133}$, $Thr^{134}$, and $Ser^{135}$; and the residues around (5 Å), the nitrogen atom of the $Lys^{520}$ amine group, are $Tyr^{150}$, $Gly^{151}$, $Gly^{153}$, and $Ser^{163}$. Overall, comparing the crystallographic NS3-MbBBI complex with the docked structure of NS3 and MbBBI fragment, it may be pointed, therefore, that the fragment of the MbBBI was able to maintain the same orientation in the active site of NS3 protease and the principal interactions in the docked model, therefore validating the proposed model of the NS3 docked with our compound.

The most simple compound of our series, the Ala-Boc ester **10a**, was docked into the active site of the NS3 protease, showing three hydrogen bond interactions with the NS3 protease (Fig. 2A/B). One hydrogen bond is between the backbone NH group of $Gly^{133}$ and one oxygen atom of the bicycle group (1.88 Å), that corresponds




**1** **2** **10a-g**

(**10a**) R=Ala-Boc, (**10b**) R=Phe-Boc, (**10c**) R=Val-Boc, (**10d**) R=Pro-Boc, (**10e**) R=His-Boc, (**10f**) R=OMeGlu-Boc, (**10g**) R=PyroGlu-Boc

**Scheme 1.** **a** HOAc/$H_2SO_4$.; **b** Boc-amino acids (**3**–**9**), DCC, $CH_2Cl_2$

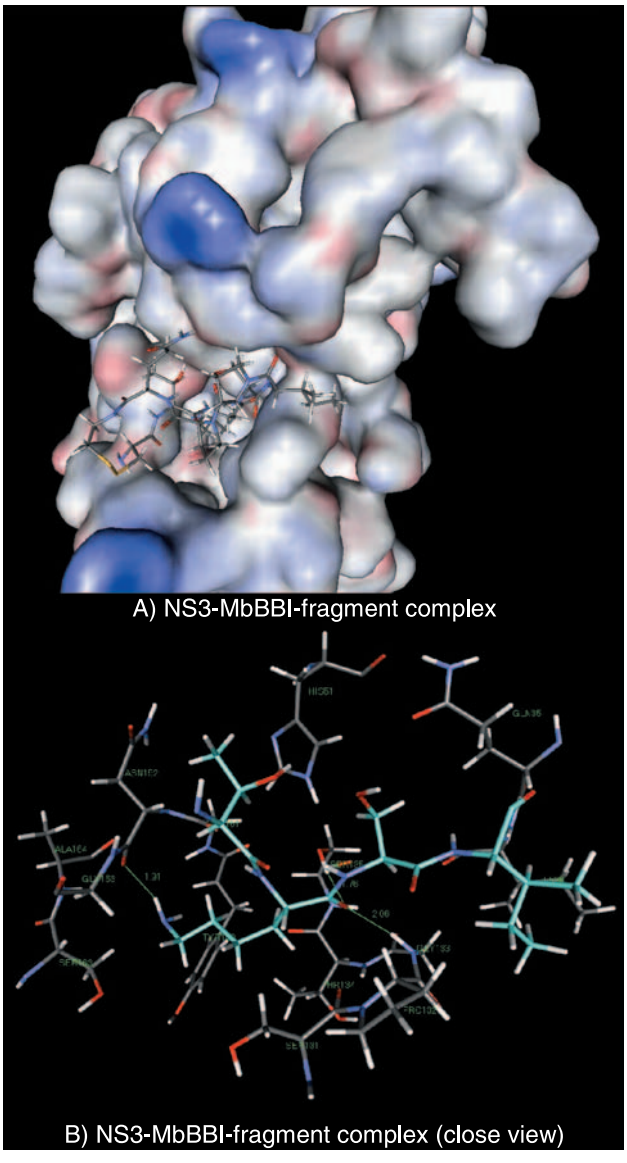



**Fig. 1.** **A** Docked structure of MbBBI-fragment into the NS3-protease active site, where the enzyme is represented with a solid surface color by electrostatic potential. **B** Close view of this complex representing only the residues P2′-P1′-P1-P2 of MbBBI-fragment and the aminoacids residues no more distant than 5 Å from this tetrapeptide fragment. The carbon atoms of the MbBBI tetrapeptided are colored in light blue

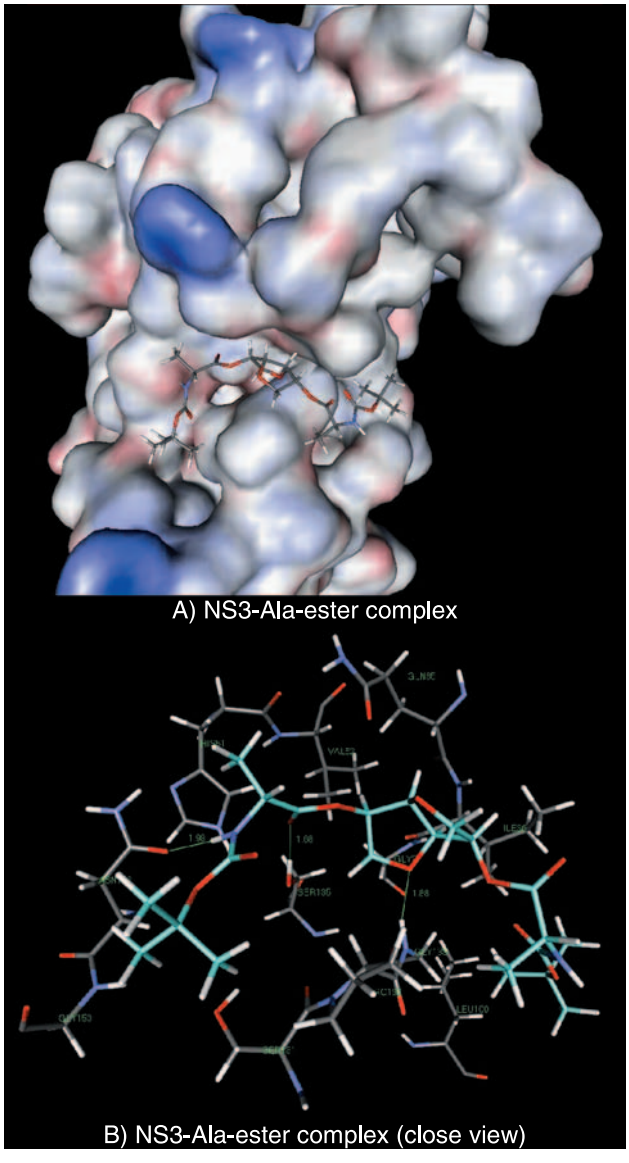



**Fig. 2.** **A** Docked structure of compound **10a** into the NS3-protease active site, where the enzyme is represented with a solid surface color by electrostatic potential. **B** Close view of this complex representing only the aminoacids residues no more distant than 5 Å from this tetrapeptide fragment. The carbon atoms of the compound **10a** are colored in light blue

to the P1′ position of compound **10a**, according to Fig. 3. The other two hydrogen bonds correspond to the P1 position: i.e., the Ala residue from compound **10a**, where one of them includes the hydroxyl group of $Ser^{135}$ (catalytic residue) and the carbonyl oxygen atom of Ala residue (1.68 Å), while the other includes the carbonyl oxygen atom of $Asn^{152}$ and NH group of Ala residue (1.96 Å). There are three residues, namely, $Ile^{36}$, $Leu^{100}$, and $Pro^{132}$, potentially capable of providing specific van der Waals interactions stabilizing the complex NS3 protease-compound **10a**. In addition, the catalytic $His^{51}$ and residues $Gln^{35}$, $Ser^{131}$, and $Gly^{153}$ make nonspecific interactions. It is clear that the putative ligand, the Ala-Boc ester compound, is well accommodated into the active site of the NS3 protease, although it should be pointed out that it does not have at P1 position a basic residue: e.g., Arg or Lys. Therefore, we are planning to obtain a new series of compounds showing basic residues in order to reach $Tyr^{150}$ residue, that stays at the bottom of the cleft at S1 position, as well as polypeptide derivatives.

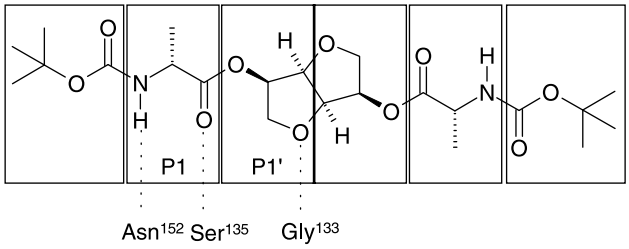



**Fig. 3.** Schematic hydrogen bonding representation between the compound **10a** and the residues of NS3-protease after docking calculations

As part of our antiviral program (Peçanha et al., 2003), we described here the synthesis of a new series of *N-t*-Boc amino acid esters of isomannide, designed as potential inhibitors of serine proteases. In order to have a model to validate the proposed model of NS3 protease docked with our compound, a preliminary molecular modeling study was developed using a peptide inhibitor (MbBBI) from the literature, the bioassay of the new compounds described here in the dengue virus test are underway.

## Acknowledgment

Financial support from CAPES, CNPq, and FAPERJ is acknowledged. We also thank Eli Lilly & Company for the crystallography data.

## References

Barany G, Albericio F (1985) Three-dimensional orthogonal protection scheme for solid-phase peptide synthesis under mild conditions. J Am Chem Soc 107: 4936–4942

Barcelo G, Senet J-P, Sennyey G (1986) Alkyl 1-chloroalkyl carbonates: Reagents for the synthesis of carbamates and protection of amino groups. Synthesis: 627–632

Bazan JF, Fletterick RJ (1989) Detection of a trypsin-like serine protease domain in flaviviruses and pestiviruses. Virology 171: 637–639

Bazan JF, Fletterick RJ (1990) Structural and catalytic models of trypsine-like viral proteases. Semin Virology 1: 311–322

Benoiton NL, Lee YC, Chen FMF (1993) Identification and suppression of decomposition during carbodiimide-mediated reactions of boc amino acids with phenols, hydroxylamines and amino acid ester hydrochlorides. Int J Pep Proteins Res 41: 587–594

Bencsik JR, Kercher T, O'Sullivan M, Josey JA (2003) Efficient, stereoselective synthesis of oxazolo[3,2-a]pyrazin-5-ones: novel bicyclic lactam scaffolds from the bicyclocondensation of 3-aza-1,5-ketoacids and amino alcohols. Org Lett 5: 2727–2730

Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, Shindyalov IN, Bourne PE (2000) The protein data bank. Nucleic Acids Res 28: 235–242

Bruix J, Cavalet X, Costa J, Ventura M, Bruguera M, Castillo R, Barrera JM, Ercilla G, Sanchez-Tapias JM, Vall M, Bru C, Rodes J (1989) Prevalence of antibodies to hepatitis-C virus in spanish patients with hepatocellular carcinoma and hepatic cirrhosis. Lancet 2: 1004–1006

Costi MP, Ferrari S (2001) Update on antifolate drugs targets. Curr Drug Targets 2: 135–166

Dietrich E, Lubell WD (2003) Efficient synthesis of enantiopure pyrrolizidinone amino acid. J Org Chem 68: 6988–6996

DISCOVER®, version 2.9.7 – User Guide. Part 1. Accelrys Inc. San Diego

El Marini A, Roumestant ML, Viallefont P, Razafindramboa D, Bonato M, Follet M (1992) Synthesis of enantiomerically pure $\beta$- and $\gamma$-amino acids from aspartic and glutamic acid. Synthesis: 1104–1108

Feng X, Edstrom A (1999) A synthetic approach to diaryl ethers using the Robinson annulation. Tetrahedron Asymmetr 10: 99–105

Gołębiowski A, Jacobsson U, Jurczak, J (1987) High pressure approach to the total synthesis of 6-epi-D-purpurosamine B. Tetrahedron 43: 3063–3066

Gomurashvili Z, Kricheldorf HR, Katsarava R (2000) Amino acid based bioanalogous polymers. Synthesis and study of new poly(ester amides) composed of hydrophobic $\alpha$-amino acids and dianhydrohexitoles. JMS-Pure Appl Chem A37: 215–227

Gorbalenya AE, Donchenko AP, Koonin EV, Blinov VM (1989) N-terminal domains of putative helicases of flavi- and pestiviruses may be serine proteases. Nucleic Acids Res 17: 3889–3897

Hayes CG (1989) The arboviruses: epidemiology and ecology, vol V. In: Monath TP (ed) CRC, Boca Raton, FL, p 59

Herranz R, Castro-Pichel J, García-López MT, Pérez C, Balzarini J, De Clercq E (1991) Synthesis of penicillamine- and cysteine-containing nucleoamino acids as potential antivirals and aminopeptidase B inhibitors. J Chem Soc Perkin Trans 1: 43–48

Houghton M, Weiner A, Han J, Kuo G, Choo Q-L (1991) Molecular biology of the hepatitis-C viruses – implications for diagnosis, development and control of viral disease. Hepatology 14: 381–388

Keller O, Keller WE, van Look G, Wersin G (1985) Tert-butoxycarbonylation of amino acids and their derivatives: N-tert-butoxycarbonyl-L-phenylalanine. Org Synth 63: 160–170

Kim S, Lee JI, Kim YC (1985) A simple and mild esterification method for carboxylic acids using mixed carboxylic-carbonic anhydrides. J Org Chem 50: 560–565

Kuntz ID, Blaney JM, Oatley SJ, Langridge R, Ferrin TE (1982) A geometric approach to macromolecule-ligand interactions. J Mol Biol 161: 269–288

Le Nguyen D, Seyer R, Heitz A, Castro B (1985) Renin substrates. Part 1. Liquid-phase synthesis of the equine sequence with benzotriazolyloxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP). J Chem Soc Perkin Trans 1: 1025–1031

Leung D, Schroder K, White H, Fang N-X, Stoermer MJ, Abbenante G, Martin JL, Young PR, Fairlie DP (2001) Activity of recombinant dengue 2 virus NS3 protease in the presence of a truncated NS3B co-factor, small peptide substrates, and inhibitors. J Biol Chem 276: 45762–45771

Li H, Sakamoto T, Kato M, Kikugawa YA (1995) A convenient *N*-protection of pyroglutamate derivatives. Synth Comm 25: 4045–4052

Muri E, Kanazawa A, Barreiro E, Greene A (2000) Asymmetric synthesis of 3$\beta$-angeloyloxy-4$\beta$-hydroxyeudesman-8-one, purported sesquiterpene from *Pluchea quitoc*. J Chem Soc Perkin Trans 1: 731–735

Murthy HMK, Judge K, DeLucas L, Padmanabhan R (2000) Crystal structure of dengue virus NS3 protease in complex with a Bowman-Birk inhibitor: implications for flaviviral polyprotein processing and drug design. J Mol Biol 301: 759–763

Ookawa A, Soai K (1987) Asymmetric synthesis of optically active *threo*- and *erythro*-pyrrolidinylbenzyl alcohol by highly stereospecific arylation of (S)-proline and the subsequent highly diastereoselective reduction of the $\alpha$-amino ketone. J Chem Soc Perkin Trans 1: 1465–1471

Peçanha EP, Figueiredo LJO, Brindeiro RM, Tanuri A, Calazans AR, Antunes OAC (2003) Synthesis and anti-HIV activity of new $C_2$ symmetric derivatives designed as HIV-1 protease inhibitors. Farmaco 58: 149–157

Pletnev AG, Putnak R, Speicher J, Wagar EJ, Vaughn DW (2002) West Nile virus/dengue type 4 virus chimeras that are reduced in neurovirulence and peripheral virulence without loss of immunogenicity or protective efficacy. PNAS 99: 3036–3041

Ye T, McKervey A (1992) Synthesis of chiral N-protected $\alpha$-amino-$\beta$-diketones from $\alpha$-diazoketones derived from natural amino acids. Tetrahedron 48: 8007–8022

Yoshifuji S, Tanaka K-I, Kawai T, Nitta YA (1986) A novel synthesis of L-pyroglutamic acid derivatives from L-proline: Utility of *N*-protecting groups for ruthenium tetroxide oxidation of cyclic $\alpha$-amino acids. Chem Pharm Bull 34: 3873–3878

Yusof R, Clum S, Wetzel M, Murthy HMK, Padmanabhan R (2000) Purified NS2B/NS3 serine protease of dengue virus type 2 exhibits cofactor NS2B dependence for cleavage of substrates with dibasic amino acids *in vitro*. J Biol Chem 275: 9963–9969

Wiggins LF (1945) The anhydrides of polyhydric alcohols. 1. The constitution of isomannide. J Chem Soc: 4–7

World Health Organization (1996) Dengue and dengue haemorrhagic fever. Report N117. World Health Organization, Geneva

Wright M, Main J, Thomas HC (2001) Treatment of chronic viral hepatitis. Chem Chemother 12: 201–212

**Authors' address:** Octavio A. C. Antunes, Instituto de Química, UFRJ, Cidade Universitária, CT Bloco A, Rio de Janeiro, RJ 21945-970, Brazil, Fax: +55 21 25627559, E-mail: octavio@iq.ufrj.br