

Amino Acid Derivatives, IV [1]: Synthesis and Antiviral Evaluation of New α -Amino Acid Esters Bearing Methyl β -D-Ribofuranoside Side Chain

Ibrahim A. I. Ali¹, Omar M. Ali², and Adel A.-H. Abdel-Rahman^{2,*}

¹ Department of Chemistry, Faculty of Science, Suez Canal University, Ismailia, Egypt

² Department of Chemistry, Faculty of Science, Menoufia University, Shebin El-Koam, Egypt

Received January 26, 2007; accepted February 28, 2007; published online June 29, 2007

© Springer-Verlag 2007

Summary. Methyl 2,3-*O*-isopropylidene- β -D-ribofuranoside was synthesized and oxidized with HIO₄ to afford the corresponding carboxylic acid. The latter was coupled with the appropriate acylated amino acids in the presence of HOBt and DDC as coupling reagents to give the corresponding amides. The methyl acetate derivative was hydrolyzed with 2 *N* KOH/*MeOH* to the corresponding carboxylic acid, which was coupled with L-glycine methyl ester to furnish the amide. Deprotection was carried out with 70% AcOH at reflux temperature. The prepared glycopeptides were tested for antiviral activity against *Herpes Simplex* virus type-1 (*HSV-1*) and hepatitis-A virus (HAV). The plaque reduction infectivity assay was used to determine virus count reduction as a result of treatment with tested compounds.

Keywords. Carbohydrates; Glycopeptides; α -Amino acids; 1-Hydroxybenzotriazole; *N,N'*-Dicyclohexylcarbodiimide.

Introduction

Among the great variety of functions assumed by carbohydrates in biological systems, the role of the carbohydrate domain of glycoproteins in molecular recognition has been a focus of particular attention for many scientific disciplines [2]. Glycoproteins play an important role in biological processes, such as tumor metastasis [3] and immunogenic recognition [4]. As promising building blocks for the artificial glycopeptides, *C*-glycosyl amino acids are of particular interest due to their stability toward chemical and

enzymatic degradation [5]. α -*C*-Glycosylglycine and its methylene chain tethered derivatives (Fig. 1, I) have been synthesized from glycosyl halides [6], glycols [7], allyl-*C*-glycosides [8], sugar nitrones [9], and sugar δ -lactones [10]. While the methodology of the C–C bond formation reactions and stereochemistry of the observed products with respect to the configuration of the anomeric center and/or the α -carbon of the amino acid moiety have been extensively studied for these synthetic *C*-glycosyl amino acids, only a small number of them have been utilized for *C*-glycosylpeptide synthesis [11]. Our interest in the synthesis of such compounds was to shed some light on their antiviral properties as a part of our program aimed at the development of new α -amino acid derivatives as antiviral agents [12].

Results and Discussion

Synthesis

A solution of dry D-ribose in acetone/2,2-dimethoxypropane/methanol saturated with HCl afforded after stirring methyl 2,3-*O*-isopropylidene- β -D-ribofuranoside [13]. Oxidation of the primary hydroxyl group in this compound using HIO₄ gave methyl 2,3-*O*-isopropylidene- β -D-ribo-1,4-furanosyl carboxylic acid (1) [14]. A suitable coupling method [15] was employed for the formation of peptides by reaction of the carboxylic acid group with an acylated amino

* Corresponding author. E-mail: adelnassar63@hotmail.com

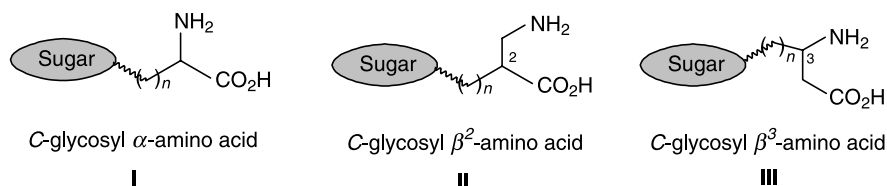
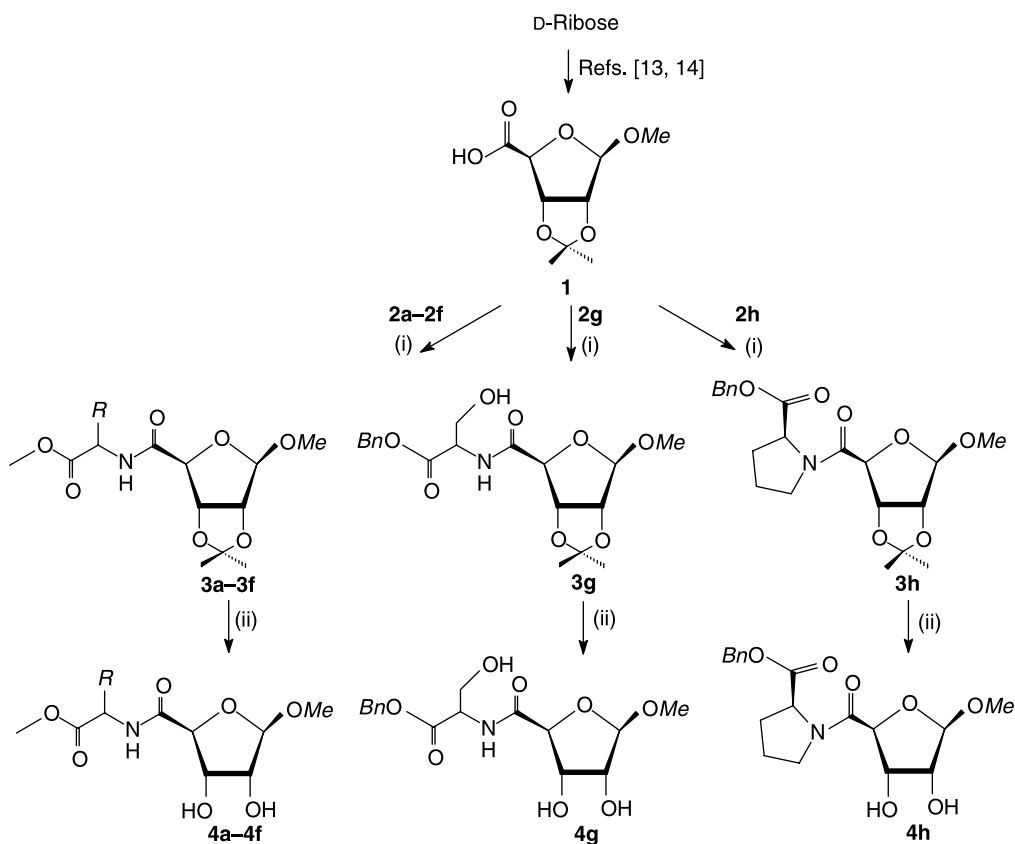


Fig. 1. Structures of C-glycosyl amino acids

acid using 1-hydroxybenzotriazole (HOBt) [16], and *N,N'*-dicyclohexylcarbodiimide (DCC) [17] as coupling reagents. HOBt is currently the most frequently used activating agent for the carboxyl group of amino acids. The procedure is fast and suppresses racemization, especially in the presence of DCC [12, 18].

Amides **3a–3h** were prepared by coupling of **1** with the appropriate acylated amino acids **2a–2h** in the presence of HOBt and DCC to yield **3a–3h** in 55–74% yields after chromatography.

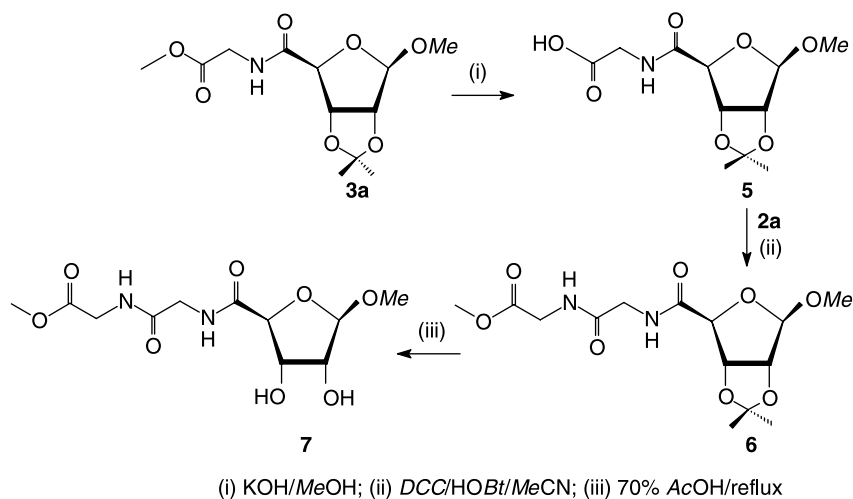
The structures of the newly synthesized compounds **3a–3h** were determined from their ^1H , ^{13}C NMR,



2-4	R	Amino Acid
a	H	L-Glycine
b	Me	L-Alanine
c	Ph	L-Phenylglycine
d	CH ₂ CH ₂ SMe	L-Methionine
e	CH ₂ CH(Me) ₂	L-Leucine
f	CH(Me) ₂	L-Valine

(i) DCC/HOBt/MeCN; (ii) 70% AcOH/reflux

Scheme 1



Scheme 2

and mass spectra. The ^1H NMR spectra showed a singlet at $\delta = 3.22\text{--}3.43$ for the *OMe*, while the isopropylidene group appeared as two singlets at the region $\delta = 1.13\text{--}1.26$ and $1.29\text{--}1.43$ ppm. The singlet at $\delta = 4.90\text{--}5.10$ corresponds to H-1 of the sugar moiety. The protons of the amino acid residues were fully assigned. ^{13}C NMR of the amides (Experimental), showed similar pattern of methyl ribofuranoside carbons resonances, meanwhile compound **3d** was selected for this study. The anomeric carbon atom (C-1) was resonated at $\delta = 86.4$ ppm, two methoxy groups resonated at $\delta = 52.4$ and 56.4 ppm, two carbonyl groups at higher field ($\delta = 169.6$ and 171.8 ppm), while the *SMe* group appeared at lower field $\delta = 15.2$ ppm.

Deprotection of compounds **3a–3h** was carried out by using 70% AcOH at reflux temperature. The crude products were purified by silica gel column affording **4a–4h** in 80–85%. The structures of the deprotected derivatives were confirmed by their ^1H , ^{13}C NMR, and mass spectra, which showed the disappearance of the isopropylidene group in all cases (Scheme 1).

Furthermore, we selected the amide **3a** as precursor for the synthesis of a new derivative to examine its antiviral activity in comparison with **4a–4h**. The methyl acetate derivative **3a** was hydrolyzed in 78% yield to the corresponding carboxylic acid **5** using 2*N* KOH in methanol. The acid derivative **5** was coupled with L-glycine acetate (**2a**) by applying the method used previously in the presence of HOBt and DCC as coupling reagents to afford **6** in 66% yield. Deprotection of **7** was carried out using

70% AcOH at reflux for 2 h. The crude product was purified by silica gel column to afford **7** in 84% yield.

Testing

The plaque infectivity assay [19] was carried out to test the prepared compounds for antiviral activity. The test was performed to include three possibilities for antiviral activity, virucidal effect, virus adsorption, and effect on virus replication for both HAV-27 and HSV-1.

For the antiviral activity against HAV-27 it was noticed that, at both concentrations 10 and $20\ \mu\text{g}/10^5$ cells, compounds **4c–4e**, **4h**, and **7** revealed the highest antiviral activity in this series of compounds and compounds **4g** revealed high activity at $10\ \mu\text{g}/10^5$ cells using amantadine (C^*) as a control. Compound **4b** showed moderate activity, while at concentration of $20\ \mu\text{g}/10^5$ cells, compounds **4a** and **4f** revealed little antiviral activity.

For the antiviral activity against *Herpes Simplex* virus-1 (HSV-1) the results revealed that compounds **4d–4f** showed the highest effect on HSV-1 at concentration $10\ \mu\text{g}/10^5$ cells, while compounds **4a–4c**, **4g**, **4h**, and **7** showed moderate activity.

Conclusions

Some of *C*-glycosyl amino acid esters were prepared and tested for antiviral activity against Hepatitis-A virus (HAV, MBB-cell culture adapted strain) and *Herpes Simplex* virus type-1 (HSV-1).

Experimental

Melting points were determined using a *Kofler* block instrument. TLC was performed on plastic plates Silica Gel 60 F₂₅₄ (E. Merck, layer thickness 0.2 mm). The detection was achieved by treatment with a solution of 15% H₂SO₄ in methanol, and heating at 150°C. NMR spectra were recorded on a Bruker AC 250 FT NMR spectrometer at 250 MHz for ¹H NMR and 62.9 MHz for ¹³C NMR with TMS as an internal standard. MALDI-MS were measured with a KRATOS Analytical Compact, using 2,5-dihydroxybenzoic acid (DHB) as matrix. The (M + Na)⁺ and (M + K)⁺ ions were peak matched using ions derived from the 2,5-dihydroxybenzoic acid matrix. The microanalyses were performed at the microanalytical unit, Universität Konstanz, Germany, and were found to agree favourably with the calculated values. Viral screening against HAV and HSV was conducted at the Environmental Virology Lab., Department of Water Pollution Research, National Research Centre, Cairo, Egypt.

General Procedure for Preparation of Methyl 2,3-O-isopropyliden-β-D-ribofuranoside Bearing Amino Acid Esters **3a–3h**

A solution of **2a–2h** (5 mmol) in 20 cm³ MeCN was cooled to –5°C. 1.10 g **1** (5 mmol), 0.70 g HOBt (5 mmol), and 1.03 g DCC (5 mmol) were added successively. The reaction mixture was stirred at 0–5°C for 2 h, then at room temperature for 16 h. Dicyclohexylurea (DCU) was filtered off and the filtrate was evaporated to dryness. The residue was dissolved in 30 cm³ ethyl acetate and extracted successively with 10 cm³ brine, 10 cm³ 5% NaHCO₃ solution, 10 cm³ 1 N HCl, followed by 10 cm³ brine, and finally with 10 cm³ H₂O. The organic layer was dried (Na₂SO₄), filtered, and evaporated to dryness. The residue was purified by silica gel column chromatography using 3% MeOH in CH₂Cl₂.

Methyl 2-(methyl 2,3-O-isopropyliden-β-D-ribo-1,4-furanosyl carbonyl)-aminoacetate (**3a**, C₁₂H₁₉NO₇)

Colorless oil (1.05 g, 73%); *R*_f = 0.26 (petroleum ether/ethylacetate, 2/1); ¹H NMR (CDCl₃, 250 MHz): δ = 1.23, 1.39 (2s, 2CH₃), 3.31, 3.58 (2s, 2OCH₃), 3.90 (dd, *J* = 5.4, 10.5 Hz, CH₂), 4.36 (d, *J* = 6.0 Hz, H-3), 4.43 (d, *J* = 6.0 Hz, H-2), 4.90 (d, *J* = 6.5 Hz, H-4), 4.99 (s, H-1) ppm; ¹³C NMR (CDCl₃, 62.5 MHz): δ = 25.2, 26.7 (2CH₃), 41.3 (CH₂), 52.5, 56.6 (2OCH₃), 82.6 (C-4), 84.7 (C-3), 86.4 (C-2), 86.6 (C-1), 112.8 (CMe₂), 170.2, 170.7 (2C=O) ppm; MS (MALDI, positive mode, Matrix: DHB): *m/z* (%) = 312 [(M + Na)⁺, 19], 328 [(M + K)⁺, 37].

Methyl 2-(methyl 2,3-O-isopropyliden-β-D-ribo-1,4-furanosyl carbonyl)-aminopropanoate (**3b**, C₁₃H₂₁NO₇)

White powder (0.91 g, 74%); *R*_f = 0.43 (petroleum ether/ethylacetate, 2/1); mp 46–48°C; ¹H NMR (CDCl₃, 250 MHz): δ = 1.14 (s, CH₃), 1.24 (d, *J* = 7.2 Hz, CH₃), 1.30 (s, CH₃), 3.31, 3.58 (2s, 2OCH₃), 4.37 (d, *J* = 5.9 Hz, H-3), 4.40 (m, CH), 4.44 (d, *J* = 5.9 Hz, H-2), 4.91 (d, *J* = 6.5 Hz, H-4), 4.96 (s, H-1), 7.05 (d, *J* = 7.5 Hz, NH) ppm; ¹³C NMR (CDCl₃, 62.5 MHz): δ = 18.1 (CH₃), 24.7, 25.9 (2CH₃), 47.7

(CH), 52.2, 56.2 (2OCH₃), 82.2 (C-4), 84.4 (C-3), 86.0 (C-2), 86.1 (C-1), 112.4 (CMe₂), 169.4, 172.8 (2C=O) ppm; MS (MALDI, positive mode, Matrix: DHB): *m/z* (%) = 342 [(M + K)⁺, 56].

Methyl 2-(methyl 2,3-O-isopropyliden-β-D-ribo-1,4-furanosyl carbonyl)-amino-2-phenylacetate (**3c**, C₁₈H₂₃NO₇)

White powder (1.30 g, 72%); *R*_f = 0.53 (petroleum ether/ethylacetate, 2/1); mp 83–85°C; ¹H NMR (CDCl₃, 250 MHz): δ = 1.22, 1.38 (2s, 2CH₃), 3.41, 3.61 (2s, 2OCH₃), 4.53 (d, *J* = 6.0 Hz, H-3), 4.55 (d, *J* = 6.0 Hz, H-2), 4.99 (d, *J* = 6.5 Hz, H-4), 5.03 (s, H-1), 5.45 (d, *J* = 7.2 Hz, CH), 7.26 (m, Ph-H), 7.68 (d, *J* = 7.3 Hz, NH) ppm; ¹³C NMR (CDCl₃, 62.5 MHz): δ = 24.9, 26.4 (2CH₃), 34.8 (CH), 52.6, 56.2 (2OCH₃), 82.6 (C-4), 84.5 (C-3), 86.6 (C-2), 86.7 (C-1), 112.6 (CMe₂), 127.1, 128.5, 128.9, 136.1 (Ph-C), 169.6, 170.8 (2C=O) ppm; MS (MALDI, positive mode, Matrix: DHB): *m/z* (%) = 388 [(M + Na)⁺, 78], 404 [(M + K)⁺, 45].

Methyl 2-(methyl 2,3-O-isopropyliden-β-D-ribo-1,4-furanosyl carbonyl)-amino-4-methylthiobutanoate (**3d**, C₁₅H₂₅NO₇S)

Colorless oil (1.3 g, 72%); *R*_f = 0.40 (petroleum ether/ethylacetate, 2/1); ¹H NMR (CDCl₃, 250 MHz): δ = 1.23, 1.39 (2s, 2CH₃), 1.88 (m, CH₂), 2.51 (s, SCH₃), 2.40 (m, CH₂), 3.41, 3.67 (2s, 2OCH₃), 4.45 (d, *J* = 5.9 Hz, H-3), 4.54 (d, *J* = 5.9 Hz, H-2), 4.58 (m, CH), 4.87 (m, H-4), 5.00 (s, H-1), 7.15 (d, *J* = 7.9 Hz, NH) ppm; ¹³C NMR (CDCl₃, 62.5 MHz): δ = 15.2 (SCH₃), 24.8 (CH₂), 26.3, 29.3 (2CH₃), 31.8 (CH₂), 51.2 (CH), 52.4, 56.4 (2OCH₃), 82.6 (C-4), 84.3 (C-3), 86.3 (C-2), 86.4 (C-1), 112.6 (CMe₂), 169.6, 171.8 (2C=O) ppm; MS (MALDI, positive mode, Matrix: DHB): *m/z* (%) = 386 [(M + Na)⁺, 78], 402 [(M + K)⁺, 34].

Methyl 2-(methyl 2,3-O-isopropyliden-β-D-ribo-1,4-furanosyl carbonyl)-amino-4-methylpentanoate (**3e**, C₁₆H₂₇NO₇)

Colorless oil (1.2 g, 69%); *R*_f = 0.66 (petroleum ether/ethylacetate, 2/1); ¹H NMR (CDCl₃, 250 MHz): δ = 0.89 (m, 2CH₃), 1.26, 1.43 (2s, 2CH₃), 1.54 (m, CH, CH₂), 3.42, 3.68 (2s, 2OCH₃), 4.12 (m, CH), 4.48 (d, *J* = 6.0 Hz, H-3), 4.58 (d, *J* = 6.0 Hz, H-2), 5.01 (d, *J* = 6.5 Hz, H-4), 5.10 (s, H-1), 6.94 (d, *J* = 8.5 Hz, NH) ppm; ¹³C NMR (CDCl₃, 62.5 MHz): δ = 21.9, 22.6 (2CH₃), 24.5, 24.8 (2CH₃), 26.3 (CH₂), 34.8, 41.8 (2CH), 50.4, 56.3 (2OCH₃), 82.6 (C-4), 84.4 (C-3), 86.4 (C-2), 86.5 (C-1), 112.6 (CMe₂), 169.6, 172.8 (2C=O) ppm; MS (MALDI, positive mode, Matrix: DHB): *m/z* (%) = 368 [(M + Na)⁺, 88].

Methyl 2-(methyl 2,3-O-isopropyliden-β-D-ribo-1,4-furanosyl carbonyl)-amino-3-methylbutanoate (**3f**, C₁₅H₂₅NO₇)

Colorless oil (1.4 g, 67%); *R*_f = 0.63 (petroleum ether/ethylacetate, 2/1); ¹H NMR (CDCl₃, 250 MHz): δ = 0.80 (d, 2CH₃), 1.22, 1.39 (2s, 2CH₃), 2.08 (m, CH), 3.43, 3.65 (2s, 2OCH₃), 4.47 (m, CH, H-3), 4.55 (d, *J* = 5.9 Hz, H-2), 4.92 (m, H-4), 5.03 (s, H-1), 7.11 (br, s, NH) ppm; ¹³C NMR (CDCl₃,

62.5 MHz): δ = 17.4, 18.5 (2CH₃), 25.2, 26.3 (2CH₃), 31.3, 34.7 (2CH), 51.8, 56.7 (2OCH₃), 83.0 (C-4), 84.4 (C-3), 86.7 (C-2), 86.8 (C-1), 112.6 (CMe₂), 169.6, 171.6 (2C=O) ppm; MS (MALDI, positive mode, Matrix: DHB): m/z (%) = 354 [(M + Na)⁺, 66], 370 [(M + K)⁺, 75].

Benzyl 2-(methyl 2,3-O-isopropylidene- β -D-ribo-1,4-furanosyl carbonyl)-amino-3-hydroxypropanoate (3g, C₁₉H₂₅NO₈)

Colorless oil (0.26 g, 65%); R_f = 0.17 (petroleum ether/ethylacetate, 2/1); ¹H NMR (CDCl₃, 250 MHz): δ = 1.23, 1.39 (2s, 2CH₃), 3.31 (s, OCH₃), 3.82 (dd, J = 3.3, 11.2 Hz, CH₂OH), 3.92 (dd, J = 7.8, 11.2 Hz, CH₂OH), 4.20 (br, s, OH), 4.46 (d, J = 6.0 Hz, H-3), 4.57 (m, CH, H-2), 5.00 (d, J = 6.5 Hz, H-4), 5.06 (s, H-1), 5.12 (m, CH₂Ph), 7.26 (m, Ph-H), 7.46 (d, J = 7.7 Hz, NH) ppm; ¹³C NMR (CDCl₃, 62.5 MHz): δ = 24.8, 26.3 (2CH₃), 34.8 (CH₂), 54.6 (CH), 56.3 (OCH₃), 67.3 (CH₂), 82.3 (C-4), 84.3 (C-3), 86.1 (C-2), 86.8 (C-1), 112.6 (CMe₂), 127.9, 128.3, 128.5, 128.6, 135.1 (Ph-C), 169.8, 170.4 (2C=O) ppm; MS (MALDI, positive mode, Matrix: DHB): m/z (%) = 434 [(M + K)⁺, 70].

Benzyl 1-(methyl 2,3-O-isopropylidene- β -D-ribo-1,4-furanosyl carbonyl)-amino-2-pyrrolidinecarboxylate (3h, C₂₁H₂₇NO₇)

Colorless oil (1.10 g, 55%); R_f = 0.17 (petroleum ether/ethylacetate, 2/1); ¹H NMR (CDCl₃, 250 MHz): δ = 1.25, 1.43 (2s, 2CH₃), 1.90 (m, 2CH₂), 3.22 (s, OCH₃), 3.61–3.90 (m, CH₂), 4.42 (m, CH), 4.55 (d, J = 5.9 Hz, H-3), 4.70 (d, J = 5.9 Hz, H-2), 4.93 (m, H-4), 5.10 (s, H-1), 5.15 (m, CH₂Ph), 7.28 (m, Ph-H) ppm; ¹³C NMR (CDCl₃, 62.5 MHz): δ = 24.3, 24.6 (2CH₃), 26.1, 28.6, 46.7 (CH₂), 54.8 (CH), 55.4 (CH₂), 58.8 (OCH₃), 66.4 (CH₂), 80.6 (C-4), 83.3 (C-3), 83.4 (C-2), 109.6 (C-1), 111.8 (CMe₂), 127.7, 127.9, 128.0, 128.2, 135.3 (Ph-C), 168.2, 171.5 (2C=O) ppm; MS (MALDI, positive mode, Matrix: DHB): m/z (%) = 444 [(M + K)⁺, 76].

General Procedure for Preparation of Methyl β -D-Ribofuranoside Bearing Amino Acid Esters 4a–4h

Compounds **3a–3h** (3 mmol) were dissolved in 5 cm³ 70% AcOH and heated under reflux for 2 h. The solvent was evaporated under reduced pressure and the residue was coevaporated two times with 3 cm³ H₂O and two times with 3 cm³ ethanol. The residual oil was purified by silica gel column chromatography using 10% MeOH in CHCl₃ to give **4a–4h** in 75–80% yields.

Methyl 2-(methyl β -D-ribo-1,4-furanosyl carbonyl)-aminoacetate (4a, C₉H₁₅NO₇)

White foam (0.60 g, 80%); R_f = 0.20 (petroleum ether/ethylacetate, 1/1); ¹H NMR (CDCl₃, 250 MHz): δ = 3.41, 3.68 (2s, 2OCH₃), 3.94 (dd, J = 5.4, 10.5 Hz, CH₂), 4.16 (m, H-3), 4.33 (m, H-2), 4.66 (m, H-4), 4.90 (br, s, 2OH), 5.01 (s, H-1) ppm; MS (MALDI, positive mode, Matrix: DHB): m/z (%) = 272 [(M + Na)⁺, 55], 288 [(M + K)⁺, 25].

Methyl 2-(methyl β -D-ribofuranosyl carbonyl)-aminopropanoate (4b, C₁₀H₁₇NO₇)

White foam (0.64 g, 81%); R_f = 0.33 (petroleum ether/ethylacetate, 1/1); ¹H NMR (CDCl₃, 250 MHz): δ = 1.22 (d,

J = 7.2 Hz, CH₃), 3.41, 3.66 (2s, 2OCH₃), 4.17–4.26 (m, CH, H-3), 4.34 (m, H-2), 4.70 (m, H-4), 4.90 (s, H-1), 7.00 (br, s, NH) ppm; MS (MALDI, positive mode, Matrix: DHB): m/z (%) = 286 [(M + Na)⁺, 35], 302 [(M + K)⁺, 19].

Methyl 2-(methyl β -D-ribo-1,4-furanosyl carbonyl)-amino-2-phenylacetate (4c, C₁₅H₁₉NO₇)

White foam (0.78 g, 80%); R_f = 0.42 (petroleum ether/ethylacetate, 1/1); ¹H NMR (CDCl₃, 250 MHz): 3.42, 3.71 (2s, 2OCH₃), 4.23 (d, J = 6.0 Hz, H-3), 4.40 (d, J = 6.0 Hz, H-2), 4.59 (d, J = 6.5 Hz, H-4), 4.95 (br, s, 2OH), 5.01 (s, H-1), 5.45 (d, J = 7.2 Hz, CH), 7.26 (m, Ph-H), 7.60 (br, s, NH) ppm; MS (MALDI, positive mode, Matrix: DHB): m/z (%) = 348 [(M + Na)⁺, 75], 364 [(M + K)⁺, 28].

Methyl 2-(methyl β -D-ribo-1,4-furanosyl carbonyl)-amino-4-methylthiobutanoate (4d, C₁₂H₂₁NO₇S)

White foam (0.79 g, 82%); R_f = 0.32 (petroleum ether/ethylacetate, 1/1); ¹H NMR (CDCl₃, 250 MHz): δ = 1.90–2.22 (m, CH₂), 2.46–2.57 (m, SCH₃, CH₂), 3.41, 3.65 (2s, 2OCH₃), 4.15 (m, H-3), 4.34 (m, H-2), 4.60 (br, s, 2OH), 4.70–4.80 (m, CH, H-4), 5.02 (s, H-1), 7.10 (br, s, NH) ppm; MS (MALDI, positive mode, Matrix: DHB): m/z (%) = 346 [(M + Na)⁺, 80], 362 [(M + K)⁺, 41].

Methyl 2-(methyl β -D-ribo-1,4-furanosyl carbonyl)-amino-4-methylpentanoate (4e, C₁₃H₂₃NO₇)

White foam (0.77 g, 84%); R_f = 0.45 (petroleum ether/ethylacetate, 1/1); ¹H NMR (CDCl₃, 250 MHz): δ = 0.87 (m, 2CH₃), 1.45–1.70 (m, CH, CH₂), 3.42, 3.63 (2s, 2OCH₃), 4.00 (m, CH), 4.18 (d, J = 6.0 Hz, H-3), 4.38 (d, J = 6.0 Hz, H-2), 4.88 (br, s, 2OH), 5.01 (m, H-4), 5.08 (s, H-1), 6.99 (br, s, NH) ppm; MS (MALDI, positive mode, Matrix: DHB): m/z (%) = 328 [(M + Na)⁺, 65], 344 [(M + K)⁺, 15].

Methyl 2-(methyl β -D-ribo-1,4-furanosyl carbonyl)-amino-3-methylbutanoate (4f, C₁₂H₂₁NO₇)

White foam (0.72 g, 83%); R_f = 0.47 (petroleum ether/ethylacetate, 1/1); ¹H NMR (CDCl₃, 250 MHz): δ = 0.79 (d, 2CH₃), 2.18 (m, CH), 3.43, 3.69 (2s, 2OCH₃), 4.17–4.38 (m, CH, H-2, H-3), 4.90 (m, H-4), 4.97 (br, s, 2OH), 5.06 (s, H-1), 7.05 (br, s, NH) ppm; MS (MALDI, positive mode, Matrix: DHB): m/z (%) = 314 [(M + Na)⁺, 77], 330 [(M + K)⁺, 35].

Benzyl 2-(methyl β -D-ribo-1,4-furanosyl carbonyl)-amino-3-hydroxypropanoate (4g, C₁₆H₂₁NO₈)

White powder (0.87 g, 82%); mp 109–110°C; R_f = 0.13 (petroleum ether/ethylacetate, 1/1); ¹H NMR (CDCl₃, 250 MHz): δ = 3.39–3.51 (m, OCH₃, CH₂OH), 4.25–4.59 (m, H-2, H-3, CH, OH), 4.89 (br, s, 2OH), 4.95 (m, H-4), 5.09 (s, H-1), 5.14 (m, CH₂Ph), 7.33 (m, Ph-H), 7.31 (br, s, NH) ppm; MS (MALDI, positive mode, Matrix: DHB): m/z (%) = 378 [(M + Na)⁺, 66], 394 [(M + K)⁺, 29].

Benzyl 1-(methyl β -D-ribo-1,4-furanosyl carbonyl)-amino-2-pyrrolidinecarboxylate (4h, C₁₈H₂₃NO₇)

White powder (0.93 g, 85%); mp 123–125°C; R_f = 0.15 (petroleum ether/ethylacetate, 1/1); ¹H NMR (CDCl₃, 250 MHz):

$\delta = 1.70\text{--}2.03$ (m, 2CH_2), $3.42\text{--}3.58$ (m, OCH_3 , CH_2), $4.20\text{--}4.45$ (m, H-2, H-3, CH), 4.80 (br, s, 2OH), 4.98 (m, H-4), 5.07 (s, H-1), 5.17 (m, CH_2Ph), 7.34 (m, Ph-H) ppm; MS (MALDI, positive mode, Matrix: DHB): m/z (%) = 388 [(M+Na)⁺, 74], 404 [(M+K)⁺, 45].

2-(Methyl 2,3-O-isopropyliden- β -D-ribo-1,4-furanosylcarbonyl)-aminoacetic acid (5, C₁₁H₁₇NO₇)

A mixture of 0.54 g **3a** (1.87 mmol), 10 cm³ MeOH, and 5 cm³ $2N$ KOH was stirred for 3 h. The reaction mixture was acidified with HCl and evaporated *in vacuo*. The residue was purified by silica gel column chromatography (petroleum ether/ethylacetate, $2/1$) to give a white foam (0.40 g, 78%); $R_f = 0.21$ (petroleum ether/ethylacetate, $1/1$); ¹H NMR (CDCl₃, 250 MHz): $\delta = 1.18$, 1.24 (2s, 2CH_3), 3.31 (s, OCH_3), 3.82 (m, CH_2), 4.32 (d, $J = 6.2$ Hz, H-3), 4.48 (s, H-4), 5.14 (s, H-1), 5.25 (d, $J = 6.2$ Hz, H-2) 11.32 (br, s, OH) ppm; ¹³C NMR (CDCl₃, 62.5 MHz): $\delta = 25.2$, 26.8 (2CH_3), 38.2 (CH_2), 52.2 (OCH_3), 83.0 (C-4), 84.3 (C-3), 86.8 (C-2), 87.1 (C-1), 112.6 (CMe_2), 175.6 (C=O) ppm; MS (MALDI, positive mode, Matrix: DHB): m/z (%) = 314 [(M+K)⁺, 66].

Methyl 2-(methyl 2,3-O-isopropyliden- β -D-ribo-1,4-furanosylcarbonyl)-(aminoacetyl)aminoacetate (6, C₁₄H₂₂N₂O₈)

A solution of 0.63 g **2a** (5 mmol) in 20 cm³ MeCN was cooled to -5°C . 1.10 g **1** (5 mmol), 0.70 g HOBt (5 mmol), and 1.03 g DCC (5 mmol) were added successively. The reaction mixture was stirred at $0\text{--}5^\circ\text{C}$ for 2 h, and at room temperature for 16 h. Dicyclohexylurea (DCU) was filtered off and the filtrate was evaporated to dryness. The residue was dissolved in 30 cm³ ethyl acetate and extracted successively with 10 cm³ brine, 10 cm³ 5% NaHCO₃ solution, 10 cm³ $1N$ HCl, followed by 10 cm³ brine, and finally with 10 cm³ H₂O. The organic layer was dried (Na₂SO₄), filtered, and evaporated to dryness. The residue was purified by silica gel column chromatography using 3% MeOH in CH₂Cl₂ to give a colorless oil (0.50 g, 66%); $R_f = 0.30$ (petroleum ether/ethylacetate, $2/1$); ¹H NMR (CDCl₃, 250 MHz): $\delta = 1.15$, 1.34 (2s, 2CH_3), 3.40 , 3.63 (2s, 2OCH_3), 3.94 (m, 2CH_2), 4.45 (d, $J = 6.2$ Hz, H-3), 4.51 (d, $J = 6.2$ Hz, H-2), 4.93 (m, H-4), 5.04 (s, H-1), 7.10 (br, s, NH), 7.25 (br, s, NH) ppm; ¹³C NMR (CDCl₃, 62.5 MHz): $\delta = 25.0$, 26.5 (2CH_3), 41.1 , 42.6 (2CH_2), 52.3 , 56.5 (2OCH_3), 82.5 (C-4), 84.4 (C-3), 86.2 (C-2), 86.4 (C-1), 112.7 (CMe_2), 169.9 , 170.1 , 170.8 ($3\text{C}=\text{O}$) ppm; MS (MALDI, positive mode, Matrix: DHB): m/z (%) = 369 [(M+Na)⁺, 22], 385 [(M+K)⁺, 45].

Methyl 2-(methyl β -D-ribo-1,4-furanosylcarbonyl)-(aminoacetyl)aminoacetate (7, C₁₁H₁₈N₂O₈)

Compound **6** (2 mmol, 0.69 g) was dissolved in 3 cm³ 70% AcOH and heated under reflux for 2 h. The solvent was evaporated under reduced pressure and the residue was coevaporated two times with 2 cm³ H₂O and two times with 2 cm³ ethanol. The residual oil was purified by silica gel column chromatography using 10% MeOH in CHCl₃ to give 0.51 g **7** as white foam in 84% yield. $R_f = 0.28$ (petroleum ether/

ethylacetate, $1/1$); ¹H NMR (CDCl₃, 250 MHz): $\delta = 3.41$, 3.67 (2s, 2OCH_3), $3.74\text{--}3.86$ (m, 2CH_2), 4.15 (m, H-3), 4.32 (m, H-2), 4.82 (br, s, 2OH), 4.98 (m, H-4), 5.08 (s, H-1), 7.00 (br, s, NH), 7.29 (br, s, NH) ppm; MS (MALDI, positive mode, Matrix: DHB): m/z (%) = 329 [(M+Na)⁺, 65], 345 [(M+K)⁺, 15].

References

- [1] Part III: Al-Masoudi NA, Al-Masoudi IA, Ali IAI, Al-Soud YA, Saeed B, La Colla P (2006) Heteroatom Chem **16**: 576
- [2] Burda P, Aebi M (1999) Biochem Biophys Acta **1426**: 239; Dwek RA (1996) Chem Rev **96**: 683; Dwek RA (1995) Science **269**: 1234; Varki A (1993) Glycobiology **3**: 97
- [3] Kobata A (1993) Acc Chem Res **26**: 319; Feizi T (1985) Nature **314**: 53
- [4] Van Dijk W, Turner GA, Mackiewicz A (1994) Glycosyl Dis **1**: 5; Van Dijk W, Van der Stelt ME, Salera A, Dente L (1991) **55**: 143; Wang Z-G, Warren JD, Dudkin VY, Zhang X, Iserloh U, Visser M, Eckhardt M, Seeberger PH, Danishefsky SJ (2006) Tetrahedron **62**: 4954
- [5] Dondoni A, Marra A (2000) Chem Rev **100**: 4395
- [6] Axon JR, Beckwith ALJ (1995) J Chem Soc Chem Commun 549; Kessler H, Wittmann V, Köck M, Kottenhahn M (1992) Angew Chem Int Ed Engl **31**: 902; McNabb SB, Ueda M, Naito T (2004) Org Lett **6**: 1911; Brown P, Eggleston DS, Haltiwanger RC, Jarvest RL, Mensah L, O'Hanlon PJ, Pope AJ (2001) Bioorg Med Chem Lett **11**: 711; Readman SK, Marsden SP, Hodgson A (2000) Synlett 1628; Simchen G, Pürkner (1990) Synthesis 525
- [7] Parrish JD, Little RD (2002) Org Lett **4**: 1439; Colombo L, di Giacomo M, Ciceri P (2002) Tetrahedron **58**: 9381; Colombo L, Casiraghi G, Pittalis A (1991) J Org Chem **56**: 3897
- [8] Gurjar MK, Mainkar AS, Syamala M (1993) Tetrahedron: Asymmetry **4**: 2343; Vincent SP, Schleyer A, Wong C-H (2000) J Org Chem **65**: 4440
- [9] Dondoni A, Junquera F, Merchán FL, Merino P, Scherrmann M-C, Tejero T (1997) J Org Chem **62**: 5484
- [10] Schweizer F, Inazu T (2001) Org Lett **3**: 4115; Gustafsson T, Saxin M, Kihlberg J (2003) J Org Chem **68**: 2506
- [11] Gobbo M, Biondi L, Filira F, Gennaro R, Benincasa M, Scolaro B, Rocchi R (2002) J Med Chem **45**: 4494; Locardi E, Stöckle M, Gruner S, Kessler H (2001) J Am Chem Soc **123**: 8189; McGarvey GJ, Benedum TE, Schmidtman FW (2002) Org Lett **4**: 3591; Liu S, Ben RN (2005) Org Lett **7**: 2385; Lundquist JJ, Debenham SD, Toone EJ (2000) J Org Chem **65**: 8245; Inaba Y, Yano S, Mikata Y (2007) Tetrahedron Lett **48**: 993
- [12] Ali IAI, Al-Masoudi IA, Saeed B, Al-Masoudi NA, La Colla P (2005) Heteroatom Chem **16**: 148; Al-Masoudi NA, Al-Masoudi IA, Ali IAI, Al-Soud YA, Saeed B, La Colla P (2006) Heteroatom Chem **16**: 576; Al-Masoudi NA, Al-Masoudi IA, Ali IAI, Al-Soud YA, Saeed B, La Colla P (2006) Acta Pharm **56**: 175

- [13] Reist EJ, Spencer RR, Wain ME, Junga IG, Goodman L, Baker BR (1961) *J Org Chem* **26**: 2821; Baker R, Fletcher HG (1961) *J Org Chem* **26**: 4605; Montgomery JA, Hewson K (1964) *J Org Chem* **29**: 3436; Abdel-Rahman AA-H, Abdel-Megied AE-S, Goda AE-S, Zeid IF, El Ashry ESH (2003) *Nucleos Nucleot Nucl* **22**: 2027
- [14] Schmidt RR, Heermann D, Jung K-H (1974) *Liebigs Ann Chem* 1856
- [15] Asagarasu A, Uchiyama T, Achiwa K (1986) *Chem Pharm Bull* **46**: 697
- [16] Davis S, Mohammed AK (1981) *J Chem Soc Perkin Trans* **1**: 2982; König W, Geiger R (1970) *Chem Ber* **103**: 788
- [17] Pennigton RM, Fischer RR (1981) *J Biol Chem* **256**: 8963
- [18] Katritzky A, Suzuki K, Singh SK (2004) *ARKIVOC* 12
- [19] Farag RS, Shalaby AS, El-Baroty GA, Ibrahim NA (2004) *Phytother Res* **18**: 30