Synthesis of acylated nucleosides and ribonic-1,4-lactones as inhibitors of trypanosomal glyceraldehyde-3-phosphate dehydrogenase (gGAPDH)

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Dedicated to Professor Al Padwa on the occasion of his 65th birthday, with regards and best wishes

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Abstract

The synthesis of a representative group of substituted nucleosides and ribonic-1,4-lactones as potential inhibitors of trypanosomal enzyme gGAPDH is described. The novel 5-acylribonic-1,4-lactones were easily obtained from ribonic lactone by a three-step procedure involving protection-deprotection protocols, in good overall yields. All compounds synthesized were subjected to inhibitory assay against gGAPDH, the most active being those carrying aromatic groups attached to the ribose ring. Other structural features responsible for the observed activities are also discussed.

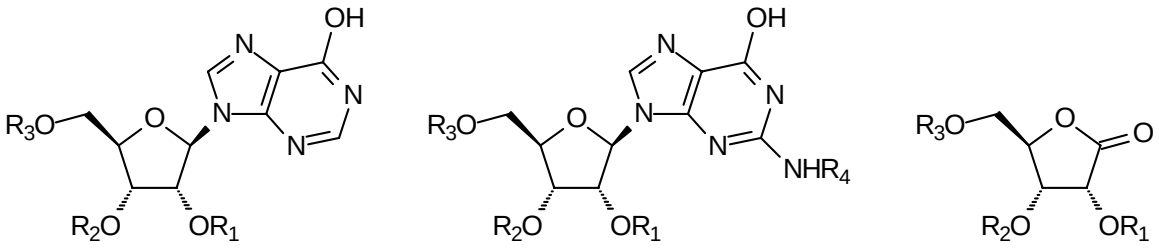
Keywords: Acyl nucleosides, ribonic lactones, 5-acylribonic-1,4-lactones, gGAPDH inhibition

Introduction

Chagas' disease is an infection caused by the protozoan *Trypanosoma cruzi* and represents a serious health problem in the American continent.[1,2] It is endemic in more than 20 countries, with 16–18 million people infected and 100 million at risk. *T. cruzi* is transmitted to humans by blood-sucking vector bugs or, more recently, by transfusion of infected blood, and no effective chemotherapy is currently available.[3]

One promising approach to combat the trypomastigote form of *T. cruzi* is through selective inhibition of enzymes or receptors that participate in important biochemical processes. Trypanosomal glycosomal glyceraldehyde-3-phosphate dehydrogenase (gGAPDH) has been selected as a potential target[4] in a structure-based drug design approach,[5] considering the

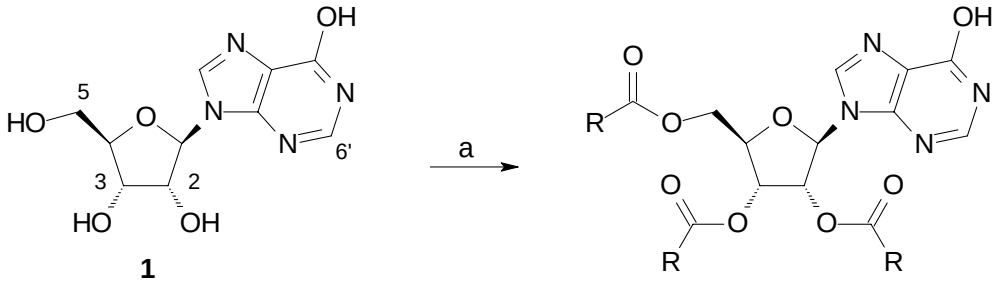
essential role of this enzyme in the metabolism of glucose by the parasite.[6] Moreover, *in silico* modeling of this pathway has indicated that the reaction catalyzed by gGAPDH is one of the preferred steps for inhibition.[7] In view of the structural differences with respect to the homologous human enzyme, the adenosine portion of the NAD^+ cofactor site in gGAPDH was mapped and chosen as the target for planning possible inhibitors against this enzyme.[5,8,9] Therefore, computational searching using the DOCK 3.5 program[10] identified a set of structures which represent potential candidates for gGAPDH inhibition. Amongst the ranked compounds, benzoyl-substituted nucleosides derived from inosine (I) and guanosine (II) were selected for chemical synthesis and biological evaluation. We report herein the preparation and inhibitory activity of the acylated nucleosides **I**, **II** and the related D-ribonic-1,4-lactones **III** as scaffolds for gGAPDH inhibitors. The development of a simple synthetic methodology for obtaining novel 5-acylribonic-1,4-lactones is also fully described.



R_1, R_2, R_3 = H, Acyl, Alkyl; R_4 = H, PhCO

Results and Discussion

A representative group of nucleosides was synthesized, starting from commercially available inosine **1** and guanosine **2**, in order to evaluate the effect of different substitution patterns at the 2-, 3- and 5-positions of the ribose ring and at the 6'-position of the purine moiety as well. The triacylated nucleosides **3**–**8** were easily obtained by treating inosine or guanosine with an excess of the respective acid chloride (or acetic anhydride) in pyridine as solvent, under mild heating (Schemes 1 and 2).[11,12] Higher temperatures promote N-benzoylation of guanosine and triacetyl guanosine, producing the peracylated compounds **9** and **10**, respectively, as previously reported (Scheme 2).[13,14]



1

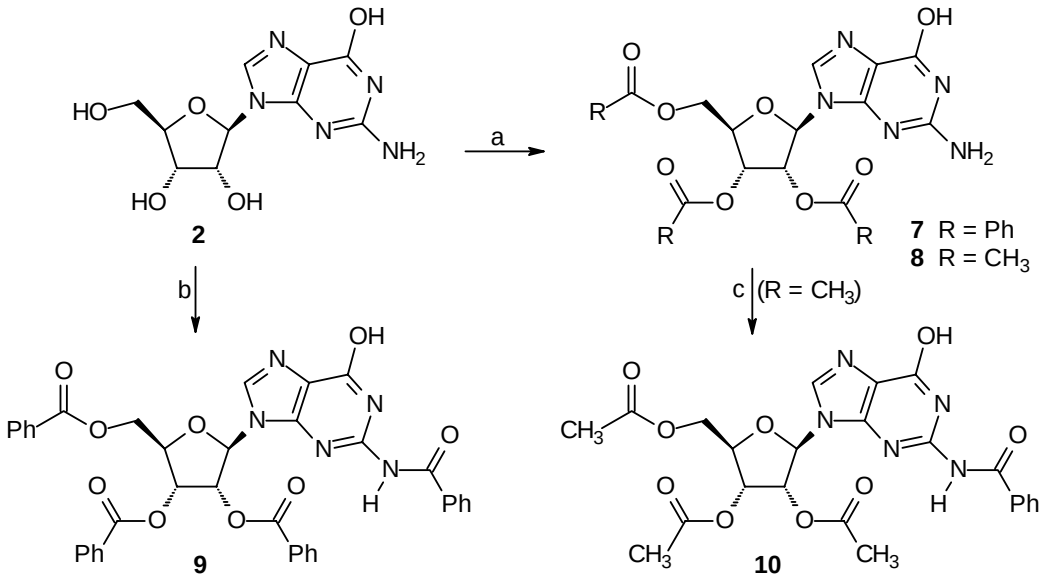
3 R = Ph
4 R = 4-ClPh
5 R = 3,5-$(NO_2)_2$Ph
6 R = CH_3

Conditions: a. ArCOCl, py, 60°C (for **3-5**: 85-98%) or Ac_2O, py, 90°C (for **6**: 95%).

Scheme 1

Selective 5-substituted nucleosides were readily obtained through protection–deprotection protocols using the 2,3-*O*-isopropylidene-inosine **11** as the common intermediate.[15] Benzoylation[15] of the 2,3-protected inosine **11** followed by acidic hydrolysis[16] of the isopropylidene group in **12** afforded 5-benzoylated inosine **13** in good overall yields (Scheme 3).

The carboxyl analogs **14–17** were obtained by firstly oxidizing the primary alcoholic function in **11** with CrO_3 in glacial acetic acid,[17] yielding the protected acid **14**, which was directly deprotected with aqueous HCl to give **15**, or esterified (via the acid chloride) to the isopropyl ester **16**. Subsequent deprotection furnished the dihydroxy ester **17** in reasonable yields (Scheme 3).



2

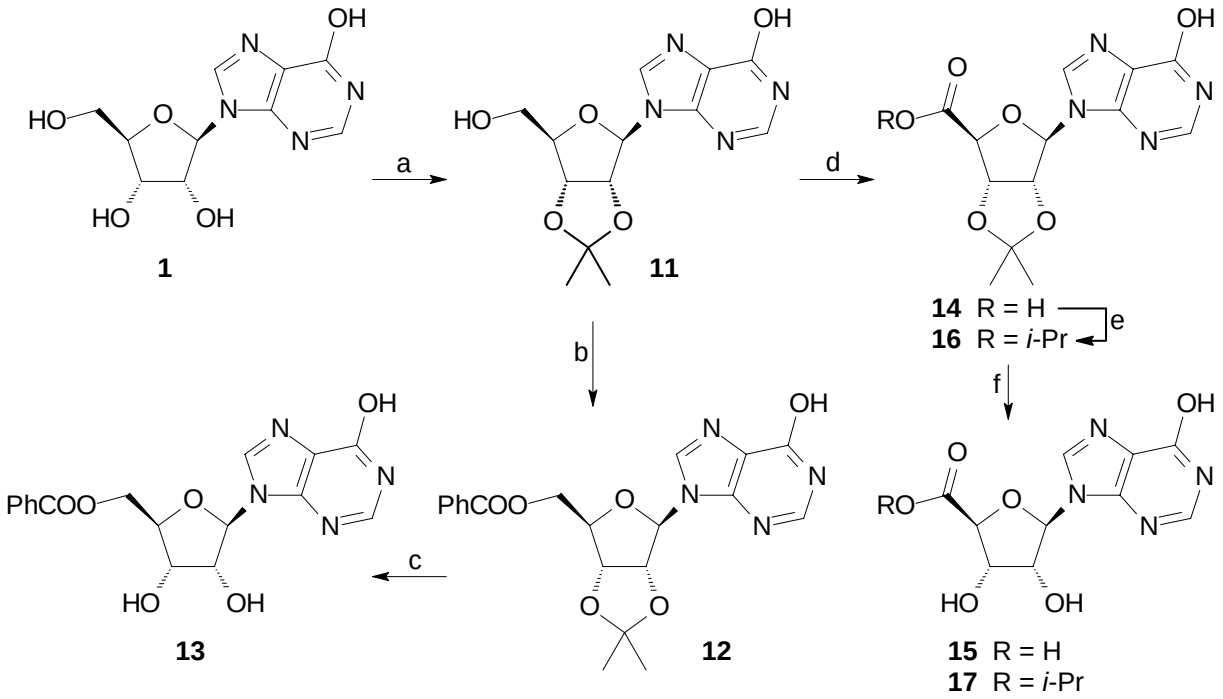
7 R = Ph
8 R = CH_3

9

10

Conditions: a. PhCOCl, py, 50-60°C (R = Ph: 75%) or Ac_2O, py, 80°C (R = CH_3: 85%); b. PhCOCl, py, 90°C (88%); c. PhCOCl, py, 75°C (86%).

Scheme 2

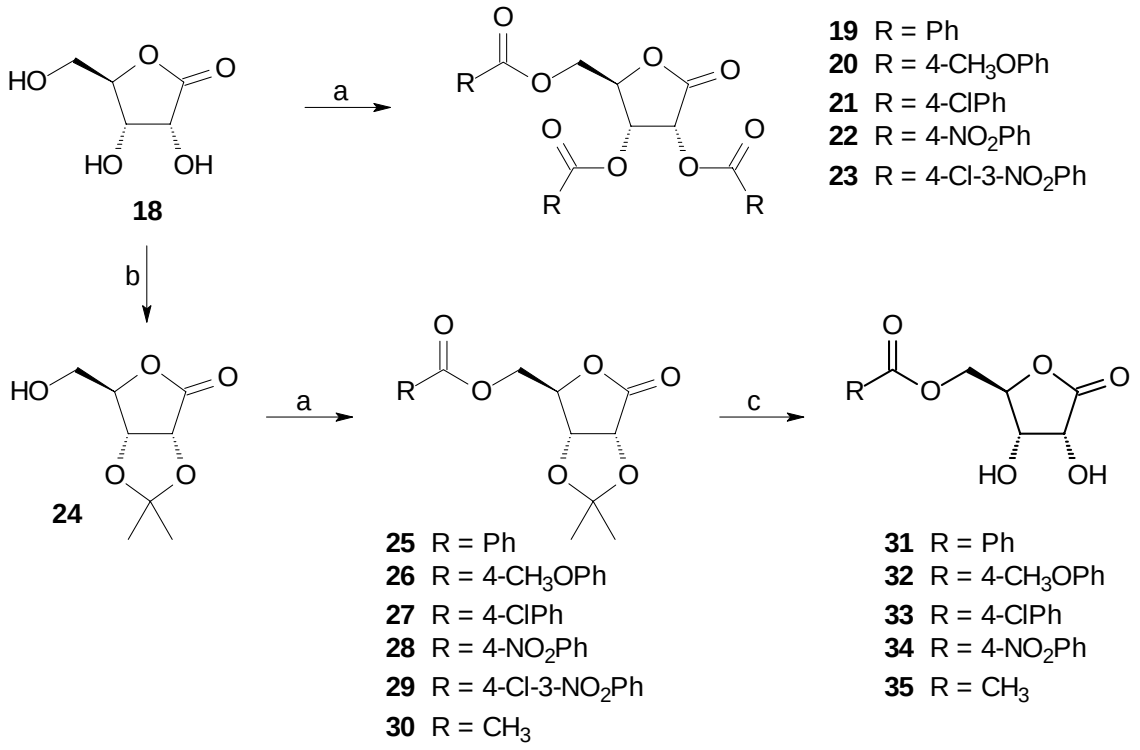


Conditions: a. *p*-TsOH, Me_2CO, rt (66%); b. PhCOCl, py, 60°C (78%); c. AcOH-H_2O (1:9), 90°C (68%); d. CrO_3, AcOH, rt (58%); e. $SOCl_2$, $(CH_3)_2CHOH$, rt (71%); f. 1M HCl, 80°C (70-75%).

Scheme 3

Carbohydrates are among the most versatile chiral pools employed in the synthesis of biologically important compounds.[18] Ribose analogs selectively substituted with acyl groups, and structurally related to the nucleosides prepared above, are good candidates for inhibitory assays as they provide a simple way to investigate the influence of a purine ring on the inhibitory activity. Moreover, carbohydrates and derivatives are usually fairly soluble in water, a required property not always found in synthetically modified nucleosides (*vide infra*). Therefore we chose the commercially accessible D-ribonic-1,4-lactone **18** as a simple scaffold for generating a representative group of acylated ribose derivatives. Ribonic-1,4-lactone **18** has been widely used as starting material for the synthesis of heterocycles and natural products.[19,20] Surprisingly, as far as we are concerned, no general method is available for the synthesis of selectively monoacylated ribonic-1,4-lactones.[21] Aside from the previously reported tribenzoyl lactone **19**,[22] no simple peracylated derivatives of ribonic-1,4-lactone are known. This fact stimulated a more detailed investigation concerning the development of simple methodologies for obtaining monoacylated and peracylated D-ribonic-1,4-lactones as synthetic targets.

A representative group of triacylribonic-1,4-lactones **19–23** was achieved by treating **18** with a four-fold excess of an acid chloride in pyridine, in excellent yields (Scheme 4). All peracylated compounds **19–23** are stable crystalline solids, easily purified by recrystallization from appropriate solvents (see Experimental Section).



Conditions: a. RCOCl, py, rt (for **19-23**: 82-95%; for **25-29**: 80-99%) or Ac_2O, py, rt (for **30**: 90%); b. Me_2CO, HCl, rt (88%); c. TFA-H_2O (1:1), 40°C or AcOH-H_2O (1:9), 90°C (54-99%).

Scheme 4

Next, we examined the preparation of a small collection of 5-acylated ribonic-1,4-lactones by a simple general method. While the more reactive primary hydroxyl group at the 5-position in **18** is selectively alkylated[23] or silylated[24] with trityl chloride or *t*-butyldimethylsilyl chloride, respectively, the initial approach for directly acylating **18** with 0.9 equiv. of 4-nitrobenzoyl chloride under mild conditions (20°C, 45 minutes) was unsuccessful. The triacyl derivative **22** was the only compound detectable in the ^{1}H-NMR of the crude reaction, being isolated in 30% yield. These results clearly indicated that a protection–deprotection protocol would be necessary to avoid acylation of both 2,3-positions. Therefore, a three-step procedure involving initial protection of the 2,3-diol- group in ribonic-1,4-lactone **18**, followed by 5-acylation and subsequent 2,3-deprotection should afford the required 5-acyl lactones.

2,3-Isopropylideneribonic-1,4-lactone **24** is a versatile compound extensively used as a synthetic building block.[19,20] The synthesis of **24** usually employs treatment of **18** with acetone in acidic media and has been thoroughly reported in the literature, although with many variations in the reaction conditions, work up and chemical yields.[21,25–29] We experienced difficulties in reproducing some of these results, and after some trial it was found that the isopropylidene lactone **24** could be produced cleanly on a multi-gram scale by first quenching the acidic reaction mixture with potassium carbonate, followed by filtering the suspension and washing the filtrate

with aqueous sodium bicarbonate. The product crystallized from ethanol in 88% yield as crystals with definite melting point (136–137°C).[26–29] 5-Acylation of the protected lactone **24** was carried out under standard conditions (ArCOCl, py), furnishing in high yields the expected 5-acyl-2,3-isopropylidene lactones **25–29** as crystalline solids. Acetylation of **24** with acetic anhydride and pyridine was effected similarly, to give the known[29] 5-acetyl derivative **30** in high yield (Scheme 4).

2,3-Diol deprotection of 5-acyl-2,3-isopropylidene lactones **25** and **28** to the respective 5-acyl-1,4-ribonic lactones was initially attempted with iodine in methanol under essentially neutral conditions,[30] but the aqueous work up required to remove the excess of reagents made the isolation of the expected 5-acylribonolactones difficult owing to their high solubility in water. Acidic hydrolysis employing volatile reagents, easily removed by reduced pressure to facilitate product isolation, were then tested with lactone **25**. While the use of 1*M* HCl at 70°C was inefficient owing to the low solubility of the starting protected lactone **25**, a complete deprotection of the 2,3-diol function could be achieved with a 10% acetic acid solution under heating,[16] giving the 5-benzoyl lactone **31** in moderate yield (Scheme 4). Milder conditions and good- to excellent yields were obtained when acetic acid was replaced by trifluoroacetic acid (TFA),[21] promoting the clean hydrolysis of the protected lactones **25–28** and **30** to the expected products **31–35**. However, it was not possible successfully to carry out the hydrolysis of protected lactone **29** owing to its low solubility in the reaction medium. Increasing the amount of TFA and raising the temperature resulted in partial decomposition of **29**, as indicated by the ^{1}H-NMR of the crude reaction mixture.

All the nucleosides and ribonic lactones prepared above were screened for their inhibitory activity against gGAPDH using an established protocol,[8] with the results presented in Tables 1 and 2. Determination of IC50 for most of the active compounds was precluded owing to their reduced solubility in the solvent system used in the assay (10% DMSO in Tris–HCl buffer, pH 8.6). While a quantitative evaluation was not possible at this time, some interesting remarks can be made from the data collected in Tables 1 and 2. Nucleosides **4**, **5** and **8**, fully substituted with aromatic groups at the 2-, 3-, and 5-positions, were the most active compounds, inhibiting 31–42% of gGAPDH at concentrations ranging from 125 to 165 μ*M* (Table 1). Moreover, the presence of polar groups (nitro and chloro) on the aromatic ring seems to increase the observed activity (compare the inhibitors **4**, **5** with inactive **3**, for example). An effect of substitution at the purine ring was also verified, with the tribenzoylguanosine **7** (containing a free amino group at the 6′-position) being one of the most active compounds, whereas the related N-benzoylated guanosine **9** and tribenzoylinosine **3** showed no activity. Compounds bearing only one benzene ring (5-acyl nucleosides **12** and **13**) were also inactive, as well as the somewhat more water-soluble carboxyl derivatives **14–17**.

Table 1. Inhibitory Activity of Acylinosine and Acylguanosine Derivatives[#]

Compound	R_1	R	Conc. (μ*M*)	Inhibition (%)
4	H	4-ClPh	145	31
5	H	3,5-$(NO_2)_2$Ph	165	42
8	NH_2	Ph	125	35

[#] Compounds considered inactive (*i.e.*, when the concentration required to inhibit gGAPDH to any extent was higher than 280 μ*M*) were omitted.

Table 2. Inhibitory Activity of Ribonic-1,4-lactones[#]

Compound	R	Conc. (μ*M*)	Inhibition (%)
20	4-CH_3OPh	255	50
22	4-NO_2Ph	115	18
23	4-Cl-3-NO_2Ph	230	23
28	4-NO_2Ph	325	4
29	4-Cl-3-NO_2Ph	215	20
34	4-NO_2Ph	335	8

[#] Compounds considered inactive (*i.e.*, when the concentration required to inhibit gGAPDH to any extent was higher than 280 μ*M*) were omitted.

Analogous results were obtained for the lactones **19–35**, the triaroyl derivatives containing polar groups attached to the aromatic nucleus (methoxy-, nitro- and chloro-) being the most active of the series (compare inhibitors **20**, **22** and **23** in Table 2 with inactive **19**, for example). Although less active than the triacyl nucleosides **4**, **5** and **8**, the inhibitory activity showed by the triacyl lactones **20**, **22** and **23** are of the same magnitude, 18–50% at concentrations ranging from 115 to 255 μ*M*. On the other hand, compounds substituted with a single aromatic group (5-acyl lactones **25–35**) were very poor inhibitors, with little, if any, activity. The exception was the 5-(4-chloro-3-nitrobenzoyl)-2,3-isopropylidene lactone, **29**, which showed moderate activity (Table 2).

Conclusions

A representative series of substituted nucleosides and ribonic lactones was prepared in good to excellent yields, using simple conditions and inexpensive reagents. Of particular interest was the synthesis of novel 5-acylribonic-1,4-lactones, easily obtained by a three-step procedure in 35–55% overall yields. Some of the synthesized nucleosides and ribonic lactones showed moderate inhibitory activity against gGAPDH. Triaroyl derivatives bearing polar groups attached to the aromatic nucleus were the most active, whereas monosubstituted compounds and those

substituted with small groups such as acetyl and isopropylidene were very poor inhibitors. Furthermore, a triacylnucleoside substituted with an amino group at the 6'-position of the purine ring showed enhanced activity. Overall, the triacyl nucleosides **4**, **5** and **8** were the most active compounds, although the inhibitory activity showed by the related triacyl lactones **20**, **22** and **23** were of comparable magnitude. These results point out the possibility for developing novel gGAPDH inhibitors using ribonic lactone derivatives as simpler structural scaffolds.

It is interesting to note that the most active compounds (nucleosides **4**, **5**, **8,** and lactones **20**, **22**, **23,** and **29**) showed, in general, limited solubility in water, while very poor inhibitors such as the carboxyl-containing nucleosides **14–17** and 5-acylribonic lactones **31–35** were highly water-soluble. Consequently, planning the next generation of potential gGAPDH inhibitors will have to take both properties into account, and detailed investigations will be reported in due course.

Experimental Section

General Procedures. All chemicals were of reagent grade and were used as received. Melting points are uncorrected. IR spectra were measured with a Perkin-Elmer FTIR-16 PC spectrophotometer using KBr for solids and film for liquid samples. ^{1}H-NMR (200 MHz) and ^{13}C-NMR (50 MHz) spectra were recorded with a Bruker AC-200F spectrometer using $CDCl_3$ or DMSO-d_6 as solvent and TMS as internal standard. Elemental analyses were performed with a CHN Perkin-Elmer 2400 by UFSC-Central Analítica, Departamento de Química, Florianópolis, SC, Brazil. Compounds **3**, **6**–**17**, and **30** were prepared according to the described methods[11–17,29]

2,3,5-*O*-Tri-(4-chlorobenzoyl)inosine (4). To a stirring suspension of 0.30 g of inosine (1.1 mmol) in 6 mL of anhydrous pyridine under N_2 at 25°C was added 0.6 mL of 4-chlorobenzoyl chloride (4.7 mmol). The resulting mixture was immediately immersed in a preheated oil bath at 55–60°C and heated for 3 hours. After cooling, the reaction was stirred at 25°C for an additional 18 hours. The excess of pyridine was distilled off, the residual oil was dissolved in CH_2Cl_2 and the organic phase was washed with 10% aqueous $NaHCO_3$ and with H_2O, dried with Na_2SO_4, filtered and concentrated to give a clear oil which solidified on standing. Recrystallization in dichloromethane–hexane afforded crystalline solid (84% yield), m.p. 147–149°C; IR: 1730, 1594 and 1268 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 4.65–4.85 (m, 3H), 6.13 (t, 1H, J = 5.5 Hz), 6.33 (m, 1H), 6.57 (d, 1H, J = 4.5 Hz), 7.50–7.60 (m, 6H), 7.85–8.00 (m, 6H), 8.10 (s, 1H) and 8.53 (s, 1H). Anal. Calcd. for $C_{31}H_{21}Cl_3N_4O_8$: C, 54.45; H, 3.09; N, 8.19; Found: C, 54.23; H, 3.21; N, 8.17.

2,3,5-*O*-Tri-(3,5-dinitrobenzoyl)inosine (5). To a stirring suspension of 0.50 g of inosine (1.85 mmol) in 10 mL of anhydrous pyridine under N_2 at 25°C was added 1.80 g of 3,5-dinitrobenzoyl chloride (7.8 mmol). The resulting mixture was immediately immersed in a preheated oil bath at 60–70°C and heated for 3 hours. After cooling, the reaction was stirred at 25°C for an additional 18 hours. The excess of pyridine was distilled off and the residual material

was triturated with ethyl acetate and methanol. The clear yellow solid obtained was filtered and thoroughly washed with water, methanol and ethyl ether to remove traces of pyridine. An amorphous solid was obtained (94% yield), m.p. 174–177°C (decomp.); IR: 1742, 1700, 1545 and 1345 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 4.85 (dd, 1H, J = 5.3 and 12.0 Hz), 4.96 (dd, 1H, J = 3.5 and 12.0 Hz), 5.11 (m, 1H), 6.34 (m, 1H), 6.47 (dd, 1H, J = 4.3 and 6.2 Hz), 6.74 (d, 1H, J = 4.3 Hz), 7.94 (s, 1H), 8.38 (s, 1H), 8.80–8.95 (m, 6H) and 9.06 (m, 3H). Anal. Calcd. for $C_{31}H_{18}N_{10}O_{20}$: C, 43.78; H, 2.13; N, 16.47; Found: C, 43.98; H, 2.08; N, 16.66.

General procedure for the synthesis of 2,3,5-*O*-triacylribonic-1,4-lactones (19–23)

A solution containing D-ribonic-1,4-lactone **18** (0.33 mmol) and an appropriated acyl chloride (1.5 mmol) in 0.5 mL of anhydrous pyridine and 0.5 mL of anhydrous $CHCl_3$ was stirred under N_2 at 25°C for 24 hours. The mixture obtained was dissolved in CH_2Cl_2 and the organic phase was washed with 5% aqueous HCl, 5% aqueous $NaHCO_3$ and H_2O, dried with Na_2SO_4, filtered and concentrated to give a clear oil which solidified on standing. Recrystallization in the solvent indicated below afforded the triacyl derivatives as crystalline products.

2,3,5-*O*-Tribenzoylribonic-1,4-lactone (19). Recrystallized in acetone–ethanol (90% yield), m.p. 148°C (lit.[22] 148°C); IR: 1772 and 1722 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 4.65-4.90 (m, 2H), 5.09 (m, 1H), 5.94 (d, 1H, J = 6.4 Hz), 6.18 (d, 1H, J = 6.4 Hz) and 7.30-8.15 (m, 15H). Anal. calcd. for $C_{26}H_{20}O_8$: C, 67.82; H, 4.38; Found: C, 67.87; H, 4.45.

2,3,5-*O*-Tri(4-methoxybenzoyl)ribonic-1,4-lactone (20). Recrystallized in acetone–ethanol (92% yield), m.p. 113–116°C; IR: 1784, 1726 and 1380 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 3.85 (s, 3H) 4.60–4.85 (m, 2H), 5.04 (m, 1H), 5.88 (d, 1H, J = 6.3 Hz), 6.13 (d, 1H, J = 6.3 Hz), 6.75–7.05 (m, 6H) and 7.78–8.10 (m, 6H). Anal. calcd. for $C_{29}H_{26}O_{11}$: C, 63.27; H, 4.76; Found: C, 63.25; H, 4.84.

2,3,5-*O*-Tri-(4-chlorobenzoyl)ribonic-1,4-lactone (21). Recrystallized in ethanol (94% yield), m.p. 148–149°C; IR: 1788 and 1726 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 4.60–4.85 (m, 2H), 5.07 (m, 1H), 5.92 (d, 1H, J = 6.2 Hz), 6.13 (d, 1H, J = 6.2 Hz) and 7.33–8.08 (m, 12H). Anal. Calcd. for $C_{26}H_{17}Cl_3O_8$: C, 55.39; H, 3.04; Found: C, 55.53; H, 3.09.

2,3,5-*O*-Tri-(4-nitrobenzoyl)ribonic-1,4-lactone (22). Recrystallized in acetone (95% yield), m.p. 165–166°C; IR: 1810, 1734, 1526 and 1354 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 4.86 (m, 2H), 5.45 (m, 1H), 6.08 (d, 1H, J = 6.2 Hz), 6.43 (d, 1H, J = 6.2 Hz) and 8.10–8.50 (m, 12H). Anal. calcd. for $C_{26}H_{17}N_3O_{14}$: C, 52.45; H, 2.88; N, 7.06; Found: C, 52.60; H, 2.93; N, 6.97.

2,3,5-*O*-Tri-(4-chloro-3-nitrobenzoyl)ribonic-1,4-lactone (23). Recrystallized in ethanol (82% yield), m.p. 144–146°C; IR: 1790, 1732, 1536 and 1354 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 4.70–4.95 (m, 2H), 5.07 (m, 1H), 5.90 (d, 1H, J = 6.3 Hz), 6.15 (d, 1H, J = 6.3 Hz) and 7.60–8.60 (m, 9H). Anal. Calcd. for $C_{26}H_{14}Cl_3N_3O_{14}$: C, 44.69; H, 2.02; N, 6.01; Found: C, 44.81; H, 2.13; N, 6.14.

2,3-*O*-Isopropylideneribonic-1,4-lactone (24). A solution containing D-ribono-1,4-lactone **18** (27.0 mmol) in 160 mL of acetone and 1.6 mL of concentrated HCl was stirred for 18 hours at 25°C. The final suspension was neutralized with anhydrous K_2CO_3 and the mixture was filtered and concentrated in vacuo. The residue obtained was dissolved in CH_2Cl_2 and the organic phase

was washed with saturated $NaHCO_3$ and H_2O, dried with Na_2SO_4, filtered, concentrated and co-evaporated with ethanol to give clear oil which solidified on standing. Recrystallization in ethanol afforded a crystalline product (88% yield), m.p. 136–137°C (lit.[27] 138–139°C). ^{1}H-NMR data were identical with those reported.[26,29]

General procedure for the synthesis of 5-*O*-acyl-2,3-*O*-isopropylideneribonic-1,4-lactones (25–29)

A solution containing 2,3-*O*-isopropylidene-1,4-lactone **24** (0.54 mmol) and an appropriate acyl chloride (0.80 mmol) in 1.0 mL of anhydrous pyridine and 1.0 mL of anhydrous $CHCl_3$ was stirred under N_2 at 25°C for 24 hours. The mixture obtained was dissolved in CH_2Cl_2 and the organic phase was washed with 5% aqueous HCl, 5% aqueous $NaHCO_3$ and H_2O, dried with Na_2SO_4, filtered and concentrated to give a clear oil which solidified on standing. Recrystallization in the solvent indicated below afforded the 5-acyl 2,3-protected lactones as crystalline products.

5-*O*-Benzoyl-2,3-*O*-isopropylideneribonic-1,4-lactone (25). Recrystallized in ethanol (99% yield), m.p. 98–99°C; IR: 1788, 1732 and 1382 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 1.40 (s, 3H), 1.51 (s, 3H), 4.48 (dd, 1H, J = 2.0 and 13.3 Hz), 4.63 (dd, 1H, J = 2.0 and 13.3 Hz), 4.79 (m, 2H), 4.92 (t, 1H, J = 2.0 Hz), 7.48 (t, 2H, J = 8.6 Hz), 7.63 (t, 1H, J = 8.6 Hz) and 7.95 (d, 2H, 8.6 Hz). Anal. calcd. for $C_{15}H_{16}O_6$: C, 61.64; H, 5.52; Found: C, 62.12; H, 5.60.

5-*O*-(4-Methoxybenzoyl)-2,3-*O*-isopropylideneribonic-1,4-lactone (26). Recrystallized in acetone–ethanol (99% yield), m.p. 99–102°C; IR: 1782, 1726, 1384 and 1170 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 1.38 (s, 3H), 1.50 (s, 3H), 4.42 (dd, 1H, J = 2.0 and 13.0 Hz), 4.68 (dd, 1H, J = 2.0 and 13.0 Hz), 4.78 (m, 2H), 4.92 (t, 1H, J = 2.0 Hz), 6.93 (d, 2H, J = 8.1 Hz) and 7.87 (d, 1H, J = 8.1 Hz). Anal. calcd. for $C_{16}H_{18}O_7$: C, 51.69; H, 4.88; Found: C, 51.60; H, 4.94.

5-*O*-(4-Chlorobenzoyl)-2,3-*O*-isopropylideneribonic-1,4-lactone (27). Recrystallized in ethyl acetate–diethyl ether (92% yield), m.p. 163°C; IR: 1782, 1726 and 1384 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 1.40 (s, 3H), 1.52 (s, 3H), 4.52 (dd, 1H, J = 2.0 and 13.5 Hz), 4.66 (dd, 1H, J = 2.0 and 13.5 Hz), 4.78 (m, 2H), 4.91 (t, 1H, J = 2.0 Hz), 7.44 (d, 2H, J = 8.8 Hz) and 7.88 (d, 2H, J = 8.8 Hz). Anal. calcd. for $C_{15}H_{15}ClO_6$: C, 55.14; H, 4.94; Found: C, 55.31; H, 4.97.

5-*O*-(4-Nitrobenzoyl)-2,3-*O*-isopropylideneribonic-1,4-lactone (28). Recrystallized in acetone–ethanol (90% yield), m.p. 158–159°C; IR: 1766, 1740, 1532, 1382 and 1354 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 1.41 (s, 3H), 1.52 (s, 3H), 4.63 (m, 2H), 4.79 (m, 2H), 4.92 (m, 1H), 8.12 (d, 2H, J = 8.9 Hz) and 8.32 (d, 2H, J = 8.9 Hz). Anal. calcd. for $C_{15}H_{15}NO_8$: C, 55.39; H, 3.04; N, 4.15; Found: C, 55.53; H, 3.09; N, 4.30.

5-*O*-(4-Chloro-3-nitrobenzoyl)-2,3-*O*-isopropylideneribonic-1,4-lactone (29). Recrystallized in ethanol (80% yield), m.p. 87°C; IR: 1796, 1720, 1538, 1380 and 1352 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 1.40 (s, 3H), 1.51 (s, 3H), 4.63 (m, 2H), 4.78 (m, 2H), 4.91 (m, 1H), 7.69 (d, 1H, J = 8.3 Hz), 8.04 (d, 1H, J = 8.3 Hz) and 8.45 (s, 1H). Anal. calcd. for $C_{15}H_{14}ClNO_8$: C, 48.47; H, 3.80; N. 3.77; Found: C, 48.54; H, 3.92; N. 3.69.

General procedure for the synthesis of 5-*O*-acylribonic-1,4-lactones (31–35)

Method A. A solution containing 5-*O*-acyl-2,3-*O*-isopropylidene-1,4-lactone (0.40 mmol) in 1.5 mL of 50% aqueous trifluoroacetic acid was stirred at 40°C for 1.5 hours. The final suspension was concentrated in vacuo and the white residue obtained was co-evaporated with ethanol to remove traces of trifluoroacetic acid. Recrystallization in the solvent indicated below afforded the 5-acyl ribonic lactones as crystalline solids.

Method B. A solution containing 5-*O*-acyl-2,3-*O*-isopropylidene-1,4-lactone (0.40 mmol) in 1.5 mL of 10% aqueous acetic acid was stirred at 90°C for 3 hours. The final suspension was concentrated in vacuo and the white residue obtained was co-evaporated with ethanol to remove traces of acetic acid and recrystallized in the appropriated solvent to give crystalline 5-acyl ribonic lactones.

5-*O*-Benzoylribonic-1,4-lactone (31). Method A, recrystallized in chloroform–diethyl ether (99% yield), m.p. 153–155°C; IR: 3482, 3276, 1770 and 1716 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 4.26 (m, 1H), 4.46 (m, 3H), 4.58 (m, 1H), 5.64 (m, 1H), 5.93 (m, 1H), 7.53 (t, 2H, J = 7.1 Hz), 7.68 (d, 1H, J = 7.1 Hz) and 7.95 (d, 2H, J = 7.1 Hz). Anal. calcd. for $C_{12}H_{12}O_6$: C, 57.14; H, 4.79; Found: C, 57.30; H, 4.91.

5-*O*-(4-Methoxybenzoyl)ribonic-1,4-lactone (32). Method B, recrystallized in acetone–ethanol (54% yield), m.p. 150–151°C; IR: 1760, 1728, 1382 and 1170 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 3.85 (s, 3H), 4.26 (m, 1H), 4.40–4.65 (m, 4H), 5.61 (m, 1H), 5.92 (m, 1H, D_2O exchange), 7.07 (d, 2H, J = 8.5 Hz) and 7.90 (d, 2H, J = 8.5 Hz). Anal. calcd. for $C_{13}H_{14}O_7$: C, 55.32; H, 5.00; Found C, 55.30; H, 5.08.

5-*O*-(4-Chlorobenzoyl)ribonic-1,4-lactone (33). Method A, recrystallized in acetone–ethanol (99% yield), m.p. 168–169°C; IR: 3392, 1768 and 1726 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 4.27 (m, 1H), 4.40–4.65 (m, 4H), 5.65 (s, 1H), 5.93 (m, 1H), 7.63 (d, 2H, J = 8.4 Hz) and 7.95 (d, 2H, J = 8.4 Hz). Anal. calcd. for $C_{12}H_{11}ClO_6$: C, 50.28; H, 3.87; Found: C, 50.15; H, 3.94.

5-*O*-(4-Nitrobenzoyl)ribonic-1,4-lactone (34). Method B, recrystallized in acetone–ethanol (65% yield), m.p. 176°C; IR: 3480, 3332, 1766, 1726, 1544 and 1348 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 4.27 (m, 1H), 4.47 (m, 2H), 4.58 (m, 2H), 5.64 (m, 1H), 5.93 (m, 1H), 8.18 (d, 2H, J = 8.6 Hz) and 8.37 (d, 2H, J = 8.6 Hz); ^{13}C-NMR (DMSO-d_6): 64.5, 68.4, 68.7, 81.9, 124.1 (2C), 130.8 (2C), 134.7, 150.5, 164.0 and 175.8. Anal. calcd. for $C_{12}H_{11}NO_8$: C, 48.49; H, 3.72; N, 4.71; Found: C, 48.60; H, 3.81; N, 4.81.

5-*O*-Acetylribonic-1,4-lactone (35). Method B, recrystallized in ethanol (80% yield), m.p. 148°C; IR: 3474, 3288, 1760, 1430 and 1386 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 2.04 (s, 3H), 4.10–4.55 (m, 5H), 5.58 (m, 1H) and 5.87 (m, 1H). Anal. calcd. for $C_7H_{10}O_6$: C, 42.22; H, 5.30; Found: C, 42.28; H, 5.06.

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