SYNTHESIS AND ANTIVIRAL EVALUATION OF THE 2′-*C*-METHYL BRANCHED DERIVATIVE OF A NUCLEOSIDE ANALOG INHIBITOR OF RNA VIRAL INFECTIONS, T-1106

Claire PIERRA[a1], Clément COUNOR[a2], Richard STORER[a3,b] and Gilles GOSSELIN[a4,c,*]

[a] *Idenix Pharmaceuticals, Medicinal Chemistry Laboratories IDENIX Sarl, Cap Gamma, 1682 Rue de la Valsière, BP 50001, 34189 Montpellier Cedex 4, France; e-mail: [1] pierra.claire@idenix.com, [2] counor.clement@idenix.com, [3] richard.storer@summitplc.com, [4] gosselin.gilles@idenix.com*

[b] *Current Address: Summit Corporation plc, 91 Milton Park, Abingdon, Oxfordshire OX14 4RY, England*

[c] *UMR 5247 CNRS-UM1-UM2 (IBMM), Université Montpellier 2, CC 1704, 34095 Montpellier Cedex 5, France*

Received April 27, 2011
Accepted May 31, 2011
Published online October 30, 2011

Dedicated to Professor Antonín Holý on the occasion of his 75th birthday in recognition of his outstanding contributions to the area of nucleic acid chemistry.

An example of a 2′-*C*-methyl branched nucleoside analogue bearing 3,4-dihydro-3-oxo-pyrazine-2-carboxamide as the base, namely 4-(2-*C*-methyl-β-D-ribofuranosyl)-3-oxo-3,4-dihydropyrazine-2-carboxamide, is reported. This compound was synthesized following a Vorbrüggen's glycosylation procedure in a few steps. When evaluated in cell culture experiments against a broad range of viruses, this compound did not exhibit any significant antiviral effect or cytotoxicity.

Keywords: T-1106; 2′-*C*-Methyl branched derivative; Antiviral evaluation; Hepatitis C virus; Nucleosides; Antiviral agents; Biological activity.

Although efficacious drugs have been developed to treat infections caused by DNA viruses[1] and to control retroviruses infections[2], no specific antiviral drugs are available for the prevention or the treatment of infections caused by the majority of RNA viruses. Yet, RNA viruses, whose more than 350 species have been described and which are classified, according to their genome structure and expression modalities, as single-stranded positive RNA ($ssRNA^+$), single-stranded negative RNA ($ssRNA^-$), and double-stranded RNA (dsRNA) viruses, are major human pathogens causing millions of deaths

doi:10.1135/cccc2011089

annually[3]. Recently, a nucleoside analogue [2-oxo-1-(β-D-ribofuranosyl)-pyrazine-3-carboxamide (**1**), T-1106; Chart 1], discovered and synthesized by Toyama Chemical Co., Ltd.[4], was shown to have antiviral activity against three viruses of the ssRNA$^+$ *Flaviviridae* family, that is, Bovine Virus Diarrhoea Virus (BVDV, a *Pestivirus*)[5], Yellow Fever (YF, a *Flavivirus*)[6], and Hepatitis C Virus (HCV, a *Hepacivirus*)[5], as well as against a virus of the ssRNA$^-$ *Bunyaviridae* family, that is, Punta Toro Virus (PTV, a *Phlebovirus*)[7]. On the other hand, and following the discovery that a series of branched-sugar ribonucleosides could act as potential chemotherapeutic agents against HCV [8] and against flaviviruses and pestiviruses[9], we have previously shown that 2′-*C*-methylribonucleoside derivatives in the pyrimidine series are potent and broad-spectrum anti-RNA virus agents[10]. In continuation of our interest in 2′-*C*-methyl-branched nucleoside derivatives[11], here we report the synthesis of 4-(2-*C*-methyl-β-D-ribofuranosyl)-3-oxo-3,4-dihydropyrazine-2-carboxamide (**2**), the 2′-*C*-methyl-branched derivative of T-1106 (Chart 1), as well as the results of its antiviral evaluations. It is noteworthy that during the course of our current work[12], the synthesis of **2** has been reported by another author[13]. However, in that communication, the approach implemented for the synthesis of **2** was slightly different from our approach, and no results of antiviral evaluations have been given.

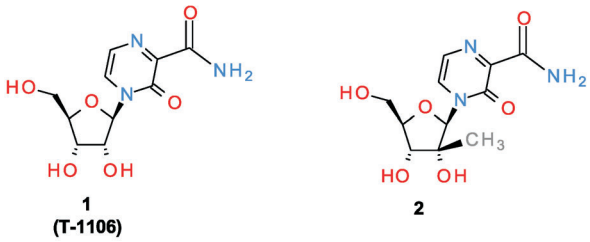
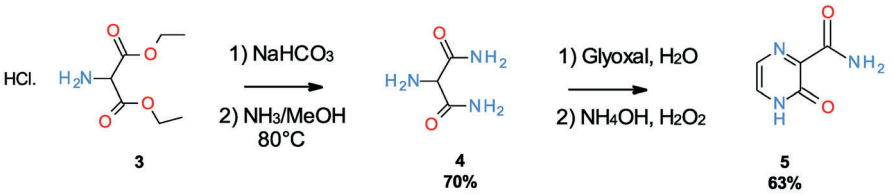


CHART 1

RESULTS AND DISCUSSION

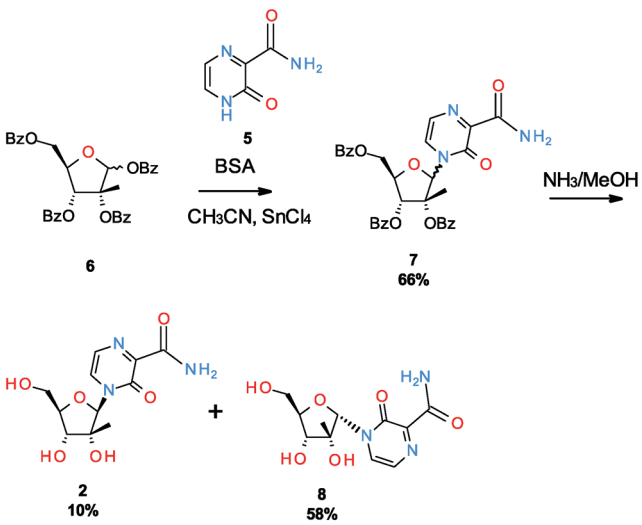
Synthesis

For the synthesis of 4-(2-*C*-methyl-β-D-ribofuranosyl)-3-oxo-3,4-dihydropyrazine-2-carboxamide (**2**), first the pyrazine base **5** was prepared in good yield following a procedure described in the literature[14], via 2-aminopropanediamide **4** [15] and starting from the commercially available diethylamino malonate **3** (Scheme 1). Then, glycosylation of **5** with 1,2,3,5-*O*-tetrabenzoyl-2-*C*-methyl-β-D-ribofuranose (**6**)[16] under Vorbrüggen-type



SCHEME 1
Synthesis of 3-oxo-3,4-dihydropyrazine-2-carboxamide (**5**)

per-silylation conditions[17] led to the benzoylated nucleoside derivative **7** as an inseparable α/β anomeric mixture (Scheme 2). Treatment with methanolic ammonia of this anomeric mixture, followed by purification on reverse phase column chromatography, allowed the isolation of the pure desired 4-(2-*C*-methyl-β-D-ribofuranosyl)-3-oxo-3,4-dihydropyrazine-2-carboxamide (**2**) and of its α-anomer (compound **8**) in 10 and 58% yields, respectively.



SCHEME 2
Synthesis of 4-(2-*C*-methyl-β-D-ribofuranosyl)-3-oxo-3,4-dihydropyrazine-2-carboxamide (**2**) and its α-anomer **8**

Antiviral Evaluations

4-(2-*C*-Methyl-β-D-ribofuranosyl)-3-oxo-3,4-dihydropyrazine-2-carboxamide (**2**) and its α-anomer **8** were evaluated in cell-based assays (following methods described in ref.[10a]) against viruses representative of three genera of the

ssRNA$^+$ *Flaviviridae* family, that is, *Pestivirus* (Bovine Virus Diarrhoea Virus), *Flavivirus* (Yellow Fever, Dengue and West Nile Viruses) and *Hepacivirus* (HCV), as well as against one genus of the ssRNA$^+$ *Picornaviridae* family, *Enterovirus* (Coxsackie Virus B2 and Poliovirus Sabin-1). Both anomers **2** and **8** were also tested against genera representative of three ssRNA$^-$ virus families, *Paramyxoviridae*, that is, *Pneumovirus* (Respiratory Syncytial Virus), *Rhabdoviridae*, that is, *Vesiculovirus* (Vesicular Stomatitis Virus), *Orthomyxoviridae*, that is, *Influenzavirus* (Influenza A), against one genus of the dsRNA *Reoviridae* family, that is, *Orthoreovirus* (Reo-1) and against viruses representative of two dsDNA virus families, *Poxviridae* (Vaccinia Virus) and *Herpesviridae* (Herpes Simplex Virus). Unfortunately, for all these viruses, the new nucleoside analogues **2** and **8** showed neither antiviral activity nor cytotoxicity at the highest concentration tested (generally 100 μM).

SUMMARY AND CONCLUSION

4-(2-*C*-Methyl-β-D-ribofuranosyl)-3-oxo-3,4-dihydropyrazine-2-carboxamide (**2**) was synthesized as an example of a 2′-*C*-methyl branched pyrazine nucleoside analogue. Unfortunately, when evaluated in cell culture experiments against a broad range of viruses, this compound exhibited no significant antiviral effect or cytotoxicity. Several factors could be responsible for the inactivity of 2-oxo-1-(2-*C*-methyl-β-D-ribofuranosyl)pyrazine-3-carboxamide (**2**), such as its inability to enter cells or to serve as a substrate for intracellular enzymes catalyzing phosphorylation, or perhaps a lack of inhibition of the viral polymerases by its triphosphate[18]. Further research, beyond the scope of this work, would be needed to support these hypotheses.

EXPERIMENTAL

General Procedure

^{1}H NMR spectra were recorded at ambient temperature on a Bruker AC 300 or 400 MHz spectrometer. ^{1}H NMR chemical shifts (δ-scale) are quoted in ppm referenced to the residual solvent peak [dimethyl sulfoxide (DMSO-d_6) set at 2.49 ppm, D_2O set at 4.79 ppm]; coupling constants *J* are given in Hz. The accepted abbreviations are as follows: s, singlet; d, doublet; t, triplet, m, multiplet. FAB mass spectra were recorded in the positive- or negative-ion mode on a JEOL DX 300 mass spectrometer operating with a JMA-DA 5000 mass data system and using a mixture of glycerol (G) and thioglycerol (T) (1:1, v/v) as the matrix. Melting points were determined in open capillary tubes with a Büchi B-545 apparatus and are uncorrected. UV spectra were recorded on a Uvikon XS spectrophotometer. Thin-layer chromatography (TLC) was performed on precoated aluminium sheets of silica gel 60 F_{254} (Merck, Art. 5554), visualization of products being accomplished by UV absorbance and by charring with 10% ethanolic sulfuric acid on heating. Column chromatography was carried out on silica gel 60

(Merck, Art. 9385). Evaporation of solvents was carried out in a rotary evaporator under reduced pressure. All moisture sensitive reactions were carried out under rigorously anhydrous conditions under argon atmosphere using oven-dried glassware. Solvents were dried and distilled prior to use and solids were dried over P_2O_5 under reduced pressure. Analytical high-performance liquid chromatography (HPLC) studies were carried out on a Waters Associates unit (multisolvent delivery system, 717 autosampler injector, 996 photodiode array detector and a Millenium data workstation) using a reverse-phase analytical column (Nova-Pak® Silica 60 Å 4 μm, C18, 150 × 3.9 mm).

2-Aminopropanediamide **4** [15]

To a solution of diethylaminomalonate hydrochloride **3** (9 g, 42.5 mmol) in water (10 ml) was added solid sodium hydrogen carbonate until pH > 7. After extraction with ethyl acetate, the organic phase was separated and evaporated under reduced pressure. Then, the residue was taken into a methanolic ammonia solution (7 N) and the reaction mixture heated in a high pressure reactor at 80 °C overnight. After evaporation of the solvent under reduced pressure, the crude residue was precipitated in methanol. The solid was filtered and washed successively with methanol and diethyl ether to afford compound **4** (3.5 g, 70%). FAB > 0 *m/z* (GT) 118 $(M + H)^+$; FAB < 0 *m/z* (GT) 116 $(M - H)^-$. ^{1}H NMR (400 MHz, DMSO-d_6): 7.40 and 7.24 2 s, 6 H, D_2O exchangeable (3 NH_2); 3.75 s, 1 H (CH).

3-Oxo-3,4-dihydropyrazine-2-carboxamide (**5**)[14]

To a suspension of compound **4** (3.5 g, 30 mmol) in water (24 ml) glyoxal (10 g, 36 mmol) was added. The reaction mixture was stirred at 90 °C for 3 h, then cooled down to room temperature. After neutralization with 15% NH_4OH solution until pH > 7, H_2O_2 was added dropwise at 0 °C and the reaction mixture was stirred at room temperature for 1 h. The precipitate was filtered, washed with acetone and crystallized in water to give the desired pure pyrazine compound **5** (2.65 g, 63%), m.p. 269–271 °C. FAB > 0 *m/z* (GT) 140 $(M + H)^+$; FAB < 0 *m/z* (GT) 138 $(M - H)^-$. ^{1}H NMR (400 MHz, DMSO-d_6): 13.30 s, 1 H, D_2O exchangeable (NH); 8.7 and 7.9 2 s, 2 H, D_2O exchangeable (NH_2); 8.1 m, 2 H (H-5 and H-6). HPLC: R_t = 0.98 min (100% H_2O over a 10 min period, flow rate of 1 ml/min), λ_{max} = 265 nm. UV (H_2O): λ_{max} = 344 nm (ε 6800), λ_{min} = 279 nm (ε 670), λ_{max} = 229 nm (ε 8000), λ_{min} = 207 nm (ε 3000).

1,2,3,5-Tetra-*O*-benzoyl-2-*C*-methyl-α-(and β)-D-ribofuranose (**6**)

This compound was prepared according to a published procedure[16]. The corresponding β-anomer was obtained by crystallization from hexanes and ethyl acetate. Yields and physico-chemical characteristics were in accordance with those reported in the literature[16].

4-(2,3,5-Tri-*O*-benzoyl-2-*C*-methyl-α-(and β)-D-ribofuranosyl)-3-oxo-3,4-dihydropyrazine-2-carboxamide (**7**)

A suspension of 3-oxo-3,4-dihydropyrazine-2-carboxamide (**5**; 300 mg, 2.1 mmol) in anhydrous acetonitrile (7.0 ml) was treated with *N*,*O*-bis(trimethylsilyl)acetamide (1.04 ml, 4.24 mmol) and brought to 90 °C for 1 h. The resulting solution was allowed to cool to ambient temperature, peracylated sugar **6** (600 mg, 1.0 mmol) was added, followed cautiously

with stannic chloride (0.41 ml, 3.5 mmol), and heated to reflux for 2 h. After addition of a cold saturated solution of hydrogen carbonate, the solid was filtered over Celite. The filtrate was diluted with ethyl acetate, washed with a solution of saturated sodium hydrogen carbonate and brine, dried over sodium sulfate, and evaporated under reduced pressure. Silica gel column chromatography of the residue using 2% of methanol in dichloromethane afforded the title compound **7** (396 mg, 66%) as a α/β (80:20) anomeric mixture. FAB > 0 *m/z* (GT) 598 $(M + H)^+$, 459 $(Sugar)^+$, 105 $(Bz)^+$; FAB < 0 *m/z* (GT) 596 $(M - H)^-$, 121 $(BzO)^-$. ^{1}H NMR (400 MHz, DMSO-d_6): 8.5–7.3 m, 19 H (H-5, H-6, Bz, NH_2); 6.9 s, 0.2 H (H-1′β); 6.83 s, 0.8 H (H-1′α); 6.01 d, 1 H *J* = 3.0 (H-3′); 5.6 m, 0.8 H (H-4′α); 5.0 m, 0.2 H (H-4′β); 4.9–4.7 m, 2 H (H-5′, H-5″); 2.2 s, 3 H (CH_3).

4-(2-*C*-Methyl-β-D-ribofuranosyl)-3-oxo-3,4-dihydropyrazine-2-carboxamide (**2**) and
4-(2-*C*-methyl-α-D-ribofuranosyl)-3-oxo-3,4-dihydropyrazine-2-carboxamide (**8**)

A solution of the α/β anomeric mixture **7** (396 mg, 0.66 mmol) in methanol (13 ml) presaturated with ammonia at 0 °C was stirred at room temperature overnight, and evaporated to dryness. The residue was purified by reverse phase column chromatography (0–5% acetonitrile in water) to provide pure **2** (19 mg, 10%) and pure **8** (109 mg, 58%) which were lyophilized in water.

β-*Anomer* **2**. FAB > 0 *m/z* (GT) 286 $(M + H)^+$, 140 $(Base + 2\ H)^+$; FAB < 0 *m/z* (GT) 284 $(M - H)^-$, 138 $(Base)^-$. ^{1}H NMR (300 MHz, D_2O): 8.14 d, 1 H *J* = 4.3 (H-6); 7.67 d, 1 H, *J* = 4.3 (H-5); 6.22 s, 1 H (H-1′); 4.1–3.7 m, 4 H (H-3′, H-4′, H-5′, H-5″); 1.01 s, 3 H (CH_3). HPLC: R_t = 3.3 min (0–50% CH_3CN in H_2O over a 15 min period, flow rate of 1 ml/min), λ_{max} = 230 nm. UV (H_2O): λ_{max} = 350 nm (ε 9900), λ_{min} = 279 nm (ε 920), λ_{max} = 228 nm (ε 10400), λ_{min} = 207 nm (ε 5600).

α-*Anomer* **8**. FAB > 0 *m/z* (GT) 286 $(M + H)^+$, 140 $(Base + 2\ H)^+$. FAB < 0 *m/z* (GT) 284 $(M - H)^-$, 138 $(Base)^-$. ^{1}H NMR (400 MHz, DMSO-d_6): 8.39 and 7.72 2 s, 2 H D_2O exchangeable (NH_2); 7.66 d, 1 H, *J* = 4.3 (H-6); 7.54 d, 1 H, *J* = 4.3 (H-5); 6.22 s, 1 H (H-1′); 5.3–4.7 m, 3 H D_2O exchangeable (OH-2′, OH-3′, OH-5′); 4.1 m, 1 H (H-4′); 3.8 m, 1 H (H-3′); 3.7 m, 1 H (H-5′); 3.5 m, 1 H (H-5″); 1.25 s, 3 H (CH_3). HPLC: R_t = 3.09 min (0–50% CH_3CN in H_2O over a 15 min period, flow rate of 1 ml/min), λ_{max} = 230 nm. UV (H_2O): λ_{max} = 355 nm (ε 8300), λ_{min} = 281 nm (ε 500), λ_{max} = 228 nm (ε 8200), λ_{min} = 205 nm (ε 3300).

We gratefully acknowledge Dr. M. Liuzzi (Idenix Senior Director Virology, Laboratory Cooperativo Idenix-Universita di Cagliari, Italy) and his collaborators for the biological results. We are indebted to K. Barry (Corporate Communications Specialist, Idenix Pharmaceuticals Inc.) for critical reading of the manuscript.

REFERENCES

1. a) De Clercq E.: *J. Med. Chem.* **2010**, *53*, 1438; b) De Clercq E.: *Adv. Virus Res.* **2009**, *73*, 1.
2. a) De Clercq E.: *Curr. Opin. Pharmacol.* **2010**, *10*, 507; b) Mehellou Y., De Clercq E.: *J. Med. Chem.* **2010**, *53*, 521.
3. a) Yang D.: *RNA Viruses: Host Gene Responses To Infections*, p. 500. World Scientific Publishing Company, Singapore 2009; b) Holmes E. C.: *The Evolution and Emergence of*

RNA Viruses, p. 254. Oxford University Press, USA 2009; c) Cann A. J.: *RNA Viruses: A Practical Approach*, p. 266. Oxford University Press, USA 2000.

4. a) Egawa H., Sugita A., Furuta Y.: Japan JP2004043371A, Kokai Tokkyo Koho 2004, p. 41; b) Furuta Y., Egawa H., Takahashi K., Tsutsui Y., Uehara S., Murakami M.: Int. Appl. WO2003015798A1, 2003, p. 146; c) Egawa H., Furuta Y., Sugita J., Uehara S., Hamamoto S., Yonezawa K.: Int. Appl. WO2001060834A1, 2001, p. 137.
5. Furuta Y., Takahashi K., Maekawa M., Maegawa H., Egawa H., Terashima N.: Presented at *44th Intersci. Conf. Antimicrob. Agents Chemother., Washington, DC, 2004*, pp. 199–200 (Abstr. F-487).
6. a) Furuta Y., Takahashi K., Shiraki K., Sakamoto K., Smee D. F., Barnard D. L., Gowen B. B., Julander J. G., Morrey J. D.: *Antiviral Res.* **2009**, *82*, 95; b) Julander J. G., Shafer K., Smee D. F., Morrey J. D., Furuta Y.: *Antimicrob. Agents Chemother.* **2009**, *53*, 202; c) Julander J. G., Furuta Y., Shafer K., Sidwell R. W.: *Antimicrob. Agents Chemother.* **2007**, *51*, 1962.
7. Gowen B. B., Wong M.-H., Jung K.-H., Smee D. F., Morrey J. D., Furuta Y.: *Antiviral Res.* **2010**, *86*, 121.
8. Sommadossi J.-P., Lacolla P.: Int. Appl. WO2001090121A2, 2001, p. 296.
9. Sommadossi J.-P., Lacolla P.: Int. Appl. WO2001092282A2, 2001, p. 302.
10. a) Benzaria S., Bardiot D., Bouisset T., Counor C., Rabeson C., Pierra C., Storer R., Loi A. G., Cadeddu A., Mura M., Musiu C., Liuzzi M., Loddo R., Begelson S., Bichko V., Bridges E., Cretton-Scott E., Mao J., Sommadossi J.-P., Seifer M., Standring D., Tausek M., Gosselin G., La Colla P.: *Antiviral Chem. Chemother.* **2007**, *18*, 225; b) Pierra C., Amador A., Benzaria S., Cretton-Scott E., D'Amours M., Mao J., Mathieu S., Moussa A., Bridges E. G., Standring D. N., Sommadossi J.-P., Storer R., Gosselin G.: *J. Med. Chem.* **2006**, *49*, 6614; c) Pierra C., Benzaria S., Amador A., Moussa A., Mathieu S., Storer R., Gosselin G.: *Nucleosides Nucleotides Nucleic Acids* **2005**, *24*, 767.
11. Pierra C., Amador A., Badaroux E., Storer R., Gosselin G.: *Collect. Czech. Chem. Commun.* **2006**, *71*, 991.
12. a) Storer R., Gosselin G., Griffon J.-F., Pierra C.: Can. Appl. CA2600359A1, 2006, p. 149; b) Sommadossi J.-P., Gosselin G., Storer R., Egan J.: Int. Appl. WO2006037028A2, 2006, p. 73.
13. Kim M. J.: *Synth. Commun.* **2010**, *40*, 2988.
14. Lee T. C., Chello P. L., Chou T. C., Templeton M. A., Parham J. C.: *J. Med. Chem.* **1983**, *26*, 283.
15. Botta M., De Angelis F., Nicoletti R.: *J. Heterocycl. Chem.* **1979**, *16*, 193.
16. Harry-O'kuru R. E., Smith J. M., Wolfe M. S.: *J. Org. Chem.* **1997**, *62*, 1754.
17. Vorbrüggen H., Ruh-Pohlenz C. (Eds): *Handbook of Nucleoside Synthesis*. John Wiley & Sons, Inc. New York 2001.
18. a) Mansour T. S., Storer R.: *Curr. Pharm. Design* **1997**, *3*, 227; b) Ichikawa E., Kato K.: *Curr. Med. Chem.* **2001**, *8*, 385.