Partial acylation of cytidine and its 2'-C-methyl analogue as a tool to functionalize the ribonucleosidic 2',3'-*cis*-diol system

Joanna Fogt,[a] Piotr Januszczyk,[a] Tomoyuki Onishi,[b] Kunisuke Izawa,[b] and Jerzy Boryski[a,*]

[a] *Institute of Bioorganic Chemistry, Polish Academy of Sciences, ul. Noskowskiego 12/14, PL-61704 Poznan, Poland*

[b] *AminoScience Laboratories, Ajinomoto Co., Inc. 1-1, Suzuki-Cho, Kawasaki-ku, Kawasaki, Kanagawa 210-8681, Japan*

E-mail: jboryski@ibch.poznan.pl

Dedicated to Professor Harri Lönnberg on the occasion of his 60th anniversary

Abstract

Precisely controlled conditions of acylation and 2',3'-O-isomerization allowed to synthesize tripivaloyl derivatives of cytidine with free 2'- or 3'-hydroxyl group in a simple manner. Acylation of 2'-C-β-methylcytidine proceeded in a different way and resulted in the formation of tripivaloyl 2'-hydroxy nucleoside, or dipivaloyl 2',3'-dihydroxy compound. All these products may be applied as key intermediates in the regioselective modification of 2',3'-*cis*-diol system.

Keywords: Hepatitis C virus, nucleoside analogs, cytidine, 2'-C-β-methylcytidine, partial acylation, *cis*-diol system

Introduction

Hepatitis C virus (HCV) is responsible for serious blood-borne infections, chronic liver diseases and, in many cases, for hepatocellular carcinoma. Since there is no established vaccine for HCV, many research laboratories have developed new therapeutic agents of anti-HCV activity. In this respect, the nucleoside analogs of the structure of 2'-C-alkyl ribofuranosides[1,2] seem to be promising candidate compounds. One of the most *in vitro* active compounds of this type is an analog of naturally occurring cytidine (**1**), 2'-C-β-methylcytidine (**2**). As it has been shown recently, the ribonucleoside **2** is a potent and selective inhibitor of *Flaviviridae* in cell culture.[3] Because there is an urgent need for new therapeutic compounds of this type, the structure of 2'-C-β-methylcytidine has been modified in different ways, and some new nucleoside analogs have been obtained, *e.g.* 3'-O-valinyl ester of 2'-C-β-methylcytidine,[3] 2'-deoxy-[1,4] and 3'-

deoxyribosides,[5] 2'-C-β-methyl-1-(β-D-xylofuranosyl)-cytosine,[5] 2'-fluoro[6] and 3'-fluoro[5] derivatives, and 3'-methoxynucleoside[5]. All these compounds derived from 2'-C-β-methylcytidine (**2**) are substituted or modified at either 2'- or 3'-hydroxyl group. Furthermore, as one may conclude from the cited literature, the most difficult synthetic steps have been related to the appropriate protection of 2',3'-*cis*-diol system prior to the final modification.

Indeed, the regioselective substitution of ribonucleosides in the 2'- or 3'-position still is a challenge to organic chemists. This requires a laborious preparation of protected compounds in which either 2'- or 3'-hydroxyl group remains unsubstituted. Fortunately, due to the invention of Markiewicz's 3',5'-O-cyclic protection, *i.e.* 3',5'-O-(1,1,3,3-tetraisopropyl-1,3-disiloxanediyl) group,[7] synthesis of nucleosides with free 2'-hydroxyl group is relatively simple, but the procedure is rather expensive.

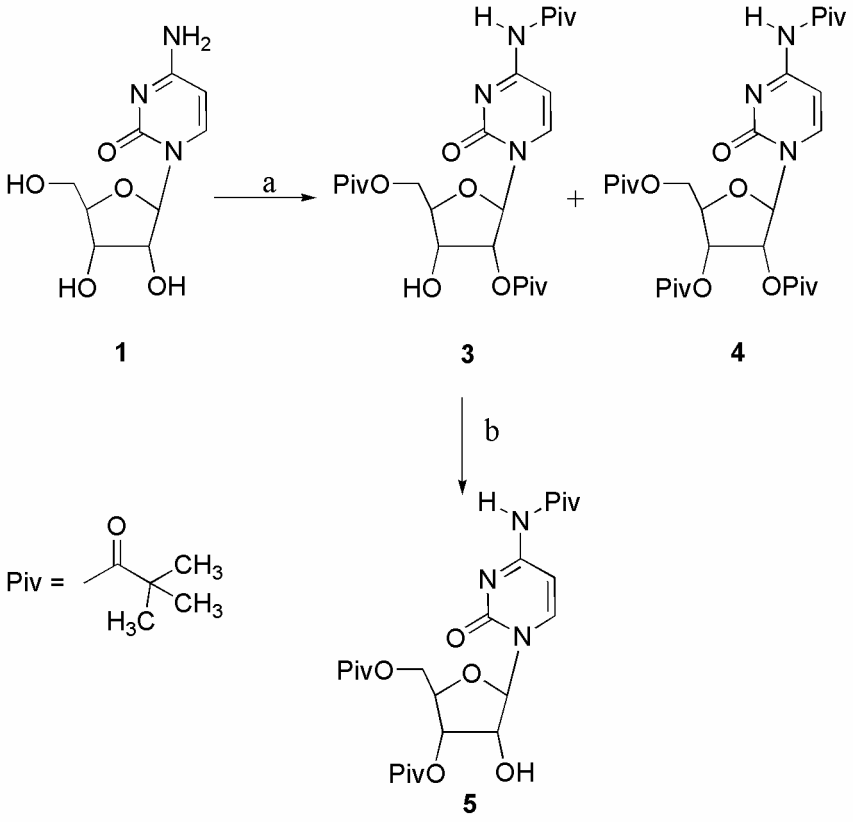
Preparation of the 3'-hydroxyl synthons is more difficult and requires an appropriate protection of 2'- and 5'-hydroxyl functions. The 2',5'-bis-substituted key intermediates can be obtained in several steps from unprotected nucleosides, either *via* partial hydrolysis of 2',3'-orthoesters and separation of the resulting 2'-O- and 3'-O-acyl derivatives,[8] or with the use of Markiewicz's reagent, followed by the 2'-O-protection, removal of the 3',5'-O-cyclic protection with fluorides, and subsequent 5'-O-protection with trityl group. Both procedures are tedious and time-consuming. Moreover, a suitable protection of exoamine groups is required for heterocyclic bases other than uracil.

However, synthesis of the 2',5'-bis-protected ribonucleosides may be accomplished in just one step by partial acylation of ribonucleosides.[9,10] The method was developed in 1980th, but it has not been used very often since that time. The procedure involves reaction of unprotected ribonucleosides with a precisely controlled amount of acid chlorides in pyridine. The observed regioselectivity of acylation can be attributed to the "unusual acidity of the 2'-hydroxyl group".[9] Therefore, the 2'-OH group undergoes acylation faster than 3'-hydroxyl. Here we describe how the latter method can be applied for partial protection of 2'-C-β-methylcytidine (**2**).

Results and Discussion

Prior to the study on partial acylation of 2'-C-β-methyl analog **2**, we performed introductory experiments applying unmodified cytidine (**1**) as a model compound (Scheme 1). We noticed that the best regioselectivity of protection was obtained in the pivaloyl series, and this was in line with the previous report.[9] In this manner, the reaction of **1** with 3.5 equivalents of pivaloyl chloride in pyridine gave $O^{2'},O^{5'},N^4$-tripivaloylcytidine (**3**) as the main product, along with $O^{2'},O^{3'},O^{5'},N^4$-tetrapivaloylcytidine (**4**) as a minor product. The reaction proceeded with high selectivity in a temperature range varying from -5 – 20 ^{o}C. The tripivaloyl cytidine derivative **3** is not a new compound. In fact, $O^{2'},O^{5'},N^4$-tripivaloylcytidine (**3**) was obtained as reported, and used without isolation in a subsequent one-pot reaction.[11] In the present work, however, we have succeeded in crystallization of the product **3** from toluene. In this way, the 3'-OH key

intermediate **3** can be now obtained as a crystalline material in a one-step procedure from cytidine (79% yield). The tripivaloyl cytidine derivative **3** can be a useful substrate in the 3'-modification reactions.

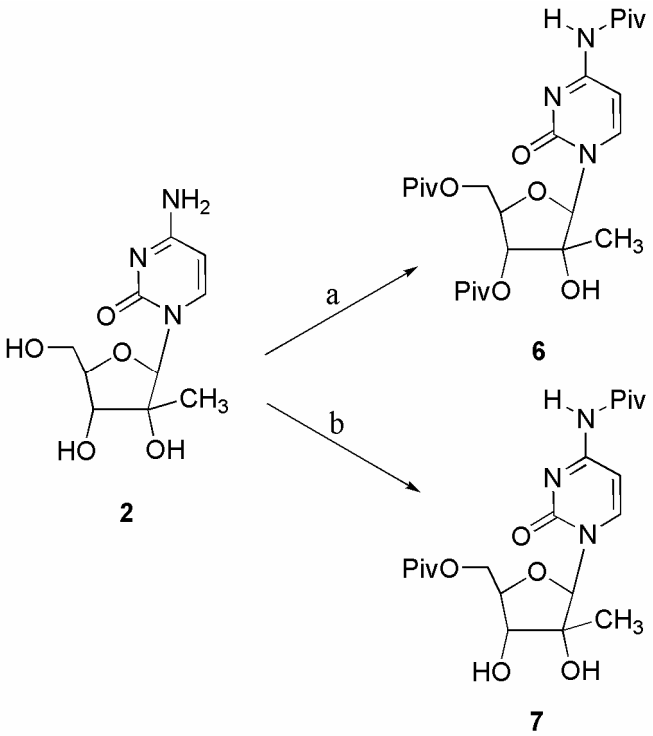


Scheme 1. *Reagents and conditions:* (a) $(CH_3)_3CCOCl$ (3.5 mol eqs.), pyridine, 4 ^oC, 20 h; (b) chloroform-methanol (4:1, v/v), silica gel, RT, 24 h.

The product **3**, however, could not be purified by chromatographic means. Although chromatography on silica gel in chloroform – methanol allowed to obtain the tetrasubstituted compound **4** for structure determination, the main product **3** underwent 2',3'-isomerization to form a mixture of **3** and $O^{3'},O^{5'},N^4$-tripivaloyl derivative **5**. Similar isomerization reactions of partially acylated ribonucleosides during chromatography were reported previously,[9] but in the present work we managed to isolate the pure 2'-OH isomer **5**. When $O^{2'},O^{5'},N^4$-tripivaloyl-cytidine (**3**) was stirred in a suspension of silica gel in methanol-containing solvents (*e.g.* chloroform – methanol 4:1, v/v), we observed the preferred formation of its isomer **5** (ratio **5**/**3** *ca.* 5:1; as proofed by ^1H NMR). The main product of isomerization, $O^{3'},O^{5'},N^4$-tripivaloyl derivative **5**, was more stable than **3** and could be purified by chromatography in aprotic solvent systems. In this manner, we managed to obtain the second isomer of tripivaloyl cytidine (**5**). This compound can be applied as a 2'-OH component for 2'-modification reactions; however, its

synthesis is slightly more laborious than the 3'-OH compound **3**, and we have not succeeded in crystallization of the isomer **5**.

In the light of presented experiments in the unmodified cytidine series, we could anticipate a similar reactivity of its close analog, 2'-C-β-methylcytidine (**2**). However, to our surprise, the treatment of **2** with pivaloyl chloride (3.5 eqs.) in pyridine gave $O^{3'},O^{5'},N^4$-tripivaloyl-2'-C-β-methylcytidine (**6**) in quantitative yield, instead of the expected $O^{2'},O^{5'},N^4$-tri-substituted product (Scheme 2). This shows that the tertiary 2'-hydroxyl group is very inert in acylation reactions and therefore, in the presence of 2'-C-β-methyl, the third pivaloyl group is being attached in the 3'-position. Unlike in the case of cytidine, any tetrapivaloyl compound has not been formed, despite of an excess of the acylating reagent. In addition, $O^{3'},O^{5'},N^4$-tripivaloyl-2'-C-β-methylcytidine (**6**) does not undergo 2',3'-O-isomerization and therefore, it may be purified by using chromatographic methods. These experimental facts further emphasize the very low reactivity of 2'-hydroxyl group in 2'-C-β-methylcytidine. According to our literature search, this is the first report on the low reactivity of the tertiary 2'-hydroxyl in the series of 2'-C-β-alkylnucleosides. However, some examples of the low reactivity of the corresponding tertiary 3'-hydroxyl have been reported in the 3'-C-β-methylribofuranosyl series.[12-14] In conclusion, compound **6** does not seem to be a promising substrate for 2'-modification.



Scheme 2. *Reagents and conditions:* (a) $(CH_3)_3CCOCl$ (3.5 eqs.), pyridine, 4 °C, 20 h; (b) $(CH_3)_3CCOCl$ (2.5 eqs.), pyridine, RT, 3 h.

To solve the problem of regioselective modification of the 2',3'-diol system in 2'-C-β-methyl-D-ribonucleosides, we decided to take advantage of this low reactivity of 2'-hydroxyl group, assuming that this position could remain unprotected during 3'-modification. To test this idea, $O^{5'}$,N^4-dipivaloyl-2'-C-β-methylcytidine (**7**), *i.e.* a compound in which the 2',3'-diol system was not protected, was synthesized in the reaction of **2** with 2.5 molar eqs. of pivaloyl chloride in pyridine at room temperature. The reaction gave a mixture of $O^{5'}$,N^4-dipivaloyl-2'-C-β-methylcytidine (**7**; 70% yield after silica gel column separation), and $O^{3'}$,$O^{5'}$,N^4-tripivaloyl-2'-C-β-methylcytidine (**6**) as a minor product. In our preliminary experiments of modifications, the dipivaloyl ribonucleoside **7** could be selectively substituted in the intermolecular reactions with other acylating reagents (*e.g.* reaction with mesyl chloride), but in some cases it underwent intramolecular reactions (*e.g.* the formation of 2',3'-isopropylidene derivative).[15]

In conclusion, the reaction of partial acylation of ribonucleosides offers a simple and inexpensive route towards new nucleoside analogs. We have developed a very simple laboratory method towards 2'(3')-monohydroxyl cytidine derivatives (**5** and **3**, respectively), which may be useful in 2'- or 3'-modification approaches. We have shown for the first time that partial acylation in the 2'-C-β-methyl ribofuranosyl proceeds quite differently than in unmodified ribofuranosyl series, and the products (**6** and especially **7**) can be useful synthons for further chemical modifications in order to obtain new biologically active compounds.

Experimental Section

General Procedures. 2'-C-β-Methylcytidine (**2**) was obtained according to Ref.[16] Melting points were determined on a Laboratory Devices Mel-Temp II micromelting points apparatus and are uncorrected. UV spectra were measured on a Beckman DU-65 spectrophotometer. The ^{1}H (300 MHz) and ^{13}C NMR (75.5 MHz) spectra were recorded on a Varian Unity 300 FT NMR 300 MHz spectrometer in DMSO-d_6 with tetramethylsilane as an internal standard, and chemical shifts are reported in δ-values (ppm). High resolution mass spectra were taken on an AMD-604 spectrometer using the LSIMS technique (Cs^+, 9 keV; in NBA). Thin-layer chromatography (TLC) was conducted on Merck silica gel 60 F_{254} plates using the following solvent systems (measured by volume): A, chloroform – methanol (95:5); B, ethyl acetate – hexane (2:1); C, ethyl acetate – toluene (2:1). For preparative short-column chromatography Merck TLC gel H 60 was used.

$O^{2'}$,$O^{5'}$,N^4-Tripivaloylcytidine (3). Pivaloyl chloride (422 mg; 0.43 mL, 3.5 mmol) was added in one portion to an anhydrous suspension of cytidine (**1**; 243 mg, 1.0 mmol) in pyridine (5 mL) at 4 ^{o}C. The reaction mixture was maintained at this temperature for 21 h. The solvent was evaporated under diminished pressure. The resulting oil was dissolved in methylene chloride (25 mL) and washed with cold saturated solution of $NaHCO_3$ (20 mL). The organic layer was separated, dried over Na_2SO_4, and concentrated to a white solid foam. The product was crystallized from toluene, yield 394 mg (79%); mp 148-149 ^{o}C; R_f 0.24 (A), 0.39 (B), 0.43 (C);

^{1}H NMR 10.47 (bs, 1H, NH), 8.07 (d, 1H, H6), 7.29 (d, 1H, H5), 5.88 (d, *J* = 3.0 Hz, 1H, H1'), 5.64 (d, *J* = 5.4 Hz, 1H, 3'OH), 5.23 (dd, *J* = 3.0, 2.4 Hz, 1H, H2'), 4.34 (dd, *J* = 12.3, 2.4 Hz, 1H, H5'a), 4.25 (m, 2H, H3', H5'b), 4.08 (m, 1H, H4'), 1.20, 1.18, 1.17 (3s, 27H, 9 x CH_3). ^{13}C NMR 178.9, 177.3, 176.3 (3 x C=O), 163.4 (C4), 154.1 (C2), 145.2 (C6), 96.1 (C5), 89.3 (C1'), 81.4 (C4'), 75.0 (C2'), 67.8 (C3'), 63.2 (C5'), 38.2, 38.2 [$\underline{C}(CH_3)_3$], 26.8 [2 x C($\underline{C}H_3)_3$], 26.3 [C($\underline{C}H_3)_3$]. HRMS calcd for $[C_{24}H_{37}N_3O_8 + H]^+$ *m/z* 496.2659, found 496.2650.

$O^{2'}$,$O^{3'}$,$O^{5'}$,N^4-Tetrapivaloylcytidine (4). Filtrates collected after several series of crystallization of **3** were combined and evaporated to an oil (3.3 g). The mixture, which contained tri- (**3**) and tetrapivaloyl (**4**) derivatives of cytidine in a ratio *ca.* 2:1, respectively, was chromatographed on a silica gel column in chloroform – methanol (98:2, v/v). The first fractions, containing chromatographically homogenous compound **4**, were evaporated to a white solid foam (1.09 g). An attempted crystallization from toluene or its mixture with hexane, chloroform or ethyl acetate failed. R_f 0.62 (A), 0.70 (B), 0.67 (C); ^{1}H NMR 10.52 (s, 1H, NH), 8.10 (d, 1H, H6), 7.32 (d, 1H, H5), 5.95 (d, *J* = 4.2 Hz, 1H, H1'), 5.51 (dd, *J* = 5.4, 4.2 Hz, 1H, H2'), 5.40 (t, *J* = 5.4 Hz, 1H, H3') 4.38 (m, 1H, H4'), 4.33 (m, 2H, H5'), 1.22, 1,19, 1.18, 1.16 (4s, 36H, 12 x CH_3). ^{13}C NMR 179.0, 177.2, 176.3, 176.2 (4 x C=O), 163.6 (C4), 154.1 (C2), 145.6 (C6), 96.4 (C5), 89.3 (C1'), 79.5 (C4'), 73.0 (C2'), 69.9 (C3'), 62.8 (C5'), 38.2 [$\underline{C}(CH_3)_3$], 26.8, 26.7, 26.6, 26.3 [4 x C($\underline{C}H_3)_3$]. HRMS calcd for $[C_{29}H_{45}N_3O_9 + H]^+$ *m/z* 580.3234, found 580.3208. Further fractions contained a 2:1 mixture of **3** and its 2'-OH isomer **5** (TLC, ^{1}H NMR).

$O^{3'}$,$O^{5'}$,N^4-Tripivaloylcytidine (5). A crystalline sample of **3** (306 mg, 0.62 mmol) was dissolved in chloroform – methanol (4:1; 20 mL) and stirred with a portion of TLC silica gel (3.5 g) at room temperature for 24 h. After this time the TLC analysis showed the presence of **3** and its 2'-OH isomer **5** in a proportion of *ca.* 1:5, respectively. Stirring for a prolonged period of time (6 days), or at increased temperature (50 ^{o}C), did not change the isomeric ratio. The silica gel was filtered off and washed with chloroform. The filtrate was concentrated to an oil and the isomers were separated on a silica gel short column in ethyl acetate – toluene (2:1, v/v). The fractions containing the preferred isomer **5** were combined and evaporated to a solid foam (210 mg, 68%). The crystallization from toluene or its mixture with hexane failed. The product obtained in this manner was usually contaminated by traces of the starting isomer **3** (2-5%; as judged by ^{1}H NMR), but it could be applied for 2'-modification reactions without further purification.[15] R_f 0.34 (A), 0.28 (B), 0.33 (C); ^{1}H NMR 10.46 (bs, 1H, NH), 8.06 (d, 1H, H6), 7.29 (d, 1H, H5), 5.86 (d, *J* = 2.4 Hz, 1H, 2'OH), 5.83 (d, *J* = 3.6 Hz, 1H, H1'), 5.01 (t, *J* = 3.9 Hz, 1H, H3'), 4.41 (dd, *J* = 3.6, 2.4 Hz, 1H, H2'), 4.29 (m, 3H, H4', H5'b), 4.08 (m, 1H, H4'), 1.20, 1.18, 1.17 (3s, 27H, 9 x CH_3). ^{13}C NMR 178.9, 177.1, 176.6 (3 x C=O), 163.3 (C4), 154.5 (C2), 145.3 (C6), 96.3 (C5), 90.9 (C5), 79.0 (C4'), 71.7 (C2'), 71.4 (C3'), 63.2 (C5'), 38.2 [$\underline{C}(CH_3)_3$], 26.8 [2 x C($\underline{C}H_3)_3$], 26.3 [C($\underline{C}H_3)_3$]. HRMS calcd for $[C_{24}H_{37}N_3O_8 + H]^+$ *m/z* 496.2659, found 496.2620.

$O^{3'}$,$O^{5'}$,N^4-Tripivaloyl-2'-C-β-methylcytidine (6). The compound was obtained as described for preparation of **3**, starting from 2'-C-β-methylcytidine (**2**; 257 mg, 1.0 mmol). The crude product was purified by silica-gel chromatography in ethyl acetate – toluene (2:1, v/v). Yield

435 mg (85%) of a white solid foam. An analytical sample was crystallized from heptane, mp 81 ^{o}C; R_f 0.36 (A), 0.32 (B), 0.34 (C); ^{1}H NMR 10.48 (bs, 1H, NH), 8.07 (d, 1H, H6), 7.33 (d, 1H, H5), 5.98 (s, 1H, H1'), 5.81 (s, 1H, 2'OH), 5.28 (s, 1H, 2'OH), 4.90 (d, *J* = 9.3 Hz, 1H, H3'), 4.38-4.24 (m, 3H, H4', H5'), 1.19, 1.17, 1.16 (3s, 27H, 9 x CH_3), 0.98 (s, 3H, 2'CH_3). ^{13}C NMR 179.0, 177.1, 177.0 (3 x C=O), 163.1 (C4), 154.5 (C2), 144.1 (C6), 96.1 (C5), 93.0 (C1'), 78.0 (C4'), 77.2 (C2'), 72.9 (C3'), 62.5 (C5'), 38.3 [$\underline{C}(CH_3)_3$], 26.8, 26.7, 26.3 [3 x C($\underline{C}H_3)_3$], 20.1 (2'CH_3). HRMS calcd for $[C_{24}H_{37}N_3O_8 + H]^+$ *m/z* 510.2816, found 510.2802.

O$^{5'}$,N^4-Dipivaloyl-2'-C-β-methylcytidine (7). Pivaloyl chloride (1.25 g; 1.23 mL, 10.0 mmol) was added in one portion to an anhydrous suspension of 2'-C-β-methylcytidine (**2**; 1.03 g, 4.0 mmol) in pyridine (40 mL). The reaction mixture was stirred at room temperature for 3 h. The solvent was evaporated under reduced pressure without heating. The resulting oil was dissolved in methylene chloride (150 mL) and washed with cold saturated solution of $NaHCO_3$ (100 mL). The organic layer was separated, dried over Na_2SO_4, and concentrated to a white solid foam. The product was purified on a silica gel column in chloroform – methanol (98:2) to give chromatographically homogenous **7**, 1.19 g (70%) as a solid foam. The preparation can be used for further modification reaction without further purification. An analytical sample was crystallized from a mixture of toluene – chloroform, mp 202-205 ^{o}C; R_f 0.11 (A), 0.08 (B), 0.08 (C); ^{1}H NMR 10.43 (bs, 1H, NH), 7.96 (d, 1H, H6), 7.31 (d, 1H, H5), 5.96 (s, 1H, H1'), 5.44 (d, *J* = 6.6 Hz, 1H, 3'OH), 5.28 (s, 1H, 2'OH), 4.35 (d, *J* = 3.6 Hz, 2H, H5'), 4.06 (m, 1H, H4'), 3.61 (d, *J* = 6.6 Hz, 1H, H3'), 1.20, 1.19, (2s, 18H, 6 x CH_3), 0.97 (s, 3H, 2'CH_3). ^{13}C NMR 178.9, 177.3 (2 x C=O), 162.9 (C4), 154.6 (C2), 144.2 (C6), 95.9 (C5), 92.2 (C1'), 79.1 (C4'), 78.0 (C2'), 72.8 (C3'), 63.4 (C5'), 38.3 [$\underline{C}(CH_3)_3$], 26.8, 26.3 [2 x C($\underline{C}H_3)_3$], 19.8 (2'CH_3). HRMS calcd for $[C_{20}H_{31}N_3O_7 + H]^+$ *m/z* 426.2240, found 426.2215.

References

1. Matsuda, A.; Takenuki, K.; Sasaki, T.; Ueda, T. *J. Med. Chem.* **1991**, *34*, 234, and references cited therein.
2. Harry-O'kuru, R. E.; Smith, J. M.; Wolfe, M. S. *J. Org. Chem*. **1997**, *62*, 1754.
3. Pierra, C.; Amador, A.; Benzaria, S.; Creton-Scott, E.; D'Amours, M.; Mao, J.; Mathieu, S.; Moussa, A.; Bridges, E. G.; Standring, D. N.; Somadossi, J.-P.; Storer, R.; Gosselin, G. *J. Med. Chem.* **2006**, *49*, 6614, and references cited therein.
4. Li, N. S.; Piccirilli J. A. *J. Org. Chem.* **2003**, *68*, 6799.
5. Pierra, C.; Amador, A.; Badaroux, E.; Storer, R.; Gosselin, G. *Collect. Czech. Chem. Commun.* **2006**, *71*, 991.
6. Clark, J. L.; Hollecker, L.; Mason, J. C.; Stuyver, L. J.; Tharnish, P. M.; Lostia, S.; McBrayer, T. R.; Schinazi, R. F.; Watanabe, K. A.; Otto, M. J.; Furman, P. A.; Stec, W. J.; Patterson, S. E.; Pankiewicz, K. W. *J. Med. Chem.* **2005**, *48*, 5504.
7. Markiewicz, W. T. *J. Chem. Res. (S)* **1979**, 24.

8. Griffin, B. E.; Jarnan, M.; Reese, C. B.; Sulston, J. E. *Tetrahedron* **1967**, *23*, 2301, and references cited therein.
9. Nishino, S.; Yamamoto, H.; Nagato, Y.; Ishido, Y. *Nucleosides Nucleotides* **1986**, *5*, 159.
10. Kamaike K.; Uemura F.; Yanakage S.; Nishino S.; Ishido, Y. *Nucleosides Nucleotides* **1987**, *6*, 699.
11. Kawana M.; Nishikawa, M.; Noritsugu N.; Kuzuhara H. *J. Chem. Soc., Perkin Trans. 1* **1989**, 1593.
12. Nutt, B. F.; Dickinson, M. J.; Holly, F. W.; Walton, E. *J. Org. Chem.* **1968**, *33*, 1789.
13. Mikhailov, S. N.; Fomitcheva, M. V. *Bioorg. Khim.* **1990**, *16*, 692.
14. Mikhailov, S. N.; Beigelman, L. N.; Gurskaya, G. V.; Padyukova, N. S.; Yakovlev, G. Y.; Karpeisky, M. Y. *Carbohydrate Res.* **1983**, *124*, 75.
15. Fogt, J.; Januszczyk, P.; Boryski, J. – unpublished data.
16. Tang, X.-Q.; Liao, X.; Piccirili, J. A. *J. Org. Chem.* **1999**, *64*, 747.

