Ribavirin derivatives with a hexitol moiety: synthesis and antiviral evaluation

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Current standard therapy for the treatment of chronic infections with hepatitis C virus consists of combination therapy with (pegylated) interferon- α and ribavirin. 1,5-Anhydrohexitol nucleoside analogues are constrained congeners known to mimic the ribonucleoside conformation. Within this series some analogues are endowed with strong antiviral properties, particularly against herpesviruses. The six-membered anhydrohexitol ring was, therefore, combined with the triazolyl

carboxamide moiety of ribavirin, thus providing a new series of ribavirin analogues. None of the newly synthesized compounds elicited any substantial antiviral activity, neither against herpes simplex virus type 1 and 2, nor against bovine viral diarrhoea virus (a surrogate for hepatitis C virus) or the yellow fever virus.

Keywords: synthesis, antiviral, ribavirin, anhydrohexitol nucleosides, HCV

Introduction

Worldwide, more than 170 million people are chronic carriers of the hepatitis C virus (HCV) and are at high risk of developing fatal liver disease. The current treatment is based on a combination therapy of (pegylated) interferon- α and ribavirin (Barnard, 2001; Scot & Perry, 2002; Wilkinson, 2001). It may be of interest to design more potent analogues of ribavirin that can be used in the combination therapy with interferon. One such analogue, EICAR (5-ethynyl-1- β -D-ribofuranosylimidazole-4-carboxamide) (Balzarini *et al.*, 1993; De Clercq *et al.*, 1991; Neyts *et al.*, 1996), is 30- to 50-fold more potent than ribavirin against various RNA viruses, including flaviviruses.

Several possible mechanisms have been reported by which ribavirin may exert antiviral activity. The 5'-monophosphate derivative of ribavirin is a potent inhibitor of IMP dehydrogenase (Sidwell *et al.*, 1979), thus leading to decreased intracellular GTP pools and as a consequence reduced viral replication. Although IMP dehydrogenase is a cellular enzyme, it can be envisaged as a suitable target for antiviral intervention because of the increased need for DNA and RNA building blocks in the virus-infected cells. Ribavirin (as is 5'-monophosphate derivative) is a well-known inhibitor of this enzyme; it is approved for the treatment of respiratory syncytial virus infections and HCV infections. EICAR, as its monophosphate form, has been shown to be a more potent inhibitor of IMP dehydrogenase than ribavirin and this correlates with a more potent antiviral activity (Balzarini *et al.*, 1993; De Clercq *et al.*, 1991).

The 5'-triphosphate metabolite of ribavirin may in addition inhibit the guanylyltransferase and viral RNA-dependent RNA polymerase (RdRp), thus leading to, respectively, (i) inhibition of viral CAP formation (at least for those viruses that acquire a CAP via the action of the guanylyltransferase); and (ii) inhibition of RdRp activity. More recently, ribavirin was also reported to be able to induce an 'error-catastrophe', whereby ribavirin-induced mutagenesis leads to less viable, or unviable, virus (Crotty *et al.*, 2002; Crotty *et al.*, 2000).

1,5-Anhydrohexitol nucleoside analogues are constrained analogues known to mimic the ribonucleoside conformation. Some of these compounds (for example, the 5-iodouracil, 5-ethyluracil, cytosine and guanine base-containing congeners, Figure 1) have previously been reported as selective inhibitors of viral replication (Verheggen *et al.*, 1993; Verheggen *et al.*, 1995). Combination of the six-membered hexitol moiety with the triazole carboxamide heterocycle, therefore, was a logical extension to the series (Figure 2).

Materials and methods

General methods

The ¹H and ¹³C NMR spectra were recorded in the solvent indicated with a Varian Gemini 200 spectrometer and for some cases a Varian 500 Unity spectrometer at 300 K, unless otherwise indicated. For ¹H spectra taken in CDCl₃ tetramethylsilane (TMS) was used as reference. For spectra

Figure 1. Chemical structure of the known antiviral agents ribavirin and EICAR, and of the most relevant anhydrohexitol nucleoside analogues (Verheggen *et al.*, 1995)

taken in DMSO- d_6 , the solvent signal at 2.5 ppm was used as reference. For the ^{13}C spectra, the solvent peaks were used as reference (for example, 77.00 ppm for CDCl_3 and 39.6 ppm for DMSO- d_6). Exact mass measurements were performed on a quadrupole/orthogonal-acceleration time-of-flight (Q/oaTOF) tandem mass spectrometer (qTof 2, Micromass, Manchester, UK) equipped with a standard electrospray ionization (ESI) interface. Samples were infused in a 2-propanol/water (1:1) mixture at 3 $\mu\text{l}/\text{min}$. Precoated Machery-Nagel Alugram® SilG/UV 254 plates were used for TLC and spots were examined with ultraviolet light and sulfuric acid/anisaldehyde spray. Column chromatography was performed on ICN silica gel (63–200 nm, 60 Å). Anhydrous solvents were obtained storing analytical grade solvents on 3 Å molecular sieves for 3 days. Methyl 1H-1,2,4-triazole-3-carboxylate was obtained from Alkemi (Belgium); 4,6-O-benzylidene-1,5-anhydrohexitol was from CMS-chemicals (UK) and methyl glucopyranoside was bought from Acros Organics (Belgium).

Purity of all compounds obtained was verified by TLC analysis, and identity was verified by MS, ^1H and ^{13}C NMR analysis. In view of the lack of meaningful antiviral activity, no elementary analysis was carried out.

Antiviral evaluation

Anti-bovine viral diarrhoea virus (BVDV, PE515 strain) activity was assessed on MDBK cells and anti-yellow fever

virus (YFV, vaccine strain 17D) activity on Vero cells. Briefly, confluent cultures grown in 96 plates were infected with 100 CCID $_{50}$ /well of virus and cultures were further incubated at 37°C for another 5 (BVDV) or 7 days (YFV), after which antiviral activity was recorded microscopically. Evaluation of activity against vesicular stomatitis virus, parainfluenza-3 virus, reovirus-1, Sindbis virus, Coxsackie virus B4, Punta Toro virus, respiratory syncytial virus, herpes simplex virus type 1 and 2, vaccinia virus was performed as described earlier (Neyts *et al.*, 1995).

Synthesis of the 1,5-anhydrohexitol nucleoside analogues

1,5-Anhydro-4,6-O-benzylidene-2-(methyl 1H-1,2,4-triazol-1-yl-3-carboxylate)-2-deoxy-D-altro-hexitol (**6**)

1,2,4-Triazole-3-methylcarboxylate (1.27 g, 10 mmol) was suspended in 50 ml of anhydrous DMF, to which was added under argon 380 mg of a 60% oil dispersion of sodium hydride (9.5 mmol) and the mixture was heated on an oil bath for 30 min at 90°C. The allitol epoxide **5** (Allart *et al.*, 1999) (703 mg, 3 mmol) was added and the mixture was heated for 15 h at 90°C, after which the reaction was cooled, quenched with sodium bicarbonate and concentrated. The residue was partitioned between 200 ml of ethyl acetate and 200 ml of 5% aqueous sodium bicarbonate, and the organics were washed twice with brine. Purification of the organic residue on 70 g of silica gel (slow gradient of CH_3OH in CH_2Cl_2 0–2%) afforded 560 mg (1.55 mmol, 52%) of the title compound **6** as a foam.

^1H -NMR (CDCl_3) δ : 8.54 (s, 1H, H5), 7.44–7.33 (m, 5H, arom-H), 5.53 (s, 1H, PhCH), 4.62 (m, 2H, H2', H3'), 4.42 (dd, 1H, $J=2.4$ and 13.6 Hz, H1'e), 4.37 (dd, 1H, $J=4.9$ and 10.2 Hz, H6'e), 4.26 (d, 1H, $J=13.2$ Hz, H1'a), 4.08 (dt, 1H, $J=4.9$ and 10.0 Hz, H5'), 4.02 (s, 3H, COOCH_3), 3.71 (t, 1H, $J=10.2$ Hz, H6'a), 3.52 (dd, 1H, $J=2.4$ and 9.8 Hz, H4') ppm;

^{13}C -NMR (CDCl_3) δ : 159.96 (CO), 154.86 (C3), 144.40 (C5), 136.75 (Ci), 129.33 (Cp), 128.31 (Co), 126.06 (Cm), 102.16 (PhCH), 76.18 (C4'), 68.78 (C6'), 67.31 (C5'), 66.92 (C3'), 64.32 (C1'), 61.13 (C2'), 52.83 (OCH_3);

ESI-MS pos.: calcd. for $\text{C}_{17}\text{H}_{20}\text{N}_3\text{O}_6$ 362.1352; found 362.1370 $[\text{M}+\text{H}]^+$.

1,5-Anhydro-2-(methyl 1H-1,2,4-triazol-1-yl-3-carboxylate)-2-deoxy-D-altro-hexitol (**8**)

The triazole methylcarboxylate analogue **6** (400 mg, 1.1 mmol) obtained in the previous preparation was dissolved in 20 ml of methanol and 1 ml of TFA was added. The mixture was stirred for 3 h, after which the mixture was evaporated and co-evaporated with dioxane. The title product partially crystallized from a methanol/toluene

mixture affording 144 mg (0.52 mmol, 48%) of the title compound.

$^1\text{H-NMR}$ (DMSO) δ : 8.76 (s, 1H, H5), 5.40 (d, 1H, $J=4.4$ Hz, 4'-OH), 4.80 (d, 1H, $J=6.3$ Hz, 3'-OH), 4.56 (t, 1H, $J=5.6$ Hz, 6'-OH), 4.50 (dt, 1H, H2'), 4.14 (dd, 1H, H3'), 4.12 (dd, 1H, $J=3.0$ and 12.7 Hz, H1'), 4.01 (dd, 1H, $J=3.4$ and 12.7 Hz, H1'), 3.64–3.57 (m, 2H, H5', H6'A), 3.52 (dd, $J_{5',6B}=4.9$ and $J_{6',6}=11.2$ Hz, 1H, H6'B), 3.44–3.39 (m, 1H, H4'), 3.30 (s, 3H, OCH_3);

$^{13}\text{C-NMR}$ (CDCl_3) δ : 159.93 (CO), 153.43 (C3), 145.55 (C5), 77.84 (C5'), 67.87 (C3'), 64.49 (C4'), 62.79 (C1'), 60.97 (C2'), 60.39 (C6'), 52.14 (OCH_3);

ESI-MS pos.: calcd. for $\text{C}_{10}\text{H}_{16}\text{N}_3\text{O}_6$ 274.1039; found 274.1048 $[\text{M}+\text{H}]^+$.

1,5-Anhydro-4,6-O-benzylidene-2-(1H-1,2,4-triazol-1-yl-3-carboxamide)-2-deoxy-D-altro-hexitol (9**)**

The methyl triazolylcarboxylate derivative **6** (530 mg, 1.47 mmol) was dissolved in 30 ml of a 2 M solution of ammonia in methanol and stirred overnight at room temperature, after which the mixture was evaporated and the residue was adsorbed on silica gel. Chromatographic purification (0–5% gradient of CH_3OH in CH_2Cl_2) yielded 380 mg (1.1 mmol, 75%) of **9** as a foam.

$^1\text{H-NMR}$ ($\text{CDCl}_3+\text{CD}_3\text{OD}$) δ : 8.59 (s, 1H, H5); 7.44–7.33 (m, 5H, arom-H), 5.52 (s, 1H, PhCH), 4.53 (m, 2H, H2', H3'), 4.39 (dd, 1H, $J=1.7$ and 13.5 Hz, H1'e), 4.30 (d, 1H, $J=13.7$ Hz, H1'a), 4.29 (dd, 1H, $J=5.3$ and 10.2 Hz, H6'e), 4.08 (dt, 1H, $J=4.9$ and 10.0 Hz, H5'), 3.70 (t, 1H, $J=10.5$ Hz, H6'a), 3.55 (dd, 1H, $J=2.0$ and 9.8 Hz, H4');

$^{13}\text{C-NMR}$ (CDCl_3) δ : 161.22 (CO), 155.89 (C3), 143.94 (C5), 136.75 (Ci), 128.35 (Cp), 127.40 (Co), 125.61 (Cm), 101.61 (PhCH), 75.85 (C4'), 68.16 (C6'), 66.36 (C5'), 65.82 (C3'), 63.47 (C1'), 61.52 (C2');

ESI-MS pos.: calcd. for $\text{C}_{16}\text{H}_{19}\text{N}_4\text{O}_5$ 347.1355; found 347.1328 $[\text{M}+\text{H}]^+$.

1,5-Anhydro-2-(1H-1,2,4-triazol-1-yl-3-carboxamide)-2-deoxy-D-altro-hexitol (1**)**

An amount of 380 mg (1.1 mmol) of the triazole carboxamide analogue **9** was dissolved in 20 ml of methanol and 1 ml of TFA was added. The mixture was stirred for 4 h, after which a precipitate was formed. After evaporation and co-evaporation with dioxane the mixture was crystallized from boiling ethanol affording 223 mg (0.86 mmol, 78%) of the title compound **1**.

$^1\text{H-NMR}$ (DMSO) δ : 8.67 (s, 1H, H5), 7.75 (s, 1H) and 7.54 (s, 1H) (NH_2), 5.40 (d, 1H, $J=4.4$ Hz, 3'-OH), 4.78 (d, 1H, $J=5.8$ Hz, 4'-OH), 4.55 (t, 1H, 6'-OH), 4.45 (dt, 1H, $J_{2',3'}=5.1$ and $J_{2',1'}=3.2$ Hz, H2'), 4.14 (dd, 1H, $J=3.2$ and 4.9 Hz, H3'), 4.12 (dd, 1H, $J=3.0$ and 12.5 Hz, H1'), 4.01 (dd, 1H, $J=3.2$ and 12.5 Hz, H1'), 3.63 (dd, $J_{5',6A}=2.9$ and $J_{6',6}=11.3$ Hz, 1H, H6'A), 3.60 (ddd, $J=2.9$, 5.0 and 7.9

Figure 2. Main target nucleoside analogues **1–4**, resulting from combination of the 1,5-anhydrohexitol moiety with the heterocyclic base 1,2,4-triazole-3-carboxamide as in ribavirin

Structures **3** and **4** have a different conformation due to the steric repulsion between the substituents on carbons 1' and 3'.

HZ, 1H, H5'), 3.52 (dd, $J_{5',6B}=5.0$ and $J_{6',6}=11.3$ Hz, 1H, H6'B), 3.38 (dd, 1H, $J_{3',4'}=3.2$ and $J_{4',5'}=7.8$ Hz, H4');

$^{13}\text{C-NMR}$ (CDCl_3) δ : 160.55 (CO), 156.53 (C3), 144.70 (C5), 77.73 (C5'), 67.67 (C3'), 64.20 (C4'), 62.62 (C1'), 60.59 (C2'), 60.21 (C6');

ESI-MS pos.: calcd. for $\text{C}_9\text{H}_{15}\text{N}_4\text{O}_5$ 259.1042; found 259.1048 $[\text{M}+\text{H}]^+$.

1,5-Anhydro-4,6-O-benzylidene-3-O-methyl-2-(methyl 1H-1,2,4-triazol-1-yl-3-carboxylate)-2-deoxy-D-altro-hexitol (7**)**

An amount of 900 mg (2.5 mmol) of the triazolidine **6** was dissolved under argon in 20 ml dry DMF and cooled on an ice bath. A 60% NaH dispersion in oil was added (120 mg, 3 mmol) and the mixture was stirred for 45 min at 0°C, after which methyl iodide (0.25 ml, 4 mmol) dissolved in 10 ml of DMF was added over 30 min. After stirring for 3 h at room temperature, the reaction was quenched with 3 ml of water and stirred further for 10 min. Following concentration, the mixture was diluted with 70 ml of ethyl acetate and washed twice with 50 ml of 5% aqueous NaHCO_3 and once with 50 ml of brine. The aqueous washes were again extracted with 50 ml of ethyl acetate. Chromatographic purification on silica gel (CH_2Cl_2 to $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$ 98:2) afforded 647 mg (1.72 mmol, 70%) of the sugar methylated product **7**.

$^1\text{H-NMR}$ (CDCl_3) δ : 8.57 (s, 1H, H5), 7.45–7.33 (m, 5H, arom-H), 5.40 (s, 1H, PhCH), 4.61 (t, 1H, $J=2.4$ Hz, H2'), 4.38 (dd, 1H, $J=2.4$ and 13.2 Hz, H1'e), 4.34 (dd, 1H, $J=5.4$ and 10.2 Hz, H6'e), 4.31 (d, 1H, $J=13.2$ Hz, H1'a), 4.23 (t, 1H, $J=5.9$ Hz, H3'), 4.11 (dt, 1H, $J=5.1$ and 9.9 Hz, H5'), 4.03 (s, 3H, COOCH_3), 3.66 (s, 3H, 3'-

OCH₃), 3.65 (t, 1H, J=10.5 Hz, H6'a), 3.44 (dd, 1H, J=2.7 and 9.5 Hz, H4');

¹³C-NMR (CDCl₃) δ: 160.02 (CO), 155.00 (C3), 144.40 (C5), 137.20 (Ci), 129.10 (Cp), 128.24 (Co), 126.09 (Cm), 102.42 (PhCH), 75.54 (C4'), 68.93 (C6'), 67.55 (C5'), 64.16 (C3'), 60.76 and 60.23 (C1', C2'), 52.79 (2xOCH₃);

ESI-MS pos.: calcd. for C₁₈H₂₂N₃O₆ 376.1509; found 376.1496 [M+H]⁺.

1,5-Anhydro-3-O-methyl-2-(1H-1,2,4-triazol-1-yl-3-carboxamide)-2-deoxy-D-altro-hexitol (2)

The foam **7** obtained in the previous preparation (408 mg, 1.08 mmol) was dissolved in 25 ml of a 2 M solution of ammonia in methanol and stirred overnight at room temperature, after which the mixture was evaporated and the residue was adsorbed on silica gel. Purification on silica gel (gradient of CH₃OH in CH₂Cl₂ 0–10%) yielded 350 mg (0.97 mmol, 90%) of **10** as a white foam.

ESI-MS pos. calcd. for C₁₇H₂₁N₄O₅ 361.1512; found 361.1534 [M+H]⁺.

Following dissolution of the foam in 25 ml of methanol, 1.25 ml of TFA were added and the mixture was stirred for 4 h at room temperature. Evaporation left an oil that was crystallized from methanol/diisopropyl ether, affording 188 mg (0.69 mmol, 71%) of the title compound **2**.

¹H-NMR (DMSO-d₆) δ: 8.70 (s, 1H, H5), 7.77 (s, 1H) and 7.56 (s, 1H) (NH₂), 5.0–4.6 (br, 2xOH), 4.66 (dt, 1H, J_{2',3'}=4.9 and J_{2',1'}=3.5 Hz, H2'), 4.15 (dd, 1H, J=3.6 and 12.5 Hz, H1'), 3.94 (dd, 1H, J=3.9 and 12.7 Hz, H1'), 3.87 (dd, 1H, J=2.9 and 4.9 Hz, H3'), 3.62 (dd, J_{5',6'A}=2.9 and J_{6',6}=11.3 Hz, 1H, H6'A), 3.60–3.54 (m, 2H, H4', H5'), 3.52 (dd, J_{5',6'B}=4.7 and J_{6',6}=11.0 Hz, 1H, H6'B), 3.42 (s, 3H, OCH₃);

¹³C-NMR (CDCl₃) δ: 160.53 (CO), 156.65 (C3), 144.93 (C5), 78.38, 77.91 (C3', C5'), 63.38, 63.12 (C4', C1'), 60.21, 58.08, 57.80 (C2', C6', OCH₃);

ESI-MS pos.: HRMS calcd. for C₁₀H₁₇N₄O₅ 273.1199; found 273.1189 [M+H]⁺.

Synthesis of the methyl pyranoside nucleoside analogues

Methyl 1,5;2,3-dianhydro-4,6-O-benzylidene-D-allo-hexopyranoside (11)

The title compound was prepared largely following literature procedures (Richtmeyer & Hudson, 1941; Rosenfeld et al., 1948).

¹H-NMR (CDCl₃) δ: 7.52–7.33 (m, 5H, arom-H), 5.56 (s, 1H, PhCH), 4.88 (d, 1H, J=2.7 Hz, H1), 4.24 (dd, 1H, J=5.0 and 10.2 Hz, H6'e), 4.08 (m, 1H, H5), 3.94 (dd, 1H, J=1.1 and 9.1 Hz, H4), 3.68 (t, 1H, J=6.9 Hz, H6'a), 3.51 (dm, 1H, J=4.4 Hz, H3), 3.48 (dd, 1H, J=2.9 and 4.4 Hz, H2), 3.47 (s, 3H, OCH₃);

¹³C-NMR (CDCl₃) δ: 137.18 (Ci), 129.20 (Cp), 128.29 (Cm), 126.32 (Co), 102.77 (PhCH), 95.35 (C1), 77.92 (C4), 68.92 (C6), 60.06 (C5), 55.82 (OCH₃), 53.10 (C2), 50.68 (C3);

ESI-MS pos.: calcd. for C₁₄H₁₇O₅ 265.1076; found 265.1082 [M+H]⁺.

Methyl 4,6-O-benzylidene-2-(methyl 1H-1,2,4-triazol-1-yl-3-carboxylate)-2-deoxy-D-altro-hexopyranoside (12)

Methyl 1H-1,2,4-triazole-3-carboxylate (5.72 g, 45 mmol) was suspended in 100 ml of anhydrous DMF, to which was added under argon 1.72 g of a 60% oil dispersion of sodium hydride (43 mmol) and the mixture was heated on an oil bath for 1 h at 90°C. The alloside epoxide **11** (3.52 g, 15 mmol) was added and the mixture was heated for 48 h at 90°C, after which TLC analysis indicated the formation of several minor products. The reaction was cooled, quenched with sodium bicarbonate and concentrated. The residue was partitioned between 200 ml of ethyl acetate and 200 ml of 5% aqueous sodium bicarbonate, the aqueous phase was extracted twice more and the organics were washed twice with brine. Purification of the organic residue on 70 g of silica gel (slow gradient of CH₃OH in CH₂Cl₂ 0–2%) afforded 1220 mg (3.12 mmol, 21%) of the title compound **12** as a foam, which was crystallized from acetone/diisopropyl ether.

¹H-NMR (CDCl₃) δ: 8.40 (s, 1H, H5), 7.46–7.34 (m, 5H, arom-H), 5.62 (s, 1H, PhCH), 5.05 (s, 1H, H1'), 4.87 (d, 1H, J=2.4 Hz, H2'), 4.45–4.39 (m, 3H, H3', H5', H6'e), 4.01 (s, 3H, COOCH₃), 3.89 (dd, J=3.4 and 9.3 Hz, 1H, H4'), 3.87 (t, J=11.7 Hz, 1H, H6'a), 3.52 (s, 3H, 1'-OCH₃);

¹³C-NMR (CDCl₃) δ: 159.81 (CO), 155.11 (C3), 144.20 (C5), 136.78 (Ci), 129.31 (Cp), 128.29 (Cm), 126.15 (Co), 102.25 (PhCH), 98.95 (C1'), 75.26 (C4'), 68.92 (C6'), 67.75 (C3'), 62.88 (C2'), 58.31 (C5'), 56.03 (1'-OCH₃), 52.82 (COOCH₃);

ESI-MS pos. calcd. for C₁₈H₂₂N₃O₇ 392.1458; found 392.1465 [M+H]⁺.

In addition 800 mg of the epoxide **11** (3.0 mmol, 20%) was recovered, and 291 mg (0.87 mmol, 6%) of the decarboxylated triazolidine **13** was isolated as a foam.

Methyl 4,6-O-benzylidene-2-(1H-1,2,4-triazol-1-yl)-2-deoxy-D-altro-hexopyranoside (13)

¹H-NMR (CDCl₃) δ: 8.22 (s, 1H, H5), 7.98 (s, 1H, H3), 7.46–7.34 (m, 5H, arom-H), 5.63 (s, 1H, PhCH), 4.93 (s, 1H, H1'), 4.75 (d, 1H, J=2.4 Hz, H2'), 4.45–4.38 (m, 2H, H5', H6'e), 4.31 (brs, 1H, H3'), 4.03 (dd, 1H, J=3 and 9 Hz, H4'), 3.87 (t, 1H, J=12 Hz, H6'a), 3.49 (s, 3H, 1'-OCH₃);

¹³C-NMR (CDCl₃) δ: 152.13 (C3), 142.97 (C5), 136.96 (Ci), 129.26 (Cp), 128.26 (Cm), 126.17 (Co),

102.19 (PhCH), 99.44 (C1'), 75.51 (C4'), 69.02 (C6'), 68.04 (C3'), 62.00 (C2'), 58.12 (C5'), 55.86 (1'-OCH₃);

ESI-MS pos.: calcd. for C₁₆H₂₀N₃O₅ 334.1403; found 334.1418 [M+H]⁺.

Methyl 4,6-O-benzylidene-2-(1H-1,2,4-triazol-1-yl-3-carboxamide)-2-deoxy-D-altro-hexopyranoside (14)

The methyl carboxylate **12** obtained in the previous preparation (500 mg, 1.28 mmol) was dissolved in 20 ml of a 2 M solution of ammonia in methanol and stirred overnight at room temperature, after which the mixture was evaporated and the residue was adsorbed on silica gel. Flash purification on silica gel (gradient of CH₃OH in CH₂Cl₂ 0–7%) yielded 450 mg of **14** (1.19 mmol, 93%) as a foam.

¹H-NMR (DMSO-d₆) δ: 8.74 (s, 1H, H5), 7.46–7.34 (m, 5H, arom-H), 5.73 (s, 1H, PhCH), 5.50 (d, 1H, 3'-OH), 5.00 (s, 1H, H1'), 4.73 (d, 1H, J=2.5 Hz, H2'), 4.25 (dd, 1H, J=5.1 and 9.5 Hz, H6'e), 4.20 (dd, 1H, J=5.3 and 9.9 Hz, H5'), 4.17 (t, 1H, J=2.8 Hz, H3'), 4.01 (dd, J=3.2 and 9.5 Hz, 1H, H4'), 3.82 (t, J=9.7 Hz, 1H, H6'a), 3.35 (s, 3H, 1'-OCH₃);

¹³C-NMR (DMSO-d₆) δ: 160.38 (CO), 156.87 (C3), 145.11 (C5), 137.79 (Ci), 128.79 (Cp), 127.92 (Cm), 126.39 (Co), 100.95 (PhCH), 98.74 (C1'), 75.02 (C4'), 68.17 (C6'), 66.47 (C3'), 63.16 (C2'), 57.53 (C5'), 54.86 (1'-OCH₃);

ESI-MS pos.: calcd. for C₁₇H₂₁N₄O₆ 377.1461; found 377.1450 [M+H]⁺.

Methyl 2-(1H-1,2,4-triazol-1-yl-3-carboxamide)-2-deoxy-D-altro-hexopyranoside (3)

The triazole carboxamide derivative **14** (404 mg, 1.07 mmol) was dissolved in 25 ml of methanol, to which was added 0.25 ml of acetic acid. The solution was degassed by bubbling nitrogen for 10 min, after which 250 mg of 10% Pd on carbon was added and the mixture was hydrogenated for 4 h on a Parr apparatus at 45 psi. The mixture was filtered, the filter was washed with hot ethanol, the volatiles were removed *in vacuo* and the residue was co-evaporated twice with dioxane. Column purification on silica gel (gradient of CH₃OH in CH₂Cl₂ 4–16%) and crystallisation from CH₃OH/toluene afforded the title compound **3** in 78% yield (242 mg, 0.84 mmol).

¹H-NMR (DMSO-d₆) δ: 8.65 (s, 1H, H5), 7.73 (s, 1H) and 7.53 (s, 1H) (NH₂), 5.04 (d, 1H, J=3 Hz, 4'-OH), 4.97 (d, J=6.8 Hz, 1H, H1'), 4.96–4.88 (m, 2H, 3'-OH, 6'-OH), 4.36 (dd, 1H, J_{2',1}=6.8 Hz, J_{2',3}=10.3 Hz, H2'), 4.08–4.02 (m, 1H, H3'), 3.86–3.80 (m, 2H, H4', H5'), 3.62 (m, 2H, H6'), 3.24 (s, 3H, 1'-OCH₃);

¹³C-NMR (DMSO-d₆) δ: 160.67 (CO), 157.11 (C3), 146.41 (C5), 99.10 (C1'), 77.41 (C5'), 67.62 (C3'), 67.07 (C4'), 62.52 (C2'), 60.97 (C6'), 55.13 (OCH₃);

ESI-MS pos.: calcd. for C₁₀H₁₇N₄O₆ 289.1148; found 289.1136 [M+H]⁺.

Methyl 4,6-O-benzylidene-3-O-methyl-2-(1H-1,2,4-triazol-1-yl-3-carboxamide)-2-deoxy-D-altro-hexopyranoside (15)

An amount of 626 mg (1.60 mmol) of the methyl carboxylate **12** was dissolved under argon in 10 ml dry DMF and cooled on an ice bath. A 60% NaH dispersion in oil was added (84 mg, 1.9 mmol) and the mixture was stirred for 45 min at 0°C, after which methyl iodide (0.16 ml, 2.6 mmol) dissolved in 10 ml of DMF was added over 60 min. After stirring for 2 h more at room temperature, the reaction was quenched with 3 ml of aqueous sodium bicarbonate and stirred for an additional 10 min. Following concentration, the mixture was partitioned between 2x70 ml of ethyl acetate and 2x50 ml of 5% aqueous NaHCO₃ and 1x50 ml of brine. Chromatographic purification on silica gel (CH₂Cl₂ to CH₂Cl₂/CH₃OH 98:2) afforded 276 mg (0.68 mmol, 42%) of the sugar-methylated product.

ESI-MS pos.: calcd. for C₁₉H₂₄N₃O₇ 406.1614; found 406.1612 [M+H]⁺.

The sugar-methylated analogue thus obtained was suspended in 15 ml of a 2 M solution of ammonia in methanol, to which was added 5 ml of dioxane, and the mixture was stirred overnight at room temperature. Thereafter, the mixture was evaporated and the residue was adsorbed on silica gel. Flash purification on silica gel (gradient of CH₃OH in CH₂Cl₂ 0–4%) yielded 228 mg (0.58 mmol, 86%) of **15** as a white foam.

¹H-NMR (DMSO-d₆) δ: 8.79 (s, 1H, H5), 7.84 (s, 1H) and 7.63 (s, 1H) (NH₂), 7.44–7.35 (m, 5H, arom-H), 5.71 (s, 1H, PhCH), 5.00 (s, 1H, H1'), 4.95 (d, 1H, J=2.0 Hz, H2'), 4.27 (dd, 1H, J=5.4 and 10.3 Hz, H6'e), 4.25 (dd, J=3.4 and 9.7 Hz, 1H, H4'), 4.19 (dt, 1H, J=5.2 and 10.0 Hz, H5'), 3.96 (t, 1H, J=2.4 Hz, H3'), 3.81 (t, J=10.0 Hz, 1H, H6'a), 3.47 (s, 3H, OCH₃), 3.35 (s, 3H, OCH₃);

¹³C-NMR (DMSO-d₆) δ: 160.38 (CO), 156.89 (C3), 145.53 (C5), 137.80 (Ci), 128.86 (Cp), 128.09 (Cm), 126.19 (Co), 101.00 (PhCH), 98.63 (C1'), 75.94 (C3'), 75.16 (C4'), 68.19 (C6'), 59.55 (C2'), 58.69 (3'-OCH₃), 58.09 (C5'), 54.94 (1'-OCH₃);

ESI-MS pos.: calcd. for C₁₈H₂₂N₄O₆Na 413.1437; found 413.1445 [M+Na]⁺.

Methyl 3-O-methyl-2-(1H-1,2,4-triazol-1-yl-3-carboxamide)-2-deoxy-D-altro-hexopyranoside (4)

The foam **15** obtained in the previous preparation (218 mg, 0.56 mmol) was dissolved in 30 ml of methanol-dioxane 5/1, to which was added 0.25 ml of acetic acid. The solution was degassed by bubbling nitrogen for 10 min, after which 250 mg of 10% Pd on carbon was added and the mixture was hydrogenated for 4 h on a Parr apparatus at 45 psi. The

mixture was filtered, the filter was washed several times with hot ethanol, the volatiles were removed *in vacuo* and the residue was coevaporated twice with dioxane. Column purification on silica gel (gradient of CH₃OH in CH₂Cl₂ 3–12%) afforded 160 mg (0.53 mmol, 94%) of the title compound as a foam partially crystallizing from methanol.

¹H-NMR (DMSO-d₆) δ: 8.72 (s, 1H, H5), 7.75 (s, 1H) and 7.56 (s, 1H) (NH₂), 5.03 (brs, 1H, 4'-OH), 4.96 (d, J=6.8 Hz, 1H, H1'), 4.94 (brs, 1H, 6'-OH), 4.48 (dd, 1H, J_{2',1'}=6.8 Hz, J_{2',3'}=10.3 Hz, H2'), 4.10 (br, 1H, H4'), 3.87 (m, 1H, H5'), 3.83 (dD, 1H, J=3.9 and 10.3 Hz, H3'), 3.64 (dAB, 2H, J=12.3 Hz, J_{5',6'}=5.9 Hz, H6'), 3.24 (s, 3H, 1'-OCH₃), 3.11 (s, 3H, 3'-OCH₃);

¹³C-NMR (DMSO-d₆ + D₂O) δ: 160.56 (CO), 157.14 (C3), 146.38 (C5), 98.98 (C1'), 77.68 (C5'), 76.01 (C3'), 63.20 (C4'), 60.83 (C6' and C2'), 56.33 (3'-OCH₃), 55.15 (1'-OCH₃);

ESI-MS pos.: calcd. for C₁₁H₁₉N₄O₆ 303.1305; found 303.1327 [M+H]⁺.

Results

Synthesis of the alitol analogue **1** and its 3'-O-methylated congener **2** is depicted in Figure 3. The route followed has been previously described for preparation of althrohexitol nucleoside monomers (Allart *et al.*, 1999). Starting from commercially available 1,5-anhydrohexitol, ring opening of the 4,6-O-benzylidene protected allitol epoxide **5** with the 3-methylcarboxylate derivative of 1,2,4-triazole furnished the althrohexitol derivative **6** in 52% yield. The site of alkylation proved difficult to ascertain, but a ghmhc spectrum (indicative of ²J and ³J couplings) displayed a C5–H2' connectivity confining the alkyl group to either N1 or N4. As no connectivity could further be found for H2' with C3, the hexitol had to reside on N1.

Further alkylation afforded 70% of the methylated nucleoside **7**. Treatment of **6** and **7**, respectively, with ammonia in methanol afforded the triazolyl carboxamide analogues **9** and **10**, respectively, which were deprotected with trifluoroacetic acid (TFA) to yield the target compounds **1** and **2**. The small 1',2'-coupling constants are indicative of the predominant axial orientation of the base moiety (⁴C₁). Alternatively, the methyl carboxylate moiety could be preserved via direct deprotection with TFA, affording **8**.

The 1'-O-methylglycosidic analogues **3** and **4** (Figure 4) could be obtained starting from ubiquitous methyl glucopyranoside, which in three steps was converted to **11** according to literature procedures (Neyts *et al.*, 1996; Richtmeyer & Hudson, 1941). Detailed NMR analysis confirmed the epoxide to possess the *allo*-configuration. Opening of the epoxide ring of the methyl 2,3-anhydro-*allo*-hexopyranoside **11** with methyl 1,2,4-triazole-3-carboxylate went

more sluggishly (48 h at 90°C) in comparison to opening of the epoxide **5**, and afforded after careful chromatography 21% of the triazolyl congener **12**, along with 20% of the starting epoxide and 6% of the decarboxylated triazolyl derivative **13**. The alkylation site could be decided on the same basis as for **6** via gHMBC NMR spectral analysis. Further treatment of **12** with ammonia in methanol to yield **14** and deprotection via hydrogenation afforded the target congener **3**. Optionally, **12** could be further methylated with methyl iodide prior to treatment with ammonia, affording **15** in 42% yield. Notable for all NMR analyses of **12** through **15** is the high field position of the C5' signal (with δ around 58 ppm), which could be explained by the formation of a distorted chair conformation due to the steric hindrance of both axial substituents on C1' and C3'.

Further deprotection of **15** via hydrogenation afforded the derivative **4**. The larger 6.8 Hz 1',2'-coupling constant for the latter compounds **3** and **4**, along with a large J_{2',3'} of 10.3 Hz, proves the equatorial orientation of the base along with equatorially oriented methoxyl substituents, yielding a ¹C₄ conformation. The steric hindrance between both methoxyl substituents precludes their axial orientation and, therefore, imposes the equato-

Figure 3. Synthetic scheme for the 1,5-anhydrohexitol analogues **1** and **2**

a) for synthesis of the allitol epoxide, see Allart *et al.*, 1999; **b**) 1,2,4-triazole-3-methylcarboxylate 3.2 eq., 3.0 eq. NaH, DMF 90°C 15 h (52%); **c**) 1.2 eq. NaH, 30 min. at 0°C, 1.6 eq. CH₃I slowly added, DMF, 3 h RT (70%); **d**) 2 M NH₃ in CH₃OH, (**9**: 75%; **10**: 90%); **e**) 5% TFA in CH₃OH (**1**: 78%; **2**: 71%; **8**: 48%).

Figure 4. Synthetic scheme for the 1,5-anhydrohexitol analogues **3** and **4**

a) C₆H₅CHO, ZnCl₂, 72 h (66%); b) 6 eq. CH₃C₆H₄SO₂Cl, pyridine, 72 h, 60°C (78%); c) NaOCH₃, CH₃OH, CH₂Cl₂ (79%); d) 1,2,4-triazole-3-methylcarboxylate 3.0 eq., 2.8 eq. NaH, DMF 90°C 48 h (12: 21% yield was obtained along with a 20% recovery of epoxide **11** and 6% of the decarboxylated triazole **13**); e) 1.2 eq. NaH, 30 min. at 0°C, 1.6 eq. CH₃I slowly added, DMF, 2 h RT (42%); f) 2 M NH₃ in CH₃OH (14: 93%; 15: 86%); g) hydrogenation with Pd/C, 4 h at 45 psi (3: 78%; 4: 78%).

rial position on the base moiety, in contrast with other hexitol nucleoside analogues.

Discussion

The anhydrohexitol nucleosides with a triazolyl carboxamide base moiety **1–4** were evaluated for a possible inhibitory effect on various viruses that are (to some extent) inhibited by ribavirin. These include the pestivirus BVDV and the flavivirus YFV. Both BVDV and YFV belong, akin to HCV, to the family of the *Flaviviridae*. However, unlike ribavirin, none of these compounds proved antivirally effective up to a concentration of 400 µg/ml. Nor could any cytotoxicity be witnessed in a number of cell lines [Vero (African green monkey kidney) cells, MDBK (Madin-Darby bovine kidney) cells, HEL (human embryonic lung) cells or HeLa (human cervical carcinoma epithelial) cells]. Only marginal activity was found for the 3'-O-methylated analogue **2** against BVDV [50% effective concentration (EC₅₀) of 64 µg/ml], while the EC₅₀ value for inhibition of

BVDV and YFV replication by ribavirin is 18 ± 5 and 28 ± 18 µg/ml, respectively. The compounds were in addition evaluated, but found inactive, against several RNA viruses (vesicular stomatitis virus, parainfluenza-3 virus, reovirus-1, Sindbis virus, Coxsackie virus B4, Punta Toro virus and respiratory syncytial virus) and DNA viruses (herpes simplex virus type 1 and 2, vaccinia virus) in the appropriate cell lines (Vero, HEL or HeLa cells).

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