

Synthesis of N,O-homonucleosides with high conformational freedom

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Dedicated to Prof Nicolò Vivona on his 70th birthday

Abstract

The 1,3-dipolar cycloaddition of vinyloxymethyl thymine with different nitrones has been exploited for the preparation of N,O-homonucleosides where the oxymethylene tether replaces the aminal linkage between the sugar moiety and the nucleobase

Keywords: Homonucleosides, 1,3-dipolar cycloaddition, nitrones, vinyloxymethyl thymine, antiviral agents

Introduction

During the past two decades, great strides have been made in the design of modified nucleoside drugs for the treatment of viral infections despite the stigma of toxicity and the development of drug resistance.^{1,2} In particular, isoxazolidine homonucleosides **3**, synthesized by cycloaddition reaction between nitrones and allyl nucleobases in a diastereoselective or enantioselective way, have emerged as an important class of carbanucleoside analogues.^{3,4} The introduction of a carbon bridge between the nucleobase and the ribose unit or the isoxazolidine mimic leads to an increased resistance to hydrolytic or enzymatic cleavage and a more conformational flexibility and rotational freedom with respect to the natural nucleosides.⁵ Moreover, nucleoside mimics, characterized by higher flexibility, seem to be beneficial for the interaction with receptors; recently, it has been demonstrated that the binding sites of many enzymes are more flexible than previously thought and, as a direct consequence, more flexible inhibitors could show better features.⁶

Oligonucleotides constructed with modified nucleosides containing a methylene or ethylene tether between the sugar moiety and the nucleobase, (compound **1** and **2** in Fig. 1), allow a lowering of the electrostatic repulsion while maintaining the ability to build Watson-Crick base pairs with unnatural DNA or RNA strand, due to a better alignment of complementary nucleobases.⁷ The creation of these artificial analogues is justified by their biological significance and by the pure scientific exploration eventually directed toward biomedical applications.⁸

Continuing our interest in the synthesis of isoxazolidine nucleosides with high conformational freedom, we report in this paper the first member of a new series of *N,O* nucleosides in which the oxymethylene tether replaces the aminal linkage between the isoxazolidine moiety and the thymine. Our target compounds were prepared by exploiting the reactivity of dipolarophile **9** in the 1,3-dipolar cycloaddition of three different nitrones: the reaction afforded to the direct construction of α - and β - oxymethylene *N,O*-nucleosides **4**.

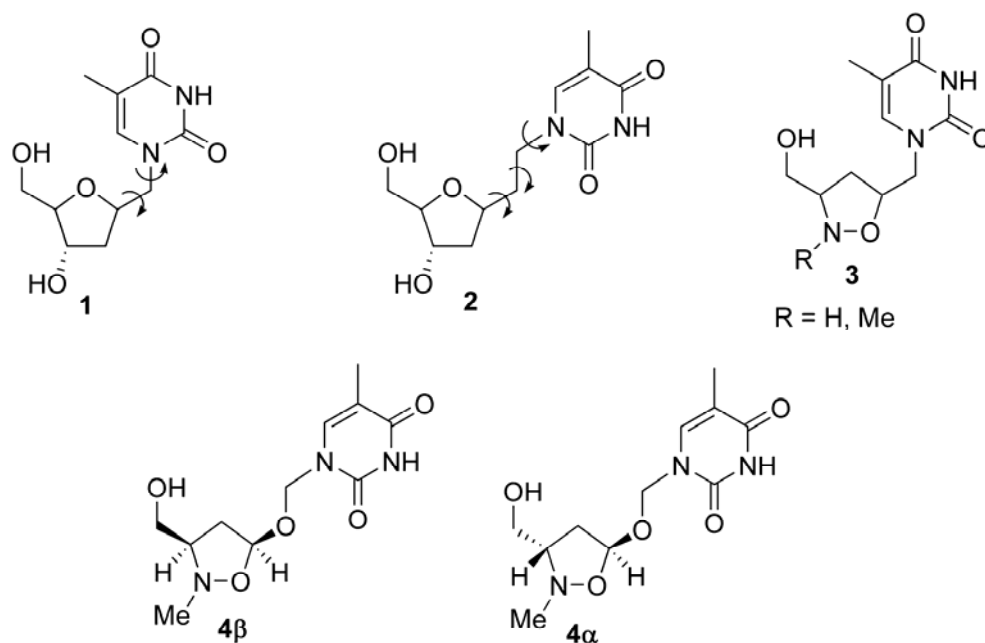
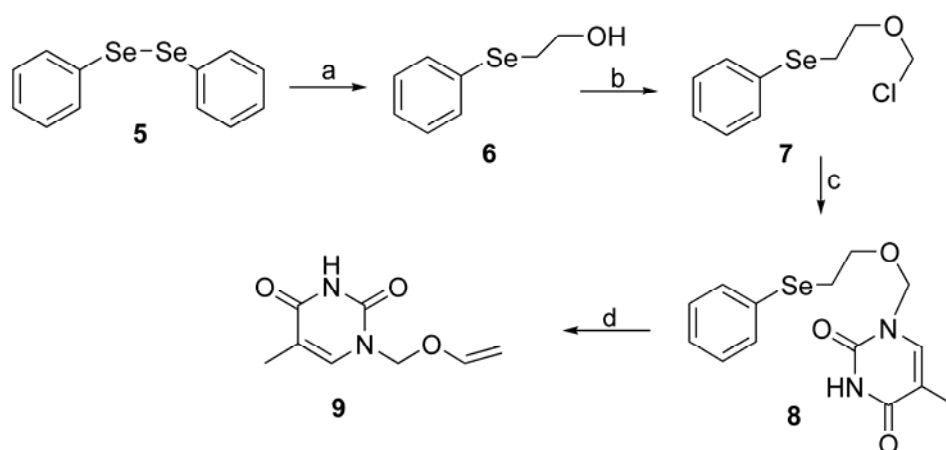


Figure 1. Homonucleosides.

Results and Discussion

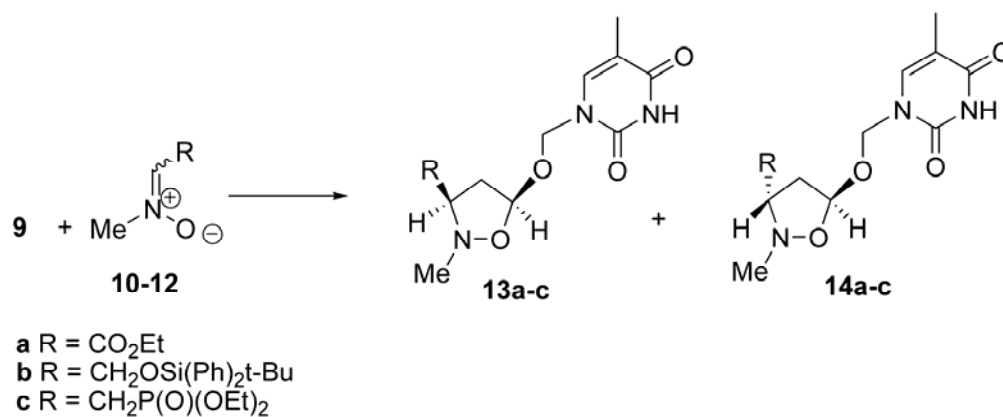
The key substrate **9** was synthesized by modifying the procedure described for the synthesis of purine derivatives (Scheme 1).⁹ Thus, the commercially available diphenyl selenide was treated with sodium borohydride to give the corresponding not isolable selenol, which *in situ* was reacted with ethylencarbonate to give compound **6**.¹⁰ The treatment of **6** with paraformaldehyde and the subsequent bubbling with HCl furnishes in a quantitative yield the intermediate **7**.⁹ The

coupling of **7** with silylated thymine, followed by treatment with sodium periodate and sodium bicarbonate, afforded the dipolarophile **9** with a yield of 70%.



Scheme 1. (a) NaBH_4 , EtOH dry, ethylencarbonate, reflux, 12h; (b) CH_2O , CH_2Cl_2 , HCl_g , 5°C , 2h; (c) BSA, thymine, TMSOTf, CH_3CN dry, r.t., 12h; (d) NaIO_4 , NaHCO_3 , MeOH, r.t., 30', then dioxane, 80°C , 2h.

A study of the optimal conditions for the 1,3-dipolar cycloaddition of compound **9** was carried out by employing nitrones **10-12**. (Scheme 2, Table 1, Entry 1-6).



Scheme 2. Cycloaddition reactions.

Table 1. Optimal conditions for 1,3-dipolar cycloaddition of **9**

Entry ^a	Nitrone	R	Conditions	Comp. α/β ratio	Yield ^d
1	10	CO ₂ Et	THF, 3g, reflux	14a/13a 3 : 1	45%
2	10	CO ₂ Et	THF, MW, 80°C, 1h, 100W	14a/13a 3 : 1	80%
3	11	TBDPSOCH ₂	THF, 72 h, reflux	14b/13b 1 : 2	35%
4	11	TBDPSOCH ₂	THF, MW, 80°C, 3h, 100W	14b/13b 1 : 2	70%
5	12	(OEt) ₂ P(O)CH ₂	THF, 72 h, reflux	N. R. ^b	-
6	12	(OEt) ₂ P(O)CH ₂	THF, MW, 80°C, 3h, 100W	I. M. ^c	10%

^a molar ratio of the dipole **10-12** and **9** is 2:1

^b no reaction

^c inseparable mixture

^d Combined yields

The reaction of nitrone **10** in THF at reflux, under traditional heating, proceeded slowly and with a low yield (entry 1). Switching to microwave irradiation at 100W for 1h at 80°C, an acceleration of the reaction time together with an increased yield was observed (entry 2). The same diastereomeric mixture of adducts **13a** and **14a** in a 1:3 ratio was obtained. The crude mixture was purified by MPLC on a Fluorosil column using as eluent a mixture of CHCl₃/MeOH (99:1) and the structures of the cycloadducts have been assigned on the basis of ¹H NMR data and confirmed by NOE experiments.

Thus, the main product **14a** shows the resonance of H_{3'} as a triplet at 3.48 ppm; the upfield proton H_{4a'} appears as a doublet of doublet at 2.62, while the downfield proton H_{4b'} appears as a doublet of doublets of doublets at 2.85 ppm. The H_{5'} proton resonates as a doublet at 5.55 ppm; the exocyclic methylene protons give rise to two doublets centered at 5.10 and 5.50 ppm. The irradiation of H_{5'} proton produced a significant enhancement for the resonances of H_{4'b} and the exocyclic methylene protons; conversely, when H_{4'b} was irradiated, a positive NOE effect was observed only for H_{5'} and H_{4'a}. Moreover, the irradiation of H_{4'a} induced a positive NOE effect for H_{4'b} and H_{3'}. These data support a *cis* relationship between H_{3'} and H_{4'a} protons and the substituent present at C-5 and allow to assign to compound **14a** a *trans* configuration. According to the results reported for similar cycloaddition¹¹ reactions of nitrone **10**, (E/Z ratio = 4:1), the stereochemical outcome of this cycloaddition reaction may be explained by considering that the major cycloadduct **14a** could be formed by the *E* nitrone reacting in an *exo* mode.

As previously reported,⁴ *Z* nitrone **11** reacts with allyl nucleobases in an *exo* mode leading to a *cis* adduct as main compound. Thus, in order to obtain the β -anomer **13b**, the reaction between the nitrone **11** and the vinyloxymethyl thymine **9** was investigated (entry 3 and 4). Anyway, a lower reactivity together with a much longer reaction time, with respect to the reaction of nitrone **10**, was observed. The ¹H NMR spectrum of the main product **13b** shows the resonance of the H_{3'} proton centered at 2.85 ppm; the methylene protons at C-4 resonate at 2.01 (H_{4'a}) and 2.45

ppm ($H_{4'b}$), while $H_{5'}$ proton resonates as a doublet centered at 5.30 ppm. The structural determination with the aid of NOE experiment was performed: a diagnostic NOE effect for proton $H_{5'}$ was observed after irradiation of $H_{3'}$. Moreover, the irradiation of the upfield proton $H_{4'a}$ induces a positive NOE effect for $H_{4'b}$, the methylene protons of the hydroxymethyl group and the vinyl proton of the thymine moiety. These data clearly indicated a *cis* configuration between the hydroxymethyl group at C-3 and the substituent at C-5 (Fig. 2)

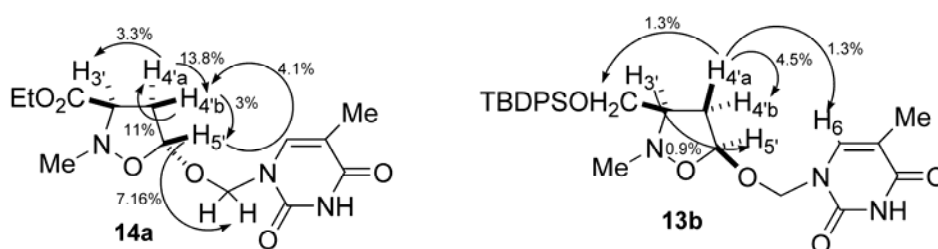


Figure 2. Selected NOEs observed for compounds **14a** and **13b**.

The synthetic scheme towards nucleosides **4** was completed by NaBH_4 reduction of the ester moiety for compound **13a** and deprotection reaction performed with TBAF for compound **13b**.

Recently, we have reported that isoxazolidine nucleosides bearing a phosphonate moiety at C-3' were able to completely inhibit the HTLV-1 infections.¹² In this context we have tried to synthesize phosphonated N,O-homonucleosides **13c** and **14c**, starting from the phosphonated nitrene **12**. However, in the cycloaddition reaction of **12** with compound **9** no significant amounts of cycloadducts were detected in the crude reaction mixture (entry 5) under traditional heating. Switching to microwave irradiation (entry 6), only a small amount of cycloadducts **13c** and **14c** is detectable in the ^1H NMR spectrum of the crude reaction mixture. Unfortunately, it was not possible to increase the yields by varying the reaction time, the microwave potency and the temperature, probably for the simultaneous acceleration of decomposition processes of nitrene **12**. Thus, compound **4** could represent the alternative substrate for the preparation of compounds **13c** and **14c** by using the previously reported procedure.¹³

Conclusions

We have explored the 1,3-dipolar cycloadditions of dipolarophile **9** with three different nitrones under conventional heating and microwave irradiation. The investigated routes allowed us to obtain α - and β -N,O-homonucleosides with an high conformational freedom. The procedure is general and may be extended to other nucleobases. Biological evaluations of the synthesized nucleosides are actually in progress.

Experimental Section

General Procedure. NMR spectra were measured on a 500 MHz Varian Unity Inova instrument in CDCl₃ as solvent. Chemical shifts are in ppm (δ) from TMS as internal standard. NOE difference spectra were obtained subtracting alternatively right-off-resonance free induction decays (FIDS) from right-on-resonance induced FIDS. IR spectra were recorded using an FTIR-8300 (Shimadzu) spectrophotometer. MS Spectra were measured with a JEOL JMS-D 300 spectrometer. Elemental analyses were performed on a Perkin-Elmer 240B microanalyzer. The microwave reactions were carried out with a Discover Focused Microwave System (CEM Corporation).

Synthesis of 5-methyl-1-[(vinylloxy)methyl]pyrimidine-2,4(1H,3H)-dione (9). To a solution of 2-(phenylselenenyl)ethanol^{10a} (20 mmol) was added paraformaldehyde (20 mmol), HCl gas was then bubbled into the solution at 5°C for 2h. The mixture was dried on sodium sulfate, filtered and the solvent was removed under reduced pressure to give the title compound (7)⁹ as a colorless oil in a quantitative yield.

To a solution of silylated thymine (20 mmol) obtained by treatment of thymine (20mmol) and *N,O*-Bis trimethylsilylacetamide (40 mmol) in CH₃CN dry (30 mL), 20 mmol of **7** solved in CH₃CN (2mL) was added and the mixture was left stirring at 0°C 10'. Then TMSOTf (20 mmol) was added and the mixture was stirred for 12 h at r.t. The solvent was then removed under reduced pressure and the residue was extracted with CHCl₃/ water. The organic phase was dried on sodium sulfate and evaporated to dryness. The residue was solved in methanol (20mL) and to the solution NaHCO₃ (1.1 mmol) and NaIO₄ (1.5 mmol). After stirring at 25°C for 30', the mixture was filtered and evaporated to dryness. The residue was then dissolved in dioxane (20 mL) and the solution was heated at 80°C for 20'. The solution was evaporated in vacuo and the residual oil was chromatographed on Florosil by using CHCl₃/MeOH 95:5 to give the compound **9** as light yellow foam (Yield 70%).

IR (KBr) ν 3380, 3176, 3040, 1685, 1605, 1360, 1250, 1100 cm⁻¹. ¹H NMR (CDCl₃) δ 1.98 (d, 3H, J = 0.97 Hz), 4.21 (dd, 1H, J = 5.0 and 1.5 Hz), 4.5 (dd, 1H, J = 1.5 and 11 Hz), 5.41 (s, 2H, CH₂O), 6.5 (dd, 1H, J = 5.0 and 11.0 Hz), 9.19 (q, 1H, J = 0.97 Hz), 8.95 (bs, 1H, NH). ¹³C NMR (CDCl₃) δ 14.5, 79.5, 87.1, 11.0, 140.3, 150.8, 153.0, 165.3.

Anal. Calcd. for C₈H₁₀N₂O₃: C, 52.74, H, 5.53, N, 15.38. Found C, 52.81, H, 5.59, N, 15.70.

Procedure for the synthesis of nucleosides 13 and 14

Method A. A solution of nitrones **10** or **11** (20 mmol) and vinylloxymethyl thymine (10 mmol) in dry THF (20 mL) was stirred at reflux for three days. The solution was then evaporated under reduced pressure and the residue was purified by Medium Pressure Liquid Chromatography on a Fluorosil column using as eluent a mixture of CHCl₃/MeOH 99:1.

Method B. A solution of nitrones **10**, **11** or **12** (10 mmol) and vinylloxymethyl thymine (5 mmol) in THF dry (8 mL) was put in a sealed tube and irradiated under microwave conditions at 100W,

80°C for the appropriate reaction time (see table 1). The removal of the solvent in vacuo afforded a crude material which was purified by MPLC by using as eluent a mixture of CHCl₃/MeOH 99:1.

(3RS,5SR)-Ethyl 2-methyl-5[(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl) methoxy]isoxazolidine 3-carboxylate (13a). White foam. Yield 20%. ¹H NMR (CDCl₃) δ 1.23 (t, 3H, *J* = 7.0 Hz), 1.95 (d, 3H, *J* = 0.95 Hz), 2.60 (dd, 1H, *J* = 7.3 and 12.9 Hz), 2.82 (ddd, 1H, *J* = 5.5, 7.3 and 12.9 Hz), 2.91 (s, 3H), 3.75 (t, 1H, *J* = 7.3), 4.21 (q, 2H, *J* = 7.0 Hz), 5.14 (d, 1H, *J* = 10.5 Hz), 5.39 (d, 1H, *J* = 10.5 Hz), 5.45 (d, 1H, *J* = 5.5 Hz), 7.12 (q, 1H, *J* = 0.95 Hz), 8.56 (bs, 1H). ¹³C NMR (CDCl₃) δ 12.3, 14.2, 39.9, 42.1, 61.7, 67.2, 74.6, 96.5, 111.3, 139.6, 150.9, 163.8, 170.1. Anal. Calcd. for C₁₃H₁₉N₃O₆: C, 49.84, H, 6.11, N, 13.41. Found C, 49.91, H, 6.17, N, 13.44. Exact mass calculated for C₁₃H₁₉N₃O₆: 313.1274. Found: 313.1276.

(3RS,5RS)-Ethyl 2-methyl-5[(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl) methoxy]isoxazolidine 3-carboxylate (14a). White foam. Yield 60%. ¹H NMR (CDCl₃) δ 1.23 (t, 3H, *J* = 7.0 Hz), 2.02 (d, 3H, *J* = 0.95 Hz), 2.62 (dd, 1H, *J* = 7.5 and 13.0 Hz), 2.85 (ddd, 1H, *J* = 5.1, 7.5 and 13.0 Hz), 2.98 (s, 3H), 3.48 (t, 1H, *J* = 7.5), 4.21 (q, 2H, *J* = 7.0 Hz), 5.10 (d, 1H, *J* = 10.5 Hz), 5.50 (d, 1H, *J* = 10.5 Hz), 5.55 (d, 1H, *J* = 5.1 Hz), 7.15 (q, 1H, *J* = 0.95 Hz), 8.50 (bs, 1H). ¹³C NMR (CDCl₃) δ 12.8, 14.5, 40.2, 42.7, 62.8, 65.2, 75.6, 97.2, 113.6, 140.3, 152.1, 165.2, 173.4. Anal. Calcd. for C₁₃H₁₉N₃O₆: C, 49.84, H, 6.11, N, 13.41. Found C, 49.90, H, 6.15, N, 13.45. Exact mass calculated for C₁₃H₁₉N₃O₆: 313.1274. Found: 313.1278.

1-[(3RS,5SR)-3-((tert-butyldiphenylsilyloxy)methyl)-2-methylisoxazolidin-5-yloxy]methyl-5-methylpyrimidine-2,4(1H,3H)-dione (13b). White foam. Yield 24%. ¹H NMR (CDCl₃) δ ¹H NMR (CDCl₃) δ 1.05 (s, 9H), 1.90 (d, 3H, *J* = 0.95 Hz), 2.01 (m, 1H), 2.45 (ddd, 1H, *J* = 4.3, 6.9 and 11.0 Hz), 2.85 (ddd, 1H, *J* = 4.3, 5.8 and 6.0 Hz), 2.85 (s, 3H), 3.60 (dd, 1H, *J* = 5.8 and 9.5 Hz), 3.62 (dd, 1H, *J* = 6.0 and 9.5 Hz), 5.0 (d, 1H, *J* = 10.0 Hz), 5.30 (d, 1H, *J* = 6.9 Hz), 5.40 (d, 1H, *J* = 10.0 Hz), 7.05 (q, 1H, *J* = 0.95 Hz), 8.25 (bs, 1H). ¹³C NMR (CDCl₃) δ 13.9, 19.8, 26.0, 39.8, 45.9, 65.2, 68.5, 74.1, 99.0, 105.1, 128.6, 130.1, 135.9, 136.8, 140.2, 147.8, 169.0. Anal. Calcd. for C₂₇H₃₅N₃O₅Si: C, 36.63, H, 6.92, N, 8.24. Found C, 36.67, H, 6.95, N, 8.31. Exact mass calculated for C₂₇H₃₅N₃O₅Si: 509.2346. Found: 509.2349.

1-[(3RS,5RS)-3-((tert-butyldiphenylsilyloxy)methyl)-2-methylisoxazolidin-5-yloxy]methyl-5-methylpyrimidine-2,4(1H,3H)-dione (14b). White foam. Yield 46%. ¹H NMR (CDCl₃) δ ¹H NMR (CDCl₃) δ 1.03 (s, 9H), 1.95 (d, 3H, *J* = 0.95 Hz), 1.98 (m, 1H), 2.58 (ddd, 1H, *J* = 4.5, 6.9 and 11.5 Hz), 2.80 (ddd, 1H, *J* = 4.8, 5.5 and 6.9 Hz), 2.81 (s, 3H), 3.62 (dd, 1H, *J* = 4.8 and 10.0 Hz), 3.75 (dd, 1H, *J* = 5.5 and 10.0 Hz), 5.02 (d, 1H, *J* = 10.0 Hz), 5.34 (d, 1H, *J* = 4.5 Hz), 5.40 (d, 1H, *J* = 10.0 Hz), 7.08 (q, 1H, *J* = 0.95 Hz), 8.24 (bs, 1H). ¹³C NMR (CDCl₃) δ 14.1, 19.5, 26.7, 40.4, 45.6, 64.9, 68.9, 73.9, 99.7, 104.8, 127.7, 129.8, 135.5, 136.1, 139.8, 148.1, 168.5. Anal. Calcd. for C₂₇H₃₅N₃O₅Si: C, 36.63, H, 6.92, N, 8.24. Found C, 36.68, H, 6.95, N, 8.29. Exact mass calculated for C₂₇H₃₅N₃O₅Si: 509.2346. Found: 509.2348.

Preparation of N,O-nucleosides 4 α and 4 β from 14a and 13a

To a solution of **13a** or **14a** (1 mmol) in dioxane/water mixture (10mL), NaBH₄ (10mmol) was added and the mixture was stirred for 6h at r.t. At the end of this time, the solvent was removed and the residue was subjected to purification by column chromatography on neutral alumina (CHCl₃/MeOH 95:5).

Preparation of N,O-nucleosides 4 α and 4 β from 14b and 13b

To a solution of **13b** or **14b** (1 mmol) in THF dry (10 mL), 1M TBAF in THF (1.1 mmol) was added and the solution was stirred at r.t. for 1h. After evaporation of the solvent in vacuo, the residue was purified by column chromatography on neutral alumina (CHCl₃/MeOH 95:5).

Reaction of 14a with NaBH₄. 1-[(3SR,5SR)-3-(hydroxymethyl)-2-methyl- isoxazolidin-5-yloxy]methyl]-5-methylpyrimidine-2,4(1H,3H)-dione (4 α). White foam. Yield 85%. δ ¹H NMR (D₂O) δ 1.95 (d, 3H, J =0.95 Hz), 2.01 (m, 1H), 2.15 (m, 1H), 2.75 (ddd, 1H, J = 4.9, 5.3 and 5.5 Hz), 2.85 (s, 3H), 3.62 (dd, 1H, J = 4.9 and 8.5 Hz), 3.70 (dd, 1H, J = 5.5 and 8.5 Hz), 5.02 (d, 1H, J = 9.8 Hz), 5.35 (d, 1H, J = 4.8 Hz), 5.40 (d, 1H, J = 9.8 Hz), 7.15 (q, 1H, J = 0.95 Hz). ¹³C NMR (D₂O) δ 12.5, 36.9, 43.1, 61.5, 66.3, 73.0, 110.0, 139.8, 150.3, 163.7. Anal. Calcd. for C₁₁H₁₇N₃O₅: C, 48.70, H, 6.32, N, 15.49. Found C, 48.75, H, 6.30, N, 15.47. Exact mass calculated for C₁₁H₁₇N₃O₅: 271.1168. Found: 271.1165.

Reaction of 13b with TBAF. 1-[(3SR,5RS)-3-(hydroxymethyl)-2-methylisoxazolidin -5-yloxy]methyl]-5-methylpyrimidine-2,4(1H,3H)-dione (4 β). White foam. Yield 90%. ¹H NMR (D₂O) δ 1.95 (d, 3H, J =0.95 Hz), 2.05 (m, 1H), 2.15 (m, 1H), 2.70 (ddd, 1H, J = 4.8, 5.0 and 5.5 Hz), 2.89 (s, 3H), 3.60 (dd, 1H, J = 4.8 and 8.4 Hz), 3.65 (dd, 1H, J = 5.0 and 8.4 Hz), 5.02 (d, 1H, J = 9.8 Hz), 5.35 (d, 1H, J = 4.5 Hz), 5.40 (d, 1H, J = 9.8 Hz), 7.10 (q, 1H, J = 0.95 Hz). ¹³C NMR (D₂O) δ 12.8, 37.2, 42.9, 61.9, 66.5, 73.8, 109.5, 140.2, 150.8, 164.0. Anal. Calcd. for C₁₁H₁₇N₃O₅: C, 48.70, H, 6.32, N, 15.49. Found C, 48.72, H, 6.30, N, 15.45. Exact mass calculated for C₁₁H₁₇N₃O₅: 271.1168. Found: 271.1170.

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