Diastereoselective Synthesis of *N,O*-Psiconucleosides, a New Class of Modified Nucleosides

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Anomeric α- and β-*N,O*-psiconucleosides were prepared by 1,3-dipolar cycloaddition of *C*-ethoxycarbonyl *N*-methyl nitrone with ethyl 2-acetyloxyacrylate, followed by Vorbrüggen nucleosidation. The synthetic scheme has been applied to all purine and pyrimidine nucleobases. Nucleosidation can proceed under kinetic and under thermodynamic control; under thermodynamic control conditions only β-nucleosides are obtained for pyrimidine derivatives and α-nucleosides for purine derivatives.

Introduction

Nucleoside analogues, in which the furanose ring has been replaced by carbo- or heterocyclic rings, have attracted considerable interest by virtue of their biological action, mainly as antiviral agents.[1] In this context, we have recently become interested in the development of new synthetic schemes for the construction of a series of *N,O*-modified nucleosides, some of which have shown promising anti-AIDS activity in vitro.[2]

The synthetic strategy we chose was based on the potential for the construction of stereochemically defined heterocyclic systems offered by 1,3-dipolar cycloaddition processes. In the same context, enantiomerically pure *N,O*-nucleosides have also been synthesized by the use of chiral nitrones, through cycloaddition reactions with vinyl nucleobases,[3] by exploiting, for each nucleobase, convenient experimental procedures for the preparation of the required dipolarophile.[4]

There are a number of natural products reminiscent of nucleosides and endowed with relevant biological activities, but containing modifications in the C-1′ position.[5] Angustmycin A (**1**) and angustmycin C (**2**) possess interesting antiviral and antimicrobial properties, whilst hydantocidin (**3**), a spironucleoside, shows herbicide features able to regulate plant growth. The C-1′-branched nucleosides (psiconucleosides) are doubly of interest: besides their potential biological activity as antiviral agents, interest in this kind of compounds is also linked to the availability of model nucleosides that might allow study of the formation and evolution of radical species generated during DNA/RNA damage.[6]

In view of this, we have recently devised a new synthetic route[7] to *N,O*-psiconucleosides in which the sugar moiety is replaced by an *N,O*-pentatomic ring, and in a previous paper we reported the first preparation of isoxazolidinylthymine and -adenine (**4** and **5**) branched at the anomeric position (Figure 1).

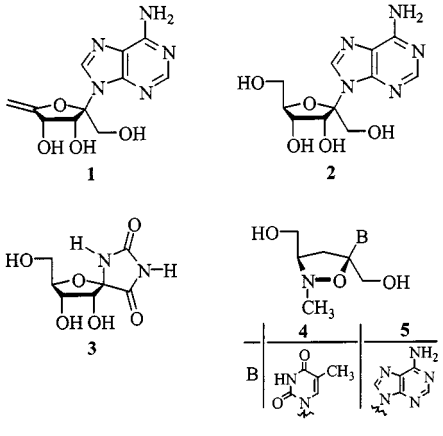

Figure 1. Modified nucleosides

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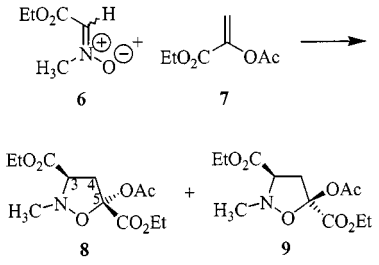
The designed reaction route appears to be very versatile; in this paper we report our recent results and the general procedure that allows the synthesis of the new classes of α- and β-*N,O*-psiconucleosides by extension of the approach to all purine and pyrimidine nucleobases. The synthetic scheme is based on cycloaddition processes between suitably substituted *C*-alkoxycarbonyl nitrones and enol acetates, and allows differently functionalizable groups to be inserted onto the heterocyclic moiety.

Results and Discussion

The cycloaddition reaction between *C*-ethoxycarbonyl *N*-methyl nitrone (**6**) and ethyl 2-acetyloxyacrylate (**7**) proceeded smoothly (at room temperature in anhydrous ether for 24 h) to give a mixture of epimeric isoxazolidines **8** and **9**, in an 8.6:1 ratio (96% combined yields). These were separated by flash chromatography (Scheme 1).

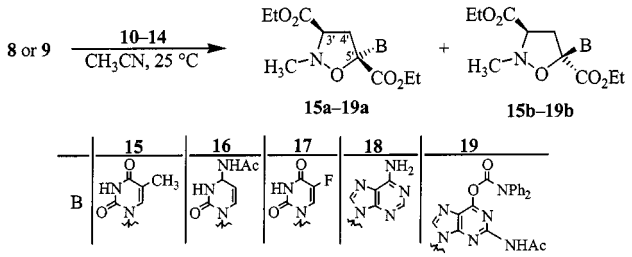


Scheme 1

Structural determinations were performed with the aid of NOE experiments. Thus, for the major *trans* isomer **8**, the NOE correlation between 3-H, the methyl group of the acetyl moiety at C-5, and 4-H^b (the more upfield resonance of the methylene protons at C-4) was clearly indicative of their *cis* relationship.[7]

Nitrone **6** exists as a mixture of (*E*) and (*Z*) isomers, with the more reactive (*E*) isomer predominating (4:1).[8] In line with similar cycloaddition processes, the major stereoisomer **8** presumably arises from the (*E*)-nitrone reacting through an *endo* transition state (TS) (with respect to the CO_2Et group). PM3 calculations confirmed this assumption, showing that the (*E*)-*endo* TS giving rise to this compound was about 1.05 kcal/mol more stable than the (*E*)-*exo* TS producing *cis* stereoisomer **9** (Table 1). This value is in satisfactory agreement with the observed **8/9** ratio.

The two cycloadducts **8** and **9** were independently coupled with silylated nucleobases[9] [thymine **10**, *N*-acetylcytosine **11**, 5-fluorouracil **12**, adenine **13**, and 2-*N*-acetyl-6-*O*-(diphenylcarbamoyl)guanine (**14**)] in acetonitrile at room temperature, in the presence of trimethylsilyl triflate or $SnCl_4$ as catalysts (Scheme 2).



Scheme 2

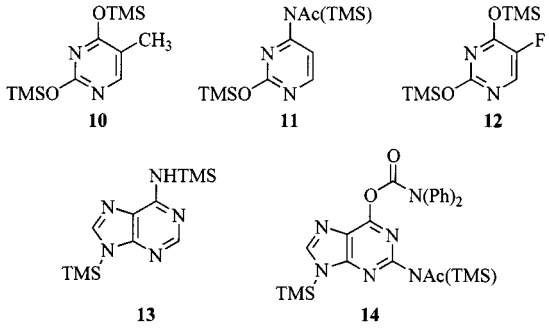


Figure 2. Silylated nucleobases; TMS = trimethylsilyl

In all cases, nucleosidation proceeded without any stereoselectivity to give α-nucleosides **15a**−**19a** and β-nucleosides **15b**−**19b** in nearly equimolar ratios, as determined on the crude reaction mixtures by ^{1}H NMR. The coupling reaction with cytosine and guanine nucleobases required prior protection of their amino groups.[10]

Table 2 lists the yields and α/β ratios obtained under various experimental conditions. The nucleosidation process required a mild acid catalysis; the highest yields were achieved by use of 0.4 equiv. of trimethylsilyl triflate, a weaker Lewis acid than $SnCl_4$. The use of equimolar amounts of $SnCl_4$ or TMSOTf was not successful, because of the competitive cleavage of the isoxazolidine ring. It was noteworthy that, when $SnCl_4$ was employed, partial production of regioisomers (N-3 for pyrimidine and N-7 for purine bases) occurred.

Table 1. PM3 calculations for 1,3-dipolar cycloaddition between **6** and **7**

Isoxazolidine	Transition state	PM3 [kcal/mol]	Calculated yield [%]	Observed yield [%]
8	(*E*)-*endo*	−191.57	82.1	86
9	(*E*)-*exo*	−190.52	13.9	10

Table 2. Reactions between isoxazolidines **8** and **9** and silylated nucleobases **10**−**14** at 25 °C

Entry	Base[a]	Conditions[b]	Obtained compd.	α/β ratio	Combined yield [%]
1	**10**	0.4 equiv. TMSOTf	**15**	1:1	60
2	**10**	1.0 equiv. TMSOTf	**15**	1:1	57
3	**10**	0.4 equiv. $SnCl_4$	**15**	1:1	35
4	**10**	1.0 equiv. $SnCl_4$	**15**	1:1	15
5	**11**	0.4 equiv. TMSOTf	**16**	1:1	40
6	**11**	1.0 equiv. TMSOTf	**16**	1:1	30
7	**12**	0.4 equiv. TMSOTf	**17**	1:2.5	61
8	**12**	1.0 equiv. TMSOTf	**17**	1:2.5	40
9	**13**	0.4 equiv. TMSOTf	**18**	1:1	30
10	**13**	1.0 equiv. TMSOTf	**18**	N.I.[c]	25
11	**13**	0.4 equiv. $SnCl_4$	**18**	1:1	25
12	**13**	1.0 equiv. $SnCl_4$	**18**	N.I.[c]	24
13	**14**	0.4 equiv. TMSOTf	**19**	1:1	15
14	**14**	1.0 equiv. TMSOTf	**19**	1:1	15

[a] Molar ratio of base/isoxazolidine = 2:1. [b] All reactions were conducted for 24 h. [c] Inseparable mixture of N-1-/N-3-pyrimidine isomers or N-7-/N-9-purine isomers.

Under the adopted experimental conditions, α and β anomers were obtained in nearly equimolar ratios, except for the 5-fluorouracil derivatives, in which the β anomer predominated with a relative α/β ratio of 1:2.5. The product distributions were sensitive to the reaction temperature, as discussed below.

Anomers were separated by flash chromatography and characterized by NMR spectroscopy. Thus, for compounds **15**−**17**, the α anomers showed two distinct multiplets centered at δ = 2.92−3.15 and δ = 3.72−3.89 for the methylene protons at C-4′, while β anomers gave rise to multiplets in the range δ = 2.83−2.98 and δ = 3.80−3.98. For purine nucleosides **18** and **19**, the same resonances were at δ = 3.99 and 2.92 and at 3.74 and 2.70 respectively (α anomers) and δ = 2.94 and 2.95 and at 3.55 and 3.95 (β anomers). The protons at C-3′ resonated as doublets of doublets at δ = 3.55−3.65 for all the β anomers, and at δ = 3.18−4.18 for all the α anomers.

The stereochemical assignments of the obtained nucleosides were performed by means of NOEDS spectroscopy. Thus, for pyrimidine nucleosides **15**−**17**, diagnostic NOE effects were detected by irradiation of the methylene protons at C-4′. In particular, irradiation of the upfield resonances of the methylene protons at C-4′ (4′-H^a) in β anomers **15b**−**17b** induced enhancement of 4′-H^b and 6-H signals, while strong NOE effects were observed for 3′-H and 4′-H^a when 4′-H^b was irradiated; these data clearly indicated a topologically *cis* relationship between 4′-H^a, the nucleoside base at C-5′ and the CO_2Et group at C-3′.

Similarly, α-nucleosides **15a**−**17a** showed NOE correlations between 3′-H, the downfield resonance at C-4′ (4′-H^b), and 6-H, thus confirming that the relative configuration of the CO_2Et groups at C-5′ and C-3′ had to be *cis*.

In the case of purine derivatives **18**[11] and **19**, diagnostic NOE effects were detected by irradiation of the methyl group on the isoxazolidine nitrogen atom; the assignment of the anomeric configuration to the α anomers **18a** and **19a** was in fact supported by the enhancements observed for 3′-H, 9-H, and the downfield resonance of the methylene protons at C-4′. Consequently, the β configuration could be assigned to **18b** and **19b**, in which irradiation of *N*-CH_3 induced positive NOE effects only on the more upfield resonances of the methylene protons at C-4′.

When the nucleosidation reaction was performed on an epimeric mixture of isoxazolidine **8** and **9** without any preventive separation, an analogous distribution of α and β anomers was obtained. These results showed that the coupling reaction between isoxazolidines and silylated bases occurred without selectivity with respect to the anomeric center. This is due to the formation of an intermediate oxonium ion, which would not be expected to have any significant facial bias, since the substituent at C-3 would not be in any close proximity to induce any steric effect upon the incoming group at C-5.

Efforts were made to improve both the diastereoselectivity of the coupling process and the chemical yield by varying the temperature and the nature of the catalyst. The nucleosidation reaction was found to be strictly dependent on the adopted reaction conditions. Thus, an increase in temperature to a value of 45 °C resulted in nearly complete diastereoselectivity, resulting in the exclusive formation of the β anomers for pyrimidine derivatives **15**−**17** and the α anomers for purine nucleobases **18**−**19**. Reaction times and amount of catalyst exerted a marked influence on the chemical yield of the process; in particular, the best yields were obtained for a reaction time of 6 h and use of 0.4 equiv. of TMSOTf as a catalyst. Table 3 shows the strong influence of temperature on the reaction pathway.

Table 3. Reactions between isoxazolidines **8** and **9** and silylated nucleobases **10**−**14** at different temperatures

Entry[a]	Base[b]	Temp. [°C]	Obtained compd.	α/β ratio	Combined yield [%]
1	**10**	35	**15**	1:5	65
2	**10**	45	**15**	0:1	80
3	**10**	80	**15**	0:1	40
4	**11**	35	**16**	1:6	60
5	**11**	45	**16**	0:1	73
6	**11**	80	**16**	0:1	35
7	**12**	35	**17**	0:1	70
8	**12**	45	**17**	0:1	85
9	**12**	80	**17**	0:1	50
10	**13**	35	**18**	3:1	35
11	**13**	45	**18**	1:0	50
12	**13**	80	**18**	1:0	10
13	**14**	35	**19**	3:1	30
14	**14**	45	**19**	1:0	45
15	**14**	80	**19**	1:0	10

[a] All reactions were performed for 6 h with 0.4 equiv. of TMSOTf as catalyst. [b] Molar ratio of base/isoxazolidine = 2:1.

The obtained results showed the distinct possibility that isomerization was occurring during product formation and indicated that equilibration towards the thermodynamically more stable compounds was possible under the reaction conditions. As previously reported,[12] nucleosidation could proceed under both kinetic and thermodynamic control through intermediate oxonium ions; the thermodynamically controlled compounds are the β anomers **15b**−**17b** and the α anomers **18a**−**19a**. For confirmation, **15a**−**17a** and **18b**−**19b** were heated at 45 °C in acetonitrile in the presence of TMSOTf and silylated base; the result was the complete formation of the isomers **15b**−**17b** and **18b**−**19b**, respectively.

We attempted an explanation of the observed data on the basis of semiempirical calculations on the relative α/β stability of compounds **15**−**19**; for comparison, all optimized structures were calculated at both PM3 (Table 4) and AMBER (Table 5) levels.

Table 4. PM3-calculated heats of formation of compounds **15**−**19**

Isoxazolidinyl nucleoside	ΔH_f [a] (α anomer)	ΔH_f (β anomer)	Calculated α/β ratio
15	−235.64	−237.98	1:52
16	−210.04	−211.65	1:15
17	−266.66	−267.63	1:5
18	−106.60	−105.67	4.8:1
19	−158.05	−157.12	4.8:1

[a] kcal/mol.

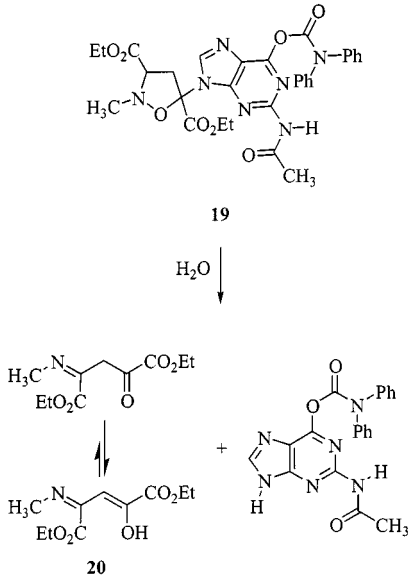
Table 5. AMBER-calculated heats of formation of compounds **15**−**19**

Isoxazolidinyl nucleoside	ΔH_f [a] (α anomer)	ΔH_f (β anomer)	Calculated α/β ratio
15	64.59	62.46	1:36
16	81.71	80.71	1:5.4
17	64.39	62.54	1:22
18	64.34	64.34	1:1
19	113.56	113.97	2:1

[a] kcal/mol.

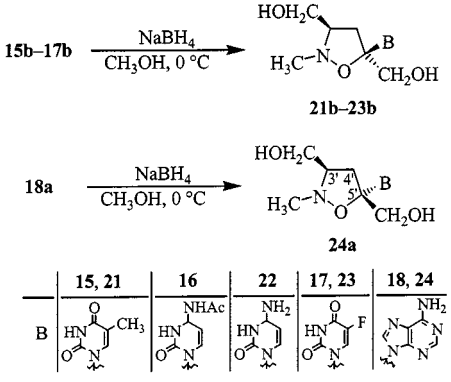
The PM3 data showed that the β anomers of compounds **15**−**17** were more stable than the α anomers, while the reverse was the case with compounds **18** and **19**, in agreement with the obtained results. Interestingly, whereas PM3 data correctly identified the observed trend, Amber did not predict the experimental results in the case of purine derivatives, particularly for compound **18**.

For purine derivatives, the experimental conditions assume particular importance: the molar ratio of catalyst in this case appears to be essential to produce the desired nitrogen substitution product (N-9). In fact, when the amount of TMSOTf exceeded 0.4 equiv., a complex mixture of different, non-isolable derivatives, probably originating from nucleosidation at the other nitrogen atoms (N-7, N-1, N-3), was obtained.[12] Furthermore, compounds **19** appeared to be unstable; they decomposed within a few hours by cleavage of the glycosidic bond and release of the nucleoside base together with diethyl 2-hydroxy-4-(methylimino)pent-2-enedioate (**20**) (Scheme 3).



Scheme 3

Finally, the synthetic scheme was completed by reduction of the ester function. Thus, reduction of compounds **15**−**18** with $NaBH_4$ in methanol afforded the target nucleosides **21**−**24** with a good yield for the thymine derivative[7] and moderate yields for the other nucleobases (30−40%, Scheme 4).



Scheme 4

Conclusion

A synthetic approach to a new class of *N,O*-psiconucleosides has been described. The reaction route appears to be versatile and easily extendable to all purine and pyrimidine nucleobases. The synthesis of both D and L isomers by use of homochiral nitrones is in progress, as well as the evaluation of the biological activity of all the synthesized α- and β-*N,O*-psiconucleosides.

Experimental Section

General Remarks: Melting points are uncorrected. NMR spectra were recorded at 500 MHz (^{1}H) and at 125 MHz (^{13}C) with a Varian Unity Inova spectrometer and are reported in ppm downfield from TMS. Thin layer chromatography was performed on Merck 60 F_{254} coated plates. Silica gel chromatography was performed with Macherey−Nagel 60 M (0.040−0.063 mm). Preparative radial chromatography was performed on glass rotors coated with Merck 60 PF_{254} silica gel (1−4 mm layer). All reactions involving air-sensitive agents were conducted under nitrogen. All reagents were purchased from Aldrich or Acros Chimica and were used without further purification. Solvents for chromatography were distilled at atmospheric pressure prior to use and dried by standard procedures.

Diethyl (3*RS*,5*SR*)-5-Acetyloxy-2-methylisoxazolidine-3,5-dicarboxylate (8) and Diethyl (3*RS*,5*RS*)-5-Acetyloxy-2-methylisoxazolidine-3,5-dicarboxylate (9): A solution of *C*-ethoxycarbonyl *N*-methyl nitrone (**6**; 3.0 g, 22.9 mmol) and ethyl 2-acetyloxyacrylate (**7**; 3.7 g, 23 mmol) in dry ether (100 mL) was stirred at room temperature for 24 h. The reaction solvents were evaporated and the residue was purified by flash chromatography (chloroform/methanol, 99:1). The product eluted first was compound **8**; 86% yield; yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.19 (t, *J* = 6.2 Hz, 6 H), 2.06 (s, 3 H), 2.87 (dd, *J* = 7.5, 13.5 Hz, 1 H, 4-H^a), 2.91 (s, 3 H, *N*-Me), 3.17 (dd, *J* = 9.0, 13.5 Hz, 1 H, 4-H^b), 3.70 (dd, *J* = 7.5, 9.0 Hz, 1 H, 3-H), 4.11−4.20 (m, 4 H). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 13.8, 14.0, 20.9, 43.0, 45.3, 61.8, 62.6, 67.6, 104.6, 168.0, 168.4, 169.5. $C_{12}H_{19}NO_7$ (289.3): calcd. C 49.82, H 6.62, N 4.84; found C 49.65, H 6.63, N 4.85. The fraction eluted second was compound **9**; 10% yield; yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.23 (t, *J* = 6.2 Hz, 6 H), 2.01 (s, 3 H), 2.85 (dd, *J* = 8.6, 14.0 Hz, 1 H, 4-H^a), 2.86 (s, 3 H, *N*-Me), 3.27 (dd, *J* = 8.5, 14.0 Hz, 1 H, 4-H^b), 3.46 (dd, *J* = 8.5, 8.6 Hz, 1 H, 3-H), 4.15−4.21 (m, 4 H). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 13.8, 14.0, 25.6, 44.0, 45.3, 61.8, 62.6, 69.1, 103.5, 168.0, 168.4, 169.5. $C_{12}H_{19}NO_7$ (289.3): calcd. C 49.82, H 6.62, N 4.84; found C 49.61, H 6.65, N 4.83.

Treatment of Isoxazolidines 8 and 9 with Silylated Nucleobases 10−14

General Procedure: A solution of isoxazolidines **8** or **9** (0.29 g, 1.0 mmol) in dry acetonitrile (5 mL) and TMSOTf (0.056 g, 0.25 mmol) was added to a stirred solution of **10−14** (2.0 mmol, obtained by standard procedures) in dry acetonitrile (70 mL). The resulting mixture was stirred either at 25 °C for 24 h, or at a different temperature (Table 3). After this period, the solution was neutralized by addition of 5% aqueous sodium bicarbonate, and then concentrated in vacuum. The aqueous layer was extracted with ethyl acetate (5 × 10 mL), and the combined organic layers were dried with sodium sulfate and filtered, and the solvents were evaporated to dryness. The residue was purified by flash chromatography with cyclohexane/ethyl acetate (3:7) for compounds **15** and **16**, with cyclohexane/ethyl acetate (5:5) for compounds **17**, and with chloroform/methanol (99:1) for compounds **18** and **19**. All products are reported in order of their column elution.

(3′*RS*,5′*RS*)-1-[3′,5′-Bis(ethoxycarbonyl)-2′-methyl-1′,2′-isoxazolidin-5′-yl]thymine (15b): 30% yield, white solid, m.p. 145−146 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.20 (t, *J* = 7.1 Hz, 6 H), 1.90 (d, *J* = 1.3 Hz, 3 H), 2.83 (dd, *J* = 9.0, 13.9 Hz, 1 H, 4′-H^a), 2.90 (s, 3 H, *N*-Me), 3.56 (dd, *J* = 8.2, 9.0 Hz, 1 H, 3′-H), 3.80 (dd, *J* = 8.2, 13.9 Hz, 1 H, 4′-H^b), 4.16 (m, 4 H), 7.46 (q, *J* = 1.3 Hz, 1 H, 6-H), 8.07 (br. s, 1 H, NH). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 12.7, 13.8, 14.0, 45.1, 45.3, 61.9, 63.2, 69.7, 92.9, 109.5, 134.6, 150.4, 164.2, 165.3, 167.9. $C_{15}H_{21}N_3O_7$ (355.3): calcd. C 50.70, H 5.96, N 11.83; found C 50.78, H 5.94, N 11.81.

(3′*RS*,5′*SR*)-1-[3′,5′-Bis(ethoxycarbonyl)-2′-methyl-1′,2′-isoxazolidin-5′-yl]thymine (15a): 30% yield, white solid, m.p. 147−150 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.20 (t, *J* = 7.1 Hz, 3 H), 1.21 (t, *J* = 7.1 Hz, 3 H), 1.91 (d, *J* = 1.3 Hz, 3 H), 2.94 (s, 3 H, *N*-Me), 2.97 (m, 1 H, 4′-H^a), 3.81 (m, 1 H, 4′-H^b), 4.18 (m, 1 H, 3′-H), 4.20 (m, 4 H), 7.45 (q, *J* = 1.3 Hz, 1 H, 6-H), 8.82 (br. s, 1 H, NH). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 12.8, 13.8, 14.2, 43.9, 45.4, 61.6, 63.2, 69.7, 93.6, 109.7, 134.7, 150.3, 164.3, 165.5, 166.3, 168.5. $C_{15}H_{21}N_3O_7$ (355.3): calcd. C 50.70, H 5.96, N 11.83; found C 50.75, H 5.95, N 11.84.

(3′*RS*,5′*RS*)-4-*N*-Acetyl-1-[3′,5′-bis(ethoxycarbonyl)-2′-methyl-1′,2′-isoxazolidin-5′-yl]cytosine (16b): 20% yield; viscous oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.23 (t, *J* = 7.1 Hz, 3 H), 1.24 (t, *J* = 7.1 Hz, 3 H), 2.28 (s, 3 H), 2.86 (dd, *J* = 8.9, 14.3 Hz, 1 H, 4′-H^a), 2.97 (s, 3 H, *N*-Me), 3.65 (dd, *J* = 8.3, 8.9 Hz, 1 H, 3′-H), 3.98 (dd, *J* = 8.3, 14.3 Hz, 1 H, 4′-H^b), 4.17 (q, *J* = 7.1 Hz, 2 H), 4.19 (q, *J* = 7.1 Hz, 2 H), 7.52 (d, *J* = 7.5 Hz, 1 H, 5-H), 8.13 (d, *J* = 7.5 Hz, 1 H, 6-H), 10.60 (br. s, 1 H, NH). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 13.7, 13.9, 24.6, 44.4, 45.3, 61.7, 63.3, 79.7, 93.7, 96.2, 143.7, 155.1, 163.4, 164.5, 167.3, 171.2. $C_{16}H_{22}N_4O_7$ (382.4): calcd. C 50.26, H 5.80, N 14.65; found C 50.22, H 5.81, N 14.64.

(3′*RS*,5′*SR*)-4-*N*-Acetyl-1-[3′,5′-bis(ethoxycarbonyl)-2′-methyl-1′,2′-isoxazolidin-5′-yl]cytosine (16a): 20% yield; viscous oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.24 (t, 3 H, *J* = 7.1 Hz), 1.24 (t, *J* = 7.1 Hz, 3 H), 2.28 (s, 3 H), 2.92 (dd, *J* = 8.5, 13.8 Hz, 1 H, 4′-H^a), 2.93 (s, 3 H, *N*-Me), 3.72 (m, 1 H, 4′-H^b), 3.73 (m, 1 H, 3′-H), 4.07 (q, *J* = 7.1 Hz, 2 H), 4.10 (q, *J* = 7.1 Hz, 2 H), 7.41 (d, *J* = 7.4 Hz, 1 H, 5-H), 8.05 (d, *J* = 7.4 Hz, 1 H, 6-H), 10.05 (br. s, 1 H, NH). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 13.7, 13.9, 25.1, 44.4, 45.3, 63.0, 65.5, 79.8, 94.2, 98.3, 143.6, 155.1, 163.4, 163.5, 167.9, 171.2. $C_{16}H_{22}N_4O_7$ (382.4): calcd. C 50.26, H 5.80, N 14.65; found C 50.20, H 5.81, N 14.63.

(3′*RS*,5′*RS*)-1-[3′,5′-Bis(ethoxycarbonyl)-2′-methyl-1′,2′-isoxazolidin-5′-yl]-5-fluorouridine (17b): 42.6% yield; yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.27 (t, *J* = 7.0 Hz, 3 H) 1.28 (t, *J* = 7.0 Hz, 3 H), 2.98 (dd, *J* = 4.8, 13.4 Hz, 1 H, 4′-H^a), 2.99 (s, 3 H, *N*-Me), 3.59 (dd, *J* = 4.8, 7.2 Hz, 1 H, 3′-H), 3.88 (dd, *J* = 4.8, 13.4 Hz, 1 H, 4′-H^b), 4.25 (q, *J* = 7.0 Hz, 2 H), 4.26 (q, *J* = 7.0 Hz, 2 H), 7.78 (d, *J* = 6.3 Hz, 1 H, 6-H), 9.70 (br. s, 1 H, NH). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 13.6, 13.8, 43.5, 45.1, 61.8, 63.3, 69.4, 92.9, 123.5 (d, *J* = 36.1 Hz), 138.3, 140.4 (d, *J* = 237.4 Hz), 149.0, 157.6, 167.6. $C_{14}H_{18}FN_3O_7$ (359.3): calcd. C 46.80, H 5.05, F 5.29, N 11.69; found C 46.73, H 5.07, F 5.28, N 11.70.

(3′*RS*,5′*SR*)-1-[3′,5′-Bis(ethoxycarbonyl)-2′-methyl-1′,2′-isoxazolidin-5′-yl]-5-fluorouridine (17a): 18.4% yield; yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.25 (t, *J* = 7.1 Hz, 3 H) 1.34 (t, *J* = 7.1 Hz, 3 H), 3.01 (s, 3 H, *N*-Me), 3.15 (dd, *J* = 5.8, 15.4 Hz, 1 H, 4′-H^a), 3.89 (dd, *J* = 5.8, 15.4 Hz, 1 H, 4′-H^b), 3.92 (dd, *J* = 4.7, 5.8 Hz, 1 H, 3′-H), 4.25 (q, *J* = 7.1 Hz, 2 H), 4.26 (q, *J* = 7.1 Hz, 2 H), 7.77 (d, *J* = 6.2 Hz, 1 H, 6-H), 9.98 (br. s, 1 H, NH). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 13.7, 14.0, 43.8, 44.7, 61.5, 63.4, 66.5, 93.7, 123.7 (d, *J* = 36.1 Hz), 137.5, 139 (d, *J* = 237.4), 149.0, 157.6, 168.0. $C_{14}H_{18}FN_3O_7$ (359.3): calcd. C 46.80, H 5.05, F 5.29, N 11.69; found C 46.74, H 5.06, F 5.29, N 11.70.

(3′*RS*,5′*SR*)-9-[3′,5′-Bis(ethoxycarbonyl)-2′-methyl-1′,2′-isoxazolidin-5′-yl]adenine (18a): 15% yield; yellow oil. ^{1}H NMR

(500 MHz, $CDCl_3$): δ = 1.09 (t, *J* = 7.2 Hz, 3 H), 1.14 (t, *J* = 7.2 Hz, 3 H), 2.92 (s, 3 H, *N*-Me), 3.74 (m, 2 H, 4′-H[a] and 3′-H), 3.99 (m, 1 H, 4′-H[b]), 4.16 (m, 4 H), 6.03 (br. s, 2 H, NH_2), 8.10 (s, 1 H), 8.26 (s, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 13.8, 14.1, 41.8, 45.7, 61.9, 63.5, 68.2, 92.7, 120.2, 149.2, 152.7, 155.4, 164.2, 168.0, 168.0. $C_{15}H_{20}N_6O_5$ (364.3): calcd. C 49.45, H 5.53, N 23.07; found C 49.49, H 5.52, N 23.05.

(3′*RS*,5′*RS*)-9-[3′,5′-Bis(ethoxycarbonyl)-2′-methyl-1′,2′-isoxazolidin-5′-yl]adenine (18b): 15% yield; yellow oil. ^1H NMR (500 MHz, $CDCl_3$): δ = 1.15 (t, 3 H, *J* = 7.1, Hz), 1.16 (t, *J* = 7.2 Hz, 3 H), 2.94 (m, 1 H, 4′-H[a]), 2.95 (s, 3 H, *N*-Me), 3.55 (dd, *J* = 9.3, 12.4 Hz, 1 H, 4′-H[b]), 3.95 (m, 1 H, 3′-H), 4.06 (m, 4 H), 5.95 (br. s, 2 H, NH_2), 8.10 (s, 1 H), 8.30 (s, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 13.8, 14.1, 41.9, 45.6, 62.1, 63.5, 68.3, 92.8, 120.3, 149.1, 152.8, 155.5, 165.3, 168.0, 168.2. $C_{15}H_{20}N_6O_5$ (364.3): calcd. C 49.45, H 5.53, N 23.07; found C 49.50, H 5.54, N 23.05.

(3′*RS*,5′*SR*)-2-*N*-Acetyl-9-[3′,5′-bis(ethoxycarbonyl)-2′-methyl-1′,2′-isoxazolidin-5′-yl]-6-*O*-(diphenylcarbamoyl)guanine (19a): 7.5% yield; yellow oil. ^1H NMR (500 MHz, $CDCl_3$): δ = 1.25 (t, *J* = 7.1 Hz, 3 H), 1.28 (t, *J* = 7.2 Hz, 3 H), 2.53 (s, 3 H), 2.70 (dd, *J* = 6.0, 11.7 Hz, 1 H, 4′-H[a]), 2.98 (s, 3 H, *N*-Me), 2.92 (dd, *J* = 6.9, 11.7 Hz, 1 H, 4′-H[b]), 3.18 (dd, *J* = 6.0, 6.9 Hz, 1 H, 3′-H), 4.20 (q, *J* = 7.1 Hz, 2 H), 4.27 (q, *J* = 7.2 Hz, 2 H), 7.17−7.38 (m, 10 H), 7.87 (s, 1 H), 8.07 (s, 1 H, 8-H). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 14.1, 14.3, 25.3, 43.4, 45.8, 61.9, 63.4, 70.0, 103.0, 126.7, 128.0, 129.5, 129.8, 141.9, 130.8, 145.5, 151.5, 153.1, 164.2, 168.1, 169.5, 169.9, 171.6. $C_{30}H_{31}N_7O_8$ (617.6): calcd. C 58.34, H 5.06, N 15.88; found C 58.30, H 5.04, N 15.89.

(3′*RS*,5′*RS*)-2-*N*-Acetyl-9-[3′,5′-bis(ethoxycarbonyl)-2′-methyl-1′,2′-isoxazolidin-5′-yl]-6-*O*-(diphenylcarbamoyl)guanine (19b): 7.5% yield; yellow oil. ^1H NMR (500 MHz, $CDCl_3$): δ = 1.26 (t, *J* = 7.1 Hz, 3 H), 1.27 (t, *J* = 7.0 Hz, 3 H), 2.50 (s, 3 H), 2.95 (m, 1 H, 4′-H[a]), 2.99 (s, 3 H, *N*-Me), 3.55 (dd, *J* = 5.9, 6.3 Hz, 1 H, 3′-H), 3.95 (m, 1 H, 4′-H[b]), 4.26 (q, *J* = 7.1 Hz, 2 H), 4.30 (q, *J* = 7.0 Hz, 2 H), 7.20−7.40 (m, 10 H), 7.70 (s, 1 H), 8.05 (s, 1 H, 8-H). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 14.1, 14.2, 25.3, 42.5, 45.8, 62.1, 63.1, 69.1, 103.2, 126.7, 128.0, 129.5, 129.8, 141.9, 130.7, 144.0, 150.2, 153.0, 164.5, 168.0, 169.9, 170.0, 171.5. $C_{30}H_{31}N_7O_8$ (617.6): calcd. C 58.34, H 5.06, N 15.88; found C 58.29, H 5.04, N 15.90.

Diethyl 2-Hydroxy-4-(methylimino)pent-2-enedioate (20): 50% yield; yellow oil. ^1H NMR (500 MHz, $CDCl_3$): δ = 1.36 (t, *J* = 5.9 Hz, 3 H), 1.38 (t, *J* = 5.9 Hz, 3 H), 3.2 (d, *J* = 5.8 Hz, 3 H), 4.34 (q, *J* = 5.9 Hz, 2 H), 4.39 (q, *J* = 5.9 Hz, 2 H), 6.28 (s, 1 H), 10.90 (br. s, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 14.0, 14.1, 32.5, 62.0, 62.6, 92.6, 94.4, 135.9, 170.0, 171.5. $C_{10}H_{15}NO_5$ (229.2): calcd. C 52.40, H 6.60, N 6.11; found C 52.46, H 6.61, N 6.10.

Treatment of Isoxazolidine Nucleosides 15−18 with $NaBH_4$

General Procedure: $NaBH_4$ (0.012 g, 3 mmol) was added at 0 °C to a stirred solution of **15**−**18** (1.0 mmol) in methanol (50 mL), and the mixture was stirred for 3 h. At the end of this period, the solvent was removed and the residue was extracted with $CHCl_3$/MeOH. The filtrates were collected and concentrated in vacuo to a volume of 1−2 mL, and loaded onto a preparative RP column (Spherisorb ODS-2, 500 μm, 4.6 × 250 nm, 7.5% MeCN in 0.1 M NH_4OAc, flow rate 1 mL/min). Fractions of pure nucleosides obtained by elution with 5% aq. MeCN were pooled, concentrated to dryness, coevaporated with EtOH (3 × 25 mL), and finally dried in vacuo to give **21**−**24** as viscous oils.

(3′*RS*,5′*RS*)-1-[3′,5′-Bis(hydroxymethyl)-2′-methyl-1′,2′-isoxazolidin-5′-yl]thymine (21b): 80% yield, viscous oil. ^1H NMR (500 MHz, $CDCl_3/CD_3OD$, 9:1): δ = 1.81 (d, *J* = 1.2 Hz, 3 H), 2.33 (m, 2 H, 4′-H), 2.73 (s, 3 H, *N*-Me), 3.44 (m, 1 H, 3′-H), 3.65 (br. s, 2 H, OH), 4.05 (m, 2 H, 3′′-H), 4.21 (m, 1 H, 5′′-H[a]), 4.24 (m, 1 H, 5′′-H[b]), 7.54 (q, *J* = 1.2 Hz, 1 H, 6-H), 8.41 (br. s, 1 H, NH). ^{13}C NMR (125 MHz, $CDCl_3/CD_3OD$, 9:1): δ = 26.04, 30.88, 46.10, 60.68, 61.30, 69.40, 102.78, 131.36, 136.26, 158.04, 166.18.

(3′*RS*,5′*RS*)-1-[3′,5′-Bis(hydroxymethyl)-2′-methyl-1′,2′-isoxazolidin-5′-yl]cytosine (22b): 40% yield, viscous oil. ^1H NMR: (500 MHz, $CDCl_3/CD_3OD$, 9:1): δ = 2.42 (dd, *J* = 9.5, 13.5 Hz, 1 H, 4′-H[a]), 2.60 (dd, *J* = 7.5, 13.5 Hz, 1 H, 4′-H[b]), 2.82 (s, 3 H, *N*-Me), 2.89 (m, 1 H, 3′-H), 3.53 (m, 2 H, 3′′-H), 3.60−3.70 (br. s, 4 H, NH_2 and OH), 3.98 (d, *J* = 12.0 Hz, 1 H, 5′′-H[a]), 4.15 (d, *J* = 12.0 Hz, 1 H, 5′′-H[b]), 5.65 (d, *J* = 7.0 Hz, 1 H, 5-H), 7.72 (d, *J* = 7.0 Hz, 1 H, 6-H). ^{13}C NMR: (125 MHz, $CDCl_3/CD_3OD$, 9:1): δ = 42.16, 45.30, 64.30, 64.40, 70.06, 93.62, 96.07, 115.99, 143.79, 149.66, 155.52.

(3′*RS*,5′*RS*)-1-[3′,5′-Bis(hydroxymethyl)-2′-methyl-1′,2′-isoxazolidin-5′-yl]-5-fluorouridine (23b): 35% yield, viscous oil. ^1H NMR (500 MHz, $CDCl_3/CD_3OD$, 9:1): δ = 2.50 (m, 2 H, 4′-H), 2.92 (s, 3 H, *N*-Me), 3.45 (m, 1 H, 3′-H), 3.50−3.65 (br. s, 2 H, OH), 3.70 (m, 2 H, 3′′-H) 3.97 (d, *J* = 11.8 Hz, 1 H, 5′′-H[a]), 4.05 (d, *J* = 11.8 Hz, 1 H, 5′′-H[b]), 7.82 (d, *J* = 6.8 Hz, 1 H, 6-H), 8.90 (br. s, 1 H, NH). ^{13}C NMR (125 MHz, $CDCl_3/CD_3OD$, 9:1): δ = 43.96, 44.05, 64.20, 64.25, 69.43, 93.52, 124.28, 137.58, 149.07, 156.94.

(3′*RS*,5′*SR*)-9-[3′,5′-Bis(hydroxymethyl)-2′-methyl-1′,2′-isoxazolidin-5′-yl]adenine (24a): 30% yield, viscous oil. ^1H NMR (500 MHz, $CDCl_3/CD_3OD$, 9:1): δ = 2.70 (d, 1 H, *J* = 8.2, 12.4 Hz, 4′-H[a]), 2.99 (s, 3 H, *N*-Me), 3.05 (m, 2 H, 3′-H and 4′-H[b]), 3.50 (br. s, 2 H, OH), 3.67 (m, 2 H, 3′′-H), 4.15 (d, *J* = 12.1 Hz, 1 H, 5′′-H[a]), 4.20 (d, *J* = 12.1 Hz, 1 H, 5′′-H[b]), 6.20 (br. s, 2 H, NH_2), 8.15 (s, 1 H, 9-H), 8.23 (s, 1 H, 3-H). ^{13}C NMR (125 MHz, $CDCl_3/CD_3OD$, 9:1): δ = 42.30, 45.15, 63.63, 63.90, 70.20, 95.40, 120.19, 149.25, 152.74, 155.50, 164.20.

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