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Isoxazolidine analogues of pseudouridine: a new class of modified nucleosides

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Abstract—A new class of modified C-nucleosides has been synthesized according to the 1,3-dipolar cycloaddition methodology. The obtained compounds are structurally related to natural pseudouridine, where the sugar moiety is replaced by an isoxazolidine ring. Different experimental conditions, and the effect of additives on the cycloaddition process, have been examined; the best results were obtained when the cycloaddition reaction was performed under microwave irradiation

1. Introduction

In the treatment of human viral diseases, nucleoside analogues have recently emerged as important therapeutic agents.[1] The majority of nucleoside analogues consist of modifications of the natural substrates in the heterocyclic base and/or the sugar moiety: the most notable structural variations are found in the furanose ring with its replacement by a acyclic chain[2] or alternative carbo-[3] or heterocyclic systems[4] to give a series of biologically interesting compounds.

Any variation on the base moiety should preserve the possibility of hydrogen bond interactions between heterocyclic bases, which are fundamental for the biological activity; as a consequence, only minor modifications of bases are present in biologically active modified nucleosides. The most remarkable of that sort of structural modification is found in *C*-nucleosides, where the typical C–N glycosidic bond is replaced by a nonhydrolyzable C–C bond.[5]

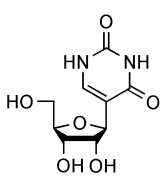

Figure 1. Pseudouridine (ψ).

Among this latter kind of compounds, pseudouridine (ψ or 5-β-D-ribofuranosyluracil, Fig. 1) plays a particularly interesting role. Pseudouridine is a ubiquitous yet enigmatic constituent of structural RNAs; although it was the first modified nucleoside to be discovered in RNA, and is the most abundant, its biosynthesis and biological role have remained poorly understood since its identification as a fifth nucleoside in RNA.[6] Through its unique ability to coordinate a structural water molecule via its free N_1–H, ψ exerts a subtle but significant rigidifying influence on the nearby sugar–phosphate backbone and also enhances base stacking.[7] These effects may underlie the biological role of most of the pseudouridine residues in RNA. The lack of pseudouridine residues in tRNA or rRNA leads to slow growth rates: such studies demonstrate that pseudouridylation of RNA confers an important selective advantage in a natural biological context.[8]

In this paper we report the synthesis of new nucleoside analogues which include modifications at the level of both the furanose ring and heterocyclic base. These derivatives, structurally related to natural pseudouridine, with the sugar moiety replaced by an isoxazolidine ring,[9] represent the first example of this kind of compounds which has not yet been reported in literature.

2. Results and discussion

The key step of the approach involves the synthesis of 5-formyluracil (**3**), which was converted into the corresponding nitrone **5** and, subsequently, into the target

Keywords: glycosidic bond; pseudouridine; cycloaddition.
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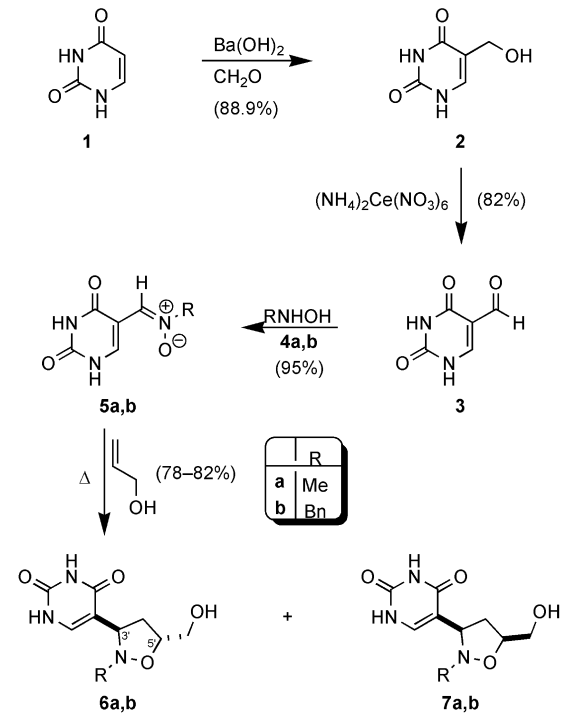
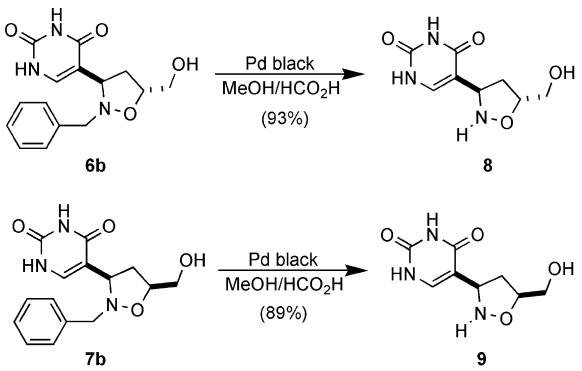


Scheme 1.



Scheme 2.

nucleoside **6** and **7** by a 1,3-dipolar cycloaddition reaction (Scheme 1).

As reported in literature,[10] the uracil (**1**), was converted into the 5-hydroxymethyl derivative **2** by treatment with 37% formaldehyde in aqueous $Ba(OH)_2$; further oxidation with ammonium cerium(IV) nitrate gave the required 5-formyluracil (**3**).[11]

The subsequent reaction with *N*-methyl and *N*-benzyl hydroxylamine hydrochlorides in the presence of sodium acetate, as a base, afforded nitrones **5a**[11] and **5b**, respectively, in *Z* configuration, as ascertained by ^{1}H NMR NOEDS analysis. A DMF solution of nitrone **5a** and allyl alcohol in excess was heated in a sealed tube at 120°C for 24 h. The cycloaddition reaction proceeded regioselectively to give, after separation by silica gel chromatography, isoxazolidines **6a** and **7a** in 44 and 34% yields, respectively (Scheme 1). The analogous reaction of nitrone **5b** with allyl alcohol in a sealed tube at 140°C for 24 h yielded isoxazolidines **6b** and **7b** in 45 and 37% yields, respectively.

The structures of the obtained compounds have been assigned on the basis of spectrometric measurements; in particular, stereochemical assignments were established by ^{1}H NOEDS. For *cis* compounds (β-derivatives) **7**, irradiation of proton $H_{5'}$ at δ=4.17 and 4.26, in compounds **a** and **b** respectively, induced a strong enhancement of $H_{3'}$ (δ=3.66 and 3.96), thus indicating a *cis* topological relationship between these protons. For *trans* compounds **6** (α-derivatives), irradiation of $H_{5'}$ at δ=3.95 and 3.97 resulted in a positive NOE effect on $H_{4'a}$, the upfield resonance of methylene protons at $C_{4'}$ (δ=2.07 and 2.09), while irradiation of the $H_{4'b}$ (δ=2.26 and 2.34 gives rise to the enhancement of $H_{3'}$ resonance (δ=3.55 and 3.86).

Compounds **6b** and **7b** were converted into the corresponding free hydroxyamino derivatives **8** and **9** by treatment with palladium black and formic acid (Scheme 2).

Table 1. Effect of the additives in the cycloaddition between **5b** and allyl alcohol

Entry		Additive	Solvent	Eq. alcohol	Temp (°C)	Time	Conversion[a] (%)	Isolated yield[a] (%)	Ratio[a]
1	Normal tube	None	Neat	200	100	48 h	<5	–	–
2		M K-10[b]	DMF	20	100	4 days	0	–	–
3		Si-W[c]	DMF	200	100	27 h	<5	–	–
4		MS[d]	DMF	100	100	3.5 days	<15	10	–
5		Si-W[c]+MS[d]	DMF	100	100	3.5 days	<15	10	–
6		Nafion[e]	DMF	100	100	3.5 days	<15	10	–
7	Sealed tube	None	Neat	200	140	24 h	100	40	1.5:1
8		M K-10[b]	DMF	20	140	2 days	<15	10	–
9		MS[d]	DMF	20	130	24 h	25	20	1:1
10		Si-W[d]	DMF	20	140	24 h	<10	5	–
11		MS[d]	Neat	200	140	24 h	100	80	1:1
12		Nafion[e]	DMF	20	140	24 h	100	80	1:1
13		Nafion[e]	DMF	20	140	12 h	40	80	1:1
14		Nafion[e]	Neat	200	140	12 h	100	80	1:1
15		M K-10[b]	neat	200	140	12 h	100	94[f]	1:1
16		None	Neat	200	MW[g]	10 min	100	85	1:1

[a] All reactions have been monitored by TLC, NMR and HPLC.
[b] M K-10: Montmorillonite K-10 (heated at 250°C for 24 h before use).
[c] Si-W: Silicotungstic acid (heated at 250°C for 24 h before use).
[d] MS: activated molecular sieves 4 Å (heated at 250°C for 24 h before use).
[e] Nafion: powder (heated at 100°C for 2 h under high vacuum before use).
[f] Only a 6% of product was obtained, the remaining 88% being the debenzylated products **8** and **9**.
[g] Microwave irradiation was conducted in a Moulinex FM 5745, a domestic oven, at 650 W.

1,3-Dipolar cycloaddition reactions might be activated by the use of Lewis acids.[12] However, we anticipated that typical Lewis acids are not compatible with nitrones **5a**,**b**, due to the high number of coordinating sites of the base moiety. With the aim of making compatible our substrates with acidity, we decided to examine several solid acids such as montmorillonite K-10, Nafion and the heteropolyacid $H_4SiW_{12}O_{40}$. The use of these additives has been successfully described in other reactions such as glycosylation[13] as an alternative to classic Lewis acid.

Nitrone **5b** was selected as model compound for these studies and the obtained results are summarized in Table 1. For the purpose of comparison, we have also performed several reactions in a normal open tube. Disappointingly, no conversion or quite low yields were obtained (Table 1, entries 1–6). Presumably, the lack of conversion was also due to the lower temperature used; however, at higher temperatures, extensive decomposition was observed.

So, we turned back to the use of sealed tubes in all the reactions. In absence of solvent (entry 7), a total conversion was observed, albeit with low chemical yields: traces of the debenzylated derivatives **8** and **9** have been detected among the by-products of the reaction. The addition of montmorillonite K-10 (entry 8), 4 Å molecular sieves (entry 9) or silicotungstic acid (entry 10) to the reaction performed in DMF did not show better results.

However, when the reaction was carried out without solvent, in the presence of 4 Å molecular sieves, a good chemical yield (80%) was obtained (entry 11). Comparable results were recorded with the use of Nafion as catalyst (entries 12–14). Under neat conditions (entry 14), 100% of conversion was obtained after 12 h, with 80% yield.

Interestingly, when montmorillonite K-10 was used as an additive under the same conditions (entry 15), the reaction led directly to the debenzylated derivatives **8** and **9** as the major compounds (88%): *N*-benzyl isoxazolidines **6b**, and **7b** were obtained in only 6% yield.

Finally, the best results were obtained when the cycloaddition reaction was performed under microwave irradiation (entry 16). In this case the reaction time was dramatically reduced (10 minutes) and the yield increased to 85%.

In conclusion, a synthetic approach based on the 1,3-dipolar cycloaddition methodology towards a new class of modified isoxazolidinyl-*C*-nucleosides has been reported. The obtained compounds are structurally related to natural pseudouridine. Tests on the biological activity of these derivatives are in progress.

3. Experimental

3.1. General

Melting points are uncorrected. NMR spectra were recorded at 500 MHz (^{1}H) and at 125 MHz (^{13}C) and are reported in ppm downfield from TMS. The NOE difference spectra were obtained by subtracting alternatively right off-resonance free induction decays (FIDS) from right-on-resonance-induced FIDS. All reagents were purchased from commercial suppliers and were used without further purification. The solvents for chromatography were distilled at atmospheric pressure prior to use and were dried using standard procedures. The HPLC purifications were made by preparative HPLC with a microsorb silica DYNAMAX-100 Å (21×250 mm) column, with a Varian Pro Star instrument. Elemental analysis were performed on a Perkin–Elmer 240B microanalyzer.

3.1.1. 5-(Hydroxymethyl)uracil (2).[10] Uracil (**1**) (25 g, 223 mmol) was added to a filtered solution of $Ba(OH)_2 \cdot 8H_2O$ (15 g, 480 mmol) in water (500 mL). A solution of 37% aqueous formaldehyde (54 mL, 720 mmol) was then added, and the reaction mixture was refluxed for a few minutes in order to dissolve uracil. After standing for 12 h at room temperature, gaseous CO_2 was bubbled into the reaction mixture in order to precipitate $BaCO_3$. After filtration, water was evaporated, and the viscous residue was dissolved at reflux in 70% ethanol (250 mL). The obtained compound **2** crystallized in the refrigerator as a pure white solid [23 g, 73%, mp 225–230°C (lit.[10] mp 220–230°C)]. Mother liquor was evaporated and the residue was purified by flash chromatography column on silica gel (chloroform/methanol, 7:3) to give 5 g of **2** (15.9%).

3.1.2. 5-Formyluracil (3).[11] 5-(Hydroxymethyl)uracil (**2**) (10 g, 70 mmol) was dissolved in water (74 mL) at 70°C. A 2 M aqueous ammonium cerium(IV) nitrate solution (81 g in 74 mL, 148 mmol) was added and the temperature raised to 90°C, under magnetic stirring. The reaction mixture was allowed to stand at this temperature until the dark red color change to light yellow (almost 1 h). After cooling, the mixture was filtered on a buchner funnel; the residue was washed with acetone and air dried to give compound **3** as white solid [8.09 g, 82% yield, mp 303–305 with decomposition (lit.[11] mp >300°C)].

3.2. Synthesis of nitrones 5

General procedure. To solution of aldehyde **3** (10 g, 71.4 mmol), in 200 mL of water, cooled to 0°C, alkylhydroxylamine hydrochloride **4** (107 mmol) and sodium acetate (8.78 g, 107 mmol) were added. The reaction mixture was warmed to room temperature and allowed to react overnight. After filtration and acetone washing, nitrone **5** was recovered as a white solid which was utilized without further purification.

3.2.1. (*Z*)-*N*-Methyl-*C*-(5-uracil) nitrone (5a).[11] 11.47 g, 95% yield, mp 285–287°C (lit.[11] mp 281–283°C).

3.2.2. (*Z*)-*N*-Benzyl-*C*-(5-uracil) nitrone 5b. (16.63 g, 95% yield, mp 267–270°C). IR (KBr) ν_{max} 3152, 3066, 3040, 3020, 2825, 1705, 1600, 1255, 1150, 880, 755 cm^{-1}. ^{1}H NMR, ($DMSO_{d6}$, 500 MHz) δ 5.05 (s, 2H, *N*-CH_2), 7.33–7.45 (m, 5H, aromatic protons), 7.84 (s, 1H, CH═N), 9.52 (s, 1H, H_6), 11.25 (bs, 2H, NH). ^{13}C NMR ($DMSO_{d6}$, 125 MHz) δ 68.8, 105.2, 126.3, 128.3, 128.4, 129.1, 134.7, 140.3, 150.3, 162.6. HRMS (EI) calcd for [M^+] $C_{12}H_{11}N_3O_3$ 245.0800, found: 245.0798. Anal. calcd for

$C_{12}H_{11}N_3O_3$: C, 58.77; H, 4.52; N, 17.13%. Found: C, 58.61; H, 4.53; N, 17.11%.

3.3. Synthesis of isoxazolidinyluridines 6 and 7

General procedure. A solution of nitrone **5** (5 mmol) and allyl alcohol (5.8 g, 6.8 mL, 100 mmol), in dimethylformamide (DMF) (100 mL), was heated, in a sealed tube, for 24 h at 120°C for **5a** and at 140°C for **5b**. DMF was evaporated at reduced pressure and the residue was purified by flash chromatography column on silica gel (chloroform/methanol, 9:1), followed by preparative HPLC [microsorb silica DYNAMAX-100 Å (21×250 mm) column, flow 3.5 mL/min] utilising a *n*-hexane/2-propanol 85:15 eluting mixture for compounds **6a** and **7a** while a mixed isocratic and linear gradient of 2-propanol (10%, 0–15 min, 10–15%, 15–20 min) in *n*-hexane for compounds **6b** and **7b**.

3.3.1. (3′*RS*,5′*RS*)-5-[5′-Hydroxymethyl-2′-methyl-1′,2′-isoxazolidin-3′-yl]uracil (6a). (500 mg, 44% yield, HPLC: t_R 37.5 min; sticky oil). IR (KBr) ν_{max} 3450–3250, 3220, 3105, 2990, 2910, 2840, 1730, 1660, 1450, 1230, 1050, 770 cm^{-1}. ^1H NMR, ($DMSO_{d6}$, 500 MHz) δ 2.03–2.09 (m, 1H, $H_{4'a}$), 2.22–2.28 (m, 1H, $H_{4'b}$), 2.49 (s, 3H, *N*-Me), 3.40–3.43 (m, 2H, C*H*$_2$OH), 3.53–3.57 (m, 1H, $H_{3'}$), 3.93–3.97 (m, 1H, $H_{5'}$), 4.75 (bs, 1H, OH), 7.25 (s, 1H, H_6), 10.79 (bs, 1H, NH), 11.10 (bs, 1H, NH). ^{13}C NMR ($DMSO_{d6}$, 125 MHz) δ 37.3, 44.2, 62.7, 62.9, 77.7, 110.7, 138.6, 151.0, 163.8. HRMS (EI) calcd for [M^+] $C_9H_{13}N_3O_4$ 227.0906, found: 227.0905. Anal. calcd for $C_9H_{13}N_3O_4$: C, 47.57; H, 5.77; N, 18.49%. Found: C, 47.43; H, 5.78; N, 18.53%.

3.3.2. (3′*RS*,5′*SR*)-5-[5′-Hydroxymethyl-2′-methyl-1′,2′-isoxazolidin-3′-yl]uracil (7a). (386 mg, 34% yield, HPLC: t_R 31.6 min; sticky oil). IR (KBr) ν_{max} 3450–3250, 3215, 3120, 3030, 2960, 2920, 2880, 1715, 1670, 1420, 1210, 1110, 760 cm^{-1}. ^1H NMR, ($DMSO_{d6}$, 500 MHz) δ 1.74–2.00 (m, 1H, $H_{4'a}$), 2.52 (s, 3H, *N*-Me), 2.59–2.65 (m, 1H, $H_{4'b}$), 3.36–3.40 (m, 2H, C*H*$_2$OH), 3.63–3.69 (m, 1H, $H_{3'}$), 4.15–4.19 (m, 1H, $H_{5'}$), 4.71 (bs, 1H, OH), 7.24 (s, 1H, H_6), 10.77 (bs, 1H, NH), 11.08 (bs, 1H, NH). ^{13}C NMR ($DMSO_{d6}$, 125 MHz) δ 37.2, 43.7, 62.8, 63.1, 77.1, 111.8, 138.1, 151.0, 163.8. HRMS (EI) calcd for [M^+] $C_9H_{13}N_3O_4$ 227.0906, found: 227.0904. Anal. calcd for $C_9H_{13}N_3O_4$: C, 47.57; H, 5.77; N, 18.49%. Found: C, 47.47; H, 5.76; N, 18.51%.

3.3.3. (3′*RS*,5′*RS*)-5-[2′-Benzyl-5′-hydroxymethyl-1′,2′-isoxazolidin-3′-yl]uracil (6b). (670 mg, 45% yield, HPLC: t_R 32.7 min; white solid: mp 194–196°C). IR (KBr) ν_{max} 3450–3250, 3220, 3105, 3030, 2995, 2925, 2855, 1720, 1665, 1440, 1230, 1045, 775 cm^{-1}. ^1H NMR, ($DMSO_{d6}$, 500 MHz) δ 2.09 (dt, 1H, *J*=7.0, 11.5 Hz, $H_{4'a}$), 2.34 (dt, 1H, *J*=8.0, 11.5 Hz, $H_{4'b}$), 3.34–3.48 (m, 2H, C*H*$_2$OH), 3.81 (d, 1H, *J*=14.0 Hz, *N*-C*H*$_2$Ph), 3.86 (dd, 1H, *J*=7.0, 8.0 Hz, $H_{3'}$), 3.88 (d, 1H, *J*=14.0 Hz, *N*-C*H*$_2$Ph), 3.95–3.99 (m, 1H, $H_{5'}$), 4.13 (t, 1H, *J*=5.5 Hz, OH), 7.21–7.32 (m, 5H, aromatic protons), 7.30 (s, 1H, H_6), 10.81 (bs, 1H, NH), 11.13 (bs, 1H, NH). ^{13}C NMR ($DMSO_{d6}$, 125 MHz) δ 36.7, 60.7, 61.2, 62.6, 78.1, 111.1, 126.8, 128.0, 128.6, 138.3, 138.5, 151.0, 163.7. HRMS (EI) calcd for [M^+] $C_{15}H_{17}N_3O_4$ 303.1219, found: 303.1221. Anal. calcd for $C_{15}H_{17}N_3O_4$: C, 59.40; H, 5.65; N, 13.85%. Found: C, 59.52; H, 5.64; N, 13.83%.

3.3.4. (3′*RS*,5′*SR*)-5-[2′-Benzyl-5′-hydroxymethyl-1′,2′-isoxazolidin-3′-yl]uracil (7b). (558 mg, 37% yield, HPLC: t_R 30.4 min; white solid: mp 190–191°C). IR (KBr) ν_{max} 3450–3250, 3210, 3115, 3025, 2970, 2940, 2895, 1720, 1665, 1430, 1200, 1115, 775 cm^{-1}. ^1H NMR, ($DMSO_{d6}$, 500 MHz) δ 1.83 (dt, 1H, *J*=5.5, 12.5 Hz, $H_{4'a}$), 2.73 (dt, 1H, *J*=8.0, 12.5 Hz, $H_{4'b}$), 3.32–3.36 (m, 2H, C*H*$_2$OH), 3.87 (d, 1H, *J*=13.5 Hz, *N*-C*H*$_2$Ph), 3.92 (d, 1H, *J*=13.5 Hz, *N*-C*H*$_2$Ph), 3.96 (dd, 1H, *J*=5.5, 8.0 Hz, $H_{3'}$), 4.23–4.29 (m, 1H, $H_{5'}$), 4.69 (t, 1H, *J*=5.8 Hz, OH), 7.21–7.34 (m, 5H, aromatic protons), 7.30 (s, 1H, H_6), 10.73 (bs, 1H, NH), 11.05 (bs, 1H, NH). ^{13}C NMR ($DMSO_{d6}$, 125 MHz) δ 36.9, 59.7, 60.5, 62.8, 77.6, 112.6, 126.9, 128.1, 128.7, 138.0, 138.0, 151.1, 163.8. HRMS (EI) calcd for [M^+] $C_{15}H_{17}N_3O_4$ 303.1219, found: 303.1222. Anal. calcd for $C_{15}H_{17}N_3O_4$: C, 59.40; H, 5.65; N, 13.85%. Found: C, 59.55; H, 5.66; N, 13.81%.

3.4. Hydrogenolysis of isoxazolidines 6b and 7b

General procedure. Isoxazolidine **6b** or **7b** (200 mg, 0.66 mmol) was dissolved in anhydrous MeOH (25 mL) and treated with anhydrous HCO_2H (2.3 mL). Palladium black (700 mg, 6.60 mmol) was added to the rapidly stirring solution. After 2.5 h TLC analysis revealed that reaction was completed. The mixture was filtered through Celite, washed with MeOH (50 mL) and concentrated in vacuo. The residue was dissolved in MeOH, and stirred with anhydrous K_2CO_3 (15 min.). After filtration through adsorbent cotton, the removal of solvent in vacuo afforded a residue which was purified by column flash chromatography on silica gel, using methanol as eluant.

3.4.1. (3′*RS*,5′*RS*)-5-[5′-Hydroxymethyl-1′,2′-isoxazolidin-3′-yl]uracil (8). (130 mg, 93% yield, sticky oil). IR (KBr) ν_{max} 3500–3300, 3215, 3100, 2985, 2920, 2850, 1725, 1665, 1450, 1220, 1040, 760 cm^{-1}. ^1H NMR, ($DMSO_{d6}$, 500 MHz) δ 1.75 (ddd, 1H, *J*=5.1, 9.6, 12.6 Hz, $H_{4'a}$), 2.06 (dd, 1H, *J*=5.9, 12.6 Hz, $H_{4'b}$), 3.20 (bs, 1H, NH), 3.55 (dd, 1H, *J*=0.8, 8.8 Hz, $H_{5''a}$), 3.93 (dd, 1H, *J*=4.4, 8.8 Hz, $H_{5''b}$), 4.30–4.36 (m, 1H, $H_{5'}$), 4.74 (dd, 1H, *J*=5.9, 9.6 Hz, $H_{3'}$), 4.91 (d, 1H, *J*=3.3 Hz, OH), 7.21 (s, 1H, H_6), 10.80 (bs, 1H, NH), 11.04 (bs, 1H, NH). ^{13}C NMR ($DMSO_{d6}$, 125 MHz) δ 42.0, 70.9, 73.2, 75.2, 113.2, 137.4, 151.2, 163.4. HRMS (FAB−) calcd for [M^+] $C_8H_{11}N_3O_4$ 213.0749, found: 213.0746. Anal. calcd for $C_8H_{11}N_3O_4$: C, 45.07; H, 5.20; N, 19.71%. Found: C, 44.86; H, 5.19; N, 19.76%.

3.4.2. (3′*SR*,5′*SR*)-5-[5′-Hydroxymethyl-1′,2′-isoxazolidin-3′-yl]uracil (9). (125 mg, 89% yield, sticky oil). IR (KBr) ν_{max} 3500–3300, 3220, 3115, 3025, 2990, 2920, 2850, 1725, 1670, 1450, 1240, 1050, 770 cm^{-1}. ^1H NMR, ($DMSO_{d6}$, 500 MHz) δ 1.62 (ddd, 1H, *J*=2.1, 5.8, 13.3 Hz, $H_{4'a}$), 2.35 (ddd, 1H, *J*=6.3, 8.5, 13.3 Hz, $H_{4'b}$), 3.30 (bs, 1H, NH), 3.61 (dd, 1H, *J*=4.4, 9.2 Hz, $H_{5''a}$), 3.70 (dd, 1H, *J*=1.1, 9.2 Hz, $H_{5''b}$), 4.25–4.33 (m, 1H, $H_{5'}$), 4.60 (dd, 1H, *J*=5.8, 8.5 Hz, $H_{3'}$), 4.96 (d, 1H, *J*=4.4 Hz, NH), 7.30 (s, 1H, H_6), 10.80 (bs, 1H, NH), 11.10 (bs, 1H, NH). ^{13}C NMR ($DMSO_{d6}$, 125 MHz) δ 42.1, 70.9, 73.4, 75.3, 114.0, 138.2,

151.4, 163.8. HRMS (FAB−) calcd for [M^+] $C_8H_{11}N_3O_4$ 213.0749, found: 213.0747. Anal. calcd for $C_8H_{11}N_3O_4$: C, 45.07; H, 5.20; N, 19.71%. Found: C, 44.91; H, 5.21; N, 19.73%.

3.5. Reactions of nitrone 5b with allyl alcohol in the presence of additives

To a solution of nitrone **5b** (122.6 mg, 0.50 mmol) and allyl alcohol (0.68 mL, 10 mmol; 3.4 mL, 50 mmol or 6.8 mL, 100 mmol; see Table 1) in DMF (10 mL), the additive (Montmorillonite K-10, 63 mg for open tube reactions and 126 mg for sealed tube reactions. Silicotungstic acid, 104 mg for open tube reactions and 150 mg for sealed tube reactions. Molecular sieves, 69 mg for both open tube and sealed tube reactions. Nafion, 66 mg for both open tube and sealed tube reactions) was added. The resulting mixture was heated at the stated temperature (see Table 1) under an argon atmosphere for the indicated time (see Table 1). After cooling to room temperature, the reaction mixture was filtered and evaporated under vacuum. The obtained residue was maintained under high vacuum (<1 mmHg) for additional 6 h and than purified by column flash chromatography on silica gel (chloroform/methanol, 9:1).

The reactions without solvent were carried out with 6.8 mL of allyl alcohol (100 mmol), using the same amounts of additive.

3.6. Reaction of nitrone 5b with allyl alcohol under microwave condition

To nitrone **5b** (122.6 mg, 0.50 mmol), allyl alcohol (6.8 mL, 100 mmol) was added, and the resulting mixture was irradiated at 650 W for 10 min. After cooling to room temperature, the reaction mixture was evaporated under vacuum and the residue purified by column flash chromatography on silica gel (chloroform/methanol, 9:1).

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