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Synthesis of a Photolabeling Probe for the Study of Antiviral Mechanism of Ribavirin

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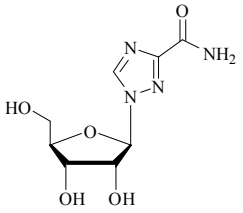
Abstract: Ribavirin has been used in urgency to treat SARS patients recently. In order to study its antiviral mechanism by photolabeling approach, we have synthesized and characterized 5-azido-1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxiamide **1** as a photolabeling probe of ribavirin. The azidotriazole nucleoside showed rapid and clean photochemical reaction, suggesting that **1** is a promising probe to study the antiviral mechanism of ribarivin by photolabeling.

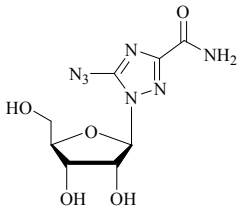
Keywords: Ribavirin, photolabeling probe of ribavirin, azidotriazole nucleoside, azole nucleosides, photolabeling.

Ribavirin (**Scheme 1**) is the first synthetic nucleoside that exhibits broad-spectrum antiviral activity against many RNA and DNA viruses[1]. It is used in combination with interferon α to treat HCV infection[2]. Recently, it is used in the treatment of SARS patients and has been found favorable responses at the early stage of infection[3]. The exact antiviral mechanism of ribavirin is not clear, although many hypotheses have been proposed since the discovery of ribavirin over 30 years ago[4]. It is very important for us to have a clear understanding of the antiviral mechanism of ribavirin in order to develop more efficacious and safe antiviral candidates for the emerging viruses, for which very few efficient therapies exist. Thus, we would like to propose a photolabeling approach[5] to study the antiviral mechanism of ribavirin.

Scheme 1 Ribavirin and the proposed photolabeling probe of ribavirin **1**



Ribavirin



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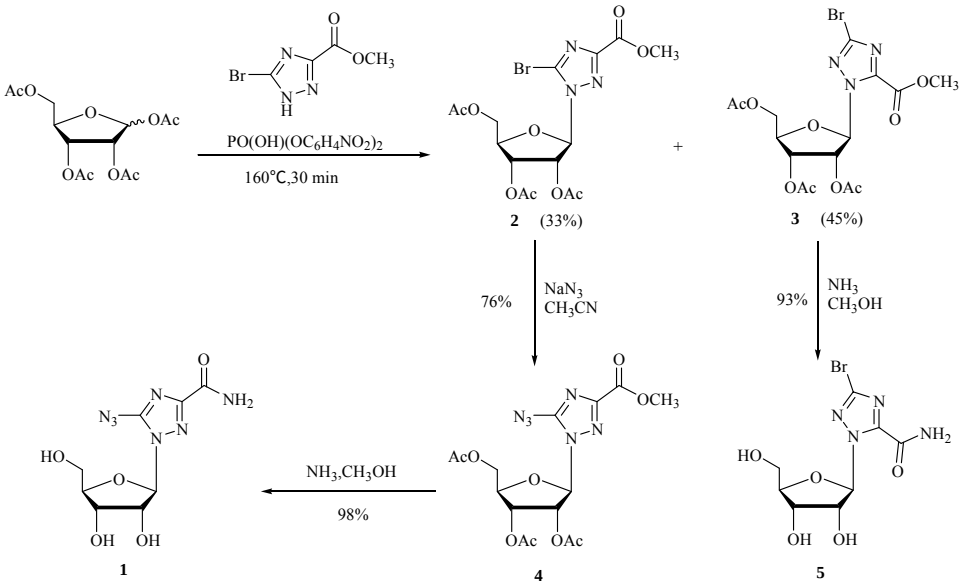
Photolabeling is becoming increasingly important and common in studying mechanism of drug action. It uses photoactivatable probes, which, by the action of light, will produce highly reactive species and bind covalently to the protein at the binding site[6]. This enables us to identify the targets of drugs, to determine the drug-target interaction, and to map the binding site on the target. In order to study the antiviral mechanism of ribavirin by photolabeling, we have designed azidotriazole nucleoside **1** (**Scheme 1**) as photolabeling probe. In this communication, we report its synthesis and characterization.

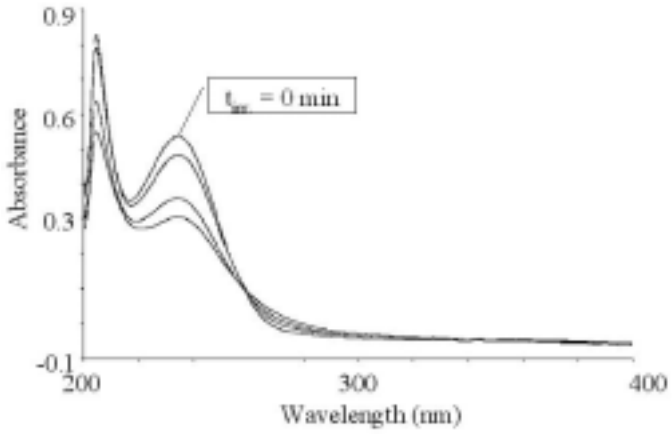
The synthesis of **1** was based on the nucleophilic substitution of the corresponding 5-bromotriazole nucleoside with NaN_3 (**Scheme 2**). The synthesis of the bromotriazole nucleosides was accomplished *via* the acid-catalyzed fusion procedure[7]. Fusion of methyl 5-bromo-1,2,4-triazole-3-carboxylate with 1,2,3,5-tetra-*O*-acetyl-D-ribofuranose at 160 °C in the presence of an acid catalyst, bis(*p*-nitrophenyl)phosphate, gave a mixture of two isomeric nucleosides: 5-bromo-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-1,2,4-triazole-5-carboxylic acid methyl ester **2** and 3-bromo-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-1,2,4-triazole-5-carboxylic acid methyl ester **3**. These two isomers were separated by column chromatography over silica gel and identified by comparing their NMR spectra with those of the corresponding chlorotriazole nucleoside analogues in the literature[8]. Further, treatment of **3** in NH_3/MeOH led to the nucleoside **5**. The structure of **5** was confirmed by single-crystal X-ray analysis[9]. Transformation of the 5-bromotriazole nucleoside **2** into the 5-azidotriazole nucleoside **4** was achieved in good yield with NaN_3 in CH_3CN. Further treatment of **4** in NH_3/MeOH provided the final product **1** in almost quantitative yield.

Scheme 2 Synthesis of the photolabeling probe **1**



All of the obtained nucleosides are new compounds and have been characterized by ^{1}H-NMR, ^{13}C-NMR, IR, UV and MS[10]. Since **1** is not ready soluble in most organic solvents, photochemical study[11] has been undertaken with **4** in cyclopentane, MeOH, ethanethiol/cyclohexane *etc.* (**Figure 1**). Preliminary results showed that the 5-azidotriazole nucleoside **4** underwent rapid and clean photochemical reaction, suggesting that **1** might be a promising probe in the photolabeling study of the antiviral mechanism of ribavirin.

Figure 1 Photoirradiation of **4** in MeOH.



In conclusion, we have synthesized and characterized 5-azido-1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxiamide **1** as a potential photolabeling probe of ribavirin. Use of this probe to study the antiviral mechanism of ribavirin by photolabeling approach is under way.

Acknowledgments

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9. X-ray data of **5** has been deposited in CCL office and in Cambridge Crystallographic Data Center with deposition No. CCDC – 219002.
10. Analytical data for compounds **1**-**5** :
1: ^{1}H-NMR δ ppm (DMSO-d_6, 300 MHz), 7.84 (s, br, 1H), 7.68 (s, br, 1H), 5.53 (d, 1H, J=5.4 Hz), 5.45 (d, 1H, J=4.2 Hz), 5.20 (d, 1H, J=5.7 Hz), 4.76 (t, 1H, J=5.7 Hz), 4.40 (dd, 1H, J=4.8,9.9 Hz), 4.14 (dd, 1H, J=4.9, 9.9 Hz), 3.87 (dd, 1H, J=4.8, 9.9 Hz), 3.50-3.55 (m, 1H), 3.34-3.48 (m, 1H). ^{13}C-NMR δ ppm (DMSO-d_6, 75 MHz), 159.8, 155.1, 150.1, 89.5, 86.6, 74.3, 71.3, 62.9. FAB-MS *m/z* 308 ($M+Na^+$), 282($M-N_2+2H+Na^+$). IR (KBr, cm^{-1}) 3453, 3398, 2938, 2171, 1692, 1606, 1518, 1474, 1291, 1087, 1052. UV (MeOH) λmax = 234 nm, ε = 10141 $M^{-1}cm^{-1}$.
2: ^{1}H-NMR δ ppm ($CDCl_3$, 300 MHz), 6.09(d, 1H, J=3.0 Hz), 5.82-5.85 (m, 1H), 5.64-5.68 (m, 1H), 4.44-4.48(m, 2H), 4.12-4.18(m, 1H), 3.97(s, 3H), 2.13 (3s, 9H). ^{13}C-NMR δ ppm ($CDCl_3$, 75 MHz), 170.5, 169.4, 169.2, 158.8, 155.6, 131.8, 89.1, 81.8, 74.4, 71.2, 63.1, 53.4, 21.2, 21.1, 21.0. FAB-MS *m/z* 467(MH^++2), 465 (MH^+), 259. IR (CH_2Cl_2, cm^{-1}) 3584, 2957, 2924, 1757, 1454, 1374, 1224, 1075. UV (EtOH), λmax = 210 nm, ε = 7160 $M^{-1}cm^{-1}$.
3: ^{1}H-NMR δ ppm ($CDCl_3$, 300 MHz), 6.89 (d, 1H, J=2.4 Hz),5.80 (dd, 1H, J=2.4, 5.4 Hz), 5.66-5.70 (m, 1H), 4.42-4.47 (m, 2H), 4.12-4.18 (m, 1H), 4.01 (s, 3H), 2.15 (s, 3H), 2.13 (s, 3H), 2.09 (s, 3H). ^{13}C-NMR δ ppm ($CDCl_3$, 75 MHz), 170.0, 169.0, 168.9, 156.5, 145.8, 140.2, 89.7, 81.2, 74.7, 71.1, 63.1, 54.3, 21.7, 21.4. FAB-MS *m/z* 466 (M^++2), 464(M^+), 259, IR (CH_2Cl_2, cm^{-1}) 3584, 2959, 2924, 1747, 1494, 1441, 1372, 1224, 1079. UV (MeOH) λmax = 240 nm, ε = 3398 $M^{-1}cm^{-1}$.
4: ^{1}H-NMR δ ppm ($CDCl_3$, 300 MHz), 5.84(d, 1H, J=3.6 Hz), 5.74-5.77(m, 1H), 5.61-5.64(m, 1H), 4.37-4.50(m, 2H), 4.14(dd, 1H, J=4.5, 12 Hz), 3.98(s, 3H), 2.14(2s, 6H), 2.12(s, 3H). ^{13}C-NMR δ ppm ($CDCl_3$, 75 MHz), 170.6, 169.5, 169.3, 159.4, 153.1, 151.4, 87.3, 81.2, 73.8, 71.1, 63.1, 53.2, 21.0, 20.9, 20.8. FAB-MS *m/z* 427(MH^+), 259, 139. IR (KBr, cm^{-1}) 3423, 2957, 2161, 1746, 1645, 1522, 1455, 1371, 1217, 1049. UV (EtOH), λmax = 238 nm, ε = 11150 $M^{-1}cm^{-1}$.
5: ^{1}H-NMR δ ppm (DMSO-d_6, 300 MHz), 8.43(s, br, 1H), 8.16(s, br, 1H), 6.68(d, 1H, J=3.6 Hz), 5.49(d, 1H, J=5.1 Hz), 5.17(d, 1H, J=5.1 Hz), 4.75(t, 1H, J=5.1 Hz), 4.35-4.36(m, 1H), 4.14-4.16(m, 1H), 3.87-3.88(m, 1H), 3.52-3.55(m, 1H), 3.40-3.44(m, 1H). ^{13}C-NMR δ ppm (DMSO-d_6, 75 MHz), 157.5, 149.7, 138.5, 91.5, 86.3, 75.1, 71.5, 63.0.
11. General procedure for photochemical reaction :
Compound **4** was dissolved in cyclopentane, MeOH, or ethanolthiol/cyclopentane, respectively, and the concentration is around 2.0×10^{-3} mol/L. The solutions were photolyzed under stirring using a 125 W Philips lamp for 0-60 minutes. Aliquots of the irradiated samples were withdrawn for UV recording.

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