Design, Synthesis, and Characterization of Photolabeling Probes for the Study of the Mechanisms of the Antiviral Effects of Ribavirin

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Ribavirin, the only small molecule available so far for treating hepatitis-C-virus infection, was recently used in an emergency context to treat patients with severe acute respiratory syndrome (SARS) in the early stages of the disease. To study the mechanisms responsible for the antiviral effects of ribavirin by using a photolabeling approach, we designed, synthesized, and characterized the azidotriazole nucleosides **1** and **2** as photolabeling probes of ribavirin. These probes were synthesized either by performing nucleophilic substitution of the corresponding bromotriazole nucleoside with NaN_3 (*Scheme 2*) or by directly coupling the azidotriazole with the protected ribose sugar (*Scheme 4*). The azidotriazole nucleosides **1** and **2** showed a fast, clear-cut photochemical reaction, which suggests that they are promising candidates for use in photolabeling studies.

Introduction. – Ribavirin (=1-β-D-ribofuranosyl-1*H*-1,2,4-triazole-3-carboxamide, *Fig. 1*) was the first synthetic nucleoside showing a broad spectrum of antiviral activity against many RNA and DNA viruses [1]. It is used for clinical treatment of patients infected with the *Lassa*-fever virus and respiratory syncytial virus. In association with interferon-α, it is the only drug available to date for treating patients infected with hepatitis C virus [2]. It was recently used to treat patients with severe acute respiratory syndrome (SARS) in mainland China, Hong Kong, and Canada, where it was particularly successful when prescribed in the early stages of the disease [3]. Since its discovery over 30 years ago, ribavirin has constituted a valuable means of treating a broad spectrum of serious viral infections worldwide. We felt that ribavirin could serve as a useful model for developing safer and more efficacious candidates with a broad spectrum of antiviral activity to deal with emerging viruses and those liable to be used in the context of bioterrorism, for which there exist very few efficient antiviral

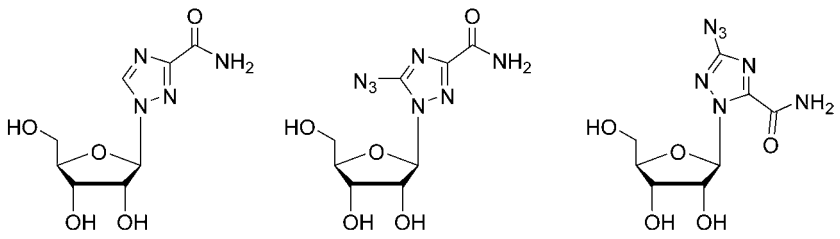


Fig. 1. *Ribavirin and photolabeling probes* **1** *and* **2**

strategies. For this purpose, it is obviously necessary in the first place to understand the molecular mechanisms of the antiviral activity of ribavirin. Although many hypotheses have been proposed to explain the mode of action of ribavirin, this is still a remarkably controversial topic [4]. The hypotheses proposed so far include inhibition of host inosine monophosphate dehydrogenase, inhibition of viral RNA polymerases and viral capping enzymes, lethal mutagenesis of viral RNA genomes, and modulation of the host immune responses [4]. Ribavirin is known to be a pleiotropic agent with many complex intrinsic mechanisms that might affect its overall antiviral properties. To study the molecular mechanisms responsible for the antiviral activity of ribavirin, we felt that photoaffinity labeling is a useful approach.

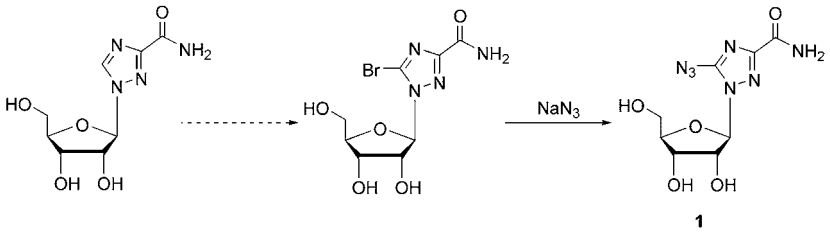
Photoaffinity labeling is an efficient method to study the interactions between biologically relevant ligands and their target macromolecules [5]. This method involves the use of photoactive probes that, when exposed to light, will produce highly reactive species such as nitrene or carbene, leading to a process of covalent cross-linkage with the protein at the binding site [6]. This can be used to identify the macromolecular targets that interact with the probes and to map the binding sites of the probes on the targets, and, thus, to study the modes of action of ligands. Azidoarenes are by far the most frequently used photoaffinity probes because they can be easily synthesized and are chemically stable in the dark and highly reactive upon irradiation [6]. To study the mechanisms of the antiviral activity of ribavirin by photolabeling procedures, we designed the azidotriazole **1** (*Fig. 1*) as a photolabeling probe. This probe has the same triazole-nucleoside structure as ribavirin, which is necessary to ensure that the probe remains biologically active, but it is substituted by an azido group at the heterocyclic triazole ring to make it photochemically reactive. Probe **2** is an isomer of **1** and is, therefore, expected to have similar properties as **1**. We report on the synthesis and characterization of both **1** and **2**.

Results and Discussion. – We tried several methods to synthesize **1**. The most straightforward one suggested to use ribavirin as starting material and to obtain probe **1** by treating the intermediate bromotriazole nucleoside **3** with NaN_3 (*Scheme 1*). However, our attempts to brominate ribavirin with either *N*-bromosuccinimide (NBS) or Br_2 under various conditions were not successful, probably because of the highly electron-deficient triazole ring in ribavirin, which, therefore, is not prone to electrophilic substitution.

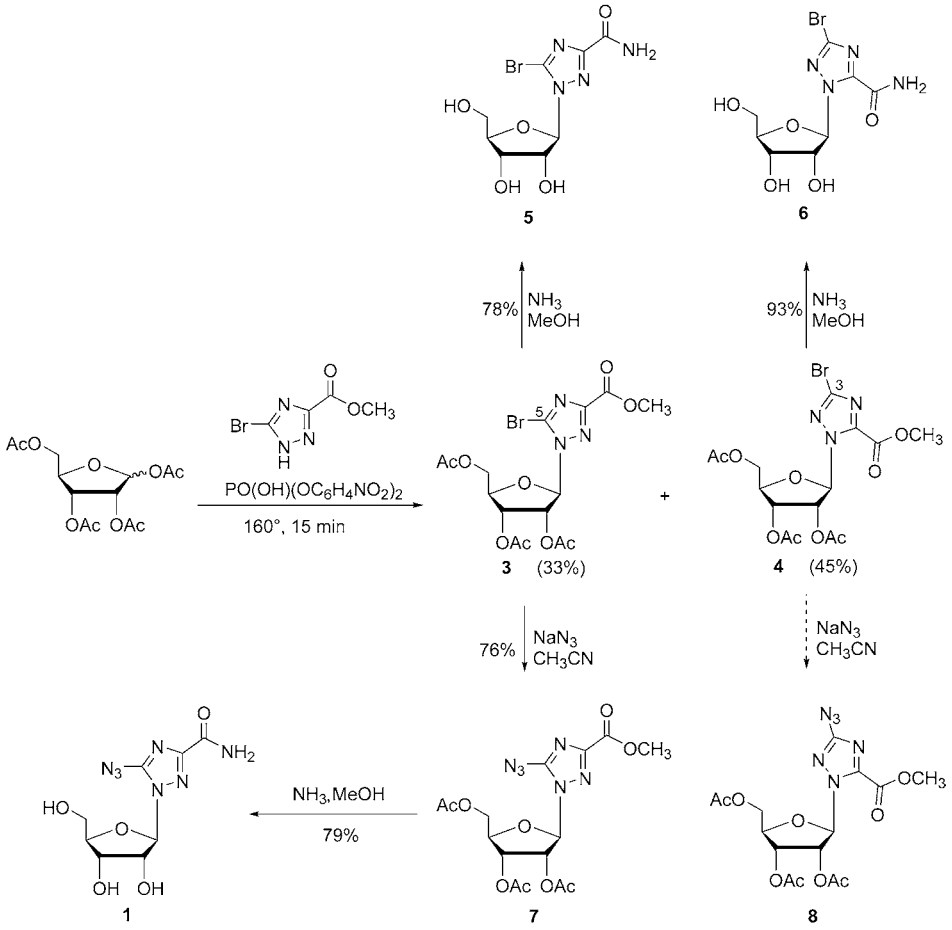
We thus performed the synthesis of the bromotriazole nucleoside **3** *de novo* by the acid-catalyzed fusion procedure [7] with methyl 5-bromo-1*H*-1,2,4-triazole-3-carboxylate and 1,2,3,5-tetra-*O*-acetyl-D-ribofuranose in the presence of the catalyst bis(4-nitrophenyl) hydrogen phosphate at 160° (*Scheme 2*). A mixture of the two isomeric bromotriazole nucleosides **3** and **4** was obtained, which were separated by column chromatography (silica gel) and identified by comparing their NMR spectra with those of the corresponding known chlorotriazole nucleosides [8] (*Table*). The structures of **3** and **4** were further confirmed by an X-ray structure analysis of the corresponding amides **5** and **6** (*Fig. 2*), which were obtained by treating **3** and **4** with NH_3/MeOH, respectively.

Nucleophilic displacement of the Br-atom in **3** with NaN_3 gave the corresponding azidotriazole nucleoside **7** in satisfactory yield when the reaction was carried out in dry

Scheme 1. *Suggested Synthesis of* **1** *Starting with Ribavirin*



Scheme 2. *Synthesis of* **1** via *Bromotriazole Nucleoside* **3**



MeCN at 80°. However, treatment of **4** with NaN_3 to give **8** was unsuccessful. It has been previously reported that nucleophilic substitution at the 3-position of such a triazole nucleoside was very difficult [9]. The difference in reactivity of the nucleophilic

Table. *^{1}H-NMR Data of Triazole Nucleoside Isomers*

	3	**7**	**4**	**8**
δ(H–C(1′)) [ppm]	6.09 (6.04)[a])	5.84	6.89 (6.87)[a])	6.88
J(1′,2′) [Hz]	3.0 (3.7)[a])	3.6	2.4 (2.6)[a])	2.7

[a]) Data of the corresponding chlorotriazole nucleosides in parentheses (from [8]).

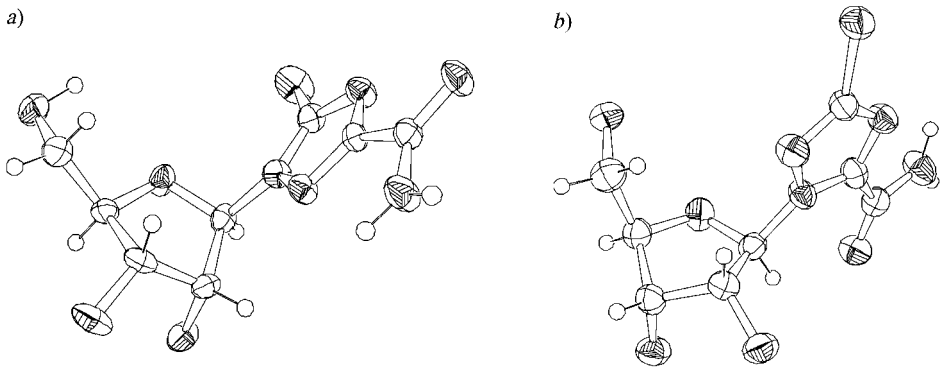
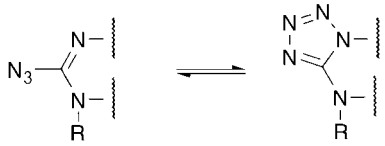


Fig. 2. *X-Ray structures* a) *of* **5** *and* b) *of* **6**

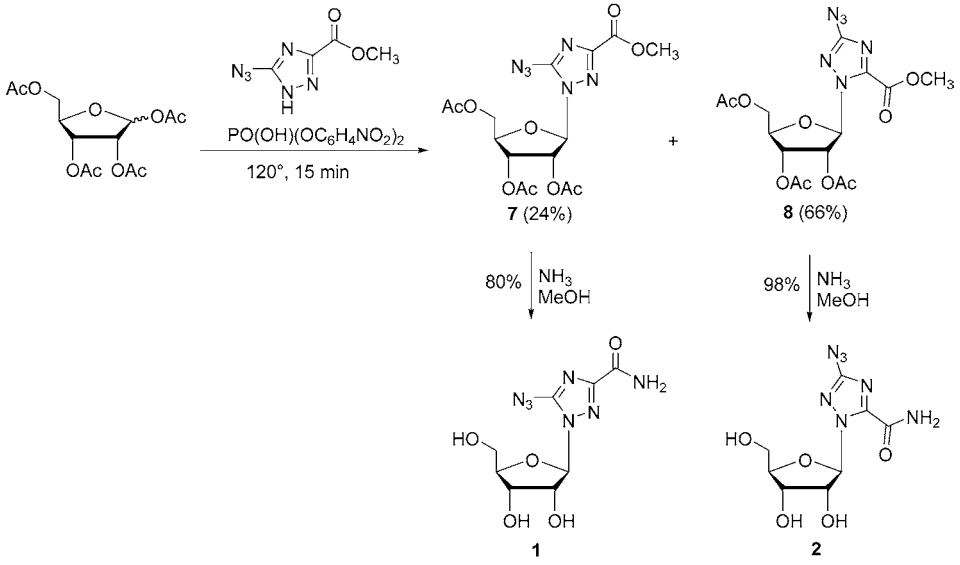
substitution at the 3- and 5-positions of the bromotriazole nucleosides **4** and **3**, respectively, may be attributed to the fact that nucleophilic substitution of **3** results in mesomeric structures favorable for stabilizing the anionic intermediate by delocalizing the charge, in contrast to the potential intermediate from **4**. Although the azido group attached to the C-atom of the azomethine bond can readily undergo ring closure and form a tetrazole (*Scheme 3*), the IR spectrum (KBr) of **7** exhibited an intense band at 2161 cm^{-1} characteristic of azides. Finally, treatment of **7** with NH_3/MeOH at room temperature resulted in deprotection of the sugar moiety and amination of the ester group to give probe **1**.

Scheme 3. *Isomerization between an Azide and Tetrazole Moiety*



Furthermore, we synthesized the azidotriazole nucleosides **1** and **2** directly by the fusion method from methyl 5-azido-1*H*-1,2,4-triazole-3-carboxylate and 1,2,3,5-tetra-*O*-acetyl-D-ribofuranose in the presence of bis(*p*-nitrophenyl) hydrogen phosphate at 120° (*Scheme 4*). To our great surprise, the azidotriazole moieties of both the starting material and the products were highly stable under the reaction conditions, resulting in a conversion yield as high as 90%. The structures of the two isomers **7** and **8** obtained were deduced by comparing their NMR spectra with those of the corresponding

Scheme 4. *Synthesis of Probes* **1** *and* **2**, *Starting with Azidotriazole, by the Fusion Procedure*



chlorotriazole nucleoside isomers reported in the *Table*. The isomer ratio **7**/**8** of 24 : 66 may be explained by steric hindrance of the relative bulky azido group adjacent to the nucleosidic bond in **7**. Therefore, **7** might be thermodynamically less favorable than **8**. After separation, treatment of **7** and **8** in NH_3/MeOH gave the products **1** and **2**, respectively.

Both **1** and **2** are stable in the dark and undergo photodecomposition when exposed to light. A photochemical study was carried out with **1** and **2** in a buffered 100-mM phosphate solution at pH 7.4 and in MeOH or EtOH. Although ribavirin has only a weak UV absorption (*Fig. 3*), both probes showed a strong absorption with molar

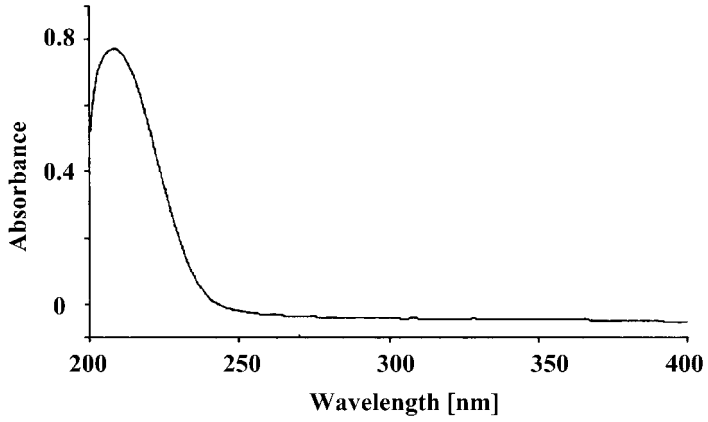


Fig. 3. *UV-Absorption spectrum* (EtOH) *of ribavirin*

absorption coefficients of 10500 $M^{-1}cm^{-1}$ (at 235 nm) for **1** and 10700 $M^{-1}cm^{-1}$ (at 229 nm) for **2**, respectively. *Fig. 4* shows the evolution of the absorption spectra of **1** and **2** on photodecomposition (irradiation at 260 nm). Irradiation of **1** and **2** quickly led to the disappearance of their absorption band, and the observed isosbestic points suggest a single photodecomposition process.

Conclusions. – The two photolabeling probes **1** and **2**, based on ribavirin, were synthesized with the aim to study the mechanisms responsible for the antiviral effects of ribavirin by using a photolabeling approach. Compound **1** was accessible by nucleophilic substitution of the corresponding bromotriazole nucleoside with NaN_3, and both **1** and **2** by directly coupling the azidotriazole with the protected ribose sugar. The photodecomposition of **1** and **2** in various solvents consistently showed a fast, clear-cut photochemical reaction, indicating that **1** and **2** are promising photolabeling probes for the study of the molecular mechanisms of the antiviral activity of ribavirin. Further investigations are under way to identify the photoproducts of **1** and **2** as well as the mechanisms involved in their photoreaction. In addition, the azidotriazoles **1** and **2** constitute a useful new class of promising photoprobes, which extends the range of the presently available photolabeling reagents.

We are grateful to Mr. *Michel Giorgi*, University of Aix-Marseilles III, for performing X-ray structure analyses of compounds **5** and **6** and for preparing *Fig. 2*. We thank Professor *André Samat* for giving us access to the irradiation equipment used here, Dr. *Arnault Heynderickx* for his help with the irradiation experiments, and Dr. *Jessica Blanc* for revising the English manuscript. Financial support from the *CNRS*, Wuhan University, *Cheung Kong Scholar Foundation*, the *Hi-Tech Research and Development Program of China* (N° 2003AA2Z3506), and *the State Key Program of Basic Research of China* (N° 2003CB114403) is gratefully acknowledged.

Experimental Part

General. The 1,2,3,5-tetra-*O*-acetyl-D-ribofuranose was prepared from inosine as described in [10]. Both methyl 5-bromo-1*H*-1,2,4-triazole-3-carboxylate and methyl 5-azido-1*H*-1,2,4-triazole-3-carboxylate were synthesized from methyl 5-amino-1*H*-1,2,4-triazole-3-carboxylate by diazotization followed by substitution with KBr and NaN_3, respectively. MeCN and MeOH were purchased from the *First Chemical Plant* in Shanghai. Flash column chromatography (FC): silica gel (200–300 mesh) from *Qingdao Ocean Chemical Plant*. UV Spectra: *Perkin-Elmer-Lambda-35* UV/VIS spectrophotometer; λ_{max} in nm, ε in M^{-1} cm^{-1} in parentheses. IR Spectra: *Avatar-360*-FT-IR spectrophotometer; in cm^{-1}. ^{1}H- and ^{13}C-NMR Spectra: at 300 MHz and 75 MHz, resp.; *Varian-Mercury-VX300* spectrometer; chemical shifts δ in ppm with $SiMe_4$ as the internal reference, *J* in Hz. MS: *ZAB-HF-3F* mass spectrometer; in *m*/*z*.

*5-Bromo-1-(2,3,5-tri-*O*-acetyl-β-*D*-ribofuranosyl)-1*H*-1,2,4-triazole-3-carboxylic Acid Methyl Ester* (**3**) *and 3-Bromo-1-(2,3,5-tri-*O*-acetyl-β-*D*-ribofuranosyl)-1*H*-1,2,4-triazole-5-carboxylic Acid Methyl Ester* (**4**). A mixture of methyl 5-bromo-1*H*-1,2,4-triazole-3-carboxylate (0.936 g, 4.55 mmol) and 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (1.45 g, 4.55 mmol) was heated to 160° in the presence of bis(*p*-nitrophenyl) hydrogen phosphate (10 mg) and kept under reduced pressure for 15 min. The resulting mixture was separated by FC (silica gel, CH_2Cl_2/acetone 30:1): **3** (0.68 g, 33%) and **4** (0.95 g, 45%).

Data of **3**: Colorless oil: R_f = 0.39 (light petroleum ether/AcOEt 1:1). ^{1}H-NMR ($CDCl_3$). 6.09 (*d*, *J* = 3.0, H–C(1′)); 5.82–5.85 (*m*, H–C(2′)); 5.64–5.68 (*m*, H–C(3′)); 4.44–4.48 (*m*, H–C(4′), H–C(5′)); 4.12–4.18 (*m*, 1 H–C(5′)); 3.97 (*s*, MeO); 2.13 (*s*, 3 Me); ^{13}C-NMR ($CDCl_3$); 170.5 (CO); 169.4 (CO); 169.2 (CO); 158.8 (CO); 155.6 (C(3)); 131.8 (C(5)); 89.1 (C(1′)); 81.8 (C(2′)); 74.4 (C(3′)); 71.2 (C(4′)); 63.1 (C(5′)); 53.4 (MeO); 21.2 (Me); 21.1 (Me); 21.0 (Me). FAB-MS: 464 (M^+, $C_{15}H_{18}BrN_3O_9^+$), 466 ($[M+2]^+$).

Data of **4**: Colorless oil: R_f 0.54 (light petroleum ether/AcOEt 1:1). UV (EtOH): 242 (3641). ^{1}H-NMR ($CDCl_3$); 6.89 (*d*, *J* = 2.4, H–C(1′)), 5.80 (*dd*, *J* = 2.4, 5.4, H–C(2′)); 5.66–5.70 (*m*, H–C(3′)); 4.42–4.47 (*m*, H–C(4′), H–C(5′)); 4.12–4.18 (*m*, 1 H–C(5′)); 4.01 (*s*, MeO); 2.15 (*s*, Me); 2.13 (*s*, Me); 2.09 (*s*, Me).

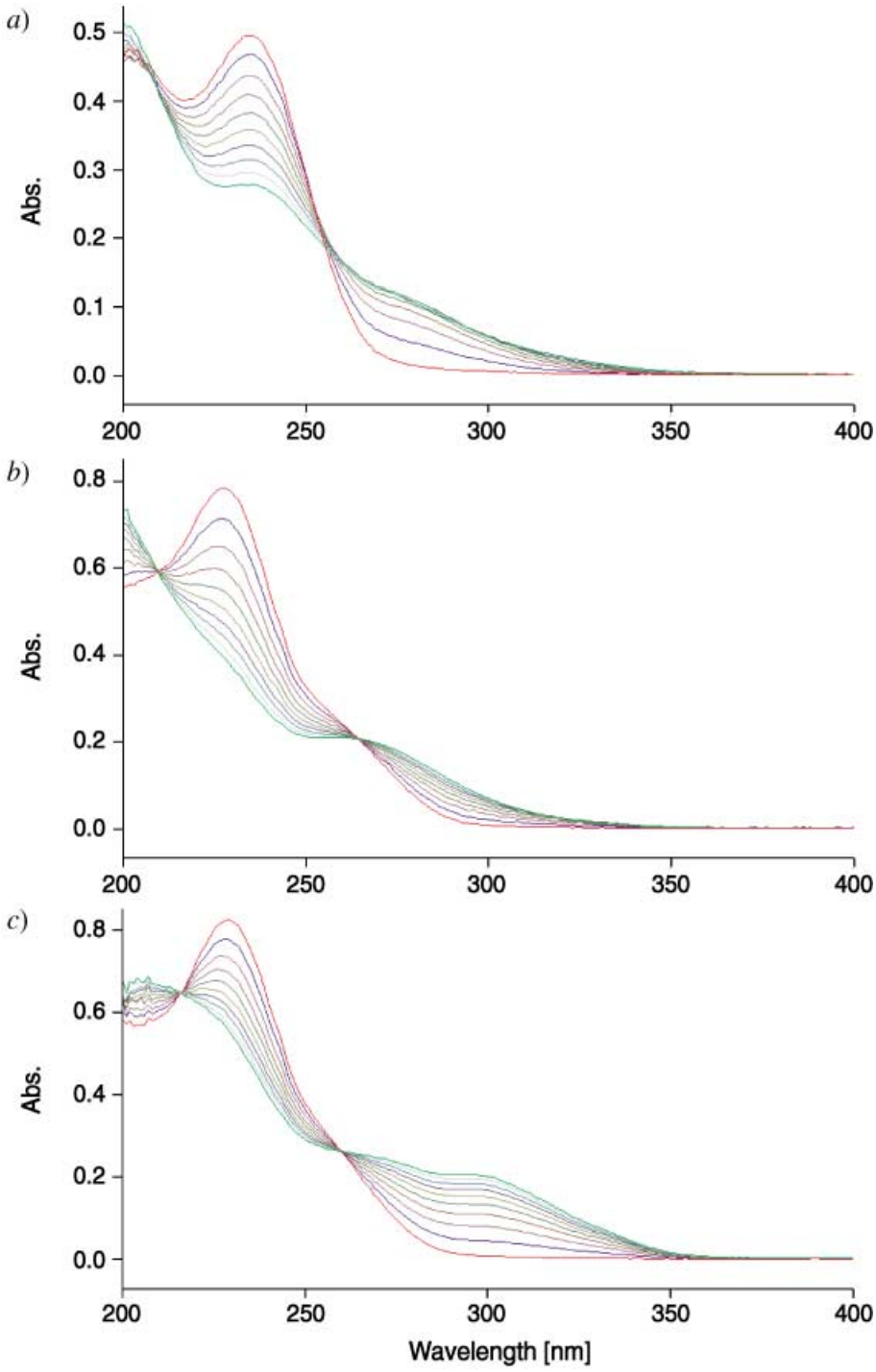


Fig. 4. *Evolution of the absorption spectra during irradiation* a) *of* **1** *in 100-m*M *phosphate buffer (pH 7.4),* b) *of* **2** *in 100-m*M *phosphate buffer (pH 7.4), and* c) *of* **2** *in MeOH*

^{13}C-NMR ($CDCl_3$): 170.0 (CO); 169.0 (CO); 168.9 (CO); 156.5 (CO); 145.8 (C(5)); 140.2 (C(3)); 89.7 (C(1′)); 81.2 (C(2′)); 74.7 (C(3′)); 71.1 (C(4′)); 63.1 (C(5′)); 54.3 (MeO); 21.7 (Me); 21.4 (2 Me). FAB-MS: 464 (M^+, $C_{15}H_{18}BrN_3O_9^+$), 466 ($[M+2]^+$).

*5-Bromo-1-β-*D*-ribofuranosyl-1*H*-1,2,4-triazole-3-carboxamide* (**5**). A soln. of **3** (0.23 g, 0.50 mmol) in MeOH sat. with ammonia (20 ml) was kept at r.t. for 16 h. The solvent was evaporated and the residue purified by FC (silica gel, CH_2Cl_2/MeOH 10:1): **5** (0.13 g, 78%). White solid. R_f 0.12 (CH_2Cl_2/MeOH 10:1). ^{1}H-NMR ((D$_6$)DMSO): 7.94 (br. *s*, 1 NH); 7.70 (br. *s*, 1 NH); 5.73 (*d*, *J* = 4.5, H–C(1′)); 5.55 (br. *s*, HO–C(2′)); 5.23 (br. *s*, HO–C(3′)); 4.74 (br. *s*, HO–C(5′)); 4.48–4.47 (*m*, H–C(2′)); 4.14–4.17 (*m*, H–C(3′)); 3.90 (*dd*, *J* = 5.1, 10.2, H–C(4′)); 3.50 (*dd*, *J* = 4.8, 11.7, 1 H–C(5′)); 3.36–3.40 (*m*, 1 H–C(5′)). ^{13}C-NMR ((D$_6$)DMSO): 160.0 (CO); 158.2 (C(3)); 132.2 (C(5)); 90.9 (C(1′)); 86.9 (C(4′)); 74.5 (C(2′)); 71.1 (C(3′)); 62.5 (C(5′)). FAB-MS: 323 (M^+, $C_8H_{11}BrN_4O_5^+$), 325 ($[M+2]^+$).

*3-Bromo-1-β-*D*-ribofuranosyl-1,2,4-triazole-5-carboxamide* (**6**). As described for **5**, with **4** (0.53 g, 1.14 mmol) in methanolic ammonia (20 ml): **6** (0.34 g, 93%). White solid. R_f 0.15 (CH_2Cl_2/MeOH 10:1). ^{1}H-NMR ((D$_6$)DMSO): 8.43 (br. *s*, 1 NH); 8.16 (br. *s*, 1 NH); 6.68 (*d*, *J* = 3.6, H–C(1′)); 5.49 (*d*, *J* = 5.1, HO–C(2′)); 5.17 (*d*, *J* = 5.1, HO–C(3′)); 4.75 (*t*, *J* = 5.1, HO–C(5′)); 4.35–4.36 (*m*, H–C(2′)); 4.14–4.16 (*m*, H–C(3′)); 3.87–3.88 (*m*, H–C(4′)); 3.52–3.55 (*m*, 1 H–C(5′)); 3.40–3.44 (*m*, 1 H–C(5′)). ^{13}C-NMR ((D$_6$)DMSO): 157.5 (CO); 149.7 (C(5)); 138.5 (C(3)); 91.5 (C(1′)); 86.3 (C(4′)); 75.1 (C(2′)); 71.5 (C(3′)); 63.0 (C(5′)). FAB-MS 323 (M^+, $C_8H_{11}BrN_4O_5^+$), 325 ($[M+2]^+$).

*5-Azido-1-(2,3,5-tri-*O*-acetyl-β-*D*-ribofuranosyl)-1*H*-1,2,4-triazole-3-carboxylic Acid Methyl Ester* (**7**) *and 3-Azido-1-(2,3,5-tri-*O*-acetyl-β-*D*-ribofuranosyl)-1*H*-1,2,4-triazole-5-carboxylic Acid Methyl Ester* (**8**). As described for **3** and **4**, with methyl 5-azido-*1*H-1,2,4-triazole-3-carboxylate (0.52 g, 3.10 mmol), 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (0.99 g, 3.10 mmol), and bis(*p*-nitrophenyl) hydrogen phosphate (10 mg) at 120°/reduced pressure (15 min): **7** (0.32 g, 24%) and **8** (0.87 g, 66%).

Data of **7**: Colorless oil: R_f 0.42 (CH_2Cl_2/acetone 30:1). UV (EtOH): 238 (11150). IR (CH_2Cl_2): 2161. ^{1}H-NMR ($CDCl_3$): 5.84 (*d*, *J* = 3.6, H–C(1′)); 5.74–5.77 (*m*, H–C(2′)); 5.61–5.64 (*m*, H–C(3′)); 4.37–4.50 (*m*, H–C(4′), 1 H–C(5′)); 4.14 (*dd*, *J* = 4.5, 12, 1 H–C(5′)); 3.98 (*s*, MeO); 2.14 (*s*, 2 Me); 2.12 (*s*, Me). ^{13}C-NMR ($CDCl_3$): 170.6 (CO); 169.5 (CO); 169.3 (CO); 159.4 (CO); 153.1 (C(3)); 151.4 (C(5)); 87.3 (C(1′)); 81.2 (C(2′)); 73.8 (C(3′)); 71.1 (C(4′)); 63.1 (C(5′)); 53.2 (MeO); 21.0 (Me); 20.9 (Me); 20.8 (Me). FAB-MS: 427 ($[M+H]^+$, $C_{15}H_{18}N_6O_9^+$).

Data of **8**: Colorless oil: R_f 0.55 (CH_2Cl_2/acetone 30:1). UV (EtOH): 230 (11043). IR (CH_2Cl_2): 2145. ^{1}H-NMR ($CDCl_3$): 6.88 (*d*, *J* = 2.7, H–C(1′)); 5.75 (*dd*, *J* = 2.7, 5.4, H–C(2′)); 5.63–5.67 (*m*, H–C(3′)); 4.40–4.46 (*m*, H–C(4′), 1 H–C(5′)); 4.14–4.20 (*m*, 1 H–C(5′)); 4.02 (*s*, MeO); 2.14 (*s*, Me); 2.12 (*s*, Me); 2.09 (*s*, Me). ^{13}C-NMR ($CDCl_3$): 170.0 (CO); 169.0 (CO); 168.9 (CO); 158.0 (CO); 156.8 (C(5)); 144.7 (C(3)); 89.5 (C(1′)); 80.9 (C(2′)); 74.6 (C(3′)); 71.1 (C(4′)); 63.4 (C(5′)); 54.2 (MeO); 21.7 (Me); 21.3(2 Me). FAB-MS: 427 ($[M+H]^+$, $C_{15}H_{18}N_6O_9^+$).

Methyl ester **7** *from* **3**. A soln. of **3** (0.158 g, 0.340 mmol) and NaN_3 (0.903 g, 1.39 mmol) in MeCN (10 ml) was refluxed for 3 days and then evaporated. The residue was separated by FC (silica gel, light petroleum ether/AcOEt 3:2): **7** (0.110 g, 76%).

*5-Azido-1-β-*D*-ribofuranosyl-1*H*-1,2,4-triazole-3-carboxamide* (**1**). As described for 5, with **7** (0.18 g, 0.41 mmol) and MeOH sat. with ammonia (20 ml): **1** (0.094 g, 79%). White solid: R_f 0.13 (CH_2Cl_2/MeOH 10:1). UV (EtOH) 235 (10504). IR (KBr): 2171. ^{1}H-NMR ((D$_6$)DMSO): 7.84 (br. *s*, 1 NH); 7.68 (br. *s*, 1 NH); 5.53 (*d*, *J* = 5.4, H–C(1′)); 5.45 (*d*, *J* = 4.2, HO–C(2′)); 5.20 (*d*, *J* = 5.7, HO–C(3′)); 4.76 (*t*, *J* = 5.7, HO–C(5′)); 4.40 (*dd*, *J* = 4.8, 9.9, H–C(2′)); 4.14 (*dd*, *J* = 4.9, 9.9, H–C(3′)); 3.87 (*dd*, *J* = 4.8, 9.9, H–C(4′)); 3.50–3.55 (*m*, H–C(5′)); 3.34–3.48 (*m*, 1 H–C(5′)). ^{13}C-NMR ((D$_6$)DMSO): 159.8 (CO); 155.1 (C(3)); 150.1 (C(5)); 89.5 (C(1′)); 86.6 (C(4′)); 74.3 (C(2′)); 71.3 (C(3′)); 62.9 (C(5′)). FAB-MS: 282 ($[M-N_2+2H+Na]^+$, $C_8H_{13}NaN_5O_5^+$).

*3-Azido-1-β-*D*-ribofuranosyl-1*H*-1,2,4-triazole-5-carboxamide* (**2**). As described for **5**, with **8** (0.64 g, 1.51 mmol) and MeOH sat. with ammonia (20 ml): **2** (0.42 g, 98%). White solid. R_f 0.16 (CH_2Cl_2/MeOH 10:1). UV (EtOH): 229 (10702). IR (KBr): 2148. ^{1}H-NMR ((D$_6$)DMSO): 8.30 (br. *s*, 1 NH); 8.13 (br. *s*, 1 NH); 6.65 (*d*, *J* = 3.6, H–C(1′)); 4.30–4.33 (*m*, H–C(2′)); 4.12–4.16 (*m*, H–C(3′)); 3.86 (*dd*, *J* = 5.1, 10.2, H–C(4′)); 3.37–3.42 (*m*, 2 H–C(5′)). ^{13}C-NMR ((D$_6$)DMSO): 157.8 (CO); 156.0 (C(5)); 148.5 (C(3)); 91.3 (C(1′)); 86.1 (C(4′)); 75.0 (C(2′)); 71.6 (C(3′)); 63.1 (C(5′)). FAB-MS: 286 ($[M+H]^+$, $C_8H_{12}N_7O_5^+$).

X-Ray Crystal Structures. Crystals were grown by slowly evaporating a soln. of **5** or **6** in MeOH. **5**: $C_{16}H_{22}Br_2N_8O_{10}$ (M_r 646.24), triclinic space group *P*1, *Z* = 1, *a* = 7.7710 (1), *b* = 8.2170 (8), *c* = 9.2610 (8) Å, α = 91.260 (5), β = 100.967(5), γ = 96.129(6)°, *V* = 576.69 (11) Å^3, MoK_α radiation, λ 0.71073 Å, 0° < θ < 29.80°, 12179 reflections, *T* 293 K, *Bruker-Nonius Kappa CCD*. The structure was solved by using direct methods

(SHELXS 97) and refined with SHELXL97 to final $R(F^2 > 4\sigma F^2) = 0.0337$ and $wR = 0.0856$ ($w = 1/[\sigma^2(F_o^2) + (0.0271P)^2 + 0.1400P]$, where $P = (F_o^2 + 2F_c^2)/3$). **6**: $C_8H_{11}BrN_4O_5$ (M_r 323.12), Orthorhombic space group $P2_12_12_1$, $Z = 4$, $a = 7.1470$ (2), $b = 7.6930$ (4), $c = 20.862$ (1) Å, $\alpha = 90.00$, $\beta = 90.00$, $\gamma = 90.00°$, $V = 1147.03$ (7) Å^3, MoK_α radiation, λ 0.71073 Å, $0° < \theta < 30.98°$, 3447 reflections, T 293 K, *Bruker-Nonius Kappa CCD*. The structure was solved using direct methods (SHELXS 97) and refined with SHELXL97 to final $R(F^2 > 4\sigma F^2) = 0.0362$ and $wR = 0.0694$ ($w = 1/[\sigma^2(F_o^2)]$).

CCDC 219296 and -219002 contain the supplementary crystallographic data for **5** and **6**, resp., of this paper. These data can be obtained, free of charge, *via* http://www.ccdc.cam.ac.uk.conts/retrieving.html (or from the Cambridge Crystallographic Data Center, 12 Union Road, Cambridge CB2 1EZ UK; fax: +44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk).

General Photolysis Procedure. Compounds **1** and **2** were each dissolved in 100-mM phosphate buffer at pH 7.4 or in MeOH or EtOH to a concentration of *ca.* 5.0×10^{-5} mol/l. The solns. (1.5 ml) were photolyzed, under stirring, by a 150-W *USHIO* Xenon short-arc lamp for 0–20 min at 20°. The absorption spectra of the irradiated samples were recorded with a *Cary* UV spectrophotometer.

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