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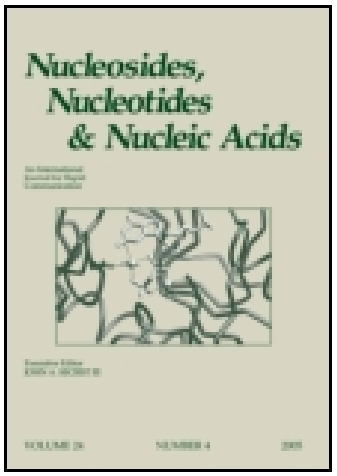

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Photochemical Behavior of 2-Azidopurine Tri-O-Acetylribonucleoside in Aqueous Solution: Unprecedented Transformation into 1-(5′-O-Acetyl-β-D-Ribofuranosyl)-5-[(2-Oxo-1,3,5-Oxadiazocan-4-Ylidene)Amino]-1H-Imidazole-4-Carbaldehyde

Krzysztof Komodziński[ab], Zofia Gdaniec[a] & Bohdan Skalski[b]
[a] Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznań, Poland
[b] Faculty of Chemistry, Adam Mickiewicz University, Poznań, Poland
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PHOTOCHEMICAL BEHAVIOR OF 2-AZIDOPURINE TRI-*O*-ACETYLRIBONUCLEOSIDE IN AQUEOUS SOLUTION: UNPRECEDENTED TRANSFORMATION INTO 1-(5′-*O*-ACETYL-β-D-RIBOFURANOSYL)-5-[(2-OXO-1,3,5-OXADIAZOCAN-4-YLIDENE)AMINO]-1*H*-IMIDAZOLE-4-CARBALDEHYDE

Krzysztof Komodziński,[1,2] Zofia Gdaniec,[1] and Bohdan Skalski[2]
[1]*Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznań, Poland*
[2]*Faculty of Chemistry, Adam Mickiewicz University, Poznań, Poland*

□ *The photochemical behavior of 2-azidopurine 2′,3′,5′-tri-O-acetylribonucleoside has been investigated in aqueous solution under aerobic and anaerobic conditions. The two major processes under anaerobic irradiation of 2-azidopurine 2′,3′,5′-tri-O-acetylribonucleoside involve unprecedented transformation into 1-(5′-O-acetyl-β-D-ribofuranosyl)-5-[(2-oxo-1,3,5-oxadiazocan-4-ylidene)amino]-1H-imidazole-4-carbaldehyde and photoreduction to respective 2-aminopurine derivative, whereas under aerobic conditions these two processes occur to a much lesser extent and photooxidation to respective 2-nitropurine derivative dominates. The structures of photoproducts formed were confirmed by NMR and high-resolution electrospray ionization mass spectral data.*

Keywords 2-Azidopurine ribonucleoside; photochemistry; modified nucleoside; photoreduction; photooxidation

INTRODUCTION

Azidopurine nucleosides, such as 2-azidoadenosine, 8-azidoadenosine, 8-azidoguanosine, and 2,6-diazidonebularine, belong to a group of the most popular photoaffinity labeling probes used for mapping nucleic acid – protein interactions.[1] One of the major advantages of the azidonucleosides as photoreactive agents is the generation of highly reactive nitrene intermediate capable of reacting in an indiscriminating manner with the surrounding functional groups and bonds present in the macromolecular system studied. Despite their common use as photoaffinity probes, the knowledge concerning photochemistry of these azidonucleosides alone is still rather

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Address correspondence to Dr. Bohdan Skalski, Faculty of Chemistry, Adam Mickiewicz University, Umultowska 89b, 61-614 Poznań, Poland. E-mail: bskalski@amu.edu.pl

limited. A better understanding of the detailed mechanisms of photochemistry of azidopurine nucleoside analogs and structures of photoproducts formed is crucial for better understanding of the mechanism of the photocrosslinking reactions and further development of the photocrosslinking technique using these type of compounds as photoprobes in molecular biology.[2] So far the photochemistry of 8-azidoadenine,[3] 2-azidoadenine,[4] 6-azidopurine,[5] and 2-amino-6-azidopurine[4] ribonucleosides in aqueous solutions has been examined. It has been shown that the photochemical behavior of these compounds is highly dependent on the position of the azide group in the purine ring system, the presence of various substituents (H or NH_2), and the presence or absence of oxygen. In the case of 8-azidoadenosine, photoreduction of 8-aminoadenosine through the formation of nitrene intermediate was observed.[3] Our recent studies on the photochemistry of a series of azidopurine nucleosides have shown that 6-azidopurine nucleoside undergoes only partial photoreduction to adenosine, and its dominant phototransformation involves purine ring expansion to give a novel imidazole-fused 1,3,5-triazepinone nucleoside, independent of the presence or absence of oxygen.[5] The purine ring expansion was also found to be a major photoreaction in the case of 6-azidopurine incorporated into an oligodeoxynucleotide chain.[6] Completely different behavior was observed in the case of near-UV irradiation of 2-azidoadenosine and 2-amino-6-azidonebularine.[4] Depending on the photoirradiation conditions, aerobic or anaerobic, both azidopurine nucleosides were found to undergo either photoreduction to respective amino derivatives or photooxidation to respective 2- and 6-nitro analogs, and no purine ring expansion product could be detected in both cases.[4]

As a part of our ongoing studies of the photochemistry of azidopurine nucleosides, we report herein the results concerning the photochemical transformations of novel azidopurine analog, 2-azidopurine tri-*O*-acetylribonucleoside **1** in aqueous solution under aerobic and anaerobic conditions.

RESULTS AND DISCUSSION

The synthesis of 2-azidopurine tri-*O*-acetylribonucleoside **1** was carried out according to the reported procedure.[4,7] An aqueous solution of **1** was irradiated at room temperature with a near-UV light ($\lambda > 300$ nm) using a high-pressure Hg lamp equipped with a 300-nm cut-off Pyrex glass filter. The progress of photoreaction was monitored by UV absorption spectroscopy and high-performance liquid chromatography (HPLC) (Figure 1). Complete disappearance of **1** was observed within 12 min of irradiation with virtually identical rates under both aerobic and anaerobic conditions. As shown in Figure 1A, irradiation of **1** under aerobic conditions resulted in

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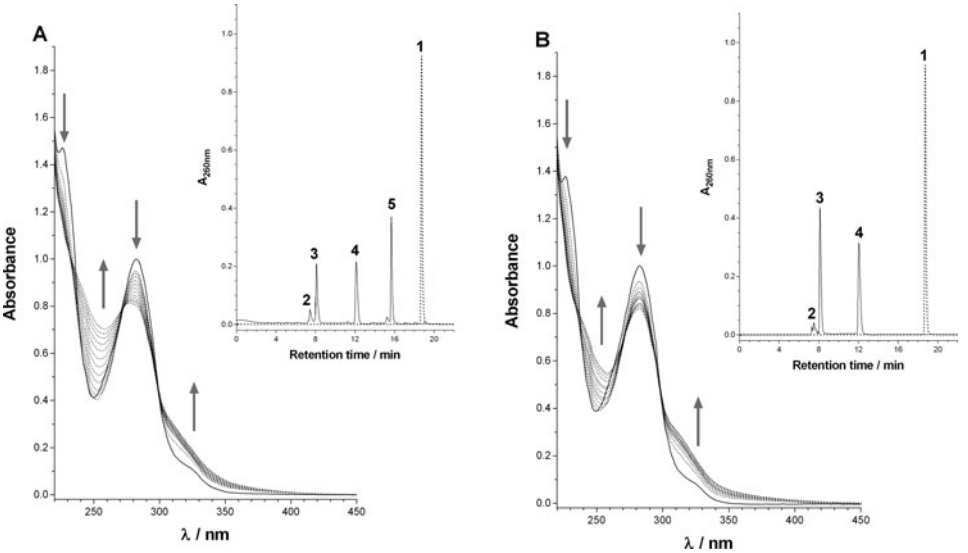

FIGURE 1 Changes in the absorption spectrum of an aqueous solution of **1** measured with 1-min increments during irradiation under (A) aerobic and (B) anaerobic conditions. The insets show HPLC analyses of the solutions before (dotted lines) and after 12-min irradiation (solid lines).

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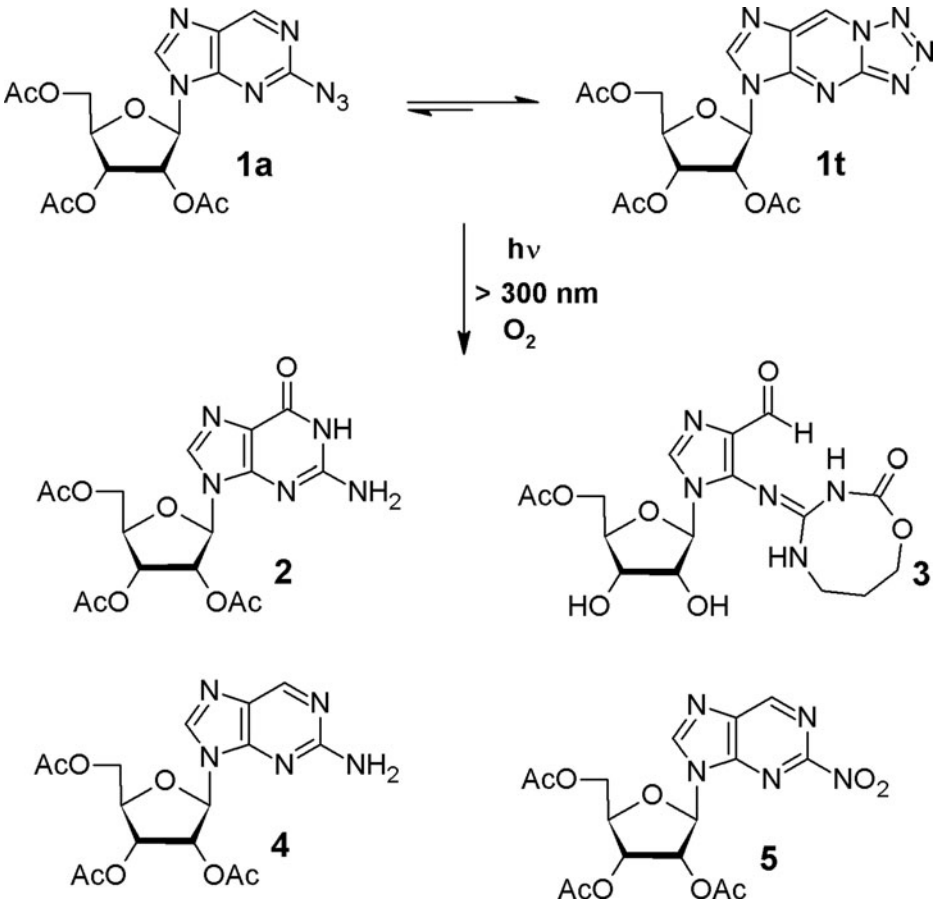
the formation of one minor **2** (∼5%) and three major products, **3** (∼20%), **4** (∼25%), and **5** (∼40%), of which **5** was clearly dominant, whereas in the absence of oxygen no formation of **5** was observed and **3** (∼45%) and **4** (∼40%) were formed as two major photoproducts (Figure 1B). The photoproducts were isolated and purified by HPLC and were fully characterized by means of spectroscopic methods (high-resolution electrospray ionization time-of-flight mass spectrometry (HR ESI-TOF MS) and NMR). The structures of all photoproducts are shown in Scheme 1.

The structure of the minor photoproduct **2** formed under both conditions was identified as 2′,3′,5′-tri-*O*-acetylguanosine in comparison (UV spectrum and HPLC) with that of an authentic sample. Similarly, the structure of photoproduct **4** as 2-amino-9-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl) purine was confirmed by comparison of its HR ESI-TOF MS, NMR, and UV spectra with those reported in the literature.[9]

The molecular formula of **5** ($C_{16}H_{17}N_5O_9$), inferred from the high-resolution ESI-TOF MS spectrum, indicated that the formation of this photoproduct involves loss of a N_2 molecule and addition of an oxygen molecule.[4] The ^{1}H NMR and ^{13}C NMR spectra of **5** showed the peak characteristics of sugar and purine residues of the molecule. In the aromatic part of ^{1}H NMR spectrum of **5**, two signals corresponding to C6-H and C8-H protons appeared at $\delta_H = 9.20$ and 8.48 ppm. In the aromatic region of ^{13}C NMR spectrum of **5**, five signals appeared, two from the methine carbon $\delta_C = 149.9$ (C6) and 147.3 ppm (C8), and three from quaternary



SCHEME 1 Azide-tetrazole tautomeric equilibrium of **1**[8] and structures of the photoproducts formed during irradiation under aerobic and anaerobic conditions.

carbons at δ_C = 154.7 (C2), 151.9 (C4), and 137.2 ppm (C5). One bond ^{1}H-^{13}C correlation was detected between C6(δ_C = 149.9 ppm) and H6(δ_H = 9.20 ppm); and C8(δ_C = 147.3 ppm) and H8(δ_C = 8.48 ppm). ^{1}H-^{13}C HMBC spectrum showed correlations of proton H6(δ_H = 9.20 ppm) with carbon atoms C2(δ_C = 154.7 ppm) and C4(δ_C = 151.9 ppm). Furthermore, typical for purine nucleosides, long-range ^{1}H-^{13}C correlations were observed for H8(δ_H = 8.48 ppm) with C4(δ_C = 151.9 ppm) and C5(δ_C = 137.2 ppm); anomeric H1'(δ_H = 6.29 ppm) with C4(δ_C = 151.9 ppm) and C8(δ_C = 147.3 ppm). The UV spectrum of this photoproduct displays absorption band centered at 272 nm and extending well above 300 nm. All the above data clearly suggest that photoproduct **5** is 2-nitro-9-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)purine. This 2-nitropurine chromophore has not been described previously in the literature.[10] As in the case of 2-amino-6-azido and 6-amino-2-azido analogs of purine ribonucleosides,[4] the most likely

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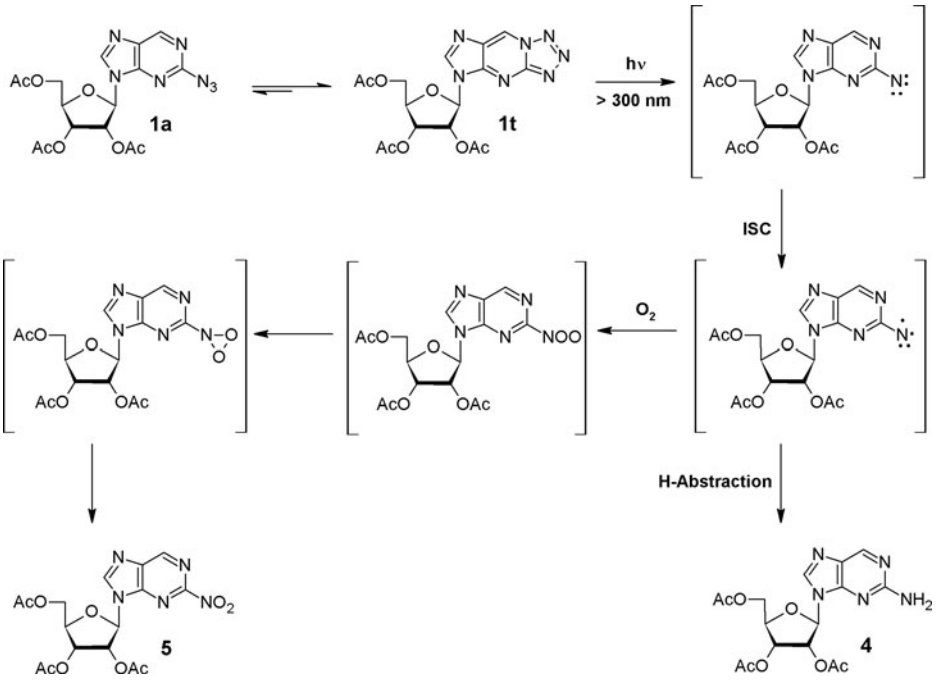
mechanism accounting for the formation of 2-nitropurine ribonucleoside involves a triplet nitrene intermediate that reacts with the O_2 molecule to give **5** (Scheme 2).



SCHEME 2 The proposed mechanism of formation of photoproducts **4** and **5**.

Based on careful analysis of NMR and MS spectral data, the structure of photoproduct **3** was established as 1-(5′-*O*-acetyl-*β*-D-ribofuranosyl)-5-[(2-oxo-1,3,5-oxadiazocan-4-ylidene)amino]-1 *H* -imidazole-4-carbaldehyde (Scheme 3).

The molecular formula $C_{16}H_{21}N_5O_8$ of photoproduct **3** was determined by high-resolution ESI-TOF MS. The ^1H and ^{13}C NMR spectra showed that the sugar moiety was deacetylated at 2′ and 3′ positions. Apart from the sugar protons, three methylene resonances were observed at $\delta_H = 4.09$ ppm (H8B), 2.69 ppm (H6B), and 1.96 ppm (H7B), and were identified as belonging to the one spin system as evidenced by ^1H-^1H COSY and TOCSY spectra. In addition, two singlets at $\delta_H = 7.66$ ppm (H2A) and 9.47 ppm (aldehyde group) appeared in ^1H NMR spectrum. The latter, substantially deshilded signal, was assigned to the proton of an H–C=O group based on the correlation of this proton and the carbon atom resonating at $\delta_C = 183.4$ ppm, i.e., in the region typical for the aldehyde carbon. Two exchangeable protons were identified at 7.78 and 7.72 ppm and were assigned to NH protons based on the one-bond ^1H-^{15}N correlation observed in the Heteronuclear Single Quantum Coherence (HSQC) spectrum. The aglycone structure was

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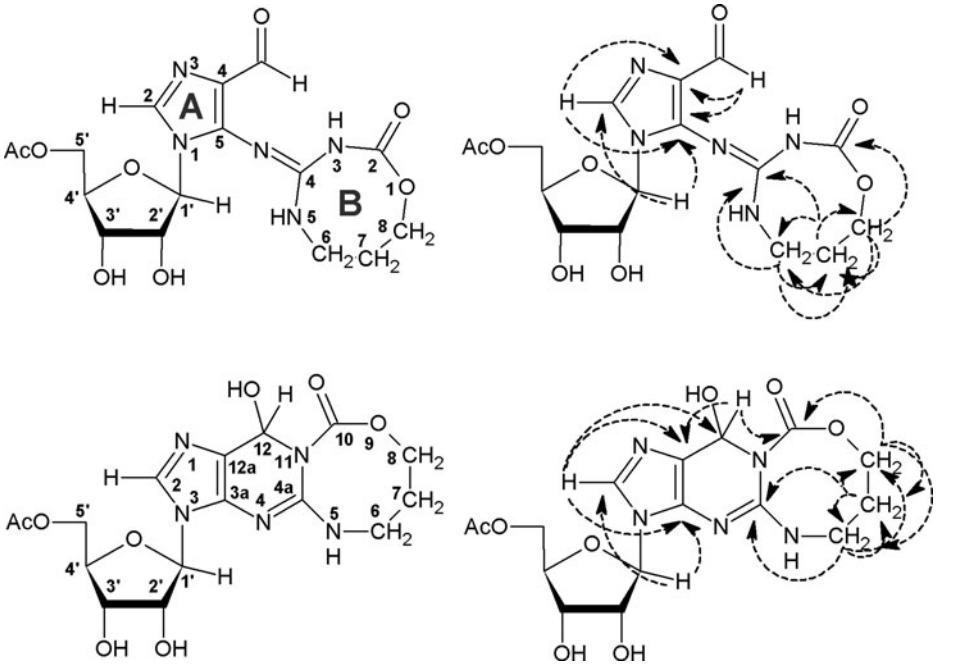

SCHEME 3 The structures and numbering of ring systems of compound **3** and the product of its cyclization in methanolic solution (**6**/**6***, see Scheme 4). The arrows show the connectivity of cross-peaks due to couplings through 2 and 3 chemical bonds observed in the ^{1}H-^{13}C HMBC experiment.

established mainly by analyzing long-range proton–carbon correlations in the ^{1}H-^{13}C HMBC spectrum shown on Scheme 3.

The presence of imidazole ring was confirmed from HMBC correlations observed between proton H2A(δ_H = 7.66 ppm) with carbon atoms C5A(δ_C = 146.6 ppm) and C4A(δ_C = 128.0 ppm) as well as between anomeric proton H1'(δ_H = 5.47 ppm) with C5A(δ_C = 146.6 ppm) and C2A(δ_C = 132.8 ppm). The resonance at δ_H = 9.47 ppm revealed HMBC correlation to C5A and C4A carbon atoms, placing the aldehyde group at C5 position of imidazole ring. ^{1}H and ^{13}C chemical shifts of methylene groups of C6B and C8B suggested that they were attached directly to the nitrogen and oxygen atoms respectively. Further evidence of attachment of C6B group to nitrogen atom could be obtained by the observation of NOE between H6 protons and NH signal at δ_H = 7.72 ppm. The H8 protons of methylene group showed long-range correlation to quaternary carbon atom at δ_C = 168.9 ppm (C2B), and H6 protons gave cross-peak to fully substituted carbon atom at δ_C = 169.0 ppm (C4B). The UV spectrum of photoproduct **3** displays an absorption band centered at 251 nm with shoulder at ca. 300 nm extending up to 380 nm (Figure 2, solid line). All the above spectral data are consistent with the proposed structure of photoproduct **3**. Considering the mechanism of the formation of this photoproduct, it can

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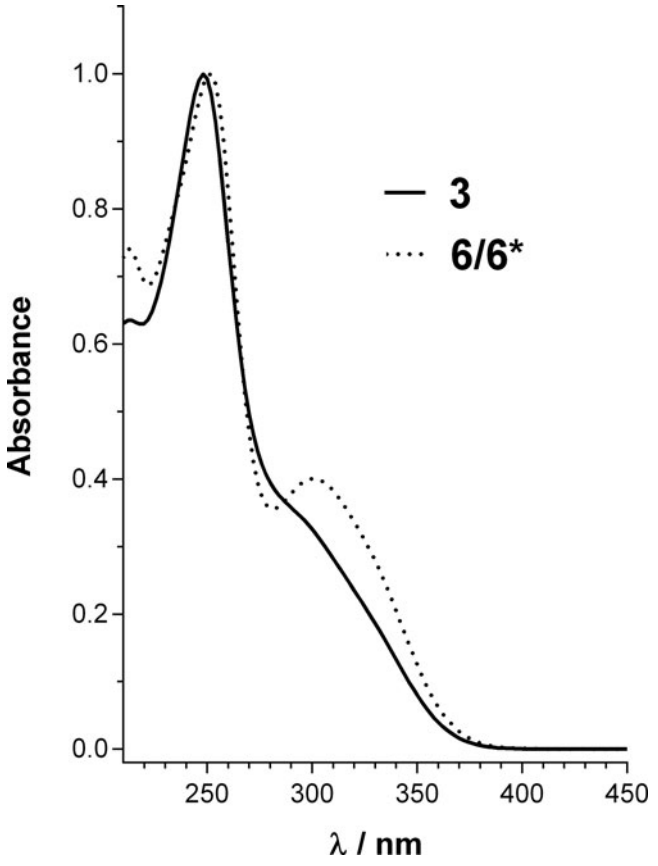

FIGURE 2 Comparison of normalized UV absorption spectra of **3** (solid line) and **6**/**6*** (dotted line).

be assumed that the key steps involve hydrolytic opening of pyrimidine ring (the presence of an aldehyde group) of the initially formed 2-nitrenopurine intermediate, following by the formation of 4-imino-1,3,5-oxadiazocan-2-one residue. The lack of two (2′, 3′) acetyl groups observed only in the case of this photoproduct indicate that they must participate in the formation of the 1,3,5-oxadiazocan-2-one ring. However, the detailed knowledge of the photochemical pathway of this unexpected transformation of **1** requires further study.

Photoproduct **3** is stable in aqueous solution, but in methanolic solution it undergoes spontaneous cyclization to form two diastereomers of a new ring-expanded nucleoside 3-(5′-*O*-acetyl-*β*-D-ribofuranosyl)-12-hydroxy-3,5,6,7,8,12-hexahydro[1,3,5]oxadiazocino[3,4-*a*]purin-10-one, **6**/**6***, (stereogenic center at C12 carbon atom) in the ratio 1:1 (Scheme 4). The transformation is quantitative and is accompanied by the appearance of a new absorption band at ca. 305 nm in UV spectrum (Figure 2, dotted line). The detailed proof of the structure is presented for diastereomer **6,**

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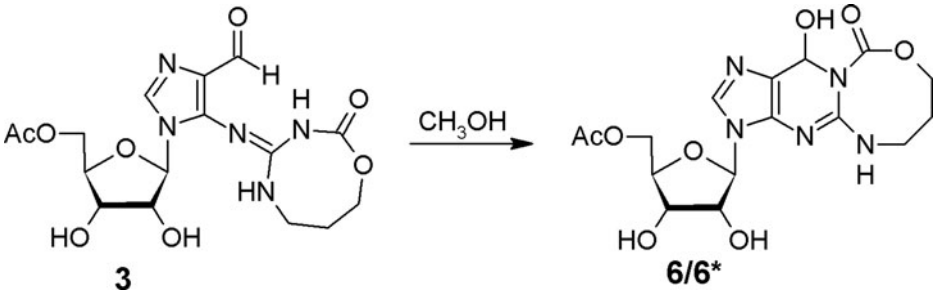
which is completely analogous as isomer **6***. The main difference between ^{1}H NMR spectra of **3** and **6** is the disappearance of resonance at $\delta_H = 9.47$ ppm (CHO) attributed to the aldehyde proton and appearance of a singlet at $\delta_H = 6.39$ ppm (H12). This new resonance gave one-bond ^{1}H-^{13}C correlation to carbon atom at $\delta_C = 88.9$ ppm (C12) and two long-range correlations to carbon atoms at $\delta_C = 115.8$ (C12a) and 150.4 ppm (C10). The ^{13}C signal at 115.8 ppm could be attributed to C12a carbon atom based on the observed HMBC correlation with H2 signal at $\delta_H = 7.50$ ppm (Scheme 3). In addition, ^{13}C signal at $\delta_C = 150.4$ ppm revealed long-range correlation with H8 methylene protons which supports the evidence for the cyclization process leading to the formation of the tricyclic ring system in compound **6**. The connectivities through 2- and 3-chemical bond atoms observed in the ^{1}H-^{13}C HMBC experiment are shown in Scheme 3. This transformation provides opportunities for the synthesis of potentially valuable novel ring-expanded nucleoside containing a 5:6:8-fused heterocyclic ring system.[11]



SCHEME 4 Transformation of **3** into diastereomers **6**/**6*** in CH_3OH.

CONCLUSIONS

In conclusion, the above results show that, depending on the irradiation conditions, the major photochemical transformations of 2′,3′,5′-tri-*O*-acetylated derivative of 2-azidopurine ribonucleoside **1** in aqueous solution involve photooxidation and photoreduction to respective 2-nitro (**5**) and 2-amino (**4**) derivatives, and unprecedented formation of 1-(5′-*O*-acetyl-*β*-D-ribofuranosyl)-5-[(2-oxo-1,3,5-oxadiazocan-4-ylidene)amino]-*1H*-imidazole-4-carbaldehyde (**3**). Photooxidation is the major process occurring under aerobic irradiation whereas in the absence of oxygen, **3** is formed as the major photoproduct. Our results enlarge the scope of the photoreactivity of azidopurine ribonucleosides used as photoaffinity labeling probes in the photocrosslinking technique and tools in synthetic organic chemistry.

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EXPERIMENTAL SECTION

Synthetic and Analytical Methods

High-performance liquid chromatography analysis was performed with Agilent 1260 Infinity system equipped with diode-array UV-vis detectors. Absorption spectra were measured using a Cary 300 Bio Varian spectrophotometer in CH_3CN. The high-resolution mass spectrometry (HRMS) analyses were performed using ESI-TOF system (Bruker). The NMR spectra were measured in $CDCl_3$ on Bruker Avance III 700 spectrometer operating at 700 MHz for ^1H and 175 MHz for ^{13}C. The QCI-P CryoProbe^{TM} 5 mm was used.

Spectral Data for Substrate

Characteristic Data for Compound 1

R_f 0.32 ($CHCl_3$:CH_3OH 95:5, v/v). UV (CH_3CN) λ_{max}/nm: 230 and 286. ^1H NMR ($CDCl_3$) δ: 8.91 (s, 1H, $H6_a$), 8.81 (s, 1H, $H6_t$), 8.09 (s, 1H, $H8_a$), 8.02 (s, 1H, $H8_t$), 6.16 (d, $J = 4.2$ Hz, 1H, H1'$_t$), 6.13 (d, $J = 4.2$ Hz, 1H, H1'$_a$), 5.79 (t, $J = 5.2$ Hz, 1H, H2'$_a$), 5.74 (t, $J = 4.8$ Hz, 1H, H2'$_t$), 5.56 (m, 1H, H3'$_t$), 5.55 (m, 1H, H3'$_a$), 4.42 (m, 2H, H4'$_{a,t}$), 4.40 (m, 4H, H5',H5"$_{a,t}$), 2.11–1.97 (s, 18H, CH_3-$Ac_{a,t}$). ^{13}C NMR ($CDCl_3$) δ: 169.6–168.4 (C=O-Ac_a), 169.3–168.5 (C=O-Ac_t), 160.3 ($C2_a$), 156.3 ($C2_t$), 151.6 ($C4_a$), 151.0 ($C4_t$), 149.6 ($C6_a$), 149.2 ($C6_t$), 142.1 ($C8_a$), 141.3 ($C8_t$), 130.9 ($C5_a$), 129.8 ($C5_t$), 88.8 (C1'$_t$), 85.1 (C1'$_a$), 81.9 (C4'$_a$), 79.2 (C4'$_t$), 73.9 (C2'$_a$), 72.1 (C2'$_t$), 70.3 (C3'$_a$), 69.4 (C3'$_t$), 62.5 (C5'$_a$), 61.9 (C5'$_t$), 19.7–19.4 (CH_3-$Ac_{a,t}$). HRMS (ESI-TOF): calcd for $C_{16}H_{17}N_7O_7Na$ $[M + Na]^+$ 442.1082, found 442.1081.

UV Irradiation

An aqueous solution (200 mL) of **1** (75 mg) was irradiated in a photoreactor with a 200 W, high-pressure immersion Hg lamp (TQ 150) equipped with a cut-off Pyrex filter ($\lambda > 300$ nm) for 12 min (anaerobic conditions: mixture was additionally degassed for 60 min under argon). The photoproducts formed were isolated and purified by preparative reversed-phase HPLC using an HPLC instrument equipped with diode-array UV-vis detector. The column was Agilent Zorbax SB-C_{18} 5.0 μm 9.1 × 150 mm, eluted with H_2O/CH_3CN using a linear gradient of 10–45% of CH_3CN over 25 min at a flow rate of 1.8 mL/min.

Spectral Data for Photoproducts

Characteristic Data for Compound 3

UV (CH_3CN) λ_{max}/nm: 251. ^1H NMR (DMSO-d_6) δ: 9.47 (s, 1H, CHO), 7.78 (s, 1H, N3-H), 7.72 (s, 1H, N5-H), 7.66 (s, 1H, H2A), 5.49 (d, $J =$

4.8 Hz, 1H, H1'), 4.26 (m, 1H, H2'), 4.25–4.10 (m, 2H, H5', H5"), 4.09 (m, 2H, H8B), 4.07 (m, 1H, H3'), 3.98 (m, 1H, H4'), 2.69 (m, 2H, H6B), 2.05 (s, 3H, CH_3-Ac), 1.96 (m, 2H, H7B). ^{13}C NMR (DMSO-d_6) δ: 183.4 (CHO), 170.1 (C=O-Ac), 169.0 (C4B), 168.9 (C2B), 146.6 (C5A), 132.8 (C2A), 128.0 (C4A), 87.3 (C1'), 81.5 (C4'), 73.9 (C2'), 70.6 (C3'), 65.7 (C8B), 64.5 (C5'), 29.9 (C6B), 25.3 (C7B), 21.0 (CH_3-Ac). HRMS (ESI-TOF): calcd for $C_{16}H_{20}N_5O_8$ $[M–H]^-$ 410.1347, found 410.1346.

Characteristic Data for Compound 4

UV (CH_3CN) λ_{max}/nm: 220, 246, and 308. ^{1}H NMR ($CDCl_3$) δ: 8.54 (s, 1H, H6), 7.68 (s, 1H, H8), 5.87 (d, $J = 5.1$ Hz, 1H, H1'), 5.83 (t, $J = 5.2$ Hz, 1H, H2'), 5.56 (t, $J = 5.1$ Hz, 1H, H3'), 4.94 (s, 2H, NH_2), 4.30 (m, 1H, H4'), 4.27 (m, 1H, H5'), 4.22 (m, 1H, H5"), 1.97–19.1 (s, 9H, CH_3-Ac). ^{13}C NMR ($CDCl_3$) δ: 170.5–169.4 (C=O-Ac), 159.8 (C2), 152.6 (C4), 150.5 (C6), 140.8 (C8), 128.8 (C5), 86.3 (C1'), 79.8 (C4'), 72.7 (C2'), 70.5 (C3'), 62.9 (C5'), 20.7–20.5 (CH_3-Ac). HRMS (ESI-TOF): calcd for $C_{16}H_{20}N_5O_7$ $[M + H]^+$ 394.1363, found 394.1378.

Characteristic Data for Compound 5

UV (CH_3CN) λ_{max}/nm: 218 and 272. ^{1}H NMR ($CDCl_3$) δ: 9.20 (s, 1H, H6), 8.48 (s, 1H, H8), 6.29 (d, $J = 5.5$ Hz, 1H, H1'), 5.73 (t, $J = 5.6$ Hz, 1H, H2'), 5.57 (t, $J = 5.3$ Hz, 1H, H3'), 4.50 (m, 1H, H4'), 4.42 (m, 2H, H5', H5"), 2.14–2.03 (s, 9H, CH_3-Ac). ^{13}C NMR ($CDCl_3$) δ: 170.2–169.6 (C=O-Ac), 154.7 (C2), 151.9 (C4), 149.9 (C6), 147.3 (C8), 137.2 (C5), 86.8 (C1'), 81.2 (C4'), 73.7 (C2'), 70.8 (C3'), 63.1 (C5'), 20.8–20.4 (CH_3-Ac). HRMS (ESI-TOF): calcd for $C_{16}H_{17}N_5O_9Na$ $[M + Na]^+$ 446.0924, found 446.0939.

Characteristic Data for Compound 6/6*

UV (CH_3CN) λ_{max}/nm: 253 and 305. ^{1}H NMR ($CDCl_3$) δ: 7.50 (s, 1H, H2), 7.47 (s, 1H, H2*), 6.39 (s, 1H, H12), 6.38 (s, 1H, H12*), 5.71 (d, $J =$ 5.0 Hz, 1H, H1'), 5.67 (d, $J = 4.7$ Hz, 1H, H1'*), 4.52 (m, 2H, H8*), 4.32 (m, 1H, H2'*), 4.31 (m, 2H, H4', H4'*), 4.30 (m, 3H, H3'*, H5', H5"), 4.29 (m, 3H, H8, H2'), 4.27 (m, 1H, H3'), 4.19 (m, 2H, H5', H5"), 3.11 (m, 2H, H6), 2.71 (m, 2H, H6*), 2.13 (m, 2H, H7), 2.11 (m, 2H, H7*), 1.99 (s, 3H, CH_3-Ac), 1.97 (s, 3H, CH_3-Ac*). ^{13}C NMR ($CDCl_3$) δ: 169.7 (C=O-Ac, C=O=Ac*), 151.1 (C4a, C4a*), 150.4 (C10, C10*), 133.8 (C2*), 133.7 (C3a, C3a*), 133.4 (C2), 116.2 (C12a*), 115.8 (C12a), 90.1 (C1'*), 89.9 (C1'), 88.9 (C12, C12*), 83.0 (C4', C4'*), 75.8 (C2'), 75.4 (C2'*), 71.5 (C3', C3'*), 70.5 (C8, C8*), 63.5 (C5' C5'*), 24.7 (C6, C6*), 23.3 (C7, C7*), 20.7 (CH_3-Ac, CH_3-Ac*). HRMS (ESI-TOF): calcd for $C_{16}H_{22}N_5O_8$ $[M + H]^+$ 412.1505, found 412.1508.

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