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Synthesis of an acyclic nucleoside analog of highly fluorescent luminarosine

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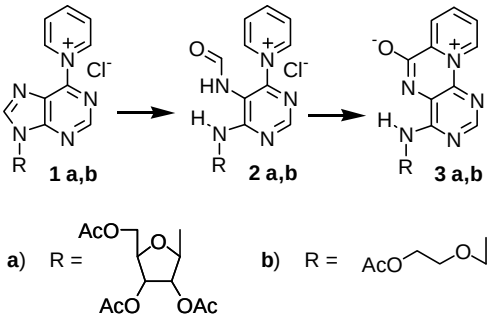
ABSTRACT

Photoirradiation of 1-{9-[(2-acetoxyethoxy)methyl]-9*H*-purin-6-yl}-pyridinium chloride (**1b**) in aqueous solution leads to two photoproducts, namely 1-{5-formamido-6-[(2-acetoxyethoxy)methylamino]pyrimidin-4-yl}pyridinium chloride (**2b**) and 1-(6-(acetoxymethyl)-5,5a,6,8-tetrahydrooxazolo[4,3-*e*]purin-4-yl)pyridinium chloride (**6**), which constitutes a new heterocyclic system. Further, photosensitized irradiation of **2b** gave the desired acyclic nucleoside analog of the highly fluorescent luminarosine **3b**.

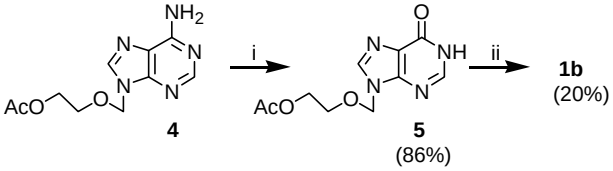
Luminarosine **3a** is the highly fluorescent nucleoside obtained by photochemical transformation of *N*-[9-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)-purin-6-yl]-pyridinium chloride **1a** (Scheme 1).[1–3] The remarkable photophysical properties of this fluorophore[4] make it very useful for various cellular studies.[5,6] Uniform labeling of cells using luminarosine dye has been demonstrated. The dye has also been used successfully to measure forskolin-stimulated I^-/Cl^- and I^-/NO_3^- exchange in cystic fibrosis transmembrane conductance regulator (CFTR)-expressing cell lines by fluorescence microscopy and microplate reader instrumentation, demonstrating substantially improved optical and cellular properties over other intracellular halide indicators.[5] The fluorophore can also be used to label DNA/RNA oligonucleotides;[7] however, its application in this field is somewhat limited, mainly due to its high susceptibility to acid-promoted anomerization reactions.[8] In order to overcome this problem and simplify the fluorescent labeling of oligonucleotides with luminarosine, we have undertaken the synthesis of an acyclic nucleoside analog **3b** of this fluorophore, in which the tri-*O*-acetylribose residue is replaced by a (2-acetoxyethoxy)methyl moiety.

Synthesis of acycloluminarosine **3b** required the preparation of *N*-[9-(2-acetoxyethoxymethyl)-9*H*-purin-6-yl]-pyridinium chloride **1b**, which was performed starting from acycloadenosine[9] **4** (Scheme 2). In the first step, compound **4** was subjected to a deamination reaction to give acycloinosine **5**,[10] which was subsequently converted into **1b** according to an earlier procedure[11] in 20% yield.

As outlined in Scheme 1, the previously described two-step photochemical synthesis of tri-*O*-acetylluminarosine (**3a**) involves transformation of the starting pyridinium salt **1a** into the imidazole ring-opened, formamidopyrimidine photoproduct **2a** in the first step. This transformation occurs upon photoirradiation of **1a** with near UV light ($\lambda > 300$ nm) in an aqueous solution (pH 5.5–6.0) in the absence of oxygen and is almost quantitative. In the second step, **2a** is converted into luminarosine by further photosensitized irradiation of the solution (pH 7.5, adjusted with $NaHCO_3$) under aerobic conditions using *N*-(9-methylpurin-6-yl)pyridinium chloride as a sensitizer. Hence, we expected that analogous photo-



Scheme 1.



i) $NaNO_2$, AcOH 50% aq, 60 °C, 2 h,
ii) pyridine, 4-$ClC_6H_4OP(O)Cl_2$, r.t., 24 h

Scheme 2.

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reactions in the case of **1b** should be possible in order to prepare the desired acyclic nucleoside analog **3b** of luminarosine.

Thus, an aqueous solution of compound **1b** ($c = 10^{-4}$ mol/dm^3) was subjected to irradiation in a 2L photochemical reactor with a high pressure immersion mercury lamp (TQ -150, Original Hanau) equipped with a Pyrex® cut-off filter ($\lambda > 300$ nm). The course of the reaction was followed spectrophotometrically and by HPLC. The reaction was carried out until almost complete disappearance of the starting pyridinium salt. HPLC analyses of the irradiated solutions (Fig. 1A and B) revealed the formation of two photoproducts with the relative amounts being dependent on the pH of the starting solution.

One of these photoproducts, which was formed as a major product when the pH of the irradiated solution was adjusted to 6.1 (Fig. 1B), was identified based on UV (Fig. 1C), NMR, and EI-MS spectral data[10] as the expected intermediate in the planned two-step photochemical synthesis of acycloluminarosine, namely *N*-{5-formamido-6-[(2-acetoxyethoxy)methylamino]-pyrimidyn-4-yl}-pyridinium chloride **2b** (Scheme 3).

Lowering the pH of the irradiated solution of **1b** to 5.0 resulted in the formation of **6** as the major product (Fig. 1A). Its UV spectrum (Fig. 1C) indicated fundamental changes in the chromophore system, but the molecular mass and elemental composition were identical with those of the substrate **1b**. The structure of this compound was established as 1-(6-(acetoxymethyl)-5,5a,6,8-tetrahydro-oxazolo[4,3-*e*]purin-4-yl)pyridinium chloride on the basis of different ^{1}H, ^{13}C, and ^{15}N NMR experiments (Table 1, see Fig. 2 for atom numbering).

From the one-dimensional ^{1}H NMR it was clear that the pyridinium cationic substituent remained intact and one of the purine protons (H-2 or H-8) had disappeared. The most profound changes occurred in the part of the spectrum consisting of three groups of signals (one singlet and two triplets), corresponding to three CH_2 groups, which were replaced by a set of six highly coupled multiplets. The multiplets corresponded to six protons, but each gave its own separate signal indicating its unique magnetic character. The most obvious explanation of this nonequivalence is the forma-

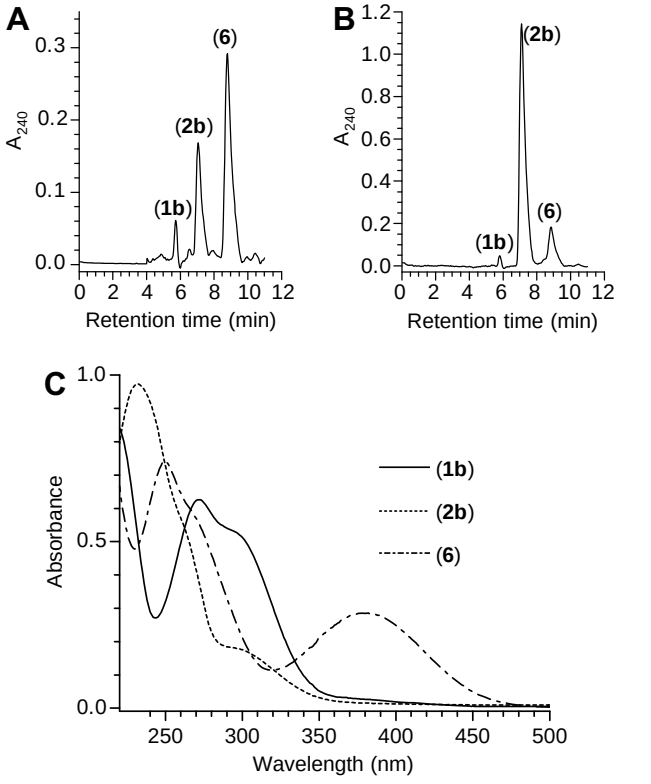
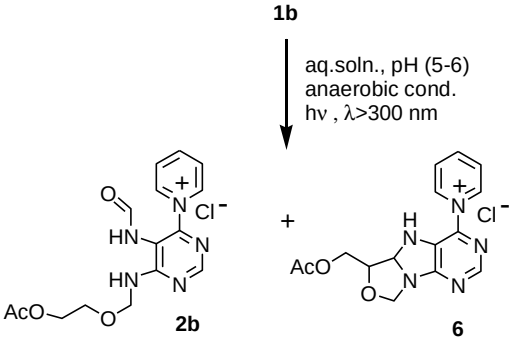


Figure 1. HPLC analyses after irradiation of **1b** in aqueous solution at pH 5.0 (A) and pH 6.1 (B), and the UV spectra (H_2O) of the starting pyridinium salt **1b** and photoproducts **2b** and **6** (C).



Scheme 3.

Table 1
NMR data for compound **6** (D_2O solution, DSS standard)

Atom position	^{1}H (400 MHz), δ	^{13}C (100 MHz), δ	^{15}N (40.5 MHz), δ	NOESY
N_{Py}			212.05	
α_{Py}	9.20 (dd, $J_{\alpha\beta}$ = 6.9 Hz, $J_{\alpha\gamma}$ = 1.1 Hz)	144.80		
β_{Py}	8.24 (pseudo-t, $J_{\alpha\beta}$ = 6.9 Hz, $J_{\beta\gamma}$ = 7.5 Hz)	131.35		
γ_{Py}	8.70 (dd, $J_{\beta\gamma}$ = 7.5 Hz, $J_{\alpha\gamma}$ = 1.1 Hz	151.04		
1			240.18	
2	8.19 (s)	151.46		
3			172.20	
4		169.07		
5		130.35		
6		136.05		
7			61.73	
8	5.88 (d, $J_{8,12}$ = 5.4 Hz)	81.14		H-10b, H-12
9			113.59	
10a	5.44 (d, $J_{10a,10b}$ = 6.3 Hz)	83.46		H-10b
10b	4.68 (d, $J_{10a,10b}$ = 6.3 Hz)			H-8, H-10a
12	4.42 (ddd, $J_{12,13a}$ = 3.6 Hz, $J_{8,12}$ = 5.4 Hz, $J_{12,13b}$ = 8.4 Hz)	78.75		H-10b, H-8, H-13a, H-13b
13a	4.34 (dd, $J_{13a,13b}$ = 12.2 Hz, $J_{12,13a}$ = 3.6 Hz)	65.63		H-13b
13b	4.17 (dd, $J_{13a,13b}$ = 12.2 Hz, $J_{12,13b}$ = 8.4 Hz)			H-13a, H-12
C=O		176.19		
CH_3	1.88 (s)	22.50		

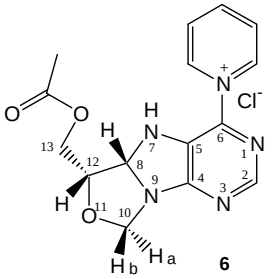


Figure 2. Numbering of atoms in photoproduct **6**.

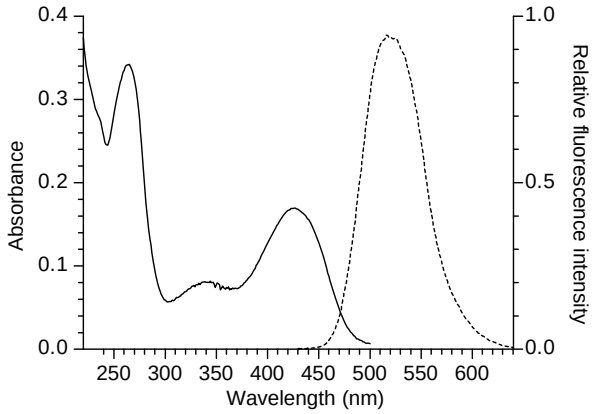


Figure 3. UV–vis absorption (solid line) and steady-state emission (dashed line) spectra of acycloluminarosine **3b** in water.

tion of an additional ring, creating an asymmetric center and thus differentiating the protons. From two-dimensional ^{1}H NMR spectra (HMBC and HSQC experiments), the presence of an X–CH_2–X–CH[CH(X)]–CH_2–X unit (X denoting a heteroatom, oxygen, or nitrogen) was established. Mutual relations between atoms could be assigned using heteronuclear correlation techniques (^{1}H–^{13}C and ^{1}H–^{15}N).

Both H-13a and H-13b showed three-bond correlations with the acetate carbonyl carbon atom as well as with C-8. Hydrogen H-12 was correlated through three bonds with C-10, and both H-10a and H-10b showed correlations with C-4, C-8, and C-12. The bridgehead position of H-8 was proved by its three-bond correlation with carbon atoms C-4, C-5, C-10, and C-13. The H-2 atom correlated with C-4 and C-6. Heteronuclear (^{1}H–^{15}N) correlation spectra showed multibond contacts of H-2 with N-1 and N-3; of H-12 with N-7; of H-8, H-10a, and H-10b with N-9; and of H-α_{Py}, H-β_{Py} with the pyridinium nitrogen. The structure of compound **6** has two stereogenic centers, which can lead to four possible diastereoisomers. NOE dipole interaction experiments established that both H-8 and H-12 interact with H-10b but not with H-10a, which implies that the three protons (H-8, H-10b, and H-12) are positioned on the same side of the oxazolidine ring, leaving H-10a on the other side. This would mean that the formation of compound **6** proceeds stereoselectively. Such high stereoselectivity and the formation of an additional ring were noted previously in a similar class of heterocycles.[12–16] HPLC analysis of the reaction mixture showed no other compound of diastereoisomeric structure.

The final step of the synthesis of acycloluminarosine (**3b**) involving photochemical transformation of **2b** (Scheme 1) was carried out according to the above-mentioned previously developed procedure.[4] Thus an aqueous solution of **2b** was neutralized with $NaHCO_3$ to pH ~ 7.5, 1.5 equiv of *N*-(9-methylpurin-6-yl)pyridinium chloride was added as the photosensitizer and the mixture was subjected to irradiation with near UV light ($\lambda > 300$ nm) under aerobic conditions. The progress of the reaction was monitored by UV–vis spectrophotometry, and irradiation was continued until no further increase in the absorption band at 428 nm, characteristic of the luminarosine chromophore, was observed. The irradiated solution was then extracted with $CHCl_3$, and the organic layer was concentrated and purified by preparative reverse phase HPLC to give the desired acycloluminarosine **3b**.[17] This compound exhibited comparable absorption and emission properties to tri-*O*-acetylluminarosine (**3a**), namely a characteristic absorption band at 428 nm and intense fluorescence at 520 nm (cf. Fig. 3). Its identity was further proved by NMR characterization.[10]

In summary, the synthesis and photochemical reactions of a novel pyridinium salt *N*-[9-(2-acetoxyethoxymethyl)-9-*H*-purin-6-yl]-pyridinium chloride (**1b**) are described. The obtained 1-(6-(acetoxymethyl)-5,5a,6,8-tetrahydrooxazolo-[4,3-*e*]purin-4-yl)pyridinium chloride (**6**) is a new heterocyclic system. The acyclic analog of luminarosine **3b** has potential application as a fluorescent label for any sequence of oligonucleotides.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2008.10.045.

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10. Spectral data:
Compound **1b**: ^{1}H NMR (D_2O), δ 2.02 (s, 3H, OAc), 3.97 (t, J = 4.7 Hz, 2H, OCH_2CH_2), 4.25 (t, J = 4.7 Hz, 2H, OCH_2CH_2), 5.98 (s, 2H, OCH_2Base), 8.46 (t, J = 7.1 Hz, 2H, β-H), 8.98 (m, 2H, H-2, γ-H), 9.22 (s, 1H, H-8), 10.01 (d, J = 5.7 Hz, 2H, α-H), ^{13}C NMR (D_2O), δ 23.00, 66.28, 70.45, 76.57, 127.67 (C-5), 131.04, 146.01, 149.23, 152.82, 152.90, 154.86, 158.30, 176.58, ESI MS m/z 314 (M^+), calculated m/z 314.12 for $C_{15}H_{16}N_5O_3^+$, UV λ_{max} (H_2O) 273, 295.
Compound **2b**: ^{1}H NMR (D_2O), δ 2.05 (s, 3H, OAc), 3.82 (t, J = 4.55 Hz, 2H, OCH_2CH_2), 4.24 (t, J = 4.55 Hz, 2H, OCH_2CH_2), 5.06 (s, 2H, OCH_2Base), 8.14 (s, CHO), 8.30 (t, J = 6.82 Hz, 2H, β-H), 8.65 (s, 1H, H-2), 8.98 (t, J = 1.13 Hz, 1H, γ-H), 9.06 (d, J = 5.7 Hz, 2H, α-H), ESI MS m/z 332 (M^+), calculated m/z 332.14 for $C_{15}H_{18}N_5O_4^+$, UV λ_{max} (H_2O) 235.
Compound **3b**: ^{1}H NMR (D_2O), δ 2.02 (s, 3H, OAc), 3.80 (t, J = 4.66 Hz, 2H, OCH_2CH_2), 4.22 (m, 2H, OCH_2CH_2), 5.20 (d, J = 7.14 Hz, 2H, OCH_2Base), 8.12 (t, J = 7.1 Hz, 1H, H-2), 8.35 (s, 1H, H-9), 8.46 (t, J = 7.1 Hz, 1H, H-3), 9.14 (d, J = 8.5 Hz, 1H, H-4), 10.22 (d, J = 6.3 Hz, 1H, H-1), ^{13}C NMR (D_2O), δ 23.70, 66.34, 68.13, 76.58, 125.18, 126.13, 127.72, 128.78, 130.88, 141.09, 149.33, 158.94, 160.59, 167.78, 171.07, ESI MS m/z 329 (M^+), calculated m/z 329.11 for $C_{15}H_{15}N_5O_4$, UV λ_{max} (H_2O) 265, 428.
Compound **5**: ^{1}H NMR (DMSO), δ 1.94 (s, 3H, OAc), 3.69 (t, J = 4.7 Hz, 2H, OCH_2CH_2), 4.07 (t, J = 4.7 Hz, 2H, OCH_2CH_2), 5.55 (s, 2H, OCH_2Base), 8.09 (s, 1H, H-2), 8.23 (s, 1H, H-8), 12.36 (s, 1H, OH).
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17. Preparation of **3b**: Compound **2b** (30 mg) was dissolved in water (1.5 L) to A_{235} = 0.75 (0.5 cm cuvette), *N*-(9-methylpurin-6-yl)pyridinium chloride (1.5 equiv) was added as sensitizer. The pH of the solution was adjusted to 7.5 with a freshly prepared saturated solution of $NaHCO_3$. The solution was irradiated in a photochemical reactor under aerobic conditions for 30 min. The course of the reaction was followed spectrophotometrically. After the irradiation, the reaction mixture was extracted with $CHCl_3$. The organic layer was evaporated in vacuo. The residue was purified by column chromatography on SiO_2 (0–10% methanol in $CHCl_3$) to afford 14 mg of the product.